Terrestrial Carbon Sequestration

Field Guide for Sampling and Analysis for Sites Remediated with Soil Amendments

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APPENDIX 1

SUGGESTED FORMAT FOR SITE INFORMATION

Appendix 1 – Suggested Format for Site Information

Info Type	Site Name
Site Location (city/state, GPS coordinates)	
Site Owner/ Responsible Party	
Site Area and Surface/Subsurface Features	
General Site Description and History (include date ranges for all human activities)	
Current Site Use	
Future Site Use	
Land Uses and Activities Surrounding the Site	
Properties of Native and Contaminated Soils (including chemical, geological, and geotechnical data, as available)	
Site Slope and Watershed	
Leaching and Runoff Potential	
Nature of Amendment(s), including origin, composition, analytical data, regulatory status/disposition (e.g., Is it a waste?)	
Alternate Management Method (How would the material be used, managed, or disposed if not used as a soil amendment?)	
Date of Amendment Applied	
Amendment Application Rate/Amount	

Appendix 1 – Suggested Format for Site Information

Info Type	Site Name
Thought Process for Selected Amendment (local availability, to stabilize zinc, etc.)	
Regulations Followed for Amendment Application	
Equipment and Methods Used for Amendment Application	
Equipment Used in Transportation (including estimates of fuel and energy use)	
Site Operation and Maintenance (O&M) Activities (including estimates of fuel and energy use)	
Climatic Variables for Site, including monthly temperature (range and average temperatures), as well as monthly total precipitation	
Site Vegetation (in area in general and noted specifically for site)	

References: *Citations for the information above should be footnoted and a reference list for the site information table should be included here.*

APPENDIX 2

EXAMPLE SAMPLING APPROACH

Appendix 2 - Example Sampling Approach for Carbon Sequestration Sites

This appendix presents an example sampling approach, including a general statistical sampling design, for sampling sites remediated with soil amendments to support carbon accounting efforts. Sampling designs for carbon accounting should be site-specific. They should be developed based on existing data for the specific site and its amendments, site-specific data needs, and site-specific data quality objectives for the study. The basic approach included in this appendix provides an example sampling approach for consideration and optimization during planning activities to assist project teams that undertake carbon sequestration studies.

The general sampling approach outlined in this appendix uses a composite sampling approach. EPA sampling guidance (EPA 2002) recommends composite sampling when:

1. A primary goal is to reduce cost by having fewer analyses;
2. The sample acquisition and handling process can be separated from the measurement process (i.e., field versus laboratory);
3. The concentrations of the target analytes can be measured accurately in the individual sample aliquots as well as in the composite, so that when the compositing process is carried out properly, the measured concentration of the analyte in the composite is expected to be equal to the average measurements made on the individual aliquots.
4. Variability among similarly formed composite samples is less than the variability of the individual aliquots; and
5. Composite sampling is compatible with the study goal of estimating a population mean, while information that is lost with compositing (e.g., concentration extremes or hot spot locations) is not needed.

These criteria are generally consistent with the goals and data needs for carbon sequestration studies at remediated sites; therefore, for the purposes of most carbon sequestration site studies, composite sampling approaches are appropriate. Site-specific teams will identify specific project goals and site-specific approaches as part of project planning.

Statistical Design

A randomized complete block design should be considered to evaluate the impact of organic soil amendments in remediating contaminated sites either devoid or very low in carbon (both above and below ground). A randomized complete block design will permit an estimate of the mean concentration for each target analyte and sample matrix of interest (along with an estimate of the error associated with the mean) to allow qualitative or statistical comparisons of data across treatment areas and over time.

Due to the high variability associated with these sites, it is suggested that each type of treatment (that is, each type of amendment application) be sampled at least three times for each sampling event. Thus, the amended site could be divided into three replicate evaluation areas (blocks). Each block is further divided into equal parts (cells), with the number of cells equal to the number of treatments plus the number of controls or reference areas. For logistical purposes (biosolids application and management), the area of a single cell should be between 50 and 100 square meters (or approximately 500 and 1000 square feet [ft^2]), equal across all treatments and across all blocks.

All treatment areas and reference areas (controls) should be represented in each of the three blocks. For example, Figure 1 shows a site with four different biosolids treatments (Treatments A, B, C, and D) and one control (no biosolids application); this site would require three blocks (rows) each with five cells (represented as five columns across the three rows in Figure 1), for a total of 15 cells. At sites where carbon sequestration pilot studies are planned, or where the locations of blocks and cells can otherwise be specified before amendment application, each treatment/control should be randomly placed in the cells within each block.

Additionally, the same treatment should not be repeated more than two times in any given row to prevent the introduction of bias from uncontrollable and unknown environmental factors. Figure 1 also illustrates these recommendations.

Three to five composite samples should be collected per treatment cell, depending on such factors as the size of treatment cell, the anticipated internal variability of the amendment or treated mixture, and other external or spatial variability (e.g., due to the amendment application process or site conditions). Due to high variability associated with slag/waste material, compositing is recommended for samples to provide a better estimate of the true cell average. For each target analyte, a mean concentration and associated error (e.g., standard deviation or upper confidence limit) can be calculated for each treatment cell based on the three to five composite sample results collected in that cell.

Project teams may wish to refine the example composite sampling approach described above for specific sites; a useful reference for refinement would be EPA's sampling design guidance (EPA 2002). Section 10.2.5 (p. 127) of this guidance provides a methodology for determining the appropriate number of composite samples and aliquots (individual samples used in the composite sample) to collect per composite sample based on data variability estimates and costs. Data variability can be estimated based on reviews of historical data or through a preliminary sampling event. If an estimation of error/variability is unavailable, it is recommended that four aliquots be combined to form each composite sample. Each composite sample represents a mean measurement of a constituent of interest for a sampled cell. The number of aliquots in each composite can be adjusted following the first round of sampling if statistical evaluation determines that the number of aliquots is insufficient or excessive for defining the true variation and therefore, the true mean concentration of the target analyte.

Previously Amended Sites

For sites where amendments have already been applied and a randomized block design is logistically impossible or impractical, the example sampling approach described above can be modified in consultation with a statistician. In cases where individual treatments have been applied to areas or parcels that are geographically non-contiguous, each area can be divided into three blocks, with three to five random composite samples collected in each of the these three blocks.

Composite Sampling Method (Treatment/Control Cell Sampling)

Three to five **randomly selected** sample locations will be flagged (located with a geographic positioning system [GPS]) in each cell of each block. A 1-meter (m) by 1-m quadrant will be located around each sample location with the flag centered in the middle of the quadrant. One aliquot will be collected from each quarter of a single quadrant from each depth of interest. The aliquots from each sample depth will be combined separately, resulting in one composite sample comprised of shallow depth aliquots and one composite sample comprised of the greater depth aliquots. These same quadrants will be sampled before treatment, at the time of treatment (Time=0), and in multiple post-application sampling events (e.g., 1-year, 3-year, and 5-year) as decided by the project team. (Only post-application sampling events will be possible at previously amended sites). If budgetary constraints require a reduction in sample numbers, an entire block can be eliminated from the design. However, given the known variability associated with these sites, reduction in the number of samples and blocks should be considered with extreme caution.

Reference Areas

At previously amended sites, reference samples from a nearby area where no soil amendments have been applied (native soil, uncontaminated) need to be collected. Other types of control samples, such as from

contaminated but untreated soils, might also be collected. Three sample cells measuring 50 to 100 square meters each are again recommended for the reference area. For each cell, three to five randomly selected locations (this number should be equivalent to the total number of samples collected per event per treatment) will be flagged (and located using a GPS). Similar to the block design sampling, a 1-m by 1-m quadrant will be located around each sample location with the flag centered in the middle of the quadrant. One aliquot will be collected from each quarter of a single quadrant from each depth of interest and combined to form one composite sample per depth. These reference quadrants will be sampled at the same times as the treatment cell samples (e.g., Time=0, 1 year, 3 years, 5 years, etc.). Statistical evaluation of Time=0 analytical results may justify a reduction in sample numbers within the reference area due to lower variability than would be expected within the contaminated areas.

Facts to Consider When Composite Sampling

According to the EPA sampling guidance (EPA 2002), composite samples must meet the following criteria:

1. Individual aliquots comprising the composite must be of equal size (volume/mass) and shape;
2. The number of aliquots comprising each composite must be the same; and
3. A single sub-sample from the well-mixed composite is selected for analysis.

If these criteria are not met, statistical analysis becomes more complicated although not impossible (Gilbert 1987). Other considerations and assumptions associated with the suggested composite sampling approach include:

1. The anticipated concentration levels for most composites will exceed detection limits of the analytical methods so that difficulties of mean estimation in the presence of non-detects are avoided;
2. Compositing will not affect sample integrity for most target analytes. However, project teams desiring to determine bulk density on in-tact cores should collect separate discrete in-tact cores for bulk density analysis at each depth of interest along with the composite samples collected in each quadrant;
3. Information regarding concentration levels for individual samples, their spatial or temporal locations, and their population variability is not considered important. Spatial correlations or correlations between the concentrations of two or more target analytes are also not considered important;
4. There are assumed to be no practical difficulties that will impede the selection of multiple aliquots according to a statistical sampling design (i.e., simple random); and
5. It is assumed that individual aliquots can be adequately homogenized. Sample matrixes that cannot be mixed into a homogeneous composite may require additional sample processing steps (e.g., sieving or grinding).

The above assumptions should be considered by project teams for their specific sites and study objectives, to assess the need to modify the recommended sampling or analytical approach outlined in this field guide, or to identify an alternate data collection approach.

REFERENCES

EPA. 2002. *Guidance on Choosing a Sampling Design for Environmental Data Collection, for Use in Developing a Quality Assurance Project Plan.* QA/G-5S. Pp 119-141.

Gilbert, R.O. 1987. *Statistical Methods for Environmental Pollution Monitoring.* John Wiley &Sons, Inc., New York.

Appendix 2 - Example Sampling Approach for Carbon Sequestration Sites

FIGURE 1

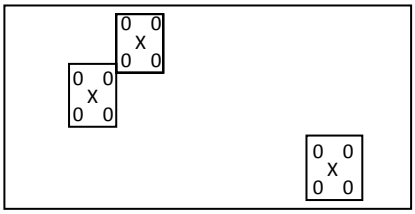
Example Sampling Approach/No Prior Application of Amendments

For this example site, the following conditions are assumed:

- Four biosolids treatments (A, B, C, D) with one control (untreated soil, or reference area)
- Sample cores to be collected to a depth of 30 centimeters (cm) and divided into 2 segments (0-15 cm and 15-30 cm)
- Treatments per depth compared for the following sampling events: pretreatment, time 0, 1 year post-treatment, 3 years post-treatment, 5 years post-treatment, and/or 10 years post-treatment (as decided by project team)
- No prior soil amendments/treatments implemented

	Cells 1-3	Cells 3-6	Cell 6-9	Cell 9-12	Cell 12-15
Block 1	Treatment C (50 to 100 m^2)	Control	Treatment B	Treatment A	Treatment D
Block 2	Treatment A	Treatment D	Treatment C	Control	Treatment B
Block 3	Control	Treatment B	Treatment A	Treatment D	Treatment C

Treatment D/Block 2/Cell 5 – Example Sampling Approach



3 random sampling locations, one 4-point composite per location:

X = random sample location within a treatment cell; sample location flagged at the center of a 1 meter by 1 meter sample quadrant; samples to be collected within these same quadrants each year of sampling.

0 = collection site of sample aliquots per depth (0-15 cm, 15-30 cm)

Calculation of number of composite samples per sampling event:

(5 treatments/control) X (3 blocks) X (3 sample quadrants) X (2 depths) = 90 composite samples

APPENDIX 3

STANDARD OPERATING PROCEDURE FOR CARBON/NITROGEN ELEMENTAL ANALYSIS

U.S. Environmental Protection Agency (EPA). 1998. GPEP TERA SOP 3.01, Version 2.00, March 31, 1998. "*Carbon/Nitrogen Elemental Analysis.*" U.S. Environmental Protection Agency National Health and Environmental Effects Research Laboratory, 200 S.W. 35th Street, Corvallis, OR 97333. Attaching Attaching: Memorandum from Mark Johnson to File, March 26, 1997. Subject: Revision of DQOs for GPEP TERA SOP 3.01.

A. Signature Page

Carbon/Nitrogen Elemental Analysis

TERA SOP Number 3.01
Version: 2.00

31 March 1998

Prepared by:

Date: 4/1/98

Rick King

Approval:

Date: 6.30.98

Mark Johnson, Task Leader

Date: 2 Jul 98

WED QA Certification

Date: 6 July 98

David M. Olszyk, Project Leader

DATE: March 31, 1998

FROM: Ricky King

TO: File

SUBJECT: Revision of SOP 3.01, Version 1.10 on C/N Analysis for TERA II Experiment

The purpose of this document is to revise experiment specific protocols and address issues contained regarding SOP 3.01. It will address changes between the projects "Effects of CO_2 and Climate Change on Forest Trees" (a.k.a TERA I) and "Interactive Effects of O_3 and CO_2 on the Ponderosa Pine Plant/Litter/Soil System (a.k.a. TERA II). Unless otherwise noted under the Experimental Differences and Revisions sections (below), the referenced SOP may be used interchangeably between the TERA I and TERA II projects.

EXPERIMENTAL DIFFERENCES

In brief, the major differences between the TERA I and TERA II experiments are as follows:

1.TERA II plant species is Ponderosa pine, TERA I had Douglas-fir
2.TERA II treatments are O_3 and CO_2, TERA I were temperature and CO_2. For both TERA I and II there were two levels of each treatment per experiment, three replicate terracosms per treatment, and two chamberless (control) terracosms.
3.TERA II has three soil horizons (A, A-C, and C), and two litter layers (Oa and Oi), TERA I had three soil horizons (A, B, C), and one litter layer.
4.TERA II has 11 tree seedlings per terracosm, TERA I had 14.
5.TERA II has only terracosms and large lysimeters, and limited field sampling and pots for comparison purposes; TERA II also had specific field plots at three sites and a specific pot study.
6.TERA II has five research tasks, TERA I had eight. However, the numbering system for the SOPs is retained as in TERA I to avoid confusion.

REVISIONS:
1. All references to project "Effects of CO_2 and Climate Change on Forest Trees" now refer to "Interactive Effects of O_3 and CO_2 on the Ponderosa Pine Plant/Litter/Soil System".

2. All references to the project and QA plans now refer to:

Project Plan: Olszyk, D.M, D. T. Tingey, M. Johnson, R. Seidler, L. Watrud, J. Weber, D. Phillips, C. Andersen, M. Cairns, W. Hogsett, S. Brown, and R. McKane. 1997. Interacting Stress and Ecosystem Health: Interactive Effects of O_3 and CO_2 on the Ponderosa Pine Plant/Litter/Soil System: Research Plan. US. EPA, National Health and Environmental Effects Research Laboratory , Western Ecology Division, Corvallis, OR.

QA Plan: Quality Assurance Project Plan. Interactive Effects of O_3 and CO_2 on the Ponderosa Pine Plant/Litter/Soil System. 1997. US. EPA, National Health and Environmental Effects Research Laboratory , Western Ecology Division, Corvallis, OR.

3. Section H. Quality Assurance / Quality Control
Reference paragraph 2, line 5 house standards now includes Ponderosa pine needles and not Douglas-fir needles.

A. Signature Page

Carbon/Nitrogen Elemental Analysis

Project SOP Number 3.01
Version: ~~6.05~~
1.00

April 26, 1995

Prepared by:

Claudia M. Wise Date: April 26, 1995
Claudia M. Wise

Ricky J. King Date: 26 April 1995
Ricky J. King

Approval:

David M. Tingey Date: 24 may 1995
David M. Tingey, Principal Investigator

Paul R. Rygiewicz Date: 12 June 1995
Paul R. Rygiewicz, Project Leader

B. Table of Contents

This copy may not contain the addenda. You can see them in QA Books assigned to buildings for common use or contact your Project QA Coordinator.

C. Introduction

Elemental analysis measurements of stems, needles, roots, litter, and soil are key indicators of carbon and plant nutrient status of conifer tree seedlings and soil nutrient pools in response to changes in their environment. Elemental analysis data for the project *Effects of CO_2 and Climate Change on Forest Trees*, will be used to track changes in carbon (C) and nitrogen (N) concentrations and C/N ratios between the above- and belowground components of forested ecosystems. The C/N elemental analysis data will be used to help answer the research questions described for Tasks 2, 5, 6, and 8 of the *Effects of CO2 and climate change on forest trees* project, and provide inputs for modeling, integration and inference tasks.

D. Objectives Statement

This Standard Operating Procedure (SOP) documents proper procedures for conducting C/N elemental analyses on plant tissue,litter material,and soil. This SOP is designed to be used in conjunction with the Quality Assurance Project Plan (QAPP) for the project *"Effects of CO2 and Climate Change on Forest Trees" (US EPA, 1993).* This SOP is based in part on the SOP "Quantitative Dynamic Flash Combustion Method for Plant Analysis", previously approved in 1992. That SOP focused on the use of the analyzer with rice leaf and ponderosa pine needle samples.

Data quality objectives (DQOs) for elemental analysis measurements of carbon and nitrogen are shown in Table 1. Experimental design provides acceptable precision, accuracy, and completeness where feasible to insure that C/N elemental analysis measurements are made consistently and are of known quality and completeness. Data will be comparable to other data analyzed using the Carlo Erba EA 1108 elemental analyzer.

Table 1. **Data Quality Objectives for Carbon and Nitrogen Concentration**

Element	Unit(s)	Method	Precision[1]	Accuracy[2]	Comp.[3]
Carbon	% by weight	Flash Combustion	5%	1%-Chemical 6%-Plant, Soil	83%
Nitrogen	% by weight	Flash Combustion	5%	1%-Chemical 6%-Plant, Soil	83%

[1] Precision based on repeated measure of every tenth sample.
[2] Accuracy based on acetanilide chemical standard and pine needle plant standard.
[3] Completeness based on data from at least two of every three chambers per treatment, and from at least two of the four treatments, i.e. data missing from no more than two of twelve

chambers.

E. List of Equipment and Reagents

Equipment and reagents used in the SOP are listed in Table 2.

Table 2. **Equipment Used for CN Analysis and Methodology to Evaluate Performance**

Instrument	Model	Standard[1]	Frequency
Elemental Analyzer	Carlo Erba EA 1108	Acetanilide ($CH_3CONHC_6H_5$ mw.135.17)	Before, after, and at regular intervals during each day's measurement
Elemental Analyzer	Carlo Erba EA 1108	Pine Needle Reference Standard(1575) NIST Approved	At regular intervals during each day's measurements
Electronic Microbalance	Sartorius XM 1000 P	Standard weights, class s	At beginning and end of each day's analysis
Computer	Epson Equity III	Not Applicable	Not Applicable

[1] Recommended standards and reference material (depends on material to be measured).

F. Sampling Procedures and Sample Custody

The study of the *Effects of CO2 and Climate Change on Forest Trees* involves careful sampling and measurement of C/N using elemental analysis to insure that changes in percent C and N can be attributed to the applied treatment, plant-to-plant variability, and microclimate in the environment where samples are collected; by minimizing variability, bias, or error in sampling and measurement. To insure representative and uniform sampling and measurement, all staff involved with measurements will be trained on proper analysis procedures and equipment. As part of their training, personnel will be given this SOP and required to follow it for duration of study.

F.1 Sample analyses schedule. Analyses will occur intermittently over course of experiment on plant and soil samples taken to evaluate dynamic changes in above and below ground carbon and nitrogen partitioning (see Figure 5.2 in Research Plan). Analysis schedule for archived samples will occur based on prioritization by project investigators.

F.2 Selection, preparation, and handling of sample containers. Samples will be processed as described in shoot growth and phenology SOP (2.01), root growth and

phenology SOP (6.01), soil biology SOP (7.01) and soil organic matter SOP (~~8.xx~~ 8.01). Summarily, plant samples are lyophilized (freeze dried) and ground in a Wiley Mill to < 60 mesh. Soil samples are air dried and pulverized to <60 mesh. Samples of plant material or soil are then placed in labeled containers. Original containers used were Wheaton polyethylene liquid scintillation vials (cat. number 66021-690) and Nalgene general purpose wide mouth high density polyethylene bottles (cat. number 16125-027). Subsequently, glass vials have been implemented to aid in reducing static electricity inherent with plastic containers. Containers with samples will be double wrapped in plastic bags with desiccant inside second bag to keep samples dry. Samples are then stored at ambient temperature in laboratory until analyses. Present SOP (3.01) assumes preparation to this point and begins with delivery of samples to C/N analyst.

F.3 Sample identification and labeling. Each sample will be labeled with the following standard information. (1) Experiment (T=terracosm; F=field; L=large lysimeter), (2) Sub Experiment designation (chamber # 1 to 14; low, middle, high site; Large Lysimeter # 1-4), (3) Treatment, (4) Treatment Replication, (5) Plant number within Terracosm, site section or lysimeter (6) Sample Type (needle (NE),stem (ST),branches (BR), etc., (7) Tissue Age (plus primary or secondary flush), (8) Julian Date and Year and (9) Individual sequential sample number if necessary. Sample information will also be entered into sample tracking database by central sample processing coordinator or designee. Carlo Erba EA-1108 elemental analyzer instrumental control and processing is based on external PC utilizing EAGER 200 software. Sample table of software package contains all essential information for identification reconstruction.

F.4 Sample Preservation, Storage, and Disposal. Prior to analyses, all samples will be stored in scintillation counter vials, or equivalent bottles as needed, and double wrapped in plastic bags with desiccant in outside bag. Samples will be stored in a dry area at room temperature in the former ERL-C Wildlife building (Rooms 110 and 112). Samples will be archived by experiment (terracosm, pots, large lysimeters, field sites) and julian date. Samples will be stored until the end of the experiment or until the data have been accepted for publication, whichever is longer. Samples will be discarded in municipal landfill when no longer needed.

F.5 Sample Tracking. A copy of the form used to track samples from delivery to analytical laboratory through archiving is presented in Appendix L-1.

G. Analytical Procedures

G.1 Overview. The "Flash Combustion Method" is a highly efficient procedure for accurate and precise measurement of elemental concentrations in plant tissue and soils. This document describes operational use of the Carlo Erba EA 1108 elemental analyzer. Procedures have been developed for analyses of plant tissues and forest soils. Representative samples of plant tissue and pulverized soil are analyzed for total carbon/ nitrogen concentrations on a percent by weight basis. Hydrogen concentrations can also be measured if water trap is removed but are not covered in this SOP.

Prepared samples are sealed in tin capsules, placed in auto sampler and subsequently dropped individually into a vertical quartz combustion tube. Sample is combusted and resulting gases passed through a catalyst then a reduction tube along with carrier gas (He). Permanent gases are separated on a porous polymer column (Porapak Q) and detected by thermal conductivity. Output signal is proportional to concentration of individual sample components. An analog signal via RS-232 protocol is routed to digitizing board for conversion and parsed to resident Carlo-Erba peripheral PC software (Eager 200) . Linear regression calibration by use of external standards is used for sample quantitation as percent nitrogen and carbon on a dry weight basis.

G.2 Sample weighing and loading. Plant materials are freeze dried prior to grinding. Soils are air dried to ambient conditions and pulverized. All samples are prepared to <60 mesh. Sample amount required for either matrix is dependent on concentration of carbon and nitrogen in sample. With every tenth sample add a replicate sample and a replicate sample plus an acetanilide spike. Spike plus sample should give about a one half scale response. Samples, standards, blanks, and spikes are loaded into autosampler (maximum 196 samples) as described in section H. then analyzed.

G.2.1 Plant material. Weigh 5-10 mg of dried ground sample into tin combustion capsules previously cleaned as described in Carlo-Erba Instruments Elemental Analyzer EA 1108 Instruction Manual Section 3.5.1. Samples are then sealed in capsules using recommended solid sample encapsulation procedures 3.5.2.

G.2.2 Soil. Soil samples typically require 10-20 mg. Sample and containers are prepared and encapsulated as described above.

G.3 Analysis procedure. Elemental analyzer recommended procedures from manufacturer concerning set-up and start-up are attached (Appendix L-2). Carlo-Erba analyzer manual will be stored ajacent to instrument in room 206 of ERL-Corvallis main building.

H. Quality Assurance/Quality Control

H.1 Calibration standards. The standard currently being used is Acetanilide ($CH_3CONHC_6H_5$, mw.135.17) purchased from Carlo-Erba (Fison's Inst). Other standards are available if necessary. Acetanilide contains theoretical values of 10.36% nitrogen, 71.09% carbon, 6.71% hydrogen, and 11.84 % oxygen. Using an electronic balance capable of microgram resolution ca 0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg, and 4.0 mg of acetanilide are weighed and placed in tin capsules. Instrument is calibrated using a Linear Regression analysis of these standards incorporated into computer software (EAGER 200).

Standards are run at beginning of every working day and at regular intervals throughout sample series being analyzed. Normally, five standards and three blanks are run at the beginning of each working day. Replicate samples plus a replicate with a standard spike added are run every tenth sample. In addition, a mid-range chemical standard (Acetanilide) and applicable NIST standards and\or house standards (Douglas Fir needles or reference soil) are run for every twentieth sample being analyzed.

For elemental analysis purposes it is neither reasonable nor necessary to establish ultimate instrument sensitivity. Maximum detection limit varies inversely with age of detector. Sensitivity of analysis required to meet specific needs within a study are established by the research team of a particular project. Lowest and highest standards used for instrument calibration are chosen based on predicted minimum and maximum concentrations for samples. These values may be dervied from combinations of literature values for similar samples, recommendations from instrument manufacturer, or prior experiments. No analytical data will be reported which does not bracket standards range by ±1 SD. Data which is low or high off scale will be re-run to achieve values within defined range. Sample values low off scale due to inherent matrix will be recorded as a "less than" value.

Standards normally give linear responses over scale range described above. Linear regression R^2 values should exceed 0.99. If correlation coefficient is less than this, standards should be considered suspect and re-run in conjunction with associated samples to insure adequate precision and accuracy. Normally, a given standard, as it is run repeatedly throughout a working day, should give a Relative Standard Deviation ([(SD/mean)x100]) of no more than 10-15 %. If instrument drift exceeds this amount standards and associated samples should be re-run or otherwise examined to insure adequate precision and accuracy. Sufficient sample will be dried and prepared for re-analysis if necessary.

A typical report with method, calibration and sample outputs are included in Appendix L-3.

H.2 Instrument Precision and Accuracy. Operating specifications for Carlo-Erba Elemental Analyzer EA 1108 are as listed in Table 3.

Table 3. **Technical Specifications for EA 1108**

Elements	C, H, N, S and/or O
Measuring Range	100 ppm to 100%
Detection Limit	10 ppm
Accuracy	< 0.3% absolute
Repeatability	< 0.2% absolute
Sample Size	0.1 to 100 mg

To insure quality data, precision is determined through use of an NIST reference materials and replicate sampling and accuracy is determined through use of a known chemical standard (Acetanilide) and spike recoveries. As a general rule, 10% of all samples will be replicated and spiked. Historic spike recovery data for this methodology indicate spike recoveries in the range of 80-120%. Acetanilide standard used in calibration of Elemental Analyzer and for use in spiked samples is currently obtained from Carlo Erba. Purity of chemical traceable to NIST standards is certified by vendor. Theoretical assay of standard is 10.36% nitrogen and 71.09% carbon.

Linearity of carbon, hydrogen, and nitrogen values are determined through linear regression analysis of calibration standards. Correlation coefficients (R^2) generally approach 0.9999 or greater. Concentration of C/N in subsequent unknown samples is based on this linear relationship.

H.3 Other methods to insure data quality. In addition to Acetanilide, pine needles (NIST #1575) are a standard reference material certified by NIST at 1.2% nitrogen. Pine needle standards obtained from NIST are not certified for percent carbon or hydrogen, however concentrations of these elements are not expected to change over time. Certification is valid for 5 years from date of shipment from NIST. Should any of the certified values change before expiration of certification, purchasers will be notified by NIST and noncertified values should become suspect. A series of NIST pine needle C/N determinations indicate an accuracy of within 5.7% for nitrogen (average of 1.27% N for 6 samples of known concentration of 1.20% N). Thus, a DQO for accuracy of at least 6% for plant standards will be established. Appendix L-4 is a sample report for a NIST pine needle sample. Previously, six standard pine needle samples were run, resulting in a RSD of 4.7% for carbon and 2.5% for nitrogen. Thus our DQO for precision will be 5% for C/N. Precision will be assessed by replicate sampling for each group from a particular experiment. A Douglas fir inhouse standard has been developed for use in verifying instrumental accuracy and precision. This standard will be used as an adjunct to NIST pine needle standard #1575 and a control chart maintained to monitor C/N values plotted over time.

