GRACEnet Sampling Protocols

I. Soil Sampling Guidelines*

Steering committee: Jeff Smith, Mark Liebig, Ron Follett, Gary Varvel, Upendra Sainju, Don Reicosky, Jim Reeves, Jerry Schuman, Hero Gollany, Ken Potter, Ray Allmaras, Wayne Honeycutt,

Assume spatial independence of soil properties and thus use a random sampling pattern. Record GPS coordinates of each sampling location and collect samples using a core method compositing a minimum of 8 cores per depth. If soil property variability is known the number of cores for compositing can be adjusted. As a rule of thumb biological soil properties have a spatial coefficient of variation (%CV) of >50%, chemical properties 25 to 45% and physical properties 15 to 40% depending upon the scale of sampling.

The required depth increments for GRACEnet sampling are 0-5 and 5-10 cm. These depths will tend to show increases in soil C and thus would be considered a minimum data set. Preferred sampling depth increments are 0-5, 5-10, 10-20, 20-30, 30-60, 60-100 cm. If near-surface stratification is not present depth increments of 10 cm to 30 cm for the third and beyond samples are adequate. During soil sampling an assessment of surface residues should be made, the mass should be determined over a 0.25 m^2 area.

A suggested mass of soil of at least 500g should be collected from each depth for the initial i.e. time zero sampling. Future soil sample mass can be adjusted for the assessments being conducted. The timing and frequency of sampling will be system dependent.

Soil samples should be kept cool in the field and during transport. Samples should be maintained at 4^0 C as much as possible during processing. First sieve each soil sample through a 2 mm sieve and remove a sample for gravimetric water content.

Soil Assessments

For the required and optional soil property measurements we will use standard methods of analysis. There are several references listed at the end of this document that can be consulted for proper procedures and step-by-step instructions.

Required measurements
- Soil organic C (combustion)
- Soil inorganic C
- Particulate organic matter C
- Soil bulk density
- Total N
- Extractable NH_4-N and NO_3-N
- Extractable P and K
- Soil pH (water)
- Electrical conductivity
- Particle-size distribution (initial sampling)

Optional measurements
- Soluble organic C (required if there is drainage in the system)
- Microbial biomass C and N
- Water-stable aggregates

Total C by mid/near infrared method (Jim Reeves)
Moisture release curve

Soil Sampling Protocol Discussion items (Developed from the October 2005 GRACEnet workshop in Fort Collins, CO):

- Soil depth increments. Increments suggested in current protocol for studies being established. On-going long-term studies with different depth increments should not change sampling scheme to correspond to GRACEnet depth increments. Depth increments sampled should encompass the depth of tillage (at least eight inch depth to include data in CQESTR). Current guidelines were set up to ensure near-surface effects of management are captured. In some cases, sampling genetic horizons at deeper depths may be more appropriate (e.g., natric horizons).
- Number of soil samples: Eight composited cores per sampling site recommended in protocol for initial sampling. However, fewer cores may be collected in certain studies where plots are small and/or treatments are not tilled.
- Frequency and timing of sampling: Beyond initial baseline sampling, frequency depends on unique attributes of management system being evaluated. The timing of sampling will depend on the investigator's knowledge of a system's variance for attributes of interest.
- Sampling management zones (row/interrow, traffic/nontraffic): Agroecosystems with controlled traffic create distinct zones within a field. Compositing an appropriate number of soil samples across different zones based on the area of each zone within a field is one approach to obtaining a representative sample.
- Sample processing: Plant materials (roots and surface residue) in soil samples need to be removed prior to analysis. Removing plant material from field-moist samples was suggested, but requires significant labor input. Once dried, soil samples should be passed through a 2 mm sieve prior to analyses. If aggregate stability measurements are to be taken, it is recommended that a separate soil sample be collected for analysis and archiving.
- Sample archiving: Archiving guidelines outlined in publication by Robertson et al. (1999) (see current protocol for full citation). Suggested amount of air-dried soil for archiving is 50 g (baseline sampling) and 10 g (previous samplings). For samples collected prior to initiation of GRACEnet, there may be a need to conduct assessments from archived samples.