Several studies have been conducted to assess EA 1108 precision and accuracy. Details are found in original SOP for Ponderosa Pine and rice. In brief, for rice, three batches (N=3) with replicate samples of rice were spiked with 1.5 mg of acetanilide. Mean spike recoveries for nitrogen were 101, 95, and 115 percent. Mean spike recoveries for carbon were 101, 97, and 113 percent. Standard deviations for nitrogen and carbon were 2 and 3 percent respectively. For Ponderosa pine: Seven batches (n=7) of replicate samples of ponderosa pine were spiked with 1.0 mg of acetanilide. Mean spike recoveries for nitrogen were 99, 99, 97, 104, 109, 110, and 110 percent. Mean spike recoveries for carbon were 100, 100, 102, 101, 104, 103, and 104 percent. Standard deviations for nitrogen and carbon were 1 and 10 percent respectively.

Methodologies for forest soil analysis have been developed. Montana Soil Standard Reference Material (SRM) 2711, not certified for carbon and nitrogen, is intended primarily for use in control charting. This soil and/or an inhouse reference forest soil will be used in plotting C/N values over time to monitor changes in instrument performance. Uncertified value of SRM 2711 percent carbon is 2.

H.4 Blanks. For the purpose of assessing normal levels of nitrogen, carbon, and hydrogen, "control" samples are collected. These samples will serve as external blanks and used as a tool for comparison of treatment effects. In addition, empty tin capsules will be analyzed along with sample analysis to indicate background contamination during processing and analysis. If measurable levels of nitrogen, carbon, and hydrogen are detected, a systematic evaluation of sample processing techniques and analytical methods will take place. Sources of capsule contamination will be identified and eliminated, if possible.

H.5 Definitions of precision, accuracy, and completeness.

Percent precision for a single plant is defined as: $(|x_1-x_2|/x_1) * 100$, where x_1 and x_2 are repeated measures on the same plant. Mean and standard deviation are calculated for all (n) precision percentages.

Percent accuracy for a single calibration is defined as: $(|y_1-y_2|/y_1) * 100$, where y_1 is standard value and y_2 is instrument reading. Mean and standard deviation are calculated for all (n) accuracy percentages.

Percent completeness for a data set is defined as: $(|z_1-z_2|/z_1) * 100$, where z_1 is the number of possible measurements and z_2 is the number of actual measurements per data set.

I. Preventive Maintenance and Corrective Action

I.1 The following preventative maintenance philosophy will apply to this SOP:

I.1.1 The C/N elemental analyzer is a very reliable instrument. In normal use over the expected life span of the instrument, very little maintenance of a highly difficult nature should be expected.

I.1.2 Personnel operating this equipment have received training from instrument manufacturer in all aspects of instrument operation and maintenance, making a service contract by manufacturer unnecessary.

I.1.3 Maintenance typically involves repacking and replacement of combustion and reduction tubes due to insufficient sensitivity. Tapered o-rings used to seal combustion and reduction tubes will be replaced on an as needed basis.

I.1.4 Recommended periodic maintenance schedule for the EA 1108 is contained in Appendix L-5. An instrument log book noting any maintenance or repair will be kept ajacent to instrument.

I.2 Balances receive annual servicing from external vendor.

I.3 Calibration of microbalance is checked before each use and written in analysis log book.

J. Data Reduction, Validation and Archiving

All information relating to sample acquisition and processing for experiment will be included in a notebook. Notebook will be retained in experimental area in main building Rm. 206, copies of pages are given to project officer. All information relating to elemental analyzer operation is retained in a notebook adjacent to instrument in room 206 of ERL-C main building.

Data is directly entered through an interface into EAGER 200 software installed in an IBM PC. Eager 200 software creates and archives a copy of standard and sample table, calibration table, and percent nitrogen and carbon report. Various report options can then be used for export to spreadsheet programs for further data manipulation (ie. to calculate spike recoveries). Flow chart for handling data is shown in Figure 1.

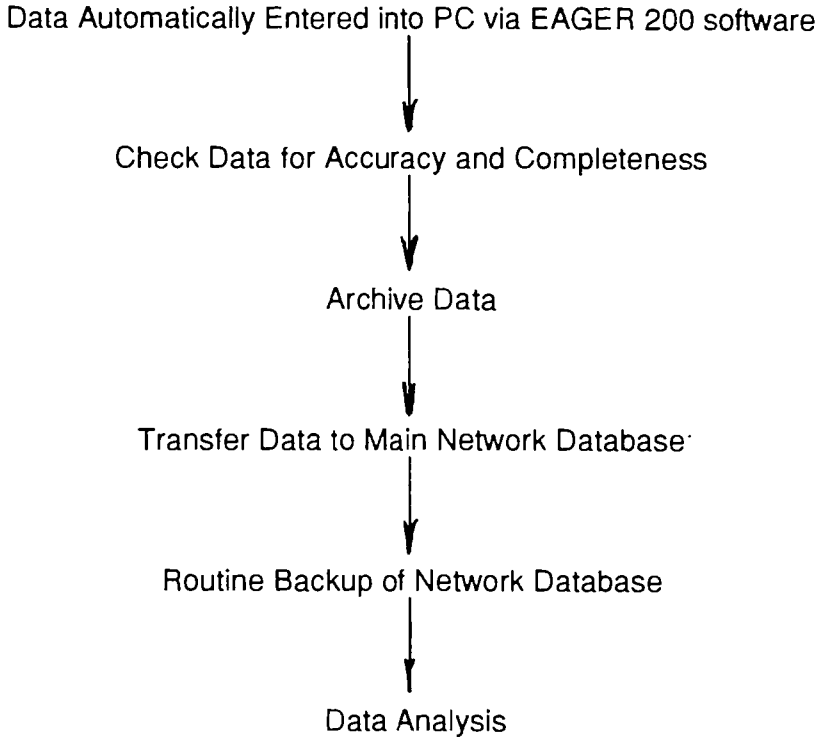
Sample report data is then checked for completeness and accuracy by analyst. Any unusual values will be rerun. Files containing raw data, sample and analytical parameters will be archived by analyst for possible subsequent retrieval .

K. References

Elemental Analyzer E.A.1108 instruction manual (Rev. A2-90), Carlo Erba.

US EPA. 1993. Effects of CO_2 and Climate Change on Forest Trees. Research Plan. ERL-Corvallis.

Figure 1. **Data flow for elemental analysis**



Appendix L-1

Sample Tracking Form - 1 page

SAMPLE L A SHEET

	Date	Signature
Delivered:		
Analyzed:		

Data Filenames

Lyophilized	Oven Dried	Exp.	Sub Exp.	Treatment	Rep.	Plant Number	Sample Type	Age	Date	CN	S	Carbon Fraction	Humus	T. Nutrients	E. Nutrients

(Column groups: Exp. through Date = **Sample Number**; CN through E. Nutrients = **Analysis Required**)

Appendix L-2

Elemental Analysis Setup and Startup Procedures – 18 pages

SECTION 3

3.1 SET-UP

3.1.1 GAS SUPPLIES

- Regulate the carrier gas pressure to 200-300 kPa at the cylinder helium supply and to a 100-150 kPa at the cylinder oxygen supply.

- Regulate the carrier gas pressure (Helium GC grade) to 50 - 100 kPa using the CARRIER pressure regulator to obtain the flowrate required (100 ml/ min ±10%). Refer to the Analytical Conditions for each configuration reported at the end of this section.

- Regulate the oxygen pressure to 100 kPa using the OXYGEN pressure regulator and adjust the needle valve to obtain at the oxygen vent on the lower part of the front central panel a flowrate between 10 - 15 ml/min

- Regulate the servo air pressure to 350-400 kPa using the SERVO AIR pressure regulator . Other inert gases may also be used as servo gas command with the only requirement that they are free of oil impurities.

3.1.2 REACTOR TUBE

- Remove the front panel of the instrument

- Remove the automatic sampler e.g. AS 200 LS (if installed) or remove the connecting joint which is placed instead of the sampler.

- Place the properly filled combustion tube (or reduction reactor, or pirolytic reactor) (see Section 2, Installation) inside the furnace through the inlet fitting (see Fig 25) making sure that the narrow-end part of the tube is directed towards the bottom part of the furnace.

- Use a viton O-ring on the upper part of the tube before you insert the tube into the furnace and a viton O-ring on the lower part of the tube after the tube has been placed inside the furnace. Refer to Fig 25 for correct order of fittings when placing the tube inside the furnace. The bottom outlet of the combustion tube is connected to the reduction tube by means of the coupling joint or directly to the column inlet when using the CHNS configuration with the appropriate fixing nut.

NOTE: *It is recommended to fit on the top of the tube the o-ring 3075 before placing the tube inside the furnace, in this ways if the tube slips, accidental damage may be prevented.*

3.1.3 ADSORBENT FILTER.

- Place the filter (properly filled as described in Section 2) into the relevant suppor using the fittings provided.

- Connect the water filter inlet to the bottom of the reduction part of the reactor (for CNS and O determinations) and the filter outlet to the chromatographic column using the stainless steel tubing and fittings provided.

3.1.3 CHROMATOGRAPHIC COLUMN.

The chromatographic column ends for oxygen determination (molecular sieve) are sealed to avoid deactivation by the effect of moisture. The columns for CHN and CHNS determinations do not necessarily require the sealing plugs and generally the column is already installed before the unit is sent out to be delivered.

To fit the column inside the oven proceed as follows:

- Remove the oven cover

- Remove the sealing plugs of column ends

- Connect the column to the filter and to the detector using 2x1 mm (o.d., i.d.) stainless steel tubing in both ends.

ANALYTICAL CONDITIONS FOR CHNS DETERMINATION.

Left Furnace Temperature	= 1000°C / 1020°C
Right Furnace Temperature	= 500°C (not used)
Oven Temperature	= 60°C
Filament Temperature	= 190°C
Carrier Flowrate	= 100 ml/min ±10%
Sample Delay Time	= 8 - 15 sec (depending on the carrier flowrate used)
Total Run Time	= 9 - 12 min

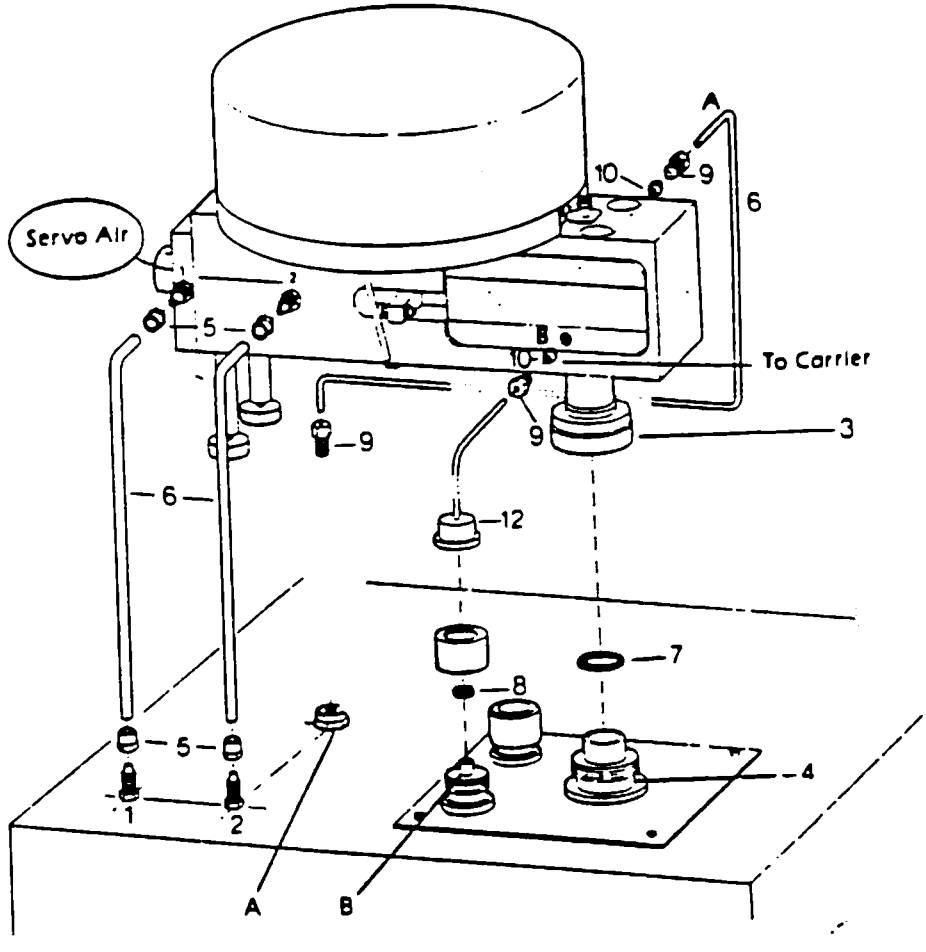


FIG. 26

3.2.2 CARRIER GAS PRESSURE LEAK CHECK

- Turn the instrument pressure regulators (see 1-2, Fig 3) OFF
- Adjust pressure of carrier gas supply to 150 kPa (cylinder)
- Cap off the carrier gas VENT outlets located on the low part of front panel
- Adjust the carrier gas pressure regulator (1-2, Fig 3) to 100 kPa measured on the corresponding gauges (3-4, Fig 3)
- Wait for 2 or 3 minutes to reach equilibrium
- Turn the carrier pressure regulators (1-2, Fig 3) OFF
- If the system maintains the set pressure for at least 3 minutes then the carrier line is leak-proof, if not, check the relevant circuit step by step until the leak is located and repaired. Repeat the pressure test procedure.

  NOTE: To check the circuit it is recommended the following procedure:

  1. Remove sampler and fit in its place the coupling joint and pressure check the base unit, if no leak is present, test the autosampler for pressure leaks according to the procedure described in Section 5.1 Maintenance.

  2. If leak is still present check bottom of reduction tube

  3. If leak persists check the analytical column outlet

- Remove the blanking caps from the VENT outlets

3.2.3 AUXILIARY OXYGEN PRESSURE LEAK CHECK

- Turn the Auxiliary Oxygen pressure regulator OFF (fully counter clockwise)
- Adjust the pressure regulator on the outlet of the oxygen supply (cylinder) to 150 kPa
- Cap off the oxygen VENT
- Adjust the oxygen pressure regulator to 100 kPa measured on the pressure gauge
- Wait for a few moments to reach equilibrium
- Turn the pressure regulator OFF (completely counter clockwise)
- If the system maintains the set pressure for about 3 minutes, then the system is

3.4 INSTRUMENT CONTROL SETTINGS

Before starting verify that the following controls are in the OFF position:

- MAINS POWER SWITCH OFF (see 1, Fig 7)
- FIL push button OFF (see 16, Fig 4)
- FURN STBY pushbutton depressed (see 15, Fig 4)

After these checks have been performed proceed as follows:

1. Switch MAIN POWER ON (see 1, Fig 7)

2. Set the control panel parameters of Left Furnace, Right Furnace Temperatures and all others according to the analytical conditions for the different configurations described at the end of Section 2 .

- SAMPLE START (see 11, Fig 4) : 8- 15 sec (refer to point 9 below and Section 5 Maintenance)
- SAMPLE STOP (see 12, Fig 4) : 50 sec
- OXY INJ STOP (see 13, Fig 4) : 60 sec
- DELAY INTEG (see 14, Fig 4) : 10 sec

3. Wait for about 60 min until the READY L.E.D. (see 7, Fig 4) lights up

4. Release the FIL push button (see 16, Fig 4), the L.E.D. (see 17, Fig 4) goes OFF and the L.E.D. (see 22, Fig 4) comes ON

5. Make sure that the CARRIER and PURGE intercepting valves are set to the ON position

6. Set the Filament Temperature range to 190°C, then the L.E.D. (see 9, Fig 4) must go OFF

7. Check that the L.E.D. (see 6, Fig 4) is glowing, if not check all parameters (e.g. all pushbuttons must be released.

8. When the built-in recorder is used, make sure that it is connected to the rear panel of the analyser as shown in Fig 15 .

Check the baseline recorder, the allowed drift up to the end of the analysis must be not greater than $\pm$ 25 ,uV.sec

The instrument is now ready for operation and after loading the samples into the a... sampler you may start the analytical cycle by pressing the MANUAL START key (see 8,

3. Start the analytical cycle and check by looking into the window on the front of the autosampler (see 2, Fig 13) how many seconds pass between the introduction of the sample into the reactor and the moment when the flash combustion occurs, when the "flash light" is seen press the MEMORY pushbutton (see 3, Fig 5).

This time in seconds is memorised on the display (see 2, Fig 5) on the front panel of the analyser.

Take 5 sec off this value and add the rest to the time set on SAMPLE START (see 11, Fig 4). For CHN samples this value may be between 8 to 15 sec and it is related to the flowrate used.

4. Start again with a new analytical cycle and check the combustion condition with the above setting using again an empty tin container.

3.5 SAMPLE PREPARATION

One of the most important parameters in quantitative determination is the degree of homogenisation of the sample to be analysed, especially when small amounts of sample of industrial or agricultural materials are used which are not totally representative of the entire batch of material.

From the elemental analysis point of view it is possible to have samples of the following types:

1. Organic/Pharmaceutical products. Use these materials as they are without any pre-treatment because they are 100 % of an homogeneous nature and they do not require sample preparation.

2. Fuels, solid/liquid samples e.g. coal, they require a sample weight of about 3 to 15 mg with a particle size between 0.2-0.5 mm diameter.

3. Soil, rock, grain, cereal, tobacco samples must be ground using the cutting or balls mills. Sample size required no less than 10 mg.

4. Protein, fat samples must be ground using the frezzing technique with addition of liquid nitrogen to the sample, the material becomes brittle allowing easier powder formation. Sample size required between 10 and 50 mg.

All type of samples that are difficult to homogenise can be ground using the liquid nitrogen technique e.g. polymers, plastics, paper, rubber and also "your difficult sample".

5. Trace analysis on metals, soils, silicates require a sample size between 10 mg up to 100 mg.

3.3.1 TYPE OF CONTAINERS AND CLEANING PROCEDURE

Tin containers for CHN/O/S and C/N determinations are shown in Fig . Containers type D/E/G and H can be used without cleaning. Generally tin containers do not need cleaning or pre-treatment, however for special applications such as trace analysis where blanks reproducibility may be critical to the results obtained, the cleaning of capsules is recommended.

CLEANING METHOD.

Place in a suitable beaker one or more sets of tin containers. Wash twice with carbon tetrachloride, then with acetone and each time discard the solvent. Wash three times with distilled water, pour most of the water out and place the beaker with the containers in a drying oven at 110°C until the tin containers are completely dry.

TREATMENT OF SILVER CONTAINERS FOR OXYGEN DETERMINATIONS.

The silver containers (type A and C of Fig) may be oxidised to silver oxide (Ag_2O) due to the presence of oxygen and moisture in the air. It is possible to reduce the amount of oxidation by heating the silver containers up to 350°-400°C until the silver oxide is decomposed, this is noted when the yellowish colour on the container surface dissappears, in some cases up to 1 hour at 400°C is required to eliminate this colouration. Th heating can be performed in a muffle or alternatively in heated quartz tube under a flo of dry air. It is necessary to keep the silver containers in tightly closed vials or inside an appropriate dissecator.

TIN CAPSULES FOR CHN/CHNS DETERMINATIONS

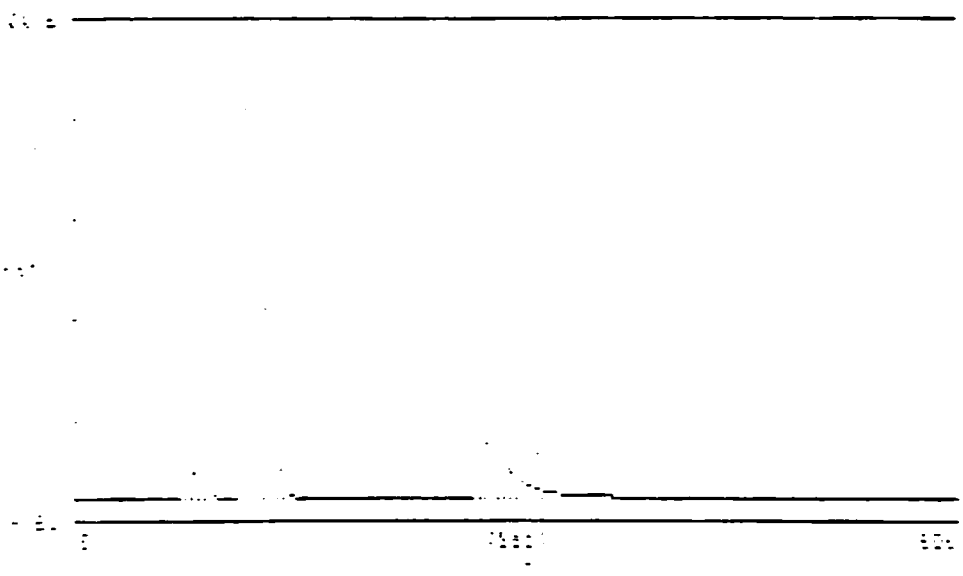
Part No.	Description	Type (Fig)	Data
240 06400 Set of 100	Tin containers for solid substances (10-100mg) Use tweezers to close containers	F	id = 5.0 mm h = 8.0 mm t = 0.02 mm w = 22 mg
24007200 Set of 50	Ultrapure tin containers for liquid/solid samples	G	i.d. = 3.5 mm h = 9.0 mm t = 0.07 mm w = 90 mg
24008810 Set of 100	Ultrapure tin containers for hygroscopic liquid samples	H	i.d. = 2.88 mm h = 6.0 mm t = 0.06 mm w = 24 mg

Appendix L-3

Sample of Calibration Standard, Method, and Sample Reports -14 pages

EAGER 200 Stripchart

Sample Ident. : 5 STD1 Filename :QA5
Analysed : 04-03-92 11:33:25 Printed :04-03-1992 11:43:38



EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (µV): 10.2
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 11:33:25 Printed : 04-03-1992 11:43:41
Sample Ident. : 5 STD1 Filename : QA5
Sample Weight : .573 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (µV)	Area (µV/Sec)	Area % (%)	Name
1	FL	67	107	82	1166.8	15082	4.13	Nitrogen
2	FL	107	234	120	20365.2	273539	74.86	Carbon
3	RS	265	545	280	2429.4	76780	21.01	Hydrogen
						3654013	100.00	

EAGER 200 Std Report

Instrument name : Instrument #1 Bline drift (µV): 10.2
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 11:33:25 Printed : 04-03-1992 11:43:41
Sample Ident. : 5 STD1 Filename : QA5
Sample Weight : .573 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

Pk. (#)	Ret Time (Sec)	Area (µV/Sec)	Elem.amount	Area Ratio	Name
1	82	15082	.593628E-01	.181363E-02	Nitrogen
2	120	273539	.407346E-00	.100000E-01	Carbon
3	280	76780	.384483E-01	.356266E-01	Hydrogen

SECTION 4

4.1 START UP

4.1.1 ANALYTICAL CYCLE

- Check that all analytical parameters settings including temperature of furnace, column oven, pressure, flowrates and time values are correctly set for the analysis required.

- Check that the autosampler is correctly loaded with the samples to be analysed.

- Press the manual START key (see 8, Fig 5) to initiate the analytical run. The L.E.D. (see 9, Fig 5) will light up. When the analyser is coupled to the Data Processor DP-200, the analysis is initiated by pressing the RUN key on the DP-200, or alternatively when the using the EAGER workstation, the start of the analysis is triggered from the computer's keyboard.

- When pressing the START key (or the run is initiated) oxygen is injected into the reactor through the bimatic valve for the period set on TIME (see 13, Fig 4)

- After the time set on TIME (see 12, Fig 4) the sample is dropped into the reactor by displacementof the autosampler slide where the sample has been loaded.

- Flash combustion takes place within a few seconds after the sample has been dropped.

- After the time set on TIME (see 12, Fig 4) the sampler slide returns to its previous position and another sample container drops into the slide cavity where it is continuously purged until the next cycle starts.

- For CHN analysis the combustion gases analysis), water and excess of oxygen enter the secondary reactor (reduction) where the excess of oxygen is retained and nitrogen oxides are reduced to elemental nitrogen. The resulting "plug" is swept into the analytical column by the carrier gas and detected by a Thermal Conductivity Detector.

- For CHNS analysis the combustion gases plus water and excess of oxygen pass to the lower part of the reactor where the excess of oxygen is retained and the oxides of nitrogen are reduced to elemental nitrogen. The resulting "plug" is swept into the chromatographic column by the carrier gas and detected by the Thermal Conductivity Detector.

- For oxygen analysis the pyrolisis gases are swept into the filter and then into the chromatographic column followed by detection with a Thermal Conductivity Detector.

- The chromatogram is printed on the built-in recorder (or external), alternatively, when using the DP-200 the chromatogram and integration report (peak area) is provided at the end of the analysis.

CHECK LIST CHN DETERMINATION (How to proceed)

1. Leak test for M and R channels.

2. Flow rate 100 -120 ml/min for Measure
   Flow rate 30 - 40 ml/min for Reference

3. Combustion reactor temperature, left side: 1050 °C
   Reduction reactor temperature, right side: 650 °C

3a. Oven temperature: 60 °C

4. Check the Flash dynamic combustion 8 - 10 sec when the M Flow rate is between 110 - 120 ml/min

5. Check the Flash combustion with an empty tin capsule.

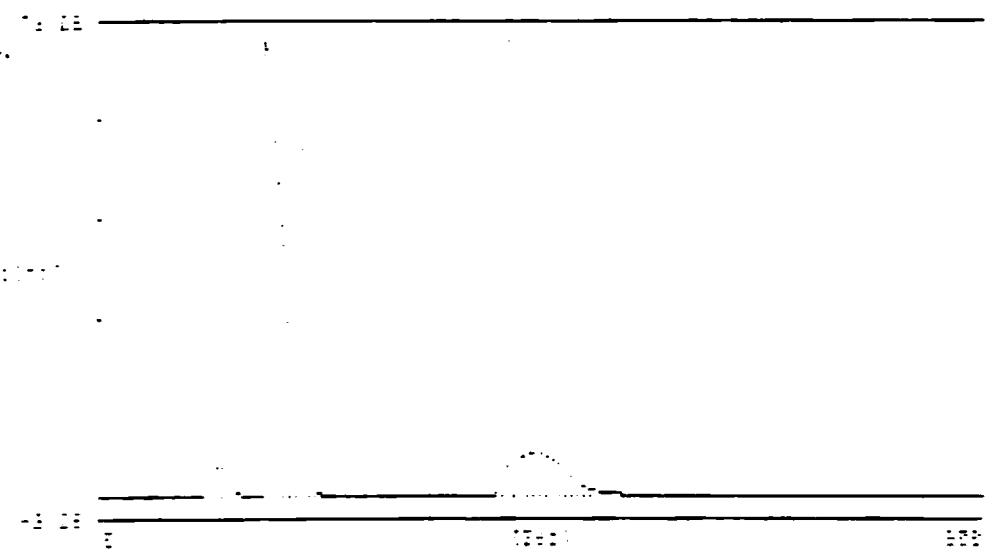
6. Check the Flash combustion with STD organic material which is included in the standard outfit (acetanilide)

7. Check the area concentration percent between CO_2/N_2; CO_2/H_2O; check that ratio is reproducible (independently of the sample size).
   If ratios are not reproducible check:
   a. combustion
   b. GC separation
   c. base line drift (maximun15/20 uv * sec)
   d. integration

8. Run STD sulfanilamide 2 or 3 times if K factor is used and then one STD each 5 or 6 unknown samples.
   If linear regression calculation mode is used standardization has to be done from 0.5 mg sample size up to 3 - 4 mg.

EAGER 200 Stripchart

Sample Ident. : 7 STD3 Filename :QA7
Analysed : 04-03-92 11:53:58 Printed :04-03-1992 12:04:11



EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (µV): 6.5
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 11:53:58 Printed : 04-03-1992 12:04:14
Sample Ident. : 7 STD3 Filename : QA7
Sample Weight : 2.102 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (µV)	Area (µV/Sec)	Area % (%)	Name
1	FU	47	67	61	8.1	117	0.01	
2	FU	67	108	83	4869.9	54998	4.00	Nitrogen
3	FU	108	264	115	68682.2	1007871	73.24	Carbon
4	FU	264	595	297	6373.9	313168	22.76	Hydrogen
						13761534	100.00	

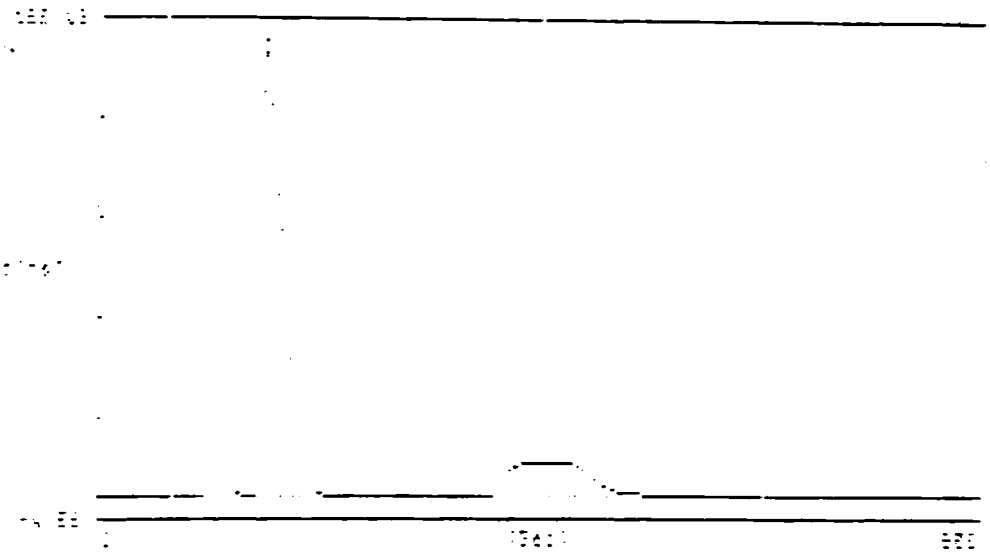
EAGER 200 Std Report

Instrument name : Instrument #1 Bline drift (µV): 6.5
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 11:53:58 Printed : 04-03-1992 12:04:14
Sample Ident. : 7 STD3 Filename : QA7
Sample Weight : 2.102 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

(#)	Ret Time (Sec)	Area (µV/Sec)	Elem.amount	Area Ratio	Name
1	83	54998	.217767E+00	.183257E+02	Nitrogen
2	115	1007871	.149431E+01	.100000E+01	Carbon
3	297	313168	.141044E-00	.321830E-01	Hydrogen

EAGER 200 Stripchart

Sample Ident. : 8 STD4 Filename :QA8
Analysed : 04-03-92 13:49:29 Printed :04-03-1992 13:59:43



EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (µV): 30.5
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 13:49:29 Printed : 04-03-1992 13:59:45
Sample Ident. : 8 STD4 Filename : QA8
Sample Weight : 3.16 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (µV)	Area (µV/Sec)	Area % (%)	Name
1	RS	19	35	30	6.7	103	0.00	
2	FU	47	67	61	13.3	195	0.01	
3	FU	67	107	84	7935.1	83341	4.02	Nitrogen
4	FU	107	264	113	92406.2	1515997	73.16	Carbon
5	FU	264	597	313	7083.4	472520	22.80	Hydrogen
						20721564	100.00	

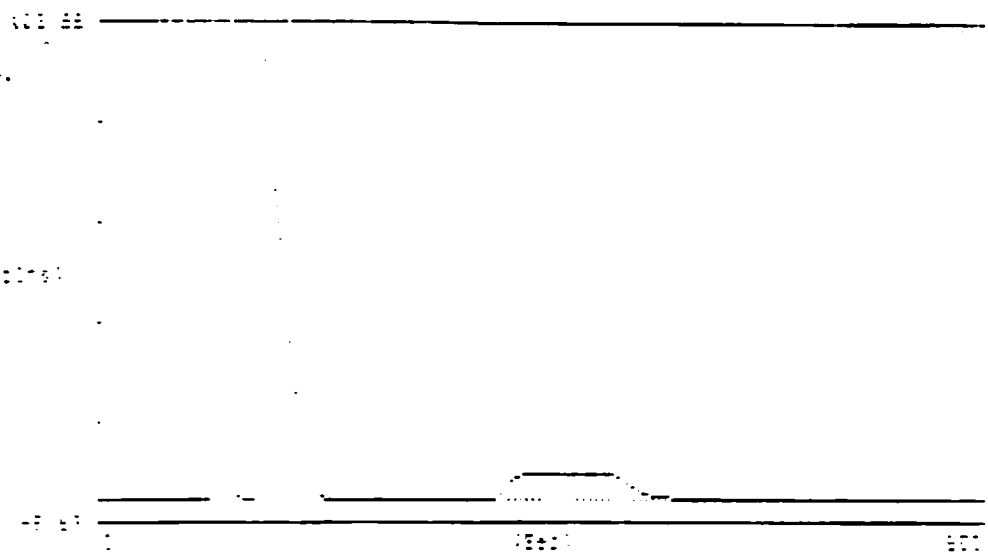
EAGER 200 Std Report

Instrument name : Instrument #1 Bline drift (µV): 30.5
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 13:49:29 Printed : 04-03-1992 13:59:45
Sample Ident. : 8 STD4 Filename : QA8
Sample Weight : 3.16 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

Pk. (#)	Ret Time (Sec)	Area (µV/Sec)	Elem.amount	Area Ratio	Name
1	84	83341	.327376E+00	.181902E+02	Nitrogen
2	113	1515997	.224644E+01	.100000E+01	Carbon
3	313	472520	.212036E+00	.320832E+01	Hydrogen

EAGER 200 Stripchart

Sample Ident. : 9 STD5 Filename :QA9
Analysed : 04-03-92 13:59:46 Printed :04-03-1992 14:09:59



EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (µV): 9.7
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 13:59:46 Printed : 04-03-1992 14:10:01
Sample Ident. : 9 STD5 Filename : QA9
Sample Weight : 4.251 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (µV)	Area (µV/Sec)	Area % (%)	Name
1	RS	19	37	29	9.1	145	0.01	
2	FU	47	67	62	16.7	257	0.01	
3	FU	67	105	84	11863.6	112766	4.04	Nitrogen
4	FU	105	263	110	116888.5	2037276	72.93	Carbon
5	FU	263	598	336	7193.4	643164	23.02	Hydrogen
						27936070	100.00	

EAGER 200 Std Report

Instrument name : Instrument #1 Bline drift (µV): 9.7
Company Name : CE Instruments Operator Ident. :
Analysed : 04-03-92 13:59:46 Printed : 04-03-1992 14:10:02
Sample Ident. : 9 STD5 Filename : QA9
Sample Weight : 4.251 Calc.method: using 'Square to Linear fit'
Standard Type : Acetanilide

(#)	Ret Time (Sec)	Area (µV/Sec)	Elem.amount	Area Ratio	Name
1	84	112766	.440404E+00	.180665E+02	Nitrogen
2	110	2037276	.302204E+01	.100000E+01	Carbon
3	336	643164	.285242E+00	.316759E+01	Hydrogen

EAGER 200 Calibration Report

nstrument name : Instrument #1
ompany Name : CE Instruments
nalysed : 04-03-92 13:59:46
ample Ident. : 9 STD5
ample Weight : 4.251
tandard Type : Acetanilide

Bline drift (µV): 9.7
Operator Ident. :
Printed : 04-03-1992 14:10:02
Filename : QA9
Calc.method: using 'Square to Linear fit'

Pk. (#)	Ret Time (Sec)	Slope (m)	Intercept (b)	Corr. Factor	Name
1	84	0.389671E-05	0.204232E-02	0.999970E-00	Nitrogen
2	110	0.148162E-05	0.207921E-02	0.999999E-00	Carbon
3	336	0.436598E-06	0.483764E-02	0.999985E-00	Hydrogen

EAGER 200 – METHOD REPORT

Method in use : Default Method
Disk & Directory : c:\chnso\STRAT\PINE
Method file name : PI070492.EAD
Instrument name : Instrument #1

Detect./Integr. parameters

Analysis time(Sec):	600
Peak Thresh. (uV):	1
Minimum area(uV / Sec):	100
Inhibit time(Sec):	5
First Sample:	90
Last Sample:	147
Sample being acquired :	118
Skim ratio:	8
Real time plot Atten. :	1
Real time plot Offset :	0

Time Events

#	Time	Events Type	Value
1	0	None	
2	0	None	
3	0	None	
4	0	None	
5	0	None	
6	0	None	
7	0	None	
8	0	None	
9	0	None	
10	0	None	
11	0	None	
12	0	None	
13	0	None	
14	0	None	
15	0	None	

	Sample name	Filename	N (%)	C (%)	H (%)
[illegible]	NBSPN	PI20	1.2902	49.5780	6.4840
[illegible]	NBSPN	PI36	1.2860	49.5031	6.5029
[illegible]	NBSPN	PI60	1.2176	52.4203	6.8455
[illegible]	NBSPN	PI38	1.2871	52.2773	6.8717
[illegible]	NBSPN	PI114	1.2752	55.4417	7.2910
[illegible]	NBSPN	PI108	1.2455	49.3260	6.4657

Statistics Calc.	Average	Standard Dev.	% Relative S.	Variance
Nitrogen (%)	1.271948	.032262	2.536427	.001041
Carbon (%)	51.44328	2.409941	4.684655	5.807812
Hydrogen (%)	6.743429	.325266	4.823459	.105798

Appendix L-4

Sample NIST pine needle standard report - 2 pages

EAGER 200 – METHOD REPORT

Method in use : Default Method
Disk & Directory : c:\chnso\STRAT\PINE
Method file name : PI070492.EAD
Instrument name : Instrument #1

Component Table

#	Time	Component name
1	82	Nitrogen
2	139	Carbon
3	247	Hydrogen
4		Oxygen
5		Sulphur

Appendix L-5

Preventive Maintenance -4 pages

SECTION 5

5.1 MAINTENANCE

5.1.1 REACTORS

The replacement of the reactor tubes should be performed periodically according to the following guidelines:

A. Configuration CHNS

- Proceed to change the copper catalyst on the lower part of the tube (reduction part) afetr 200 analyses which after this number of samples has been oxidised to copper oxide. To do this use the scraping stainless steel tool provided in the standard outfit of the instrument. First remove the autosampler (if installed) and fixing nuts, remember that you do not need to switch off the unit to cool down the instrument, it is only necessary to handle the tube by the two ends which are at room temperature.
CAUTION : the reactor tube central part is red hot, be very careful not to touch this part!

- After you have replaced the copper proceed to remove the inorganic ashes (e.g. tin dioxide) deposited on the top part of the reactor (oxidation part) which have accumulated after 200 analyses. Use the scraping tool to break down and remove the ashes by inverting the tube. Beware of the tube central part which is RED HOT!

- When 1000 analyses have been carried out it is necessary to replace the oxidation catalyst completely. In this case you must cool down the reactor before handling it.

- When replacing the packing materials (e.g. copper, oxidant or removing the ashes) recommended to replace the o-ring seals (top and bottom ends) at the same time and a test for pressure leaks should be also performed when repacking or replacing the reactor tube (refer to Section 3, paragraph 3.2)

B. Configuration CHN

- Every 200 analyses proceed to remove the ashes above the oxidation catalyst using the scraping tool. Remember that you can do this operation without cooling down the unit (refer to configuration A)

- Replace the copper in the reduction tube every 500 analyses

- Replace the oxidation catalyst in the combustion tube every 1000 analyses

- When replacing the packing material remember to replace the o-ring seals and to perform a leak-test of the flow pattern as described in Section 3, paragraph 3.2

C. Configuration O

- Proceed to replace the catalyst of the pirolysis tube every 200 analyses

D. Replacement of Filter packing

The filter packing must be replaced every 300 analyses

E. Chromatographic Column

The column packing material consists of Poropak QS 50-80 mesh and Molecular Sieve 5 A (oxygen analysis) and their average life is about 2 years.

F. Bimatic Switching Valve

The switching valve normally lasts a full year without the need for viton diaphragm replacement. In the event of fault this viton membrane may have to be replaced.

5.1.2 AUTOSAMPLER AS 200-LS

The autosampler requires from time to time a replacement of the sampling slide (see 11 Fig 27). The dismounting and reassembly operation of the autosampler MUST be performed with great care and under very clean operating conditions because the presenc of even the smallest solid particle in the sampling slide mechanism may compromise th performance of the autosampler.

Date: 3.26.97 (Correction to Precision Formula made on 5.13.08)

From: Mark Johnson

To: File

Subject: Revision of DQOs for GPEP SOP 3.01 (Carbon/Nitrogen Elemental Analysis), Version 1

After collecting data for more than a year using SOP 3.01, Version 1, it has become apparent that it is necessary to modify the DQOs specified in the SOP. The rationale for making these changes is that after evaluating this data the initial DQOs cannot be generally applied to all the samples that are being routinely analyzed. The primary reason is that many of the samples are either very low in C or N, or in both C and N. Consequently, the accuracy and precision at these low levels were out of limits set by the initial DQOs, but are not unacceptable. Additionally, values of C less than or equal to 0.5 % and N values less than or equal to 0.1 % will not be reported because these values are at or near the lower limit of detection and cannot be reliably analyzed.

Table 1, below, shall be now be used in place of Table 1 (page 3) in SOP 3.01, Version 1.

Table 1. Revised Data Quality Objectives for Carbon and Nitrogen Concentration

Element	Units	Method	Precision[1]	Accuracy[2]	Complete-ness[3]
C	% by weight	flash combustion	90 % for C > 0.5 % N.A.[4] for C ≤ 0.5 %	± 10 % for C > 0.5 % N.A.[4] for C ≤ 0.5 %	85 %
N	% by weight	flash combustion	85 % for N > 0.2 % N.A.[5] for N ≤ 0.1 %	± 15 % for N > 0.1 % N.A.[5] for N ≤ 0.1 %	85 %

[1] Precision is based upon repeated analysis of every tenth sample and calculated from the data from the replicated samples as a percent: Precision (%) = 100 - Coefficient of variation (%)

[2] Accuracy based upon recovery of a standard reference material and reported as a percent.

[3] At least 85 out of 100 samples submitted for C and N analysis from the TERA project will be successfully analyzed for C and N.

[4] Values of C less than or equal to 0.5 % will be reported as < 0.5% because these values are at or near the lower limit of detection and cannot be reliably analyzed by this technique.

[5] Values of N less than or equal to 0.1 % will be reported as < 0.1% because these values are at or near the lower limit of detection and cannot be reliably analyzed by this technique.

Calculating Precision

Precision is based upon repeated analysis of every tenth sample and is calculated from the data from these replicated samples as a percent. The formula for calculating the precision is: Precision (%) = 100 - Coefficient of variation (%), where the coefficient of variation (CV) is equal to the standard deviation of the two replicate analyses divided by the mean of the two numbers. The equation for calculating precision is the following:

Precision (%) = 100 - CV(%)

$$= 100 - \left[\left(\frac{\text{standard deviation}}{\text{mean}} \right) \times 100 \right]$$

$$= 100 - \left[\left(\frac{\left(\sqrt{\frac{n\sum x^2 - \left(\sum x\right)^2}{n(n-1)}} \right)}{\left(\frac{\sum x}{n} \right)} \right) \times 100 \right]$$

where: n = number of replicate samples

x = percent carbon or nitrogen concentration for each replicate sample

Example calculation for C precision using data from Carlo Erba:

Table 2. Example Data for Calculating Precision

Sample Type	Sample Number	Replicate Number	% C
Soil	T04MSO	1	2.313
Soil	T04MSO	2	2.349

Precision (%) = 100 - CV(%)

$$= 100 - \left[\left(\frac{\text{standard deviation of Rep 1 and Rep 2}}{\text{mean of Rep 1 and Rep 2}} \right) \times 100 \right]$$

$$= 100 - \left[\left(\frac{0.025456}{2.331} \right) \times 100 \right]$$

= 100 - [1.092]

Precision (%) = 98.908%

Calculating precision for N analyses uses the same equations but uses the data obtained for N content.

Calculating Accuracy

Accuracy is based upon recovery of a standard reference material that is spiked or added to routine sample and is reported as a percent. For this analysis percent accuracy and percent recovery are synonymous. To estimate accuracy every tenth sample is be spiked with a standard reference material. The spiked sample is usually the third QA sample; the first two being the replicate samples used for calculating precision. The mean C and N concentrations of the replicate samples are used in calculating accuracy.

To date three kinds of standard reference materials have been used on the TERA project: pine needles, soils, or chemical standards (e.g., acetanilide). It is best that the sample being analyzed and the added standard material have similar carbon and nitrogen levels and have similar matrices (i.e., use standard pine needles to spike plant tissue samples or standard soil to spike soil samples). There are occasions (e.g., a new type of sample is to be analyzed that standard reference material with proper C or N levels or matrix is not available) when chemical standards may be used. The current batch of Standard Pine Needles has a reported value of nitrogen at 1.2 %, but doesn't have a reported value for carbon. In this case we use the long-term carbon mean, which is for the last 134 Pine Needle samples analyzed (as of 3.5.97), which is 49.939 % carbon (s.d. = 0.647 and C.V. = 1.296). We use a Canadian Reference Soil (#2) which has reported values nitrogen of 0.22 % and carbon of 4.8 %.

The equation for calculating accuracy for carbon analysis is the following:

$$\text{Accuracy (\%)} = \left\{ \frac{\left[\left(\frac{\text{wt}_{\text{stnd}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\%\text{C}_{\text{stnd}}}{100}\right)\right] + \left[\left(\frac{\text{wt}_{\text{sample}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\text{Mean }\%\text{C}_{\text{sample reps}}}{100}\right)\right]}{\left(\frac{\%\text{C}_{\text{sample+stnd}}}{100}\right)} \right\} \times 100$$

where "wt" refers to the weight of either a standard reference material (wt_{stnd}) or the weight of the sample (wt_{sample}). Percent C_{stnd} ($\%C_{stnd}$) refers to the concentration of carbon of the added standard reference material on a dry weight basis. Mean $\%C_{sample\ reps}$ is the mean carbon concentration of the two replicated samples that were analyzed for calculating precision. Percent $C_{sample+stnd}$ ($\%C_{sample+stnd}$) is the concentration of carbon of the mixed sample and standard reference material. For samples with carbon contents greater than 0.5% (by weight), acceptable recoveries (accuracy) can range from 90% to 110% (see Table 1).

Example calculation for C analysis:

The replicate data used to calculate precision is also used in calculating accuracy. Additional information on weight of sample and weight of standard reference material added (spiked) to the sample, and C content of the reference material (obtained from samples analyzed on the Carlo Erba).

Table 3. Example Data for Calculating Accuracy

Sample #	Sample Type[1]	Wt Sample (mg)	Wt. Std. Ref. Material (mg)	Total Wt. (mg)	% C of Std. Ref. Material[2]	% C of Replicate Samples[3]	% C of Sample + Std. Ref. Material
T04MSO	soil	7.745	7.755	15.500	4.8	2.609	3.882

[1]Sample Type determines what type of Standard Reference Material is added. In this case the sample type is "soil", therefore, a reference soil was used as the Standard Reference Material

[2]This data is either the reported value for the standard reference material or, in the case of a standard reference material that does not have a reported value, it is the long-term mean value obtained by running the standard reference material on the Carlo Erba.

[3]This data is the mean of two replicate samples of the un-spiked sample.

$$\text{Accuracy (\%)} = \left\{ \frac{\left[\left(\frac{\text{wt}_{\text{stnd}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\%\text{C}_{\text{stnd}}}{100}\right)\right] + \left[\left(\frac{\text{wt}_{\text{sample}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\text{Mean }\%\text{C}_{\text{sample reps}}}{100}\right)\right]}{\left(\frac{\%\text{C}_{\text{sample+stnd}}}{100}\right)} \right\} \times 100$$

$$\text{Accuracy (\%)} = \left\{ \frac{\left[\left(\frac{7.755}{15.500}\right)\left(\frac{4.8}{100}\right)\right] + \left[\left(\frac{7.745}{15.500}\right)\left(\frac{2.609}{100}\right)\right]}{\left(\frac{3.882}{100}\right)} \right\} \times 100$$

$$\text{Accuracy (\%)} = \left\{ \frac{(0.025) + (0.013)}{(0.039)} \right\} \times 100$$

Accuracy (%) = 95.4 %

The equation for calculating accuracy for nitrogen analysis is the following:

$$\text{Accuracy (\%)} = \left\{ \frac{\left[\left(\frac{\text{wt}_{\text{stnd}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\%\text{N}_{\text{stnd}}}{100}\right)\right] + \left[\left(\frac{\text{wt}_{\text{sample}}}{\text{wt}_{\text{sample}} + \text{wt}_{\text{stnd}}}\right)\left(\frac{\text{Mean }\%\text{N}_{\text{sample reps}}}{100}\right)\right]}{\left(\frac{\text{Mean }\%\text{N}_{\text{sample+stnd}}}{100}\right)} \right\} \times 100$$

where "wt" refers to the weight of either a standard reference material (wt_{stnd}) or the weight of the sample (wt_{sample}). Percent N (%N) refers to the concentration of nitrogen of the added standard material on a dry weight basis. Mean $\%N_{sample\ reps}$ is the mean nitrogen concentration of the two replicated samples that were analyzed for calculating precision. Percent $N_{sample+stnd}$ ($\%N_{sample+stnd}$) is the nitrogen concentration of the mixed sample and standard reference

material. For samples with nitrogen contents greater than 0.1% (by weight), acceptable recoveries (accuracy) can range from 85% to 115 % (see Table 1).

Calculating Completeness

Completeness is measure of the number of samples in a given sample set that are analyzed. For C and N elemental analysis the DQO for completeness is 85%. To the analyst completeness is calculated for each batch of samples. For this analysis batches are limited to within kinds, or types, of samples. For example, during TERA Spring and Fall coring events plant (needles and roots), soil and litter samples are collected. The 4 kinds of samples are prepared in different ways and are grouped into 4 batches: needles, roots, litter and soil. Completeness is then calculated for each batch of samples from specific sampling events. Compleness is calculated using the following equation:

$$\text{Completeness (\%)} = 100 - \left\{\left[(n_{total} - n_{analyzed})/n_{total}\right] \times 100\right\}$$

where n_{total} is equal to the total number of samples in a batch and $n_{analyzed}$ is equal to the total number of samples successfully analyzed within the given batch.

Example calculation: $n_{total} = 196$ and $n_{analyzed} = 180$

Completeness (%) = 100 - {[(196 - 180)/196] x 100} = 91.8 %

Troubleshooting

From time to time a DQO may not be met. When this occurs with either the precision or accuracy DQOs, the analyst must first determine the cause and extent of the problem. Likely sources of the problem include: sample or spike were misweighed, instrument malfunction, or the wrong type of spike or sample was used. Since the precision and accuracy of every 10th sample is evaluated, it's easy to determine where problems have occurred in a run. For example, if there are 60 samples in a batch and the precision and accuracy DQOs are met for the first 4 (1, 2, 3 and 4) check samples but not for the last two (5 and 6), then it's likely that the last 20 samples in the batch are suspect. One approach is to repeat the precision and accuracy samples (check samples 5 and 6 in this example) and two or three samples just before them in the run. If precision and accuracy DQOs are met and the rerun samples also meet the precision DQOs, then one could assume that the remaining samples not rerun are acceptable. If the result of the rerun is that the precision and accuracy DQOs are met but the samples do not meet the precision DQOs then all of the samples from between check samples 4 and 6 are suspect. Because it takes a day to weigh and rerun the samples and a day to evaluate the results, it's best to rerun all of the potentially suspect samples again.

Potential instrument malfunctions are beyond the scope of this addenda and are addressed in SOP 3.01 and in the applicable service manuals.

In general, the completeness objective will be met. In the event that it is not met, the analyst will work to rectify the problem. If they cannot, they will report the problem to the principal investigator in charge of the chemistry section or the project leader.

APPENDIX 4

METHODS FOR INORGANIC/ORGANIC CARBON FRACTIONATION

1. Harris, D., W.R. Horwath, C. van Kessel. 2001. "Acid Fumigation of Soils to Remove Carbonates Prior to Total Organic Carbon or Carbon-13 Isotopic Analysis." *Soil Sci. Soc. Am. J.* 65:1853–1856.

2. Ussiri and Lal. 2008. "Method for Determining Coal Carbon in the Reclaimed Mine Soils Contaminated with Coal." *Soil Sci. Soc. Am. J.* 72(1):231-237.

As of June 2010, these articles can be obtained at the Soil Science Society of America Journal Web site, by entering the Year, Volume and First Page under the Specify Citation option. The Web site is available at: http://soil.scijournals.org/search.dtl.

APPENDIX 5

METHOD

FOR BULK DENSITY MEASUREMENT

U.S. Department of Agriculture (USDA). 2004. USDA Natural Resources Conservation Service. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report. No. 42. Version 4.0. Rebecca Burt, Editor.

Manual available from: U.S Department of Agriculture, Natural Resources Conservation Service Web Site: http://soils.usda.gov/technical/lmm/. Accessed on March 30, 2010.

Bulk Density (3B)
Soil Cores (3B6)
Field-State (3B6a)

1. Application

Bulk density is used to convert data from a weight to a volume basis; to determine the coefficient of linear extensibility; to estimate saturated hydraulic conductivity; and to identify compacted horizons. Procedure 3B6a determines the bulk density value of a moist soil core of known volume. Field bulk density (Db_f) offers the opportunity to obtain relatively cheaply bulk density information without the expense incurred to obtain water retention. Db_f is particularly useful if the soil layers are at or above field capacity and/or the soils have low extensibility and do not exhibit desiccation cracks even if below field capacity.

2. Summary of Method

A metal cylinder is pressed or driven into the soil. The cylinder is removed extracting a sample of known volume. The moist sample weight is recorded. The sample is then dried in a oven and weighed.

3. Interferences

During coring process, compaction of the sample is a common problem. Compression can be observed by comparing the soil elevation inside the cylinder with the original soil surface outside the cylinder. If compression is excessive, soil core may not be a valid sample for analysis. Rock fragments in the soil interfere with core collection. Dry or hard soils often shatter when hammering the cylinder into the soil. Pressing the cylinder into the soil reduces the risk of shattering the sample.

If soil cracks are present, select the sampling area so that crack space is representative of sample, if possible. If this is not possible, make measurements between the cracks and determine the aerial percentage of total cracks or of cracks in specimen.

4. Safety

No known hazard exists with this procedure.

5. Equipment

5.1 Containers, air-tight, tared, with lids
5.2 Electronic balance, ±0.01-g sensitivity
5.3 Oven 110°C
5.4 Sieve, No. 10 (2 mm-openings)
5.5 Coring equipment. Sources described in Grossman and Reinsch (2002).

6. Reagents

None

7. Procedure

7.1 Record the empty core weights (CW).

7.2 Prepare a flat surface, either horizontal or vertical, at the required depth in sampling pit.

7.3 Press or drive core sampler into soil. Use caution to prevent compaction. Remove core from the inner liner, trim protruding soil flush with ends of cylinder, and place in air-tight container for transport to laboratory. If soil is too loose to remain in the liner, use core sampler without the inner liner and deposit only the soil sample in air-tight container. Moisture cans can also be pushed directly into a prepared face. For fibrous organic materials, trim sample to fit snugly into a moisture can.

7.4 Dry core in an oven at 110°C until weight is constant. Record oven-dry weight (ODW).

7.5 Measure and record cylinder volume (CV).

7.6 If sample contains rock fragments, wet sieve sample through a 2-mm sieve. Dry and weigh the rock fragments that are retained on sieve. Record weight of rock fragments (RF). Determine density of rock fragments (PD).

8. Calculations

$$Db = (ODW - RF - CW)/[CV - (RF/PD)]$$

where:
Db = Bulk density of < 2-mm fabric at sampled, field water state (g cm^{-3})
ODW = Oven-dry weight
RF = Weight of rock fragments
CW = Empty core weight
CV = Core volume
PD = Density of rock fragments

9. Report

Bulk density is reported as g cc^{-1} to the nearest 0.01 g cm^{-3}.

10. Precision and Accuracy

Precision and accuracy data are available from the SSL upon request.

11. References

Grossman, R.B. and T.G. Reinsch. 2002. Bulk density and linear extensibility. p. 201-228. *In* J.H. Dane and G.C. Topp (eds.) Methods of soil analysis, Part 4. Physical methods. Soil Sci. Am. Book Series No. 5. ASA and SSSA, Madison, WI.

APPENDIX 6

STANDARD OPERATING PROCEDURES

FOR ABOVE AND BELOW GROUND BIOMASS CHARACTERIZATION

1. EPA. 1994. EPA ERT SOP 2034, Rev. 0.0, November 17, 1994. *"Plant Biomass Determination."* EPA Environmental Response Team, Edison, NJ.

2. EPA. 2004. Root Cores SOP, Version 1.0, January 29, 2004. *"Standard Operating Procedure for Collecting and Processing Soil and Fine Tree Samples."* EPA National Health and Environmental Effects Research Laboratory, 200 S.W. 35th Street, Corvallis, OR 97333.

U. S. EPA ENVIRONMENTAL RESPONSE TEAM

STANDARD OPERATING PROCEDURES

PLANT BIOMASS DETERMINATION

CONTENTS

SUPERCEDES: SOP #2034; Revision 0.0; 01/24/92; U.S. EPA Contract 68-03-3482.

1.0 SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) describes the method for determining biomass of herbaceous plant tissues. This analysis along with other plant physiological and toxicological techniques will be used to assess the impact of contaminants on primary productivity. This method can be used to normalize analytical data, such as contaminant, protein, or nutrient content. That is, tissue concentrations must be given on a per unit of dry weight basis for valid comparisons. In order to compare the concentration of a specific component in a sample with the concentration of that same component in another sample, a common basis for the comparison must be provided. For instance, if the sample weight is the same for both samples a comparison on this basis might be valid in some situations. However, if one sample is half water and the other is dry, then a calculation would have to be made to account for this difference. The amount of the component in question is therefore often expressed per unit of the dry weight of the sample because dry weight is a substantially uniform standard. This is called "normalizing" for the tested component. Included below are procedures for obtaining representative samples, quality assurance/quality control measures, and proper documentation of sampling activities.

These are standard (i.e., typically applicable) operating procedures which may be varied or changed as required, dependent upon site conditions, equipment limitations, or limitations imposed by the procedure. In all instances, the ultimate procedures employed should be documented and associated with the final report.

Mention of trade names or commercial products does not constitute U.S. Environmental Protection Agency (U.S. EPA) endorsement or recommendation for use.

2.0 METHOD SUMMARY

Above ground portions of plants will be collected from a plot using clippers. They will be weighed with a spring scale, in the field if possible (fresh weight), dried for 24-48 hours at 80^{o}C (constant weight), cooled in a desiccator jar, and reweighed (dry weight).

This procedure will be used during the growing season. Samples can also be separated into species and/or organ types to determine partitioning of energy, depending on the goals of the study.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

Plants will be placed in resealable plastic bags, kept cool, weighed as soon as possible, and dried following the weighing. If the plants cannot be weighed for fresh weight in the field, they must be transported to the lab or other appropriate facility in plastic bags on wet ice and weighed within 24 hours.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

There are several potential problems and interferences that may occur when sampling for plant biomass.

1. Site access must be obtained.

2. Additional impacts may occur before and during the sampling period such as drought and other climatic extremes. Other non-contaminant related impacts that can mask the effects of contaminants may include site disturbance by humans.

3. Microclimatic differences on a site such as shade and moisture, soil factors, nutrients, and topographic variation will affect plant growth and possibly mask the effects of contaminants.

4. This is a destructive method and may be undesirable on some sites.

5. This procedure can only be carried out during the growing season. Also, differences in the times when various species germinate and become dominant within a growing season may bias the results.

6. Results may also be biased if the root portions of plants of different species vary greatly in their proportion of the total biomass. Roots may also be samples but this is a tedious process requiring that all root material be extracted from the soil, and all soil be removed from the roots.

5.0 EQUIPMENT/APPARATUS

Equipment needed for plant population sampling include:

- Stakes
- Clippers
- Plastic bags
- Paper bags
- Aluminum weighing dishes
- Ice chest
- Weighing scale
- Drying oven
- Desiccator jar and desiccant
- Sharpies for labelling bags
- Spring scale
- Documentation supplies (data sheets, sample labels, Chain of Custody records and seals, logbook, pens)

6.0 REAGENTS

A desiccant such as calcium chloride-based pellets will be placed in the desiccator jar to absorb moisture. Reagents may be utilized for decontamination of sampling equipment. Decontamination solutions are specified in ERT/REAC SOP #2006, Sampling Equipment Decontamination.

7.0 PROCEDURES

7.1 Site Preparation

7.1.1 Plant Population Survey

The site will first be characterized and species of interest chosen according to ERT/REAC SOP #2037, Terrestrial Plant Community Sampling. Plots will be marked with stakes. Samples to be analyzed will be collected from each randomly selected plot laid out according to the site sampling plan. If woody plants are encountered in a plot, this plot must be eliminated and a new plot selected that contains no woody species.

7.1.2 Sample Collection

The plants will be cut at ground level, weighed as soon as possible after cutting, placed in labelled plastic bags, and kept cool until drying in the laboratory. If wet, the plants must be wiped dry using paper toweling before weighing. Tissue may be separated into species or further to organ groups (stems, leaves, etc.) and weighed separately depending on the goals of the study.

7.2 Laboratory Analysis

7.2.1 Tissue Processing

Plant tissue will be placed in paper bags or aluminum weighing dishes (depending on sample size) in a drying oven set at 80°C. The tissue will be dried for 24 - 48 hours, cooled in a desiccator jar, and reweighed (dry weight). The tissue will be weighed at 4 to 8 hour intervals, replacing the material in the oven between weighings, until no more water weight is lost (i.e., to a constant weight). Care must be taken not to cook or char the material. If oven space is limited, materials can be held refrigerated for no more than one week prior to drying. Less succulent tissues may be left to dry at room temperature in open paper bags before completing the process in the oven. It is important not to allow the samples to decay before drying.

8.0 CALCULATIONS

$$\textit{Water Content} = \textit{Fresh Weight} - \textit{Dry Weight}$$

$$\textit{Standing Biomass} = \frac{\textit{Dry Weight (of above ground tissues)}}{\textit{Plot Area}}$$

9.0 QUALITY ASSURANCE/QUALITY CONTROL

There are no specific quality assurance activities which apply to the implementation of these procedures. However, the following QA/QC procedures apply:

1. All data must be documented on field data sheets or within field/site logbooks.
2. At least one uncontaminated reference site will be sampled for comparison to the contaminated areas.
3. A sample plan, including numbers and sample size, will be diagrammed before sampling.
4. QA Work Plan will be outlined before sampling.
5. All deliverables will receive a peer review prior to release, and 10% of the calculations will be rechecked.
6. All instrumentation must be operated in accordance with operating instruction as supplied by the manufacturer, unless otherwise specified in the work plan. Equipment checkout and calibration activities must occur prior to sampling/operation and they must be documented.

10.0 DATA VALIDATION

The data generated will be reviewed according to the Quality Assurance/Quality Control considerations listed in Section 9.0. The data will be statistically analyzed.

11.0 HEALTH AND SAFETY

The preparation of a Health and Safety Plan is required prior to any field activity and must be approved by the REAC Health and Safety Office or designee. When working with potential hazardous materials, follow U.S. EPA, OSHA, and corporate health and safety procedures.

When sampling on a site known or suspected of contamination, all precautions must be taken to safeguard the samplers.

12.0 REFERENCES

Brower, J.E. and J.H. Zar, 1984. Field and Laboratory Methods for General Ecology. William C. Brown, Publishers, Dubuque, Iowa.

Godbold, Douglas L., A. Hutterman, 1988. Inhibition of Photosynthesis and Transpiration in Relation to Mercury-Induced Root Damage in Spruce Seedlings. Physiologia Planetarium. 74:270-275.

Teramura, Alan H., M.C. Perry, J. Lydon, M.S. McIntosh, E.G. Summers, 1984. Effects of Ultraviolet-B Radiation on Plants during Mild Water Stress III. Effects on Photosynthetic Recovery and Growth in Soybean. Plant Physiology. 60:484-492.

TITLE AND SIGNATURE PAGE

Standard Operating Procedure for Collecting and Processing Soil and Fine Tree Samples

Prepared by: _____ Date: 1.29.04
Mark G. Johnson, PI

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David T. Tingey, PI

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Mark G. Johnson, Project Leader

Approved by: _____ Date: 3 June 04
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U.S. Environmental Protection Agency
National Health and Environmental Effects Laboratory
Western Ecology Division

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PROCEDURES

1.0 Scope and Application

1.1 This Standard Operating Procedure (SOP) is applicable to the collection of root and soil samples from open and closed canopy forests. It describes how to collect samples in typically 20 cm depth increments (although lesser increments can also be used) using a hand auger/soil corer and how to separate the roots from the soil. Because of the size of the soil corer (~5 cm diameter) used, this procedure is best suited for the collection of roots less than 10 mm in diameter and not for those of larger diameters. This procedure also describes methods for separating roots from soil and for processing root and soil samples. While this Procedure was specifically developed for forested systems it is easily adapted to other systems such as grasslands or agricultural systems. Note that the core depth increments are set by the scientific requirements of the research and not this SOP.

2.0 Summary of Method

2.1 A hand-powered soil corer is rotated into the soil in successive 20 cm (or required) increments at designated locations. With a downward force the rotational motion of the corer causes the serrated edge of the core cup to cut through soil, roots and other materials. When the appropriate depth is reached the corer is extracted from the soil and the core cup is emptied into a labeled Ziploc bag. The bag is placed in a cooler and transported back to the laboratory. At the lab the root/soil sample is quantitatively transferred to a fine mesh (2 mm openings) sieve. Soil particles pass through the openings in the sieve and the roots and other coarse fragments are retained on the sieve. The roots are removed from the sieve and are washed free of soil particles with water. Using a metric ruler the roots are sorted by diameter and placed labeled paper envelope by diameter classes. The root samples are dried at 65°C for 48 hours. The dried roots are transferred to a tared weighing boat or weighing paper. Their dry weight is determined and recorded. The root sample is transferred back to a fresh envelope and archived for further analyses. The soil can be dried or discarded, depending on whether or not it is needed.

3.0 Health and Safety Warnings

3.1 Tree root samples are generally collected in forest settings that are often remote and require good physical conditioning to access. Environmental factors such as wind, rain, hot or cold air temperatures need to be considered when collecting these samples. The Field Health and Safety Plan developed for the Forest Indicators Research Project (Forest Ecosystem Indicators: Monitoring, Assessment, Prediction (FEIMAP)) shall be followed when collecting these samples.

4.0 Objectives Statements

The general Data Quality Objectives for the procedures described herein are listed in Table 1. These were established to insure consistent collection and handling of soil and root samples. There are two completeness objectives for this SOP and they are described in the sections below.

Table 1: Data Quality Objectives for Soil/Root Coring and Root Processing

Parameter	Equipment	Units	Precision[1]	Accuracy[2]
Coring Depth Guide Position	Metal metric tape measure	cm	± 0.1 cm	NA[3]
Root Diameter	Plastic Metric Ruler	mm	± 0.1 mm	NA[3]
Root Dry Weight	Digital Balance	g	CV < 10%	CV < 10%

[1]Precision is based on repeated measures of depth, length or weight.
[2]Accuracy is determined using calibration standards
[3]Accuracy standards do not exist for these parameters

4.1 Root Sample Collection:

One metric of data quality used in this SOP is completeness, a measure of the amount of samples collected and analyzed relative to the design specification. The data quality objective is to achieve at least 90% completeness in the collection of the target number of samples. The number of samples (target number of samples) that are planned for collection at any particular site is specific for that site and depends upon how the site was laid out. Prior to going to collect the samples a site plan is developed and the number of target samples is determined (i.e., samples to be collected at 16 locations = 16 target samples). For example, to meet the 90% completeness objective for sample collection at the Water Hole Field Site in Olympic National Park where there are 18 target samples, at least 16 of the 18 sites need to be sampled to meet this completeness objective. While not ideal, meeting the 90% sample collection completeness objective provides the minimum amount of data to describe root distribution or soil properties at a given site. The depth to which samples are collected at any given point depends upon the depth of the soil, the size and amount of coarse fragments or roots. This, however, does not affect the completeness objective. If one sample or four core segment samples are collected at a single coring location, then that location has been collected. Appendix A contains a description of all the field sites and shows the tentative locations of all the target sample sites.

4.2 Root/Soil Sample Processing

There is no “gold standard” method for root extraction from soil samples to provide an accuracy comparison. Similarly, homogeneous standard samples for roots in soil are unavailable. Split samples are also not feasible due to the heterogeneity of the distribution of roots within soil volumes. For this reason, the primary data quality objective here is completeness of sample processing since quantitative accuracy and

precision assessments are not possible. The second completeness objective has to do with the processing of soil/root segments once they have been collected. This completeness objective is set at 95%. Using the Water Hole site again as an example, if we collect 4 soil/root segments at each of the 18 core locations then we will have collected 72 soil/root segments. To meet the processing completeness objective of 95% means that 68 (72 x 0.95) of the segments must be processed to meet this objective.

5.0 List of Equipment

5.1 Root/soil sampling:

pre-labeled zip-lock bags
Sharpie
T-handled soil corer
5 cm diameter, metal, serrated tip, soil core cups (~30 cm in length)
coring depth guides
small C-clamp
metal, metric tape measure
small hammer
flexible spatula
straight spatula
knife
clippers
long screwdriver
cutting board
sample collection log sheets - see Appendix B
coolers
Blue Ice

5.2 Sample processing:

soil sieve – 2.0 mm openings
sieve pan that fits under the with 2.0 mm openings
dishpans
squeeze water bottle with water
forceps
plastic metric rulers
beaker
cutting board
large plastic weighing boats
razor blades
labeled paper (coin) envelopes
drying oven, 65°C
balance with at least 0.0001 g precision
sample analysis log sheets - see Appendix C

6.0 Forest Indicators Field Sites

6.1 General Location Description

The root samples were collected at 12 of the Forest Indicators Field Sites to obtain a measure of the standing stock of medium to fine roots. The Indicators Field Sites in Oregon lay on a general transect from east of the Cascade Mountains, across the Cascades and to the Coast Range. There are 8 field sites along this transect. Four additional sites are located in Washington in Olympic National Forest. Table 2 contains information about each site including forest type and root core collection schedule. Appendix A has a plot map for each field site and a brief description of the site and directions to the target coring locations.

6.2 Attributes of the Forest Indicators Field Sites Relative to Root Core Sample Collection:

Table 2. Forest Indicators Field Sites

Indicators Field Site	Dominant Forest Species	Sampling Date	Target Number of Samples	Target Sampling Depth (cm)
Oregon Transect				
Cascade Head Stand 3	Sitka Spruce	August 1999	16	80
Cascade Head Stand 14	Douglas fir	August 1999	16	80
Falls Creek	Douglas fir	July 1998	15	80
Moose Mountain	Douglas fir	June 2002	18	80
Soapgrass Mountain	Douglas fir	July 1998	18	60
Toad Creek	Douglas fir	July 1998	15	80
Meto lius	Ponderosa Pine	July 1999	16	80
Juni per	Juniper	June 2001	25	100
Olympic National Park				
Irely Lake	Douglas fir	June 1999	16	80
East Twin	Sitka Spruce	June 1999	16	80
Deer Park	Douglas fir	July 1999	16	80
Waterhole	Douglas fir	July 2001	18	80

7.0 Sampling Procedures and Sample Custody

7.1 Identifying Sampling Points

Using a map of the field site to be sampled, a decision is made where to locate the sampling points. For example, if a field site has 16 subplots, collecting one set of soil/root cores per plot is one approach. The sampling point may be the center of the subplot or a point 3 meters from one randomly selected subplot corner. The main criterion in developing a sampling plan is that sampling points should be located in representative areas so that the resulting samples capture the variation in

root and soil properties. Following the decision on how to locate the sampling points these points need to be transferred to the plot. Generally, the points will be located relative to a permanent plot marker or reference (e.g., rebar plot corner markers). Using various combinations of tape measures, compasses or other measuring devices (e.g., a total station) the tentative sampling points are located and identified with a colored pin flag. Often the subplot and core number are written on the pin flag (e.g., Plot 13, Core 2 could be written as 13-2 on the pin flag). It is recommended that the entire set of tentative sampling points be identified prior to coring although not required.

7.2 Adjustments to Coring Locations

It may occur that adjustments to some of the tentative coring locations will need to be made. For example, a tentative coring point is located according to the coring design plan, where a tree stump, log, large rock or some sort of obstruction prevents collection of an un-biased sample. In these cases the coring point will need to be moved. Often an offset of 1 meter is sufficient. Any adjustments or deviations to the original coring location (as developed in the coring design plan) need to be recorded on the data sheets. For example, "Coring location 13-2 was moved 1 meter due east from the original point due to the presence of a gopher hole." If possible, the location of the new point should be measured or quantified in some manner. These kinds of adjustments may need to be made for a number of coring locations.

Even if a coring location looks suitable, it may need to be moved once the actual coring has started due to a buried object (e.g., coarse root, rock, cavity in the soil, etc.). As before, if the coring location is moved, the location of the new location should be noted.

7.3 The Coring Procedure

Coring for root and/or soil samples is a relatively easy procedure. For this work EPA scientists have designed custom coring tools as shown in Figure 1. The primary tool is a metal T-handled soil corer that has a 5 cm diameter, metal, serrated tip, soil core cup (~30 cm in length) attached at the end. The coring cup is held in place by a spring plunger on the head of the T-handled corer that locks into an index hole in the coring cup. A spring plunger tool is used to compress the spring to retract the plunger. The cup can now be removed with a gentle twisting motion. It is important to keep the contact between the core cups and the head of the T-handled corer clean. Fine particles and rust can make core cup installation and removable difficult.

Prior to coring the depth of coring has to be set on either the coring cup or on the shaft of the T-handled coring tool. Figure 2 shows the two types of depth guides that can be used. These guides have stops (a.k.a. "wings") that when they reach the mineral soil surface signal that final depth of the core has been reached. For

example the stops shown in Figure 2A are attached to a coring cup and set to 20.0 cm by hooking a metal tape measure on the serrated end and measuring 20.0 cm to the bottom surface of the stops. The stops are set by tightening the stainless steel hose clamps. The other depth guide (shown in Figure 2B) is set by hooking the metal tape over the serrated end of the coring cup and setting the bottom surface of the stops. A small "C" clamp is used to secure the depth guide. This procedure is best done by placing the top of the T-handled coring tool on the soil surface with the coring cup point upward. It is also acceptable to use a permanent marker or tape to indicate the desired coring depth on the coring cup or handle. In this case it's recommended that one member of the coring team be in a position to clearly view the location of these marks so that they may tell the person doing the coring when the desired depth has been reached.

Collecting the First Core Segment: The goal of this step is to collect a cylinder of soil of known length containing roots. A team of two people is best for collecting soil and root cores. The team should proceed to one of the previously identified coring locations and, depending on the specific objectives of the research, may remove the forest floor litter layer down to the top of the mineral soil or include the litter layer in part of the first soil core. [mark we did not separate the litter layer in any of our samples] If the litter layer is removed it's best to clear an area somewhat larger in diameter than the coring guides to reduce the amount of debris that can fall into the coring hole as sample sections are removed. Before coring the length of the T-handled coring tool should be adjusted to a height so that downward pressure can easily be applied the entire length of the core being collected (usually 20 cm). [The T-handled coring tool has an adjustable shaft inside the T-handle. The length is adjusted by removing the bolt and wing-nut that hold the two pieces together, and either extending or shortening the handle to the proper length, and reinserting the bolt and replacing the wing-nut.] To collect the first core section, place the serrated end of the coring cup on the mineral surface. Apply a gentle downward pressure on the T-handled coring tool and slowly twist the coring cup into the soil by turning the coring tool in a clockwise direction. The serrated teeth on the coring cup will cut roots and soil. Application of downward pressure should stop when the depth guides touch the mineral soil surface.

Extracting the Core Cup from the Soil: The goal of this step is to pull the coring tool with core cup attached from the soil, with the soil and root sample within the core cup, without knocking debris into the hole. Place a cutting board (see Figure 3) near the coring hole before extracting the core cup from the soil. This will receive the core cup when it is removed from the soil. To extract the core cup containing soil and roots, apply an upward pressure on the T-handled coring tool while twisting with a back-and-forth motion. As the serrated end of the core cup nears the soil surface, slow the procedure down and gently remove the entire core cup from the hole and place it on the cutting board. With one person holding the T-handled coring tool vertically the other person should use the spring plunger tool (Figure 2A) to retract the spring plunger. They should now grasp the core cup and

with the help of the other person twist the T-handled coring tool to remove the core cup.

Removing Soil and Roots from the Core Cup: The goal of this step is to quantitatively transfer all the soil and roots within the core cup to a labeled zip-lock bag. With the core cup removed use clippers or a knife to clip or cut any roots that protrude beyond the core cup. Discard these protruding root segments. Carefully slip the core cup into a labeled zip-lock bag. Use a small hammer to tap the side of the core cup, using care not to change the shape of the core cup. For dry or light soils this is often all that is needed to remove the soil and roots from the core cup and transfer them to the bag. For heavy or damp soils more aggressive methods may be needed such as using a knife or a long screwdriver. When collecting samples on heavy soils the inside walls of the core cup will need to be carefully cleaned with a flexible spatula (see Figure 3) to insure that all the soil and roots are removed from the core cup and transferred to the zip-lock bag. The cutting board should be scraped with the straight spatula to transfer any soil and roots that fell out of the core during the core cup removal process. The labeled zip-lock bag should be sealed when all the soil and roots are transferred to the bag. The labeled bag should be placed in a cooler with ice or blue ice and transferred to either a refrigerator (~4 °C) or a freezer (<0 °C) for longer-term storage. Keep the soil samples refrigerated continuously from time of collection to time of processing in the laboratory. It is crucial that a permanent label is placed on each sample bag so that the sample can clearly be linked to the origin of the sample. It's recommended that a label with a permanent marker or an adhesive backed label be placed on the bag that indicates the date, location, and depth increment of the sample. The data sheets can be used to provide additional information and documentation.

Collecting the Second and Subsequent Core Segments: The goal of this step is to collect a cylinder of soil of known length containing roots deeper in the soil profile. With a core cup in place, move the adjustable depth guide into place with the metal tape measure. Before coring the length of the T-handled coring tool should be adjusted to a height so that downward pressure can easily be applied the entire length of the core being collected. To collect the second and subsequent core sections, insert the coring cup into the hole or void created by the removal of previous core sections. Care should be taken to avoid scraping the sides of the hole. When the serrated edge of the core cup is in contact with the un-cored soil (the surface adjacent to the bottom-end of the previous core section), begin applying a gentle downward pressure on the T-handled coring tool and slowly twist the coring cup into the soil by turning the coring tool in a clockwise direction. Application of downward pressure should stop when the depth guides touch the mineral soil surface [or the marked depth on the handle or coring cup is reached]. The core cup should be extracted following the procedure described above. These procedures should be repeated until all of the core segments are collected.

7.4 Exceptions to the Coring Procedure

There are times when uniformly shallow soils are encountered. For example, bedrock is encountered at approximately 30 cm below the mineral soil surface. Moving to another location will not provide additional soil depth for collection samples. In this situation, one 20 cm core segment can be collected and one ~10 cm core segment. The height of the last core segment is determined by measuring the length of the core cup not occupied by soil and subtracting this from the overall length of the core cup (mean length = 29.0 cm). The un-occupied length is determined by holding the core cup with soil and roots in it on the cutting board and inserting the metal tape measure into the core. The tape is brought into contact with the top of the core segment. While applying a gentle pressure the un-occupied length is read off the tape to the nearest 0.1 cm. Four values are to be determined, on in each of the four quadrants of the core cup. The four values are recorded on the Sample Collection Log Sheets (see Appendix B). Later the mean of these 4 values will be subtracted for the core cup length to calculate the length of the soil core collected. An alternate method to obtain the length of the core segment that is less than 20 cm is to measure the depth of the cored hole. This is done by inserting the metal tape into the core hole until it reaches the bottom of the hole. The depth of the hole is read off the tape at the top of the hole (i.e., the surface of the mineral soil). This depth is recorded on the Sample Collection Log Sheet. The depth of the last core segment is determined by subtracting the sum of the full-length (e.g., three 20 cm core segments is equal to 60 cm) cores preciously collected. The mean of 4 values provides a better estimate of the actual depth of the cored soil than a single measure.

Another exception exists when the soils being sampled contain a large proportion of coarse fragments. For example, the 0 – 20 cm section may be easily collected. However, it is not possible to collect the 20 – 40 cm section because of a rock at 20 cm. In this case, moving the entire core up to 1 meter to another location (recorded on the Sample Collection Log Sheet) may produce a location that allows the collection of a full set of core sections. Another acceptable option is to excavate the soil surrounding the first hole down to 20 cm to create a flat surface at 20 cm. The 20 – 40 cm core section may now be collected by slightly moving the core cup to a new location a few centimeters away from the original hole and away from the rock that prevented additional coring in the original hole. This procedure may need to be repeated for the 40 – 60 cm core section. It becomes difficult to excavate a sufficiently large enough hole to collect samples at depths deeper than 60 cm. The use of a tile probe may help to locate sites where coring is likely to be successful.

There are soil conditions in which soils are either dry, loose-grained (e.g., beach sand), or both that are to be sampled. In these instances the core cup is twisted into the soil and the soil around the core cup is excavated by one of the coring team members while the other team member holds the T-handled corer steady in a vertical position. The straight metal spatula is inserted at the bottom (serrated end of core cup) of the core cup. The spatula is used to hold the soil and root sample in

the core cup while the T-handled corer, core cup, and sample are transferred to the cutting board. The sample usually almost falls out of the core cup. The contents of the core cup are transferred to a labeled zip-lock bag. As in the previous paragraph, the soil surrounding the first core is excavated down to 20 cm and the 20 – 40 cm core section collected. The step of excavating the soil surrounding the 20 – 40 cm core is repeated and the straight spatula is inserted at the bottom or the core cup. Often, the deeper a soil is cored, the more moist and cohesive the soil matrix becomes. While this procedure can be repeated for the 40 – 60 cm core section, it is likely that the soil and root sample will remain in the core cup following the routine coring procedure. The same is likely to be true for subsequent core segments. If this is not true, then the spatula technique should be repeated to depths that can be adequately excavated.

8.0 Analytical Procedures

Once the soil/root samples have been collected the samples may be processed in one of several ways depending on the kinds of final samples desired. If root biomass is the only desired outcome, then the samples should be wet-sieved. If soil and root samples are desired, then the samples should dry-sieved to separate the soil from the roots. Once separated, the roots should be washed and both the soil roots dried. High purity, distilled and de-ionized (DI) water or reverse osmosis (RO) water should be used if the roots are to be analyzed for total elemental content. Tap water may be used if root mass (biomass) and C and N content are to be determined. However, it is recommended to use DI or RO water for the final rinse for these roots too.

8.1 Separating Roots from Soil by Wet Sieving

1. Place the 2 mm sieve in a dishpan. Transfer the entire sample from its zip-lock bag onto the sieve. Rinse the bag with a squeeze bottle of water and pour it into the sieve, to insure sure that no soil or roots remain in the bag. [Note: If the samples were frozen, they should be thawed at room temperature for several hours before processing. Only thaw the number of samples that can be completely processed in one day.]
2. Spray water from a squeeze bottle, or rubber tubing from a faucet, onto the soil with a back and forth motion to wash the soil particles through the sieve, leaving behind the roots and coarse fragments larger than 2 mm. [Note: A sediment trap should be used to keep soil particles from going down any drains. When the trap is full it can be dumped into the trash or on the waste soil pile near the TERA facility.] Using forceps or gloved fingers, pick off all the roots that are held by the screen. Transfer these to a clean cutting board.
3. Invert the sieve in a second clean dishpan, and tap several times to dislodge the coarse fragments and any remaining roots. Inspect the sieve carefully and remove any remaining roots and transfer them to the cutting board with the other roots. If needed, carefully rinse the sieve with water into the dish pan. Transfer any roots that are dislodged by this process to the cutting board.

4. Inspect the root to make sure that they are free of soil particles. In not, transfer the ones that need to be cleaned to a beaker of clean water and agitate them with the forceps to remove any remaining soil particles. Once clean, use the forceps to transfer the roots back to the cutting board.
5. Use the plastic ruler to measure the diameter of the roots. Roots are typically sorted into three diameter classes: < 1.0 mm, 1.0 to 2.0 mm, and > 2.0 mm, however, other classes can be used if the experiment requires it. Use a sharp razor blade to cut roots at the point where their diameters are 1.0 and 2.0 mm. Sort the root segments into the distinct diameter classes. Large plastic weighing boats are useful for holding the different diameter classes of roots. These should be labeled with "< 1.0 mm", "1.0 to 2.0 mm", and "> 2.0 mm" with the sharpie.
6. When all the roots are sorted by diameter class they are quantitatively transferred to labeled paper envelopes and closed with a staple. Label these envelopes with the sample ID that includes the coring site location information (e.g., Juniper Field Site, Core #10, 20 – 40 cm depth increment), and the root diameter classification ("< 1.0 mm", "1.0 to 2.0 mm", and "> 2.0 mm").
7. If the roots are to be freeze-dried the envelopes should be placed in a freezer. If they're to oven dried they should be placed in a forced air drying oven set to 65°C. Dry the roots for at least 48 hours. Record the time the samples went in the drying oven in order to make sure that they are not removed prematurely. If the root samples are to be freeze-dried, follow the procedure described by ISIRF EP.05, Version 1.0, Labconco Freeze Drier Operation.
8. Following standard experimental procedures as outlined in TERA EP.00 (EPA 1997b), weigh the dried root samples. First, place a piece of weighing paper on the balance and re-zero the balance. Then, pour the dried root sample onto the weighing paper. Tear the envelope apart to make sure that all roots have been removed. Weigh the roots and record the weight on the log sheet (Appendix C).
9. Following weighing, place each root fraction in a new paper envelope for archiving or processing for chemical analysis. As before, label these envelopes with the sample ID that includes the coring site location information (e.g., Juniper Field Site, Core #10, 20 – 40 cm depth increment), and the root diameter classification ("< 1.0 mm", "1.0 to 2.0 mm", and "> 2.0 mm").

8.2 Separating Roots from Soil by Dry Sieving

Following this procedure, produces separate soil, root and coarse fragment samples from a single soil/root sample.

1. Nestle together the sieve with the 2 mm openings and the sieve pan (sieve pan on bottom). Transfer the entire sample from its zip-lock bag onto the sieve. Make sure that the contents of the zip-lock bag are completely transferred to the sieve. [Note: If the samples were frozen, they should be thawed at room temperature for several hours before processing. Only thaw the number of samples that can be completely processed in one day.]
2. Gently shake the sieve and pan in a back and forth motion. Gloved fingers can be used to break up soil aggregates and push the soil through the sieve. Use forceps or

gloved fingers to pick roots off the sieve. Knock the roots against the sieve to remove as much of the adhering soil as possible. Transfer roots to a beaker of water to wash off remaining soil particles as described above. The roots are processed following the steps outlined above.

3. Once all the soil has passed through the sieve and the roots have been removed, move the sieve over a dishpan and spray water from a squeeze bottle, or rubber tubing from a faucet, onto the > 2 mm coarse fragments and wash them free of soil particles. When clean, the coarse fragments can be transferred to a labeled envelope for drying and water and soil residue in the dishpan poured into a sediment trap.
4. The soil in the sieve pan is quantitatively transferred to a labeled zip-lock bag or labeled soil sample bag. The soil may now be analyzed as "fresh" or "field moist" soil, freeze-dried or oven dried to stabilize them for subsequent analyses.

9.0 Quality Assurance / Quality Control

To insure that information is properly documented and recorded, standard Sample Collection Log Sheets (Appendix B) and Sample Analysis Log Sheets (Appendix C) have been developed. Range checks for valid data entries will be performed in a SAS program as described below in Section J.

10.0 Preventive Maintenance and Corrective Action

Preventive maintenance and calibration of the scales used for weighing the roots takes place on an annual basis. Before and after each weighing session, level the balance, if necessary, and place a standard 1 g weight on the balance to check its calibration (see TERA EP.00 for details). If the weight indicated wavers, try placing a cardboard box around the balance as a windscreen. If the scales are not functioning properly, corrective action will be taken to have them repaired. Other available scales which meet the measurement specifications may also be used in this case.

11.0 Data Reduction, Validation and Sample Archiving

Once the data have all been entered on the sample analysis log sheets, they will be entered into a Microsoft Excel file. All entries will be proofread. A comma-separated-value (.CSV) format file will be created by Excel and read into a SAS program. This SAS program will perform range checks to insure that all data entries are within a valid range of values. The dried root samples in paper envelopes will be stored in Room 108, Plant Ecology Building.

Figure 1: T-handled coring tool with coring cup and adjustable depth guide.

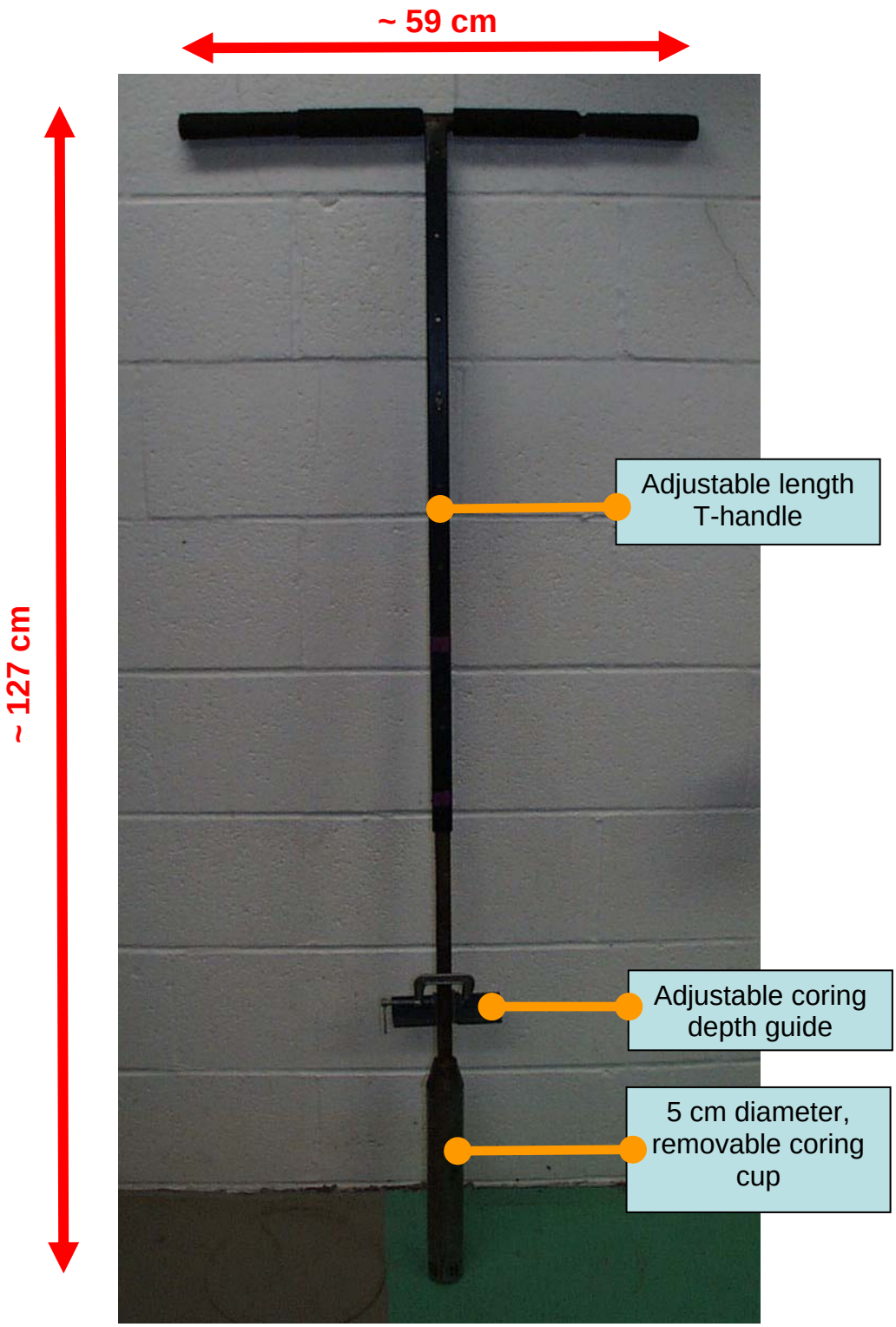
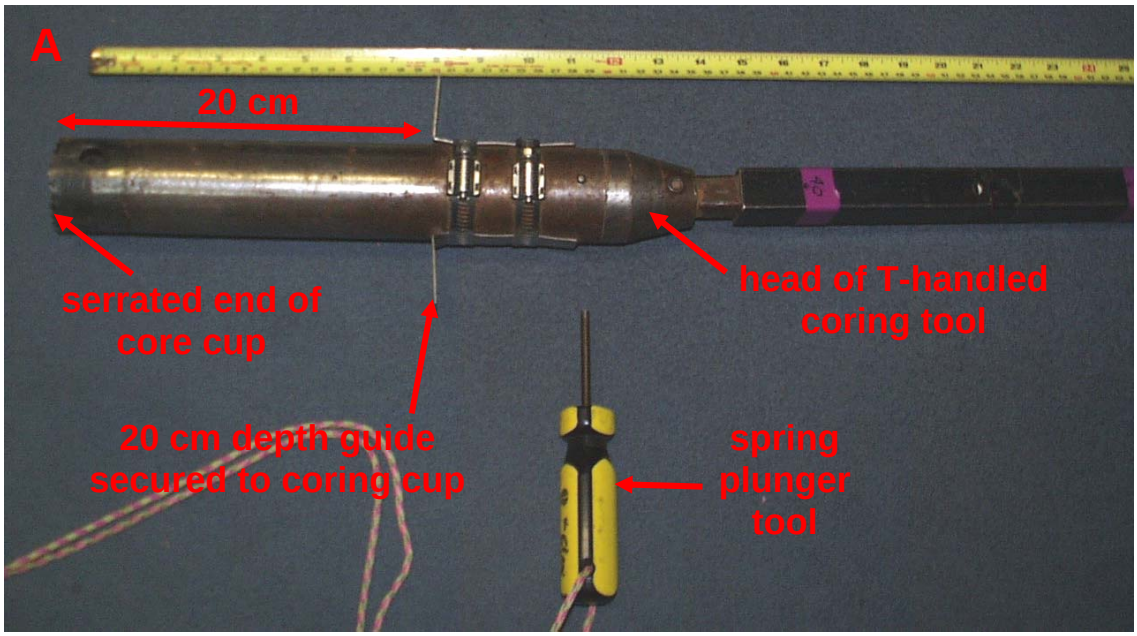


Figure 2: Coring Depth Guides – A) Attached to coring cup; and B) Attached to shaft of T-handled coring tool.



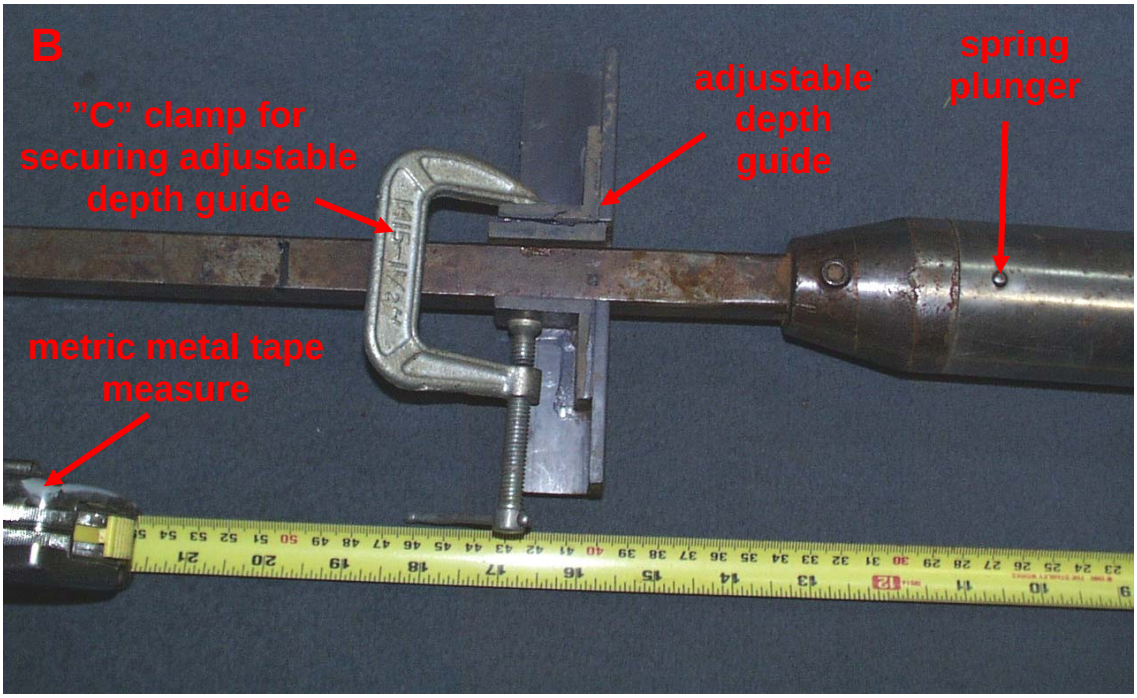
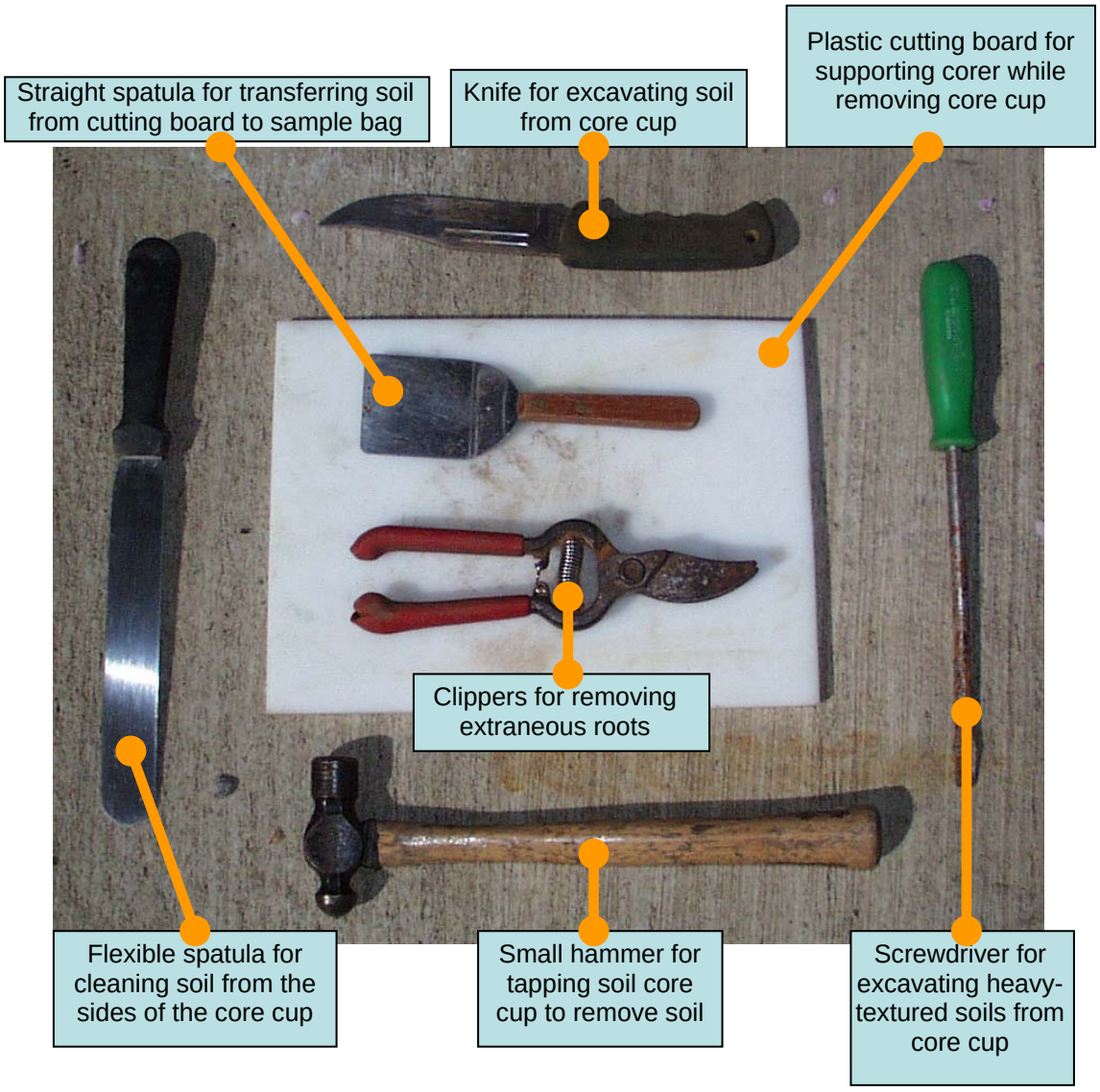
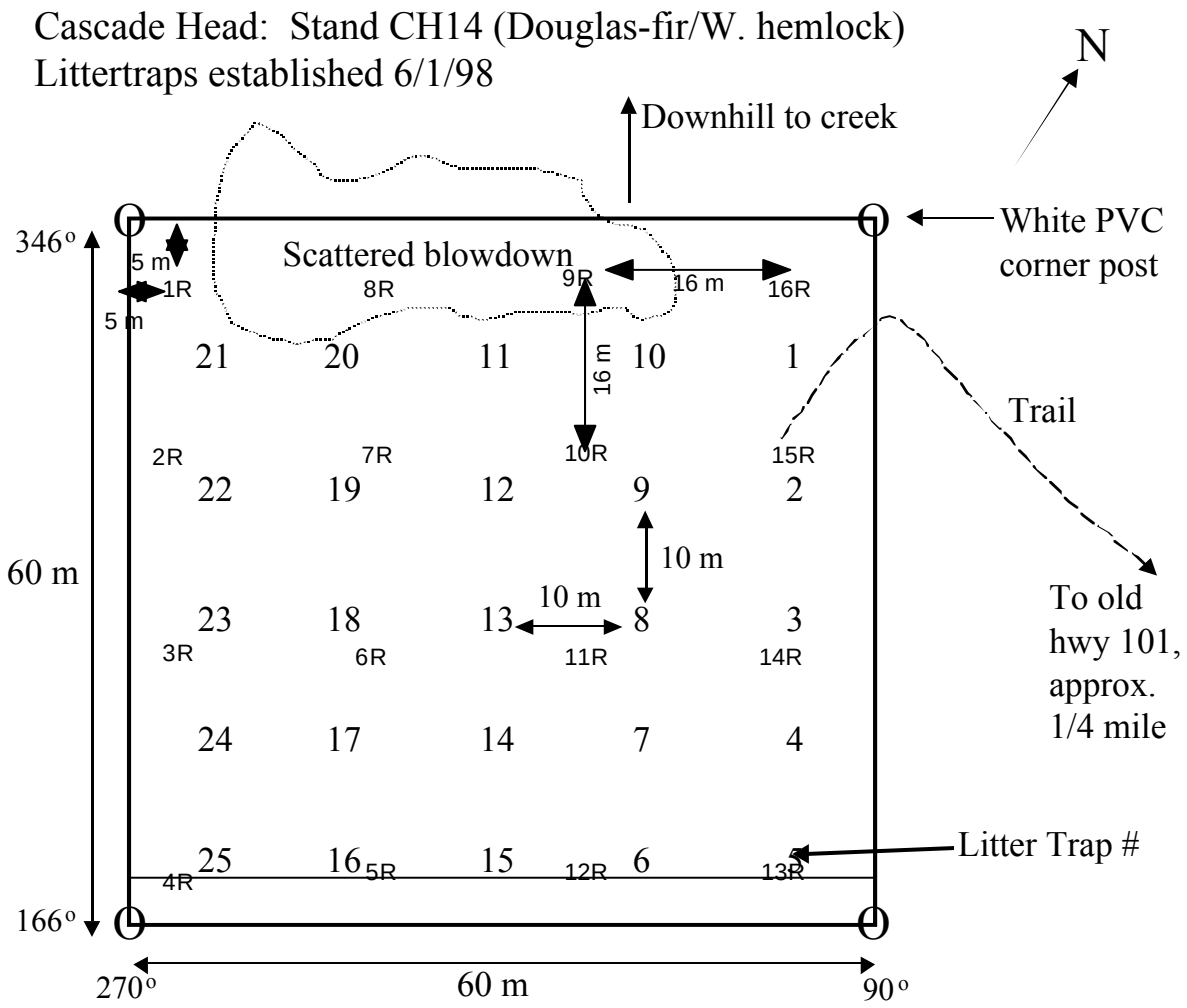


Figure 3: Tools for Removing Soil/Root Samples from Soil Coring Cup



Appendix A: Site Maps for Indicators Project Field Plots

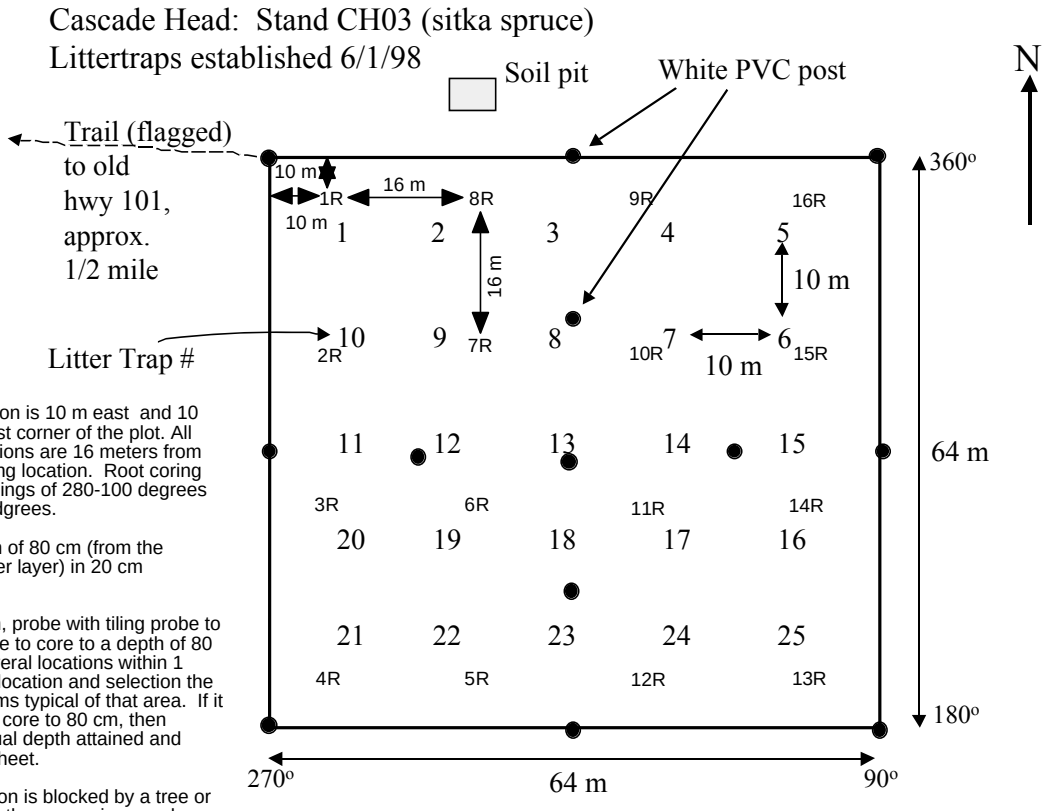


First coring location is 5 m east and 5 south of northwest corner of the plot. All subsequent locations are 16 meters from the previous coring location. Root coring locations on bearings of 270-90 degrees and 360-180 dgrees

Core to the depth of 80 cm (from the surface of the litter layer) in 20 cm sections.

At coring location, probe with tiling probe to see if it is possible to core to a depth of 80 cm. If not try several locations within 1 meter of sample location and selection the location that seems typical of that area. If it is not possible to core to 80 cm, then measure the actual depth attained and note on QA log sheet.

If sampling location is blocked by a tree or obvious foot path then move in a random pattern to a suitable location near by.



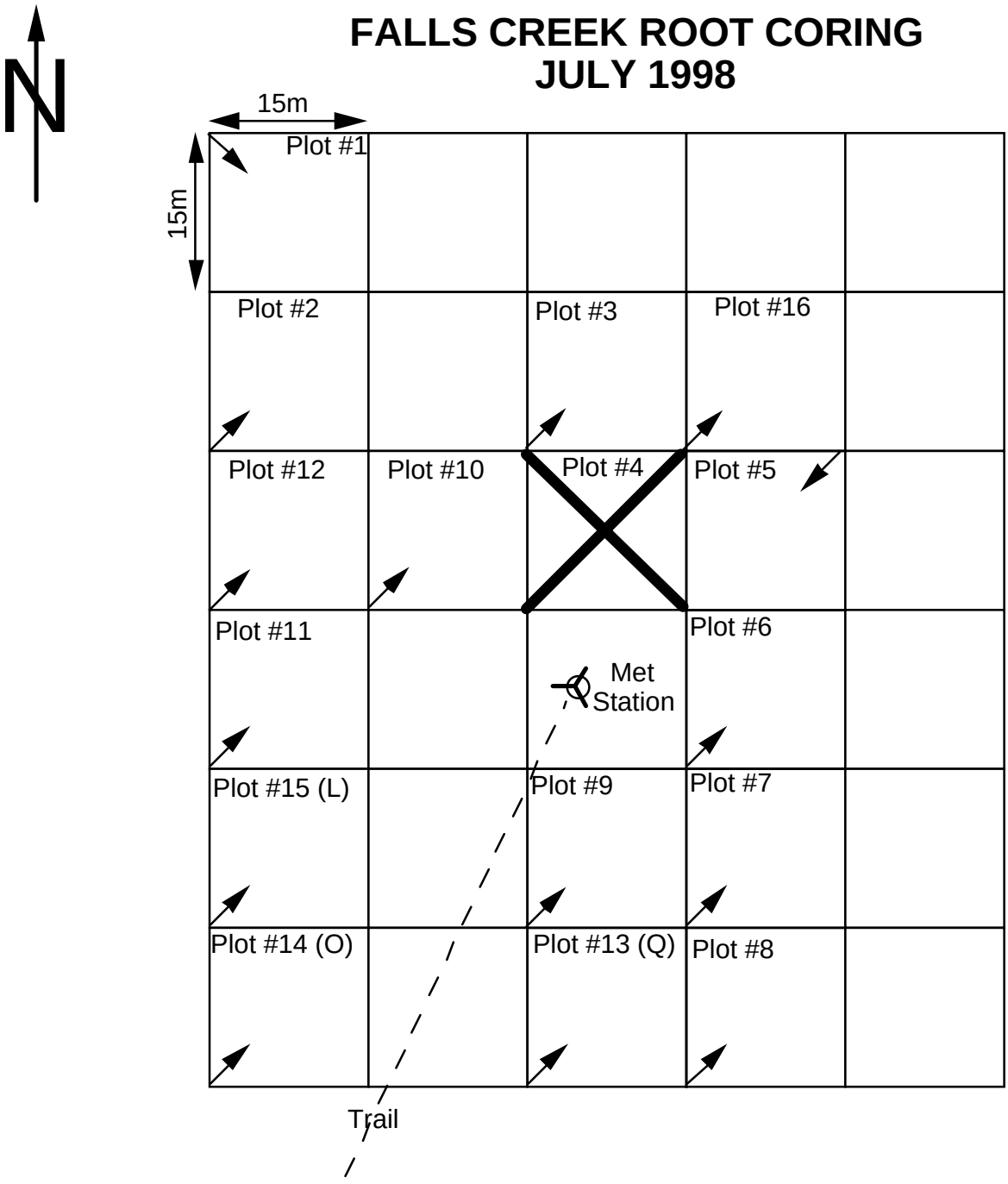
First coring location is 10 m east and 10 south of northwest corner of the plot. All subsequent locations are 16 meters from the previous coring location. Root coring locations on bearings of 280-100 degrees and 190 and 10 dgrees.

Core to the depth of 80 cm (from the surface of the litter layer) in 20 cm sections.

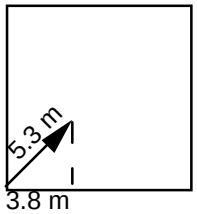
At coring location, probe with tiling probe to see if it is possible to core to a depth of 80 cm. If not try several locations within 1 meter of sample location and selection the location that seems typical of that area. If it is not possible to core to 80 cm, then measure the actual depth attained and note on QA log sheet.

If sampling location is blocked by a tree or obvious foot path then move in a random pattern to a suitable location near by.

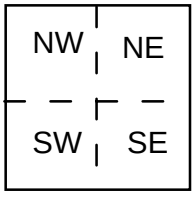
FALLS CREEK ROOT CORING
JULY 1998



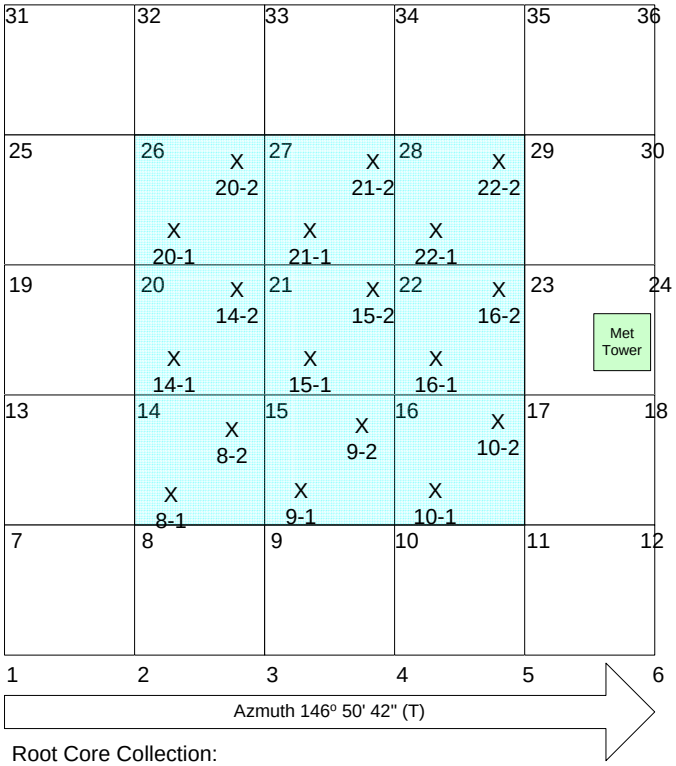
Distance from corner of subplot to coring location



Four quandrants in each subplot



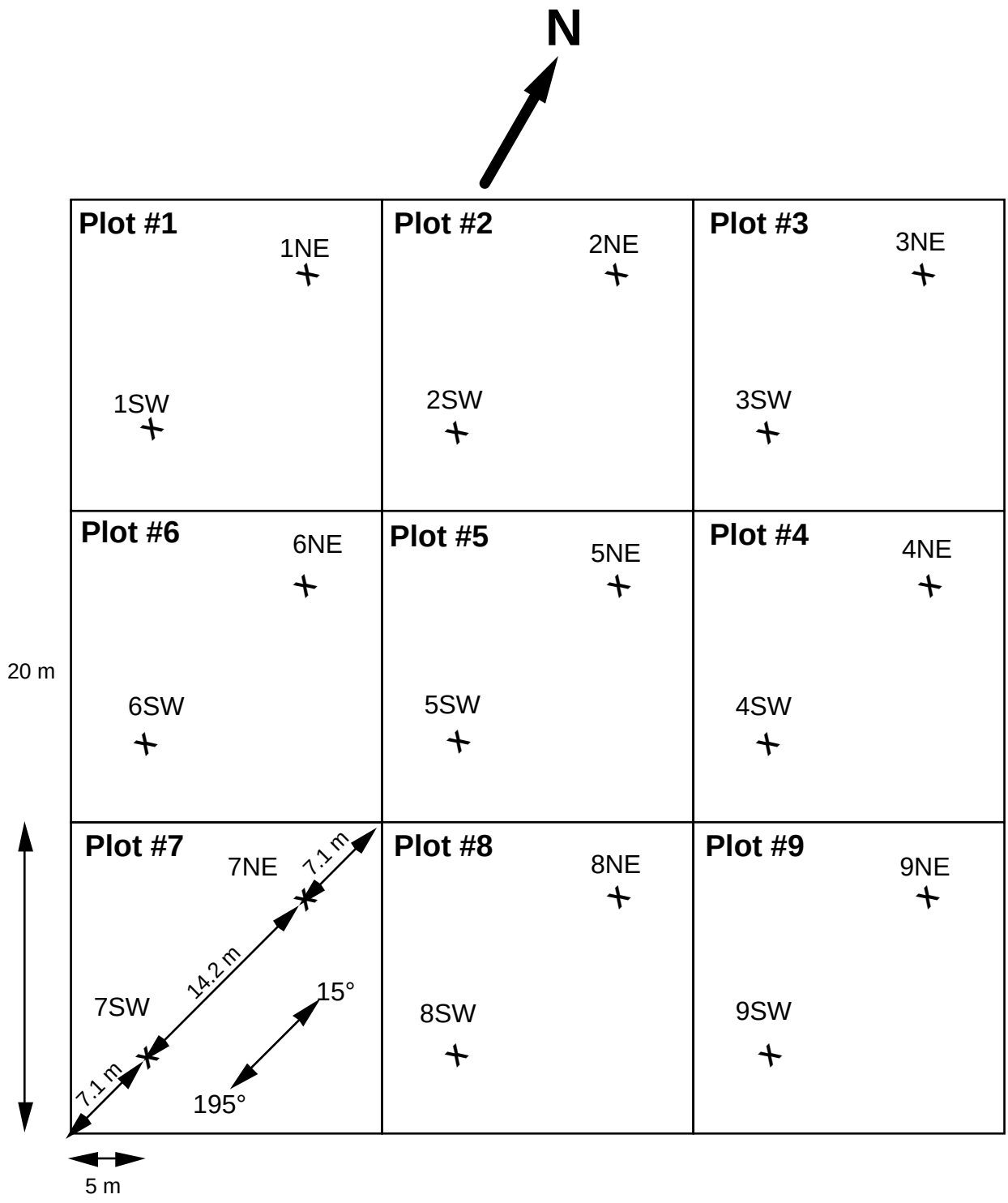
Root Coring
Moose Mountain
June 2002



Root Core Collection:
The coring will focus on the center 9 plots as they have a buffer around them. The plots are not number, hence coring in locations will be based on the number of the corner post. Corner posts 8, 9, 10, 14,15,16,20,21, and 22 will be used to identify the coring locations. The first sample will be collected 7 m (hd) from the corner post at an azimuth of 100º (T) and the second will be collected 21 m (hd) from the corner post on the same azimuth. There will be be 2 cores collected in each plot. The samples will be identified in relation to the corner post. The samples at 7 m are #1 and samples at 21 m are #2. For example, the core numbering for post 8 are 8-1 and 8-2.

Cores are collected in 20 cm sections measured from the surface of the litter layer to a depth of 80 cm. There will be be 4 separate samples collected at each coring locations.

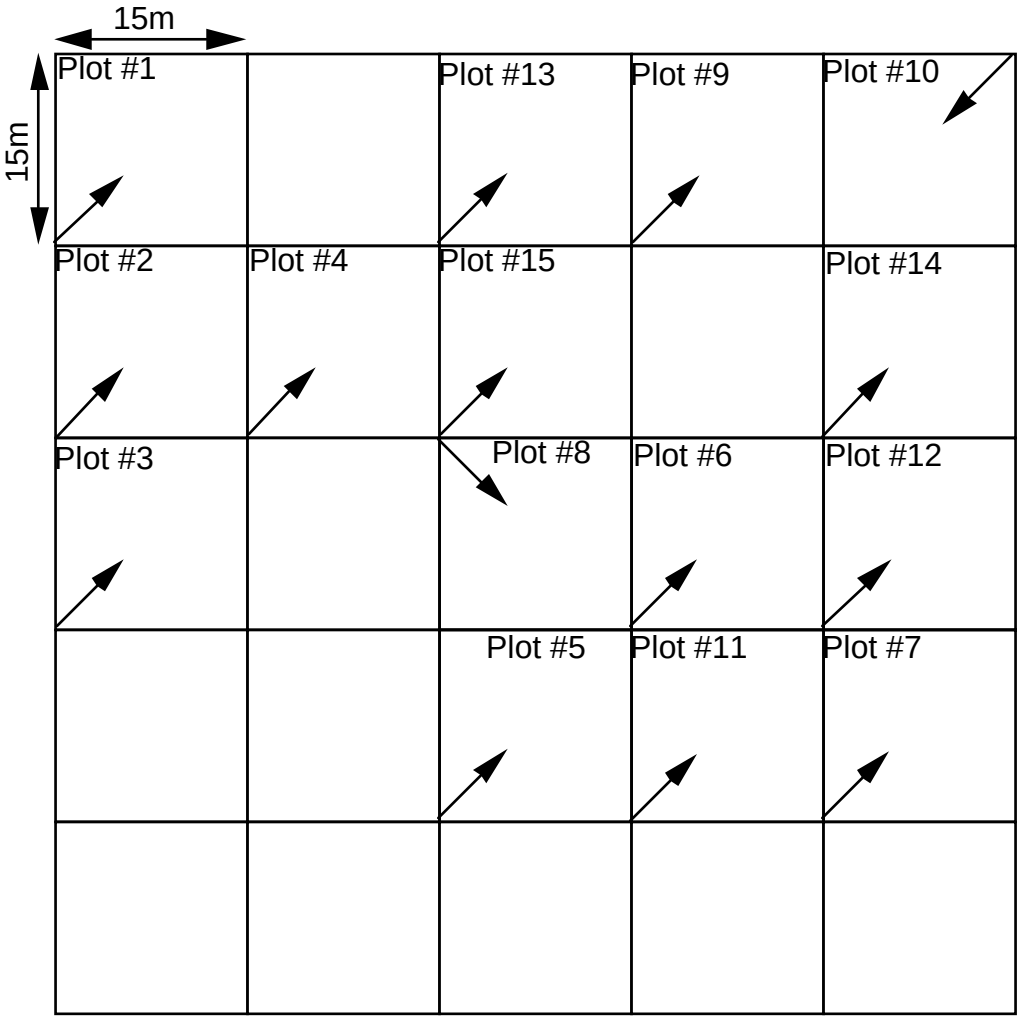
If sampling location is blocked by a tree or obvious foot path then move in a random pattern to a suitable location near by.



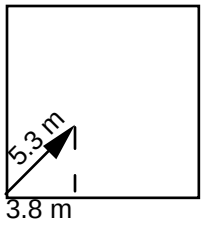
Root Coring
Soapgrass Mountain
July 1998

cored to 60 cm

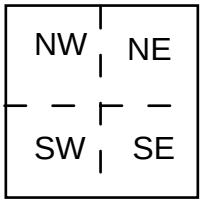
TOAD CREEK ROAD ROOT CORING
JULY 1998

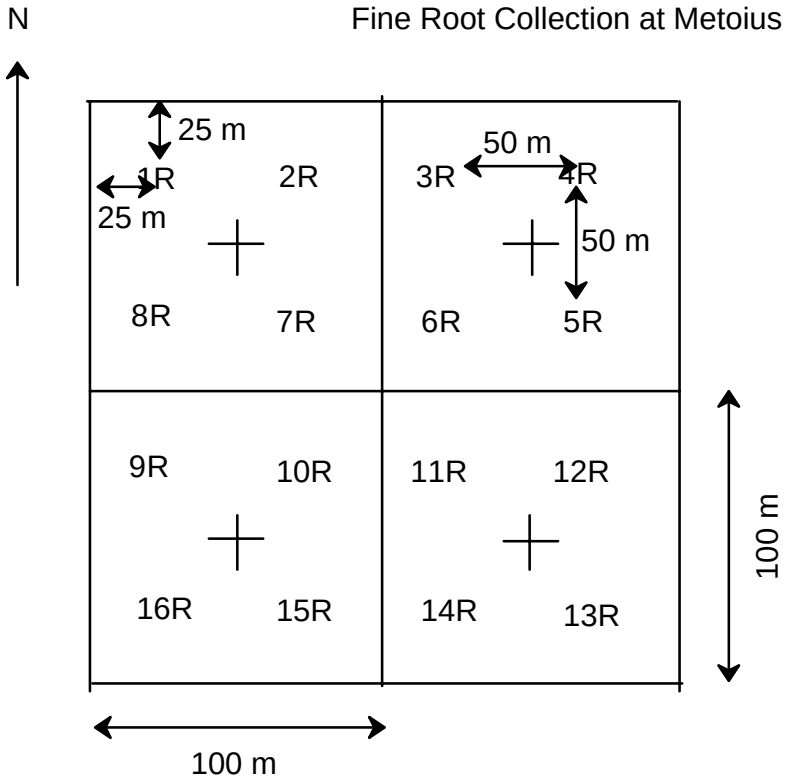


Distance from corner of subplot to coring location



Four quandrants in each subplot





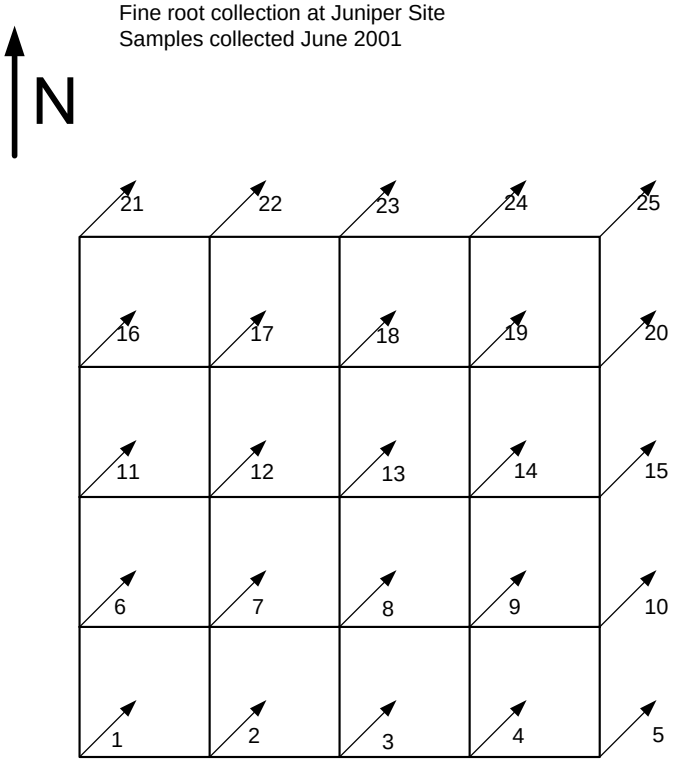
First coring location is 25 m east and 25 south of northwest corner of the plot. All subsequent locations are 50 meters from the previous coring location.

Core to the depth of 80 cm (from the surface of the litter layer) in 20 cm sections.

At coring location, probe with tiling probe to see if it is possible to core to a depth of 80 cm. If not try several locations within 1 meter of sample location and selection the location that seems typical of that area. If it is not possible to core to 80 cm, then measure the actual depth attained and note on QA log sheet.

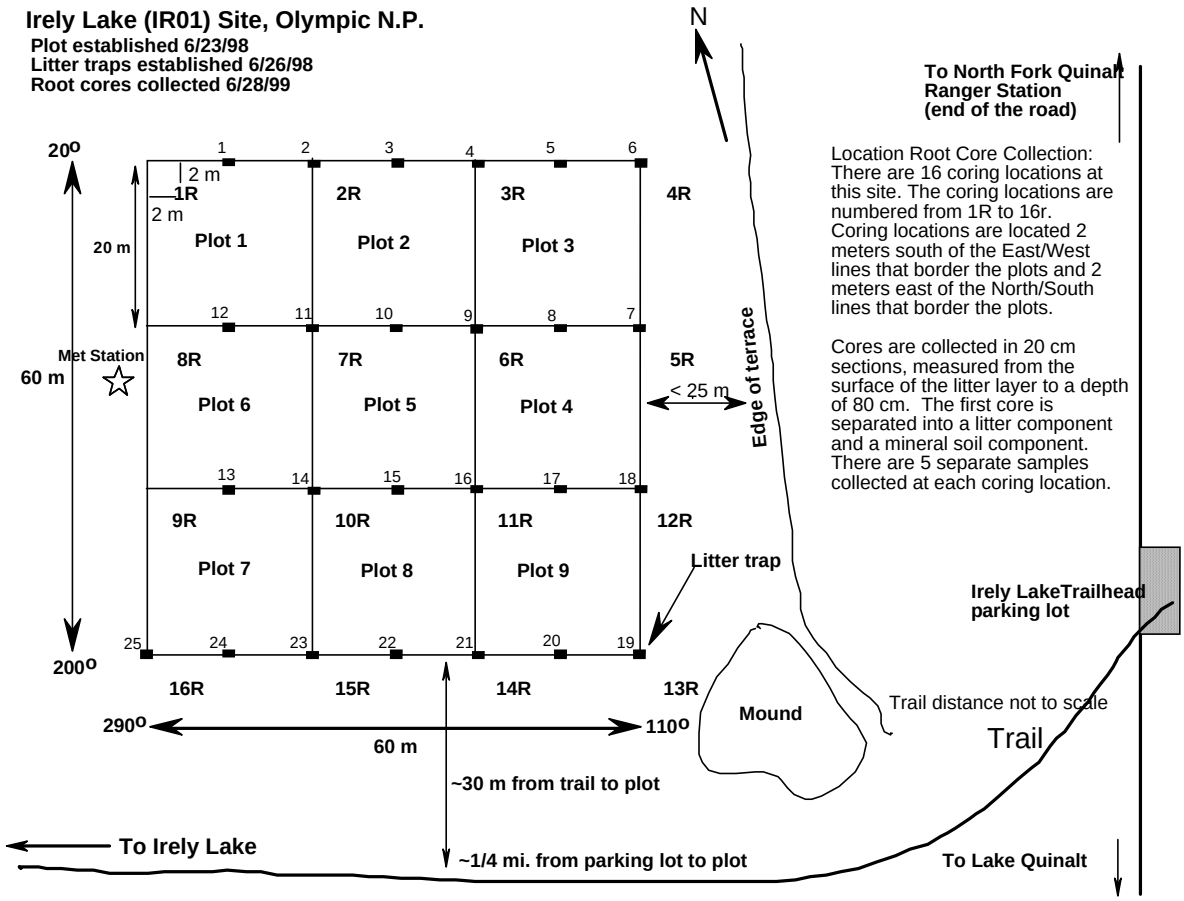
If sampling location is blocked by a tree or obvious foot path then move in a random pattern to a suitable location near by.

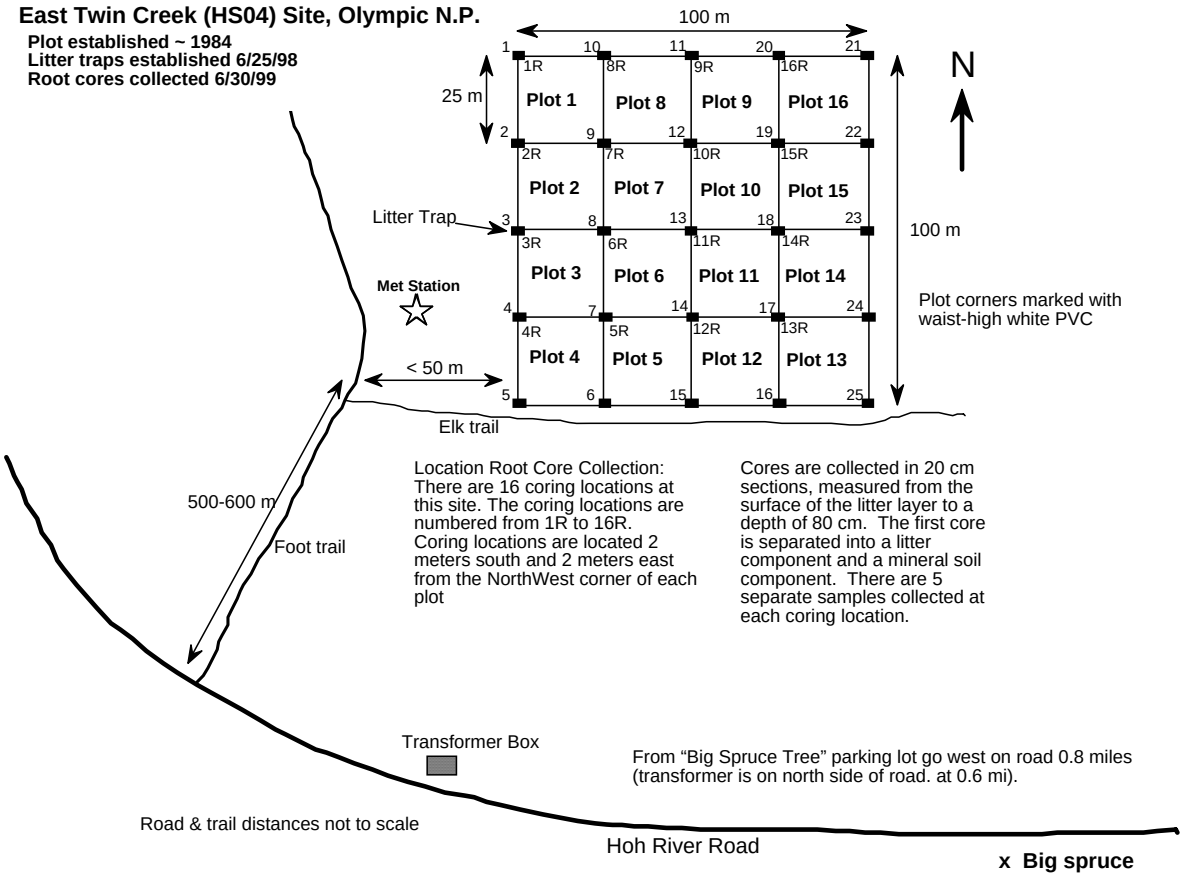
Fine root collection at Juniper Site
Samples collected June 2001



Coring procedure: Root/soil samples were collected at 25 locations at the Juniper site. From each corner (the south west corner of each subplot), the coring site was located 14 meters (this is the same as going 10 m N and then 10 m E) at a 45 degree angle from the corner. The arrow marks the location of each sample point. Samples were collected in 20 cm segments. Samples were collected until rocks prevent further downward progress then the coring hole was moved within ~30 cm distance and coring continued until rock was struck again. At that point we measured the coring depth and did not attempt to core further.

To insure uniform spacing of samples, the coring sites always went the same direction which meant that sample on the east and north edges extended beyond the plots.


Irely Lake (IR01) Site, Olympic N.P.
Plot established 6/23/98
Litter traps established 6/26/98
Root cores collected 6/28/99
N
To North Fork Quinalt Ranger Station (end of the road)
Location Root Core Collection: There are 16 coring locations at this site. The coring locations are numbered from 1R to 16r. Coring locations are located 2 meters south of the East/West lines that border the plots and 2 meters east of the North/South lines that border the plots.
Cores are collected in 20 cm sections, measured from the surface of the litter layer to a depth of 80 cm. The first core is separated into a litter component and a mineral soil component. There are 5 separate samples collected at each coring location.
20°
200°
290°
110°
2 m
2 m
20 m
60 m
60 m
1R
2R
3R
4R
5R
6R
7R
8R
9R
10R
11R
12R
13R
14R
15R
16R
Plot 1
Plot 2
Plot 3
Plot 4
Plot 5
Plot 6
Plot 7
Plot 8
Plot 9
Met Station
< 25 m
Edge of terrace
Litter trap
Irely LakeTrailhead parking lot
Mound
Trail distance not to scale
Trail
~30 m from trail to plot
~1/4 mi. from parking lot to plot
To Irely Lake
To Lake Quinalt

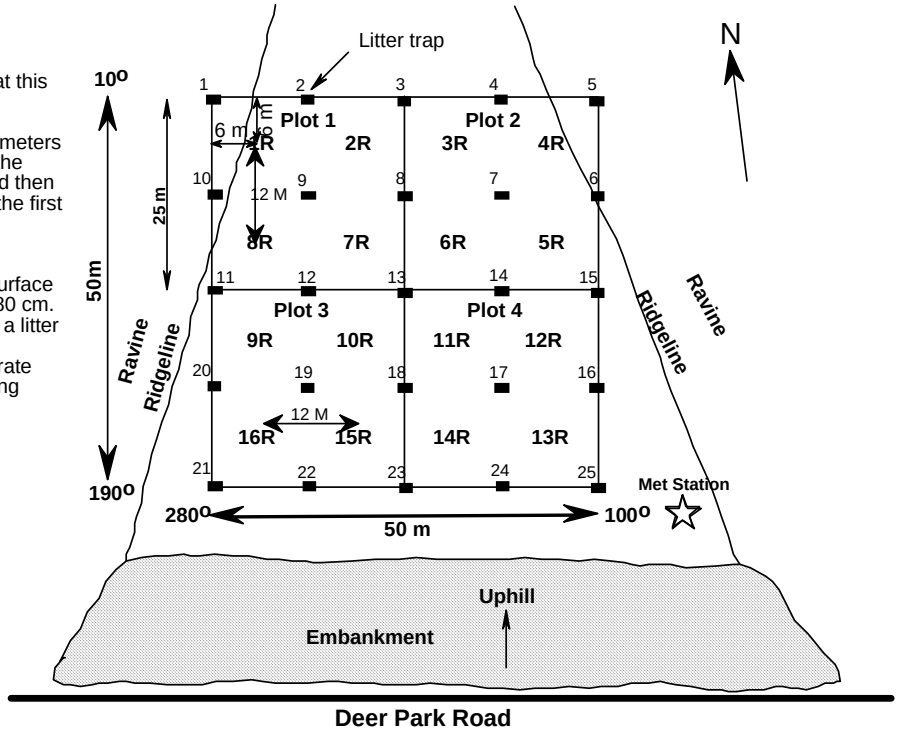

East Twin Creek (HS04) Site, Olympic N.P.
Plot established ~ 1984
Litter traps established 6/25/98
Root cores collected 6/30/99
100 m
25 m
N
Plot 1
Plot 8
Plot 9
Plot 16
Plot 2
Plot 7
Plot 10
Plot 15
Plot 3
Plot 6
Plot 11
Plot 14
Plot 4
Plot 5
Plot 12
Plot 13
Litter Trap
Met Station
100 m
Plot corners marked with waist-high white PVC
< 50 m
Elk trail
500-600 m
Foot trail
Location Root Core Collection: There are 16 coring locations at this site. The coring locations are numbered from 1R to 16R. Coring locations are located 2 meters south and 2 meters east from the NorthWest corner of each plot
Cores are collected in 20 cm sections, measured from the surface of the litter layer to a depth of 80 cm. The first core is separated into a litter component and a mineral soil component. There are 5 separate samples collected at each coring location.
Transformer Box
From "Big Spruce Tree" parking lot go west on road 0.8 miles (transformer is on north side of road. at 0.6 mi).
Road & trail distances not to scale
Hoh River Road
x Big spruce

Deer Park (DP01) Site, Olympic N.P.

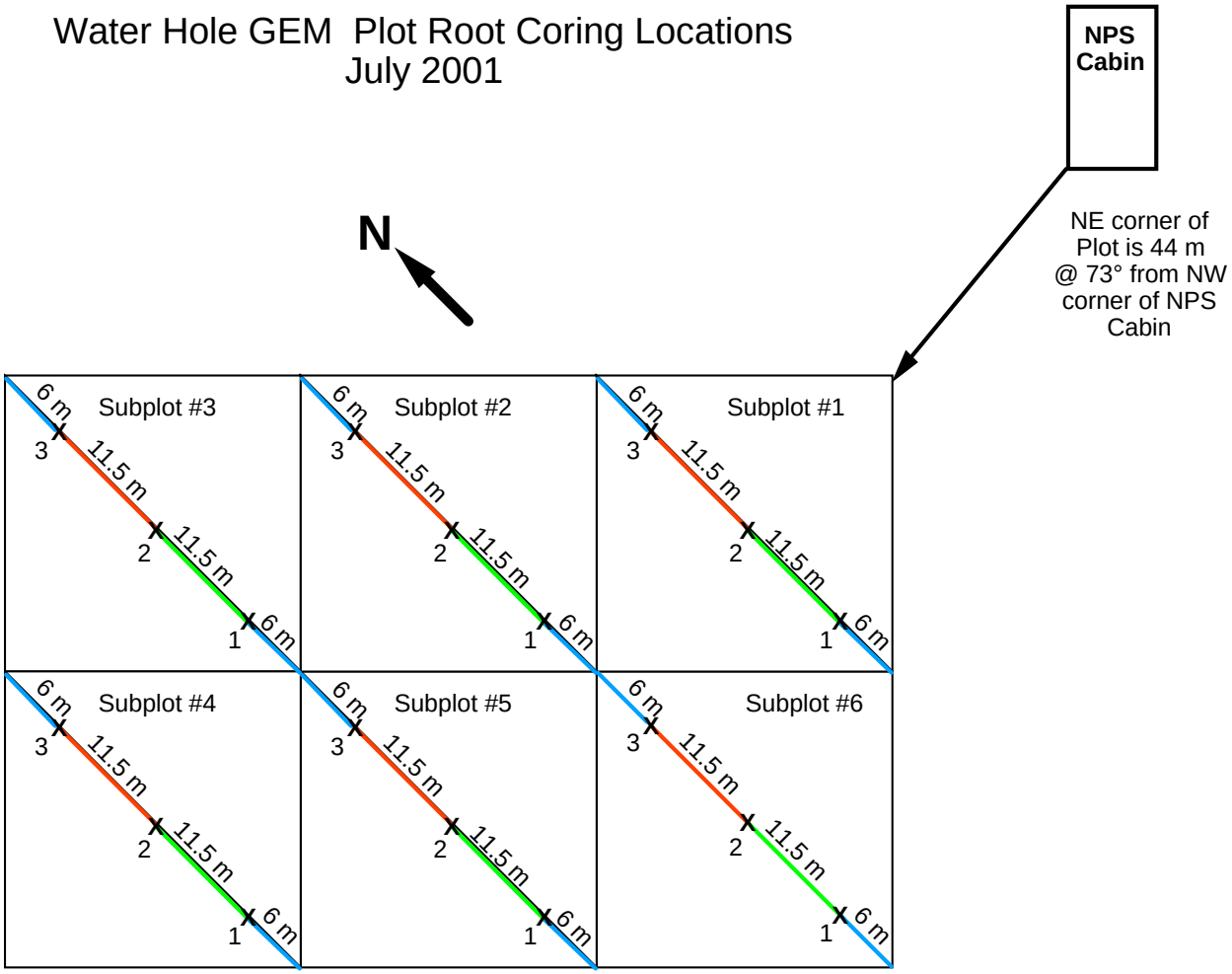
Plot established 6/24/98
Litter traps established 6/24/98
Root cores collected 7/1/99

Location Root Core Collection:
There are 16 coring locations at this site. The coring locations are numbered from 1R to 16R.
Coring locations are located 6 meters south and 6 meters east from the NorthWest corner of Plot 1 and then are located at 12 meters from the first location in Plot 1

Cores are collected in 20 cm sections, measured from the surface of the litter layer to a depth of 80 cm. The first core is separated into a litter component and a mineral soil component. There are 5 separate samples collected at each coring location.



Water Hole GEM Plot Root Coring Locations
July 2001



Appendix B: Example Field Coring Check Sheet

Site: U.S. EPA Indicators Project Water Hole Field Site
Date: July 21, 2001
Coring Team Members: M.G. Johnson, D.T. Tingey

Subplot	Core Location	0 – 20 cm	20 – 40 cm	40 – 60 cm	60 – 80 cm	Max Coring Depth (cm)	Notes
1	1						
1	2						
1	3						
2	1						
2	2						
2	3						
3	1						
3	2						
3	3						
4	1						
4	2						
4	3						
5	1						
5	2						
5	3						
6	1						
6	2						
6	3						

Appendix C: Example Sample Analysis Log Sheet Page __ of __

Site: U.S. EPA Indicators Project Water Hole Field Site
Sample Type: Roots

Date	Analysts[1]	Site Code[2]	Subplot	Core Location	Depth Increment	Root Diameter Class (mm)	Root Dry Weight (g)	Replicated Root[3] Dry Weight (g)	CV of Repeated Weights
		WHF	1	1	0 – 20 cm	0 – 1			
		WHF	1	1	0 – 20 cm	1 – 2			
		WHF	1	1	0 – 20 cm	> 2			
		WHF	1	1	20 – 40 cm	0 – 1			
		WHF	1	1	20 – 40 cm	1 – 2			
		WHF	1	1	20 – 40 cm	> 2			
		WHF	1	1	40 – 60 cm	0 – 1			
		WHF	1	1	40 – 60 cm	1 – 2			
		WHF	1	1	40 – 60 cm	> 2			
		WHF	1	1	60 – 80 cm	0 – 1			
		WHF	1	1	60 – 80 cm	1 – 2			
		WHF	1	1	60 – 80 cm	> 2			
		WHF	1	2	0 – 20 cm	0 – 1			
		WHF	1	2	0 – 20 cm	1 – 2			
		WHF	1	2	0 – 20 cm	> 2			

[1]Initials of analysts weighing roots
[2]MTF = Metolius, TCF = Toad Creek Forest, FCF = Falls Creek Forest, JPF = Juniper Forest, MMF = Moose Mountain Forested, SGF = Soapgrass Forest, ETF = East Twin Creek Forest, ILF = Irely Lake Forest, DPF = Deer Park Forest, WHF = Water Hole Forest, CH14 = Cascade Head 14 (Douglas fir), CH03 = Cascade Head 03 (Sitka Spruce)
[3]For QC purposes 10% of the samples are reweighed and the CV of the repeated weights is calculated. CV's greater than 5% indicate are not acceptable and must be reweighed.

APPENDIX 7

PROTOCOL

FOR GAS FLUX MEASUREMENT

U,S. Department of Agriculture (USDA). 2003. USDA-Agricultural Research Service (ARS) GRACEnet. Chamber-based Trace Gas Flux Measurement Protocol. April 24.

USDA-ARS GRACEnet
Chamber-based Trace Gas Flux Measurement Protocol

April 24, 2003

Trace Gas Protocol Development Committee[1]

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Greg McCarty mccartyg@ba.ars.usda.gov
John Baker jbaker@soils.umn.edu

Scope:

1. This protocol only addresses N_2O and CH_4 flux measurement methodology. The reactivities of other gasses of interest such as NO_x O_3, CO, and NH_3 will likely dictate that separate chambers and associated instrumentation be employed. CO_2 can also be included as an analyte with this protocol, however, when plants are present, interpretation of CO_2 data is complicated.

2. This protocol adopted chamber-based flux methodology (the least expensive option available) in order to allow inclusion of as many sites as possible. Since micromet techniques are expensive, they will be used at only locations with current micromet capability (Minnesota, Iowa, others?).

3. In deciding on a chamber design, our goal was the adoption of methodology which is sensitive, unbiased, has low associated variance, and allows accurate interpolation/extrapolation over time and space. Because of our inability, at this time, to *precisely* assess the extent of bias associated with a given chamber design and sampling protocol under the range of conditions which might exist, we have adopted our 'best guess' protocol. Assessment, refinement and/or modifications of the protocol may continue in the future. At some sites this may include evaluation of chambers against micromet fluxes or performing comparisons of alternate chamber designs. Recognizing that any measurement technique will have disadvantages, the best we can do at this time is to select a technique which minimizes potential problems. To facilitate the adoption of a common technique, it is important to attain a common understanding of the potential shortcomings associated with chamber-based flux measurement techniques. The following section discusses some of these issues.

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[1]Questions or comments on the protocol can be directed to Tim Parkin (parkin@nstl.gov).

Background

Mosier (1989) reviewed the key issues related to chamber techniques for gas flux measurement. These are summarized below along with recommendations to minimize potential problems.

1. Soil Disturbance:	-Soil disturbance upon installation
	-Longer term microclimate effects
Recommendations:	-Use temporary/portable chambers.
	-Install permanent chamber anchors at least 24 h prior to flux determinations.
	-Anchors or collars should be as short as possible to minimize micro environment perturbations.
	-Move chamber anchors if soil microclimate effects are observed.
2. Temperature perturbations:	-Influence biological activity
	-May cause physical absorption or dissolution of dissolved gasses.
Recommendations:	-Use insulated, reflective chambers.
	-Keep deployment time as short as possible.
3. Pressure perturbations:	-Wind may cause pressure-induced mass flow over chamber collar
	-Closed chamber may reduce natural mass flux.
	-Sampling effects may induce mass flow
Recommendations:	-Use vented chamber.
	-Use skirted chambers
4. Humidity perturbations:	-Gas solubility changes (probably a minor effect)
	-Humidity increases in the chamber may result in dilution of the gas of interest and resulting underestimate of the flux
	-Changes in humidity may impact biological activity (minor)
Recommendations:	-Keep chamber deployment short
	-Measure relative humidity changes inside chamber to correct for dilution effects from water vapor.
5. Temporal Variability:	-Diurnal variations. There is some evidence in the literature that diurnal variations (up to a factor of 10) in soil gas flux follow diurnal temperature fluctuations, however, this characterization is not consistent.
	-Daily variation. Day-to-day variation may be highly dependant upon rainfall, fertility, tillage or freeze thaw events.
	-Seasonal variation. Spring and Winter fluxes can be substantial and need to be considered.
Recommendations:	-Measure flux at times of the day that more closely correspond to daily average temperature (mid morning, early evening).
	-Apply a temperature correction algorithm to measured fluxes when time-of-day temperature induced biases might be present.

-Measure fluxes 3 to 4 times/week, all year long.
-Stratify sampling to account for episodic events.

6. Spatial Variability: -Can be extremely high. Coefficients of Variation associated with chamber-based fluxes commonly exceed 100%.

Recommendations: -Use chambers with larger footprint to minimize small scale variability.
-Use as many chambers as possible.

7. Gas Mixing : -It is generally assumed that molecular diffusion is sufficiently rapid within the chamber headspace such that homogeneous gas concentrations exist when sampling. However, this may not necessarily be true if large amounts of vegetation are present or the chamber volume:surface area is large (Livingston and Hutchinson, 1995).

Recommendations: -If it is deemed that mixing of the headspace gas is necessary, there are a couple of options.
-1. Chambers can be fit with small fans. A 12 VDC computer fan will run on a 9 volt transistor radio battery and is a cost effective way of incorporating a fan into a chamber design. Computer fans can be obtained from Action Electronics, Santa Anna, CA. Phone: (800) 563-9405, www.action-electronics.com. Example of a 12vdc fan from this company is part # 108idc12vdcs1b. Cost: ~ $7.00
-2. A gas manifold within the chamber attached to the sampling port can be used. The manifold has a single port on one end (which extends out the top of the chamber) and multiple ports on the other end which accept narrow teflon tubing (e.g., 1/16") that extend into the chamber. The narrow tubing from each of the multiple inner ports is extended to different points inside the chamber, so that when the sample is collected, gas is pulled from multiple points in the chamber. Manifolds can be purchased from Small Parts, Inc. 800-220-4242, www.smallparts.com. An example part no. is TCM-13-20/4-10 (description = Tubing Manifold 13G inlet 20G outlet).

Given these considerations, there have been a number of different chamber-based methods proposed in the literature. Below are provided our best recommendations. See referenced literature for additional details.

Recommended Protocol

General:
Gas flux will be measured by static chambers deployed on the soil surface for a period of no more than 60 min. During chamber deployment, samples of the chamber headspace gas will be removed at regular intervals, and stored for later analysis by gas chromatography. Specific recommendations on chamber design, gas sampling and analysis, and flux calculations are provided below. Investigators are encouraged to examine the referenced literature underlying these recommendations.

Chamber Design
Minimum Requirements:

1. Flux chambers should be fabricated of non-reactive materials (stainless steel, aluminum, PVC, polypropylene, polyethylene, or plexiglass.)
2. Material should be white or coated with reflective material, (Mylar, or painted).
3. Chambers should be large enough to cover at least 175 cm^2 of the soil surface, and have a target height of 15 cm (height can be decreased to increase sensitivity or increased to accommodate plants).
4. Chambers should contain a vent tube, at least 10 cm long and 4.8 mm in diameter (e.g. 1/4" stainless steel tubing). See Fig. 1 for details.
5. Chambers should have a sampling port to enable the removal of gas samples. Possible options include: butyl rubber stopper (Alltech # 95256), or nylon/polyethylene stopcock (ColeParmer # A-30600-000 : Qosina, #99705 or #99717).

Recommended Design:
Two part chamber consisting of a permanent anchor, driven at least 8 cm into the soil and extending no more than 5 cm above the soil surface, and a cap which contains the vent tube and sampling port. Anchors are fabricated so that they can accommodate the flux chamber during measurement phase. Anchors and chambers made of 8"(or larger) diameter PVC. Alternatively, anchors can be made of thin-walled stainless steel or aluminum to minimize physical disturbance upon insertion. The vent tube is necessary to avoid pressure pertubations (and subsequent mass flow) when chambers are installed and when gas samples are collected. Schematics of two potential chamber designs are presented and photographs of a variety of

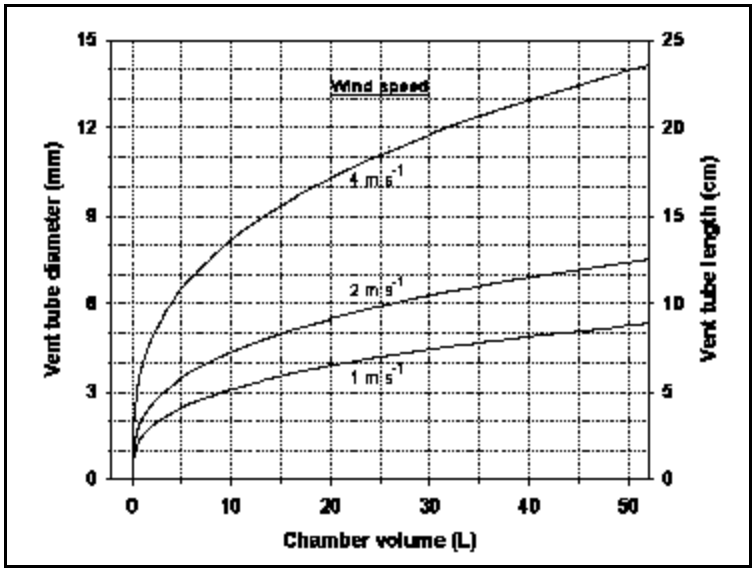

Figure 1. Optimum vent tube diameter and length for selected wind speeds and enclosure volumes as described by Hutchinson & Mosier (1981).

chambers in operation are provided in Appendices 3 and 4.

Chamber Deployment

Anchors : As indicated above, anchors should be installed at least 8 cm into the ground and extend no more than 5 cm above the surface. Permanent anchors should be installed at least 24 h prior to first flux measurement. There are no fixed guidelines regarding how long anchors can (or should) be left in place. In cultivated systems, chamber anchors are typically removed prior to cultivation, planting, or fertilizer application, then replaced. In grassland studies anchors have been left for over 10 years with no apparent deleterious effects. One advantages of leaving anchors in place is that soil disturbance and root damage are minimized. However, there have been reported problems with microclimate effects within the anchors left in place for extended periods. For example changes in humidity or shading can cause algal growth, and in heavy or compacted soils ponding of rainwater can occur. This is not a desirable situation. It will be up to the investigator to determine how often chambers should be moved.

Plants:

If the goal of this project is to quantify ecosystem contributions to net trace gas flux, then ideally, plants should be included inside chambers during flux determinations. There is some information indicating that N_2O emissions may be facilitated by living plants (Chang et al., 1998; Chen et al, 1999; Smart and Bloom, 2001). However, inclusion of plants presents an interesting problem. With regard to sensitivity, inclusion of plants would likely dictate that chamber height be increased, but an increase in chamber height results in a corresponding decrease in sensitivity (i.e. increase in minimum detectable limit, see below). Significant reductions in sensitivity might, in some cases, result in all the flux measurements being below the detection limit. In such cases, it is advisable to also measure bare soil fluxes (i.e. between rows in row-crop agriculture) using shorter chambers which have higher sensitivity. Results could then be reported as fluxes within a range of the bounds established by the two measurements. If it is not feasible to include plants (at all growth stages) at least chambers should be deployed both within and between rows (in row crop agriculture). Alternatively, chambers with a larger foot print and therefore providing more representative coverage of the ecosystem under study, can be used.

Sample numbers:

Trace gas fluxes exhibit a high degree of spatial variability. Thus, the more chambers, the better. Variability may also be a function of chamber size, and may be reduced by using larger chambers. Recommendation for minimum number is 2 chambers per treatment in plot scale studies. In landscape or field scale studies it is recommended that 'similar' landscape elements be identified and a stratified sampling design employed, whereby samples are stratified by landscape element, soil type, or vegetation (Livingston and Hutchinson, 1995). In situations where identifiable hot spots may occur (e.g. urine patches in a grazed system) a stratified sampling may have to be developed to account for this. Gilbert (1987) gives some sampling guidelines when hot spots exist.

Sampling frequency:

Trace gas fluxes exhibit a high degree of temporal variability. Thus, the more frequently measurements are made, the better. There are several elements to temporal variability that must

be considered: diel or diurnal variations, seasonal variations, and variations induced by perturbation (e.g. tillage, fertility, irrigation/rainfall, thawing). Flux measurements should be made mid-morning of each sampling day to minimize biases associated with diurnal variations. However, a Q_{10} temperature correction procedure may applicable to adjust rates determined at different times. The temperature correction procedure assumes that temperature variations are the primary factor driving diurnal flux variations, thus the temperature correction adjusts the measured flux to the average daily soil temperature. To account for perturbation effects it is recommended that fluxes be measured as soon as possible after the perturbation (such as rainfall, tillage, or fertility event), then daily for the next several days during and following the specific event. During the remainder of the year gas flux measurements should be made at regular time intervals (1, 2 or 3 week intervals) as resources allow.

Gas sampling

Fluxes are measured by determining the rate of change of trace gas concentration in the chamber headspace. In most cases trace gas concentrations are determined by physically removing a gas sample from the chamber headspace for analysis in the laboratory. Gas samples should be withdrawn at regular intervals during the chamber deployment. Chambers should be in place no longer than 60 minutes. The shorter time the deployment time, the better, but deployment must be long enough so that sensitivity is not compromised. At least 3 time points are required for flux calculation: time 0, and two additional points, equally spaced in time (e.g. 0, 30 60 min. or 0, 20, 40 min). *[Note: Sampling is performed at regular intervals to facilitate flux calculation by Eq. 1 (below). However, more samples can be collected, and sampling does not have to be at regular intervals if the stochastic model of Petersen et al., (2001) is used.]* Sampling is performed by inserting a polypropylene syringe into the chamber septa and slowly removing a gas sample. Mixing of headspace gas by pumping the syringe before sampling is not recommended as pumping may cause pressure perturbations and/or excess dilution of headspace gas by entry of outside air through the vent tube. The gas volume removed at each time point is dictated by the specific gas analysis technique to be used. Typically, from 5 to 30 ml are removed. If the syringe is equipped with a stopcock, the sample can be stored directly in the syringe. Alternatively, the gas sample can be transferred to a previously evacuated glass vial sealed with a grey butyl rubber septum. If this option is selected, excess gas is usually injected into the evacuated vial (relative to the vial volume) to produce an overpressure. This overpressure facilitates the subsequent removal of a gas sample for analysis. Brooks (1993) evaluated several storage protocols and found that red rubber stoppers such as found on commercially available evacuated blood vials were the worst. Parkin has observed that red rubber stoppers are reactive to methane. However, others report no problems with coated red

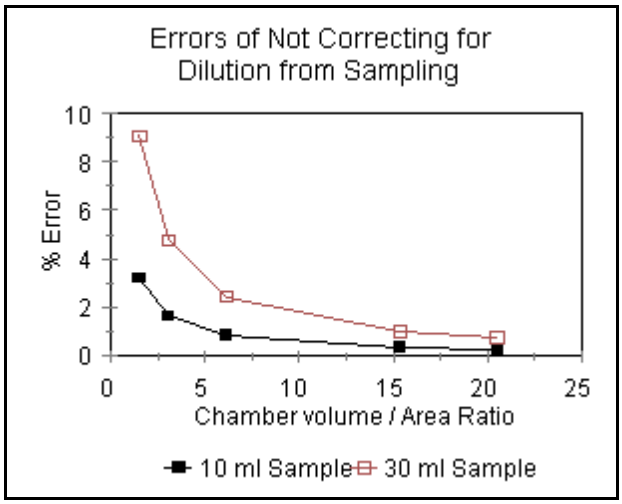

Figure 2. Percentage underestimation of flux rate due to headspace dilution as a result of sampling, presented as a function of chamber geometry and gas sample size.

rubber stoppers. Details of gas sampling and analyses are noted in Mosier et al. (1991, 1996). It should be noted that each time a headspace gas sample is removed from the chamber outside air flows into the chamber through the vent tube. This results in a dilution of the analyte in the chamber headspace. The error associated with this dilution effect is a function of both the sample volume withdrawn and the chamber Volume/Surface Area ratio (Figure 2). Correction for this dilution effect should not be necessary for chamber Volume/Surface Area ratios >10 and sample volumes < 30 ml. An example of a gas sampling protocol is presented in Appendix 2.

Gas Analysis

Samples should be run as soon as possible after collection. Gas chromatography will be used for analysis of N_2O and CH_4 (Electron capture detector for N_2O and Flame ionization detector for CH_4). Specific method of gas sample injection into the GC will depend upon the specific instrumentation available at each location. However, it is recommended that the GC be fit with a sample valve to minimize injection error. To account for problems associated with GC drift it is recommended that: 1) samples from individual chambers be run in sequence (e.g. t_o, t_1, t_2,) rather than segregating all the samples by time (e.g. all the to samples run together) and ii) standards be run periodically throughout the sample run (e.g. every 10 to 20 samples).

Standards:

Standards should be prepared each sampling time. Standards should be handled in a manner similar to samples with regard to collection and storage. Preferably samples should be prepared in the field (i.e. injected into glass vials, or collected in syringes). Several different standard concentrations should be run, as detector response may be nonlinear. The range of standards should bracket the concentrations found in samples. Examples: N_2O; 0.1, 1.0 and 10 ppm. CH_4; 0.5, 1, 2, 10 ppm. Standard curves are then used to convert the GC output of the samples into units of ppm. Certified standard gasses can be obtained from Scott Specialty Gas (www.scottgas.com) or Scott Marian.

Data Analyses:

Flux Calculation:

Fluxes are calculated from the rate of change of the concentration of the analyte of interest in the chamber headspace. Since the units associated with the gas standards are typically ppm(v), when the standard curve relationship is applied to calculate gas concentrations of the samples, the resulting unit of the analyte is also ppm(v). Volumetric parts per million (ppm(v)) has units of uL trace gas L^{-1} total gas.

If the rate of change of headspace trace gas concentration is constant (ppm(v) vs. time data is linear) then linear regression can be used to calculate the slope of the concentration vs. time data. The slope of the line is the trace gas flux. Thus, a regression of ppm(v) vs. minutes will result in a slope with units of ppm(v) min^{-1}. Multiplying the slope by the chamber volume (L) and dividing by the chamber surface area (m^2) will result in a flux with units of uL trace gas m^{-2} min^{-1}

If the rate of change of headspace trace gas concentration is not constant (ppm(v) vs. time data is curvilinear) then linear regression is not appropriate. Curvilinear concentration data with time is

attributed to a build up of the analyte concentration in the chamber headspace, which alters the diffusion gradient and the resulting flux. To account for this effect, Hutchinson and Mosier (1981) proposed an algorithm as an alternative to linear regression (Eq. 1).

$$f_o = V(C_1 - C_0)^2 / [A^* t_{1^*} (2^*C_1 - C_2 - C_0)] * \ln[(C_1 - C_0)/(C_2 - C_1)] \qquad \text{Eq. [1]}$$

where f_o is the flux at time 0, V is the chamber headspace volume (L), A is the soil surface area (m^2), C_0, C_1, and C_2 are the chamber headspace gas concentrations (ppm(v)) at time 0, 1, and 2, respectively, and t_1 is the interval between gas sampling points (min). The resulting units of f_o are: uL trace gas m^{-2} min^{-1}

It should be noted that this correction algorithm only works if $[(C_1 - C_0)/(C_2 - C_1)] > 1$ and if time points are equally spaced.

As an alternative to Eq. 1 for calculating a flux from curvilinear data, Pedersen et al. (2001) has proposed a stochastic diffusion model. The reported advantages of the Pedersen model are: i) a more rigorous treatment of gas diffusion theory, ii) there is no requirement for equi-spaced data points, and iii) it can accomadate more than 3 data points, iv) it provides an assessment of goodness of fit, and v) it has a lower failure rate than Eq. 1 . This technique will not be described in detail here, however, the computer model can be obtained from S.O. Petersen at Soren.O.Petersen@agrsci.dk .

Regarding linear regression, it should be realized, that in deciding whether to use linear regression or a non-linear model, a strict criteria for goodness of fit should be established for the linear model. Simulation data shows that even slight deviations from linearity can have a dramatic influence on the calculated flux (Fig. 3).

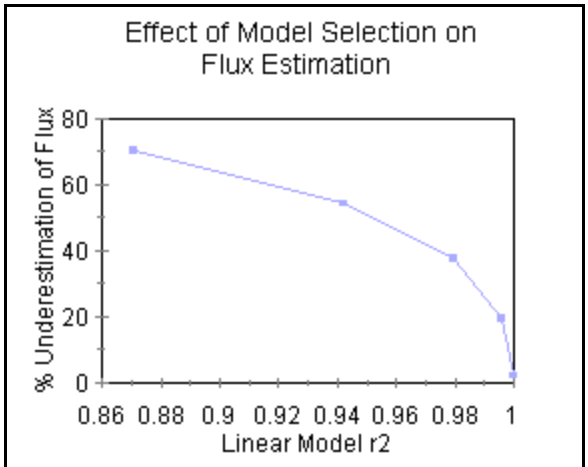

Fig. 3. Percentage underestimation of flux from linear regression as compared to non-linear analysis from Eq. 1

Flux calculations from linear regression or the non linear models described above produce values with units of uL trace gas m^{-2} min^{-1}. An additional calculation has to be performed in order to covert flux values from a volumetric basis to a mass basis. To perform this conversion the ideal gas law must be invoked (Eq. 2)

$$PV = nRT \qquad \text{Eq. [2]}$$

where P = pressure, V = volume, n = the number of moles of gas, R = the gas law constant, and T = temperature.

The ideal gas law quantifies the relationship between pressure, volume, mass and temperature of a gas.

When the value of R = 0.08206 L atm Mol^{-1} $^oK^{-1}$ is used, units of P, V, n and T have corresponding units of Atm, Liters, Moles, and ^{o}K., respectively. The goal of applying Eq. 2 is to convert uL trace gas to uMol trace gas. To do this, one must have knowledge of both the air temperature and atmospheric pressure. A table relating elevation and atmospheric pressure is provided. For example, at an altitude of 1000 ft., and at an air temperature of 20^{o}C, we can calculated from Eq. 2 that 1 uL of trace gas contains 0.0401 uMol of trace gas (see calculation box above). Thus, multiplying the calculated flux with units of uL trace gas m^{-2} min^{-1}, by 0.0401 gives flux units of uMol trace gas m^{-2} min^{-1}. (Note above that ^{o}K=(273+ ^{o}C).

1 uL trace gas * 0.965 atm / ((0.08206 L atm Mol^{-1} $^oK^{-1}$) * (273 + 20)^{o}K) * 1 L/10^6 uL * 10^6 uMol/Mol

Sample calculation to convert uL gas to uMol. (Note: conversion from ^{o}C to ^{o}K by adding 273)

Relationship between altitude and atmospheric pressure.

Alt (ft)	mm Hg	psi	atm
0	29.92	14.7	1.000335
1000	28.86	14.18	0.964949
1320	28.54	14.02	0.954061
2000	27.82	13.67	0.930244
2640	27.14	13.33	0.907107
3000	26.81	13.17	0.896219
3960	25.77	12.66	0.861513
4000	25.84	12.69	0.863555
5000	24.89	12.22	0.831571
5280	24.47	12.02	0.817961
6000	23.98	11.78	0.801629
6600	23.25	11.42	0.777131
7000	23.09	11.34	0.771687
7920	22.15	10.88	0.740384
8000	22.22	10.91	0.742426
10560	20.11	9.88	0.672334

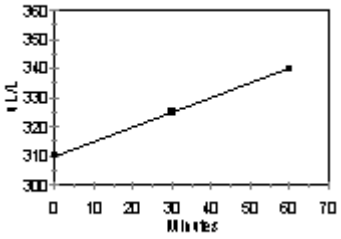

Fig. 3

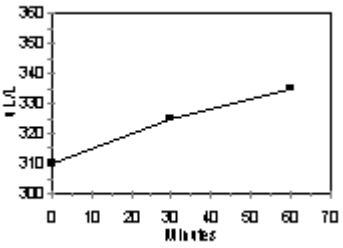

Fig 4

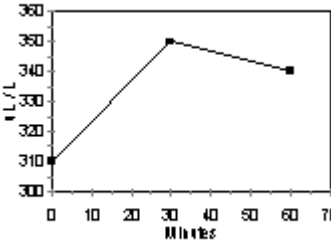

Fig. 5

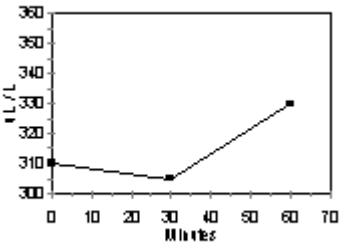

Fig. 6

Noisy Data

The change in chamber headspace trace gas concentration over time typically will be linear or curvilinear as shown in Figs. 3 and 4. In theses situations linear regression or the non-linear diffusion based models can be used to calculated the flux. However, often concentration with time data are noisy and time course data are obtained similar to those shown in Figs. 5 and 6 (Anthony et al., 1995). Determination of a flux from noisy data often requires investigator judgement. Several possibilities exist for flux estimation from noisy data including: 1) linear regession using all the points, 2) calculation of the slope from points 1 and 2, 3) slope calculation from points 1 and 3, or 4) slope calculation from points 2 and 3. If the investigator cannot discount outliers based on experience and judgement of past performance of the site or chamber, the most conservative

approach would be to adopt option 1. If noisy data proves to be a persistent problem, evaluation of GC precision, chamber design, and/or sampling protocols should be performed. Also, collection of more points during chamber deployment may help in discriminating outliers and may also yield improved estimates if the Pedersen stochastic model is applied.

Minimum Detection Limit

Often field fluxes are low, thus it is important to have an idea of the minimum detection limit (MDL). The MDL is a function of the sampling and analytical precision as well as the chamber volume and surface area. Sampling + analytical precision is determined by calculating the standard deviation of many standards on the gas chromatograph (n>20). Because instrument precision is usually a function of concentration, the standards used should contain trace gas concentrations at or near ambient levels. From analysis of large numbers of standards, precision is determined to be +-2 standard deviations of the mean. This delta ppm (2*std dev), along with specific information on the chamber volume, surface area, and chamber deployment time is used to compute the MDL as described below.

MDL = 2*std.dev uL/L * Chamber Volume (L) / Chamber Footprint (m^2) / total deployment time (min).

Units for the above computation of the MDL are uL trace gas m^{-2} min^{-1}. To convert to uMol m^{-2} min^{-1} the universal gas law must be used.

Quality Assurance /Quality Control:

Standards and standardization:

It has been reported that Scott Standard Gases may differ substantially from their stated concentrations. An alternative source of certified standard gasses is Scott Marian (these are still only +/- 2% at best). If a network of ARS sites is going to be established, it is suggested two tanks of very high quality standards containing CO_2, CH_4 and N_2O be purchased from NOAA at the cost of about $3500 + new regulator (assuming that ARS will come up with some funds). These tanks should be shipped around for people top check their GC calibrations and their standard tanks. In the interim, Ft. Collins is arranging to have one of these standard tanks made, and there may be a possibility to distribute samples of this standard in vials to different locations on a limited basis. This known standard gas would then be used to standardize gas tanks at each location. Alternatively, it has been suggested that ARS fund a trace gas analysis lab where all samples are analyzed. At this point in time agency funds do not exist to support this proposal. Details of these activities will be worked out at a future date.

Stopper Reactivity:

Currently, gray butyl rubber septa or stoppers appear to be the least reactive to N_2O and CH_4, however, there have been reports that different batches of gray butyl rubber may differ regarding their reactivity. It is recommended that individual investigators perform their own assessment of trace gas reactivity with each new batch of stoppers, regardless of the type of stoppers used. A suggested protocol for this is:

1: Prepare 60 vials with standard gas. This will be the test set.

2. Immediately after these vials are prepared run 20 of these samples.

3. After 1 day of storage (at room temperature and pressure) run 20 vials from the test set prepared on day 0, and prepare and run 20 newly prepared vials with the same standard used to prepare the test set.
4. After 1 week of storage, run the final 20 vials from the test set along with 20 vials freshly prepared.
5. Evaluate: 1) Changes in average concentration as a function of storage. 2) Changes in precision (i.e. standard deviations) as a function of storage.

Syringe Reactivity/Carryover:
Plastic syringes will leak over time. If gases are stored at any length of time in syringes equipped with stopcocks, a similar test of storage efficacy should be performed with each new batch of syringes. Polypropylene syringes are not inert, however, cross-contamination due to carryover is usually not a problem unless high concentrations are sampled, and if syringes are flushed with air between use. Similarly, if syringes are reused, the investigator might want to perform an assessment of trace gas carryover.

Ancillary Measurements
In addition to the measurements prescribed by soil sampling protocol additional measurements are recommended.

At time flux is measured:
Air temperature
5 cm Soil Temperature
Soil Water content (0-6 cm) gravimetric, capacitance (Theta Probe), or TDR.

At time of chamber installation:
Bulk density, texture, organic C and N .
Chamber headspace volume (average chamber height at several locations within the chamber multiplied by the chamber surface area)
Soil Nitrate and Ammonium (0-10 cm). **Note:** It is desirable that soil nitrate and ammonium be determined throughout the year at time intervals deemed appropriate by the individual investigator as dictated by resource availability and plot constraints.

Weather data - rainfall, air temperature, relative humidity, solar radiation.

Advice and Consultation
Several investigators involved in GRACEnet have experience in trace gas analysis and flux measurement. These people have agreed to serve as resource contacts for investigators with questions on GC set up, soils chambers, gas sampling, flux calculation, field variability, and data interpretation.

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Appendix 1. Example of Trace gas Flux Sampling Procedure

- Set of 12 Anchors placed in pairs (in-row and inter-row) -

For each set of 12 Chambers:

1. Lay out Chambers, Vials, Syringes by each anchor
2. Install 5 cm temperature Probes (1 in each plot). Air temperature and chamber temperature probes in first plot only.
3. Take ambient Gas Sample
4. Start Measurement (t 0) - Start Stop Watch
   a. Record Temperatures
      1. Place chamber on anchor #1 (vent facing downwind)
      2. Remove 10 ml gas sample
      3. Inject sample into vial
      4. Flush syringe with Air 2x
      5. Place chamber on anchor #2
      6. Remove 10 ml gas sample
      7. Inject sample into vial
      8. Flush syringe with air 2x
   b. Move to next pair of chambers in plot
      1. Record time on stop watch
      2. Place chamber 3 on anchor
      3. Remove 10 ml gas sample
      4. Inject into vial
      5. Flush syringe with Air 2x
      6. Place chamber 4 on anchor
      7. Remove 10 ml gas sample
      8. Inject into vial
      9. Flush syringe with air 2x
   c. Move to next plot
      1. Record Temperatures
      2. Repeat steps 4b.1 through 4b.8 (above)
   d. Repeat step 4c until all 12 chambers are in place and have been sampled for time 0
5. First Time Point (t 1)
   a. Move to position 1 (chamber 1)
      1. Record Soil Temperatures, record chamber temperature and air temperature.
      2. Insert syringe into chamber septa
      3. When stopwatch shows t-1 time (e.g. 20 minutes), remove 10 ml Gas sample
      4. Inject gas sample into appropriate vial
      5. Flush syringe 2x
      6. Move to next chamber, repeat steps 5a.2 - 5a.5, above.
      7. Continue until all chambers have been sampled for time 1
5. Second and third time points (t 2 and t-3)
   a. same as step 5 above.
6. Remove all chambers, Move to next set of 12 anchors. Repeat steps 1-5
7. When all plots have been done, one person collect all chambers and place in truck other person take soil moisture readings in each plot (4 measurements/plot)

Appendix 2: Suppliers

Sample Vials and Stoppers:

Option 1: Glass serum vials 6.0 ml (22 x 38 mm) and butyl rubber stoppers and aluminum crimps: Alltech, 2051 Waukegan Rd, Deerfield, IL 60015 (vial stock # 98768, butyl rubbber stoppers stock # 95256). These vials fit in the custom autosampler described by Arnold et al., 2001.

Option 2. Exetainers, screw cap 12 ml vials that have a butyl rubber septa-same idea as the serum vials and butyl rubber stoppers-just cheaper and more or less disposable-can buy new screw caps and septa relatively cheaply. Exectainers are purchased through Labco Limited (Brow Works, Copyground Land, High Wycombe, Buckinghamshire. HP123HE, United Kingdon (phone 44-1494-459741) (fax: 44-1494-465101) (Email: sales@labco.co.uk or enquiries@labco.co.uk) The cost is about $275/1000 vials. Our new CombiPal autosampler (purchased through Varian with a new GC and data system uses these vials. Exetainer vials recommended by Reynald Lemke at Swift Current. The Canadians have 4 of these instruments running-the autosampler has the capacity for 200 samples per batch.:

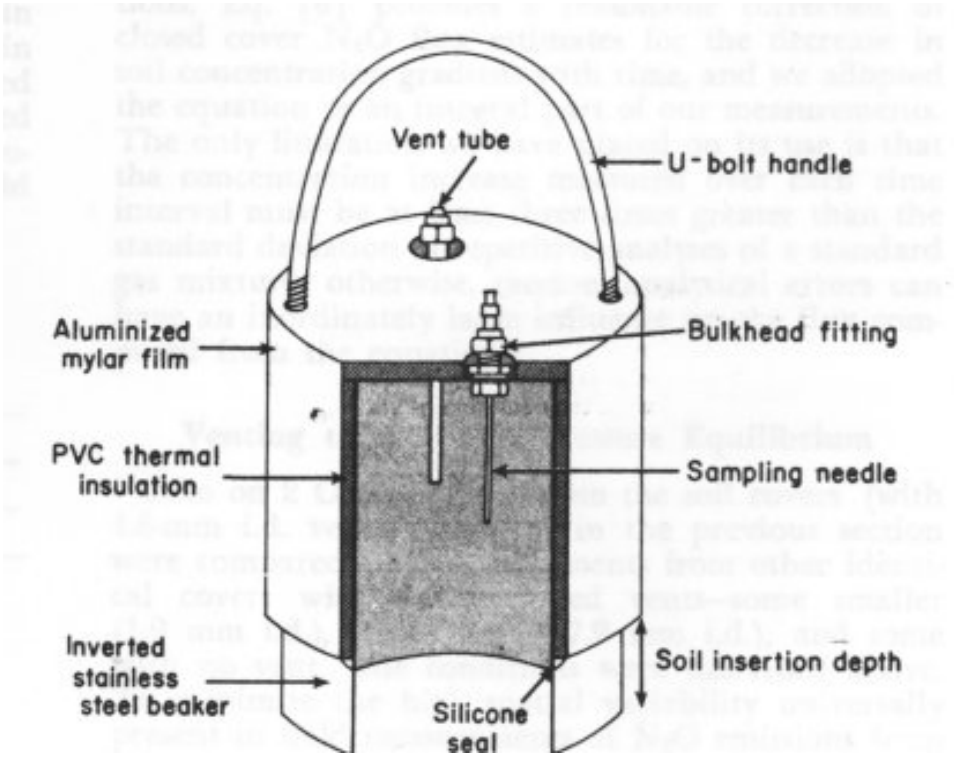
Standard gases
Scott Speciality Gas http://www.scottgas.com/. Standards come certified at +- 5%, however, actual concentrations may be suspect.
Scott Marian.

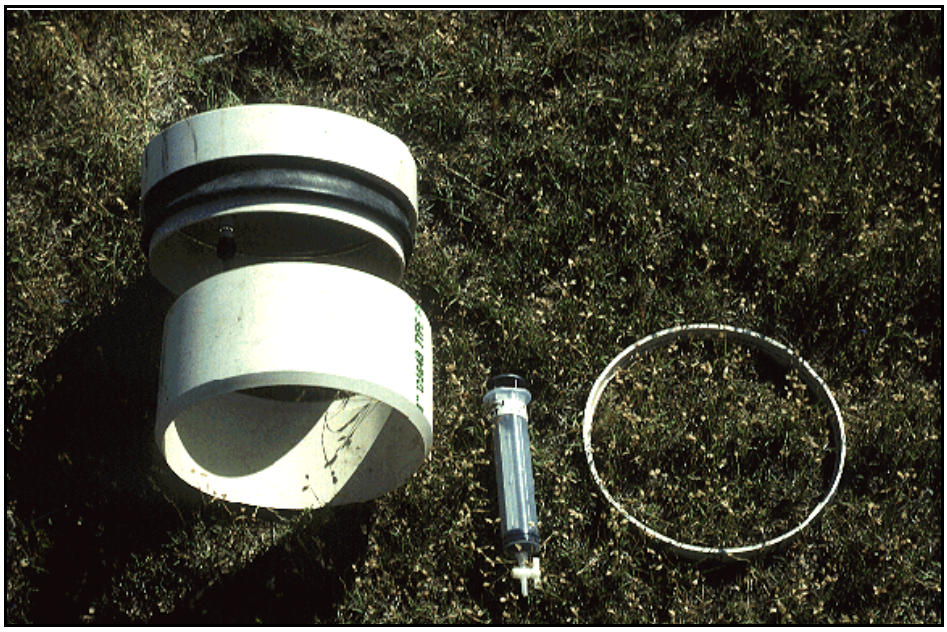
Syringes: Beckton-Dickenson (obtained from most laboratory supply companies)
Syringe stopcocks: (ColeParmer # A-30600-000 : Qosina, #99705 or #99717).

Reflective Tape:
Industrial Tape Connection: http://www.tapeconnection.com/
Silver 0.9 mil Metalized Mylar Polyester Film with a brilliant, vibrant mirror-like finish; coated with an aggressive long lasting acrylic adhesive system. 2"x72yards Mylar Film Tape
Alternative to 3M #850; Ideal #505; Tesa #4137; TLC #CT941M; Venture #1555CW
PRICE: $32.70/roll

Gas Manifolds:
Small Parts, Inc. 800-220-4242, www.smallparts.com. An example part no. is TCM-13-20/4-10 (description = Tubing Manifold 13G inlet 20G outlet).

Recirculating fans:
Computer fans can be obtained from Action Electronics, Santa Anna, CA. Phone: (800) 563-9405, www.action-electronics.com. Example of a 12vdc fan from this company is part # 108idc12vdcs1b. This fan is 25 mm x 25 mm x 10 mm and can be run on a 9 volt transistor radio battery.





PVC soil anchor and chamber used by Mosier.

Rectangular chambers used by Mosier

Example of temporary/portable chamber used by Parkin. Chamber has an attached polethylene skirt held in place on the soil surface with a length of chain. As shown, the chamber is monitoring soil CO2 flux by recirculating gas through an infrared analyzer. Gas samples can be withdrawn through septum in top of chamber for N2 and CH4 analyses.

Large skirted chamber used for CO2 flux from corn/soil system. Applicability of chamber for N2O and CH4 flux measurements has not been tested.

Appendix 4. Schematic Drawings of Chambers

Round PVC Chamber Description:

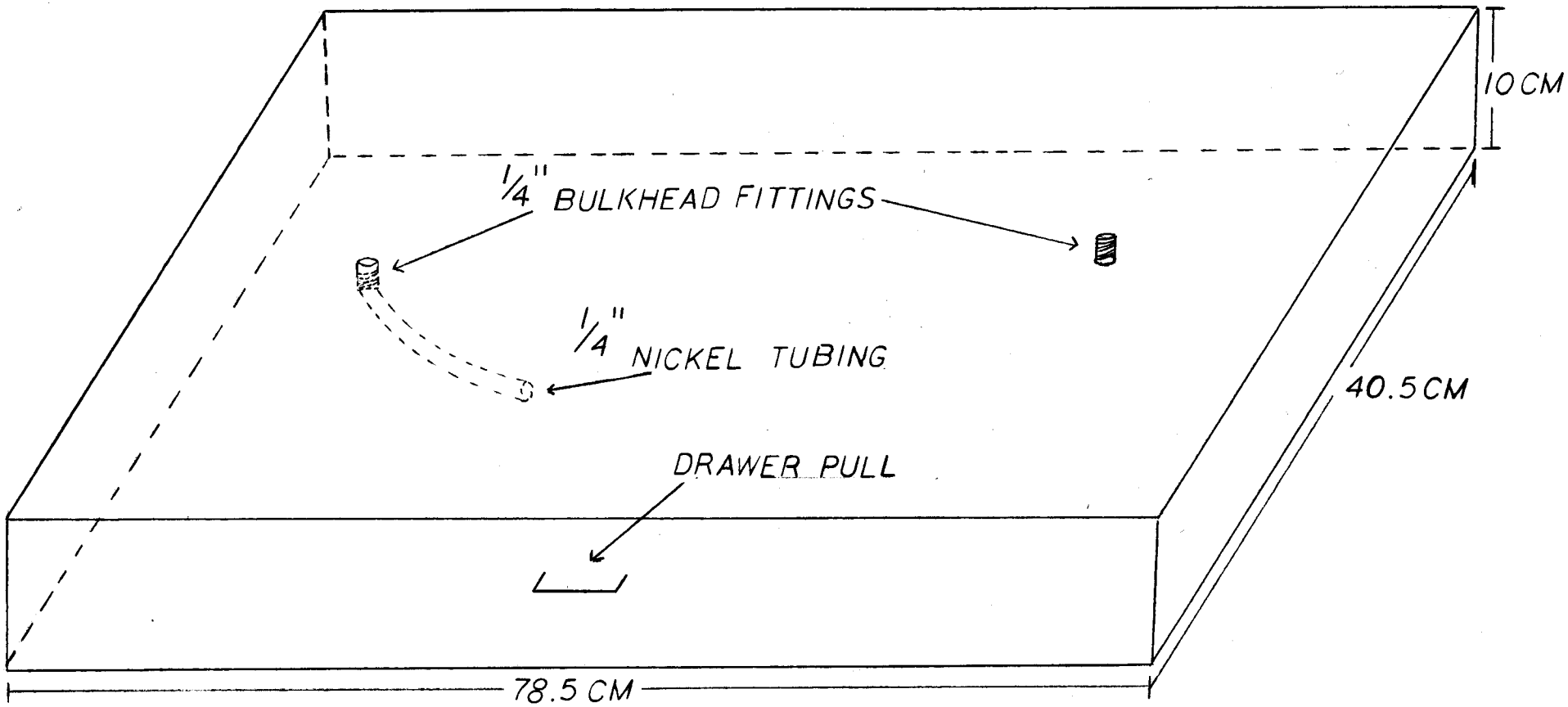
Anchor: Made from PVC pipe, 15 – 30 cm diameter. It can be tapered on the bottom for easier insertion into the soil. We typically insert the anchor 8-9 cm into the soil. The chamber can fit onto the anchor, either flush (resting on the anchor), inserted into the anchor, or if an end cap is used, fit over the anchor. A seal is made using an approximately 5 cm wide tire inner tube.

Chamber: The chamber can be made from a PVC pipe end cap of the appropriate size or a piece of PVC pipe with a top made from sheet PVC or plexiglass that is cut to fit and cemented into place. Two holes, to accommodate swagelock fittings are drilled and tapped in each chamber top.

Rectangular aluminum Chambers: Made from sheet aluminum. Can be made any size to fit the field situation.

Anchors: Made from sheet aluminum with a trough to hold water welded on top. The anchors are inserted 10 cm into the soil.

Chamber: Made from sheet aluminum to desired dimensions. Two holes, to accommodate swegelock fittings for vent tube and gas collection septum are drilled and tapped in each chamber top.

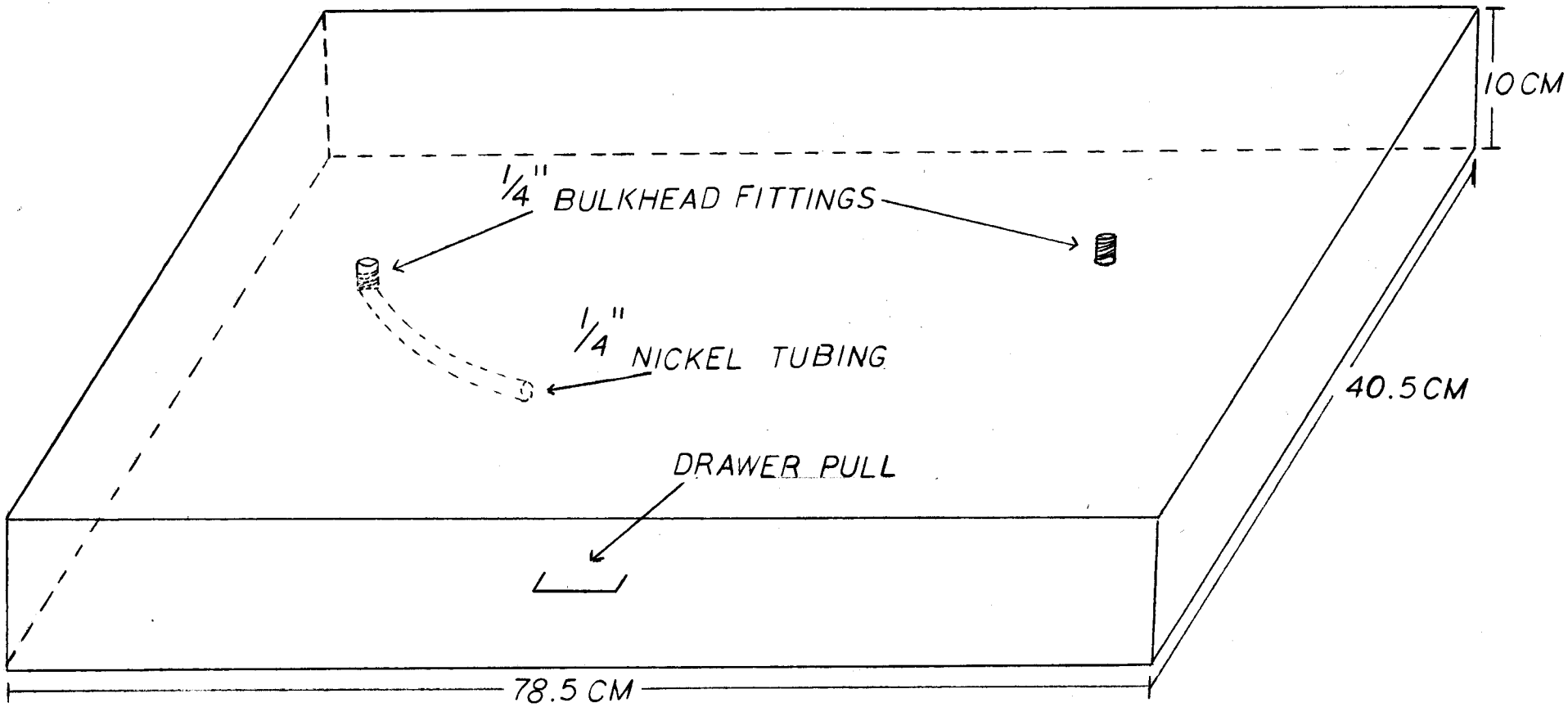


CHAMBER MADE OF 1 SHEET 1/8" ALUMINUM

INSULATED WITH CORKBOARD AND MYLAR TAPE

DRAWING 1 OF 2

TRACE GAS CHAMBER	3/32" = 1 CM
USDA-ARS-SPNR	3-2003



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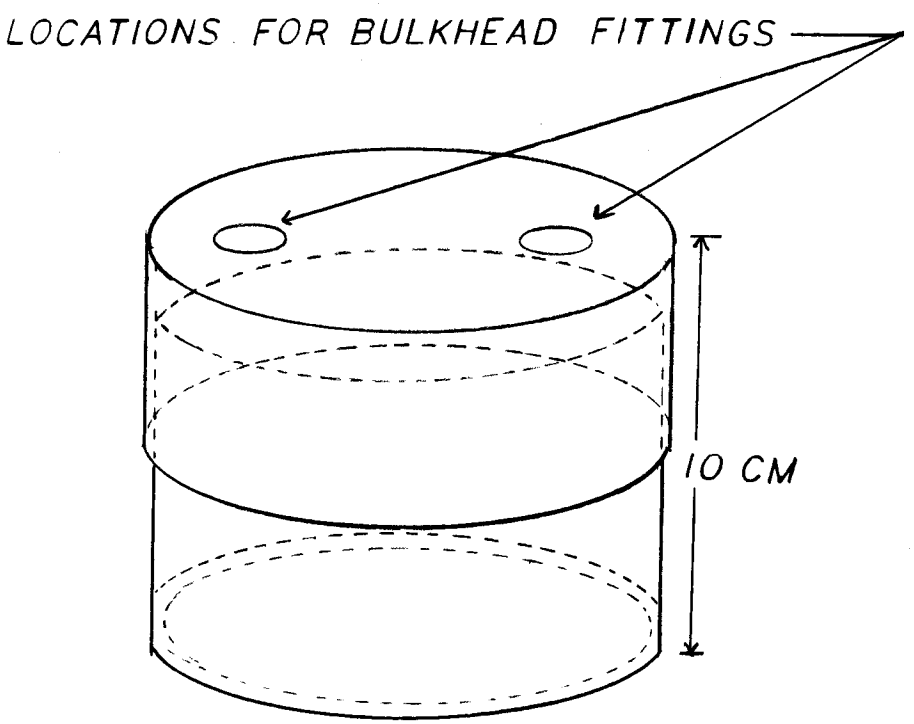
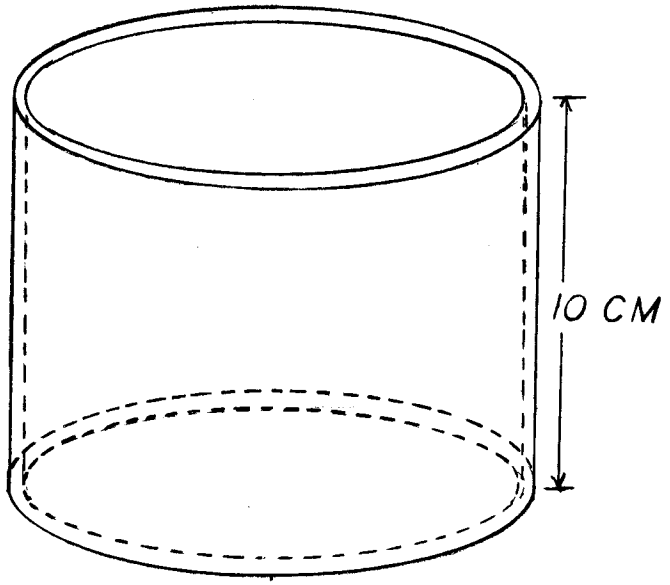
DRAWING 1 OF 2

TRACE GAS CHAMBER	3/32" = 1 CM
USDA-ARS-SPNR	3-2003



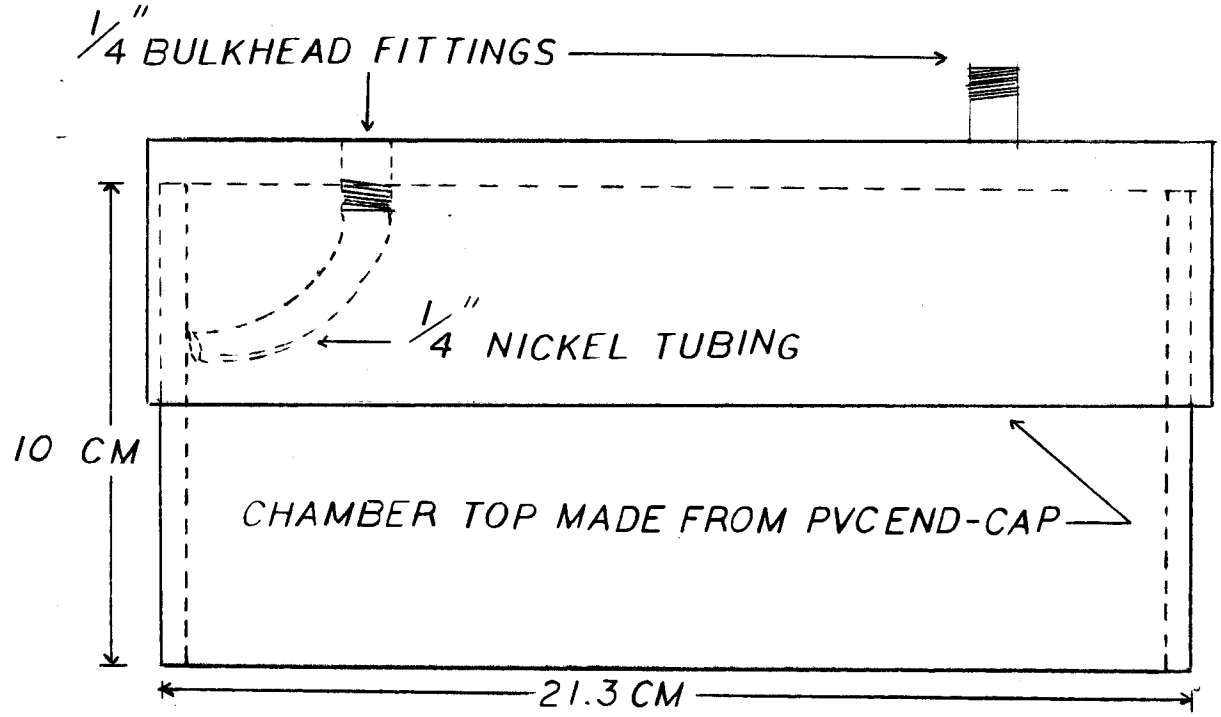
CHAMBER MADE FROM PVC PIPE AND MATCHING END CAP



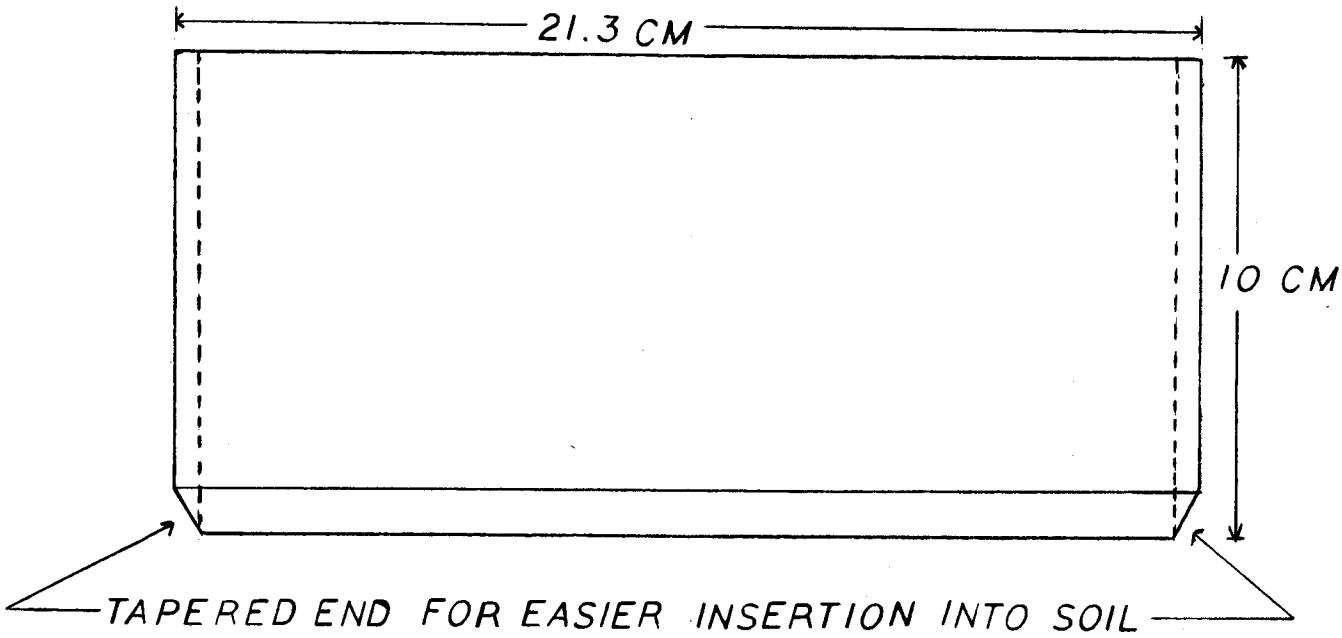
PVC RING MADE FROM SAME PIPE AS CHAMBER

DRAWING 1 OF 2

CHAMBER AND ANCHOR	NOT TO SCALE
USDA ARS SPNR	3 - 2003



- PVC CHAMBER AND RING MADE FROM SCHEDULE 40 PIPE
- COVER TOP AND SIDES WITH MYLAR TAPE
- SEAL CHAMBER AND RING WITH AN INNER TUBE GASKET



DRAWING 2 OF 2

PVC CHAMBER AND RING SET	1/4" = 1 CM
USDA-ARS-SPNR	3 - 2003