Method citations for inclusion in protocol and based upon October 2005 GRACEnet workshop discussion items:

- Compliant cavity method for soil bulk density – stony and sandy soils (required measurement):

- *USDA-NRCS. 2004. Compliant Cavity (3B3). p. 98-100. In: R. Burt (ed.) Soil survey laboratory methods manual. Soil survey investigations report no. 42, version 4.0. USDA-NRCS National Soil Survey Laboratory, Lincoln, NE.*
- Particulate organic matter (required measurement):
    - *Cambardella, C.A., and E.T. Elliott. 1992. Particulate organic matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56:777-783.*
    - *Gregorich, E.G., and B.H. Ellert. 1993. Light fraction and macroorganic matter in mineral soils. p. 397-407. In M.R. Carter (ed.) Soil Sampling Methods and Analysis. Can. Soc. Soil Sci. Lewis Publ. Boca Raton, FL.*
- Wet aggregate stability (optional measurement):
    - *Kemper, W.D., and R.C. Rosenau. 1986. Aggregate stability and size distribution. p. 425-442. In: Klute, A., (Ed.) Methods of soil analysis. Part 1 – Physical and mineralogical methods. 2nd ed. SSSA Book Series No. 5. SSSA and ASA, Madison, WI.*
- Soil microbial biomass (optional measurement):
    - *Jenkinson, D.S., and D.S. Powlson, 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8: 209-213.*

    - *Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19:703-707.*
    - *Islam, K.R., and R.R. Weil. 1998. Microwave irradiation of soil for routine measurement of microbial biomass carbon. Biol. Fertil. Soils. 27:408-416.*
- Guidelines for sampling forest soils; Kathy O'Neil (Beaver, WV) will provide.
    - Kathy O'Neil (Beaver, WV) will provide. Guidelines for steep landscapes

Original Methods Citations:

Carter, M.R. (ed.). 1999. Soil sampling and methods of analysis. Lewis Publ. Boca Raton, FL.

Doran, J.W., and A.J. Jones (ed.). 1996. Methods for assessing soil quality. SSSA Spec. Publ. 49. SSSA, Madison, WI.

Klute, A. (ed.). 1986. Methods of soil analysis. Part 1 – Physical and mineralogical methods. 2nd ed. SSSA Book Series No. 5. SSSA and ASA, Madison, WI.

Lal, R., J.M. Kimble, R.F. Follett, and B.A. Stewart (eds.). 2001. Assessment methods for soil carbon. Lewis Publ. Boca Raton, FL.

Robertson et al. (ed.). 1999. Standard soil methods for long-term ecological research. Oxford Univ. Press. New York.

Sparks, D.L. (ed.). 1996. Methods of soil analysis. Part 3 – Chemical methods. SSSA Book Series No. 5. SSSA and ASA, Madison, WI.

Weaver et al. (ed.). 1994. Methods of soil analysis. Part 2 – Microbiological and biochemical methods. SSSA Book Series No. 5. SSSA and ASA, Madison, WI.

* GRACEnet Workshop Soils Protocol Discussion (Notes and Follow-up October 7, 2005).

II. Plant Sampling Guidelines

Steering committee: Jane M-F Johnson. Ron Follett, Jack Morgan, Jean Reeder, Don Reicosky, Diane Stott, Lew Ziska, Jeff White

This document provides guide lines for sampling plants shoots and roots, with additional guidelines and references for determining plant quality.

General Plant sampling guidelines

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

The amount of total C and N in the biomass should be determined. It is recommended that ash-free biomass be determined (NREL, 2005). Additional optional quality assessment protocols are discussed below.

Plant aboveground sampling issues for quantifying weeds

In general, most plant sampling techniques that are used for crops or native species apply equally well to invasive species. However, the patchiness of weed infestations may sometimes require slightly different approaches to select representative samples. The sampling methodology and accounting for the mass of weeds (weight/unit area) will depend upon the nature of the experimental plots and the field environment. In small plot studies, sampling could be based on the overall sampling scheme for the crop species, and then separation of the weeds from the crop. Individual weeds types should be considered, including height and perhaps distance from the row. If in a more extensive area, then a

transect method might be quite satisfactory. If in a pasture or other large field, then it might be reasonable to use a random method (systematic random points). This extensive sampling could be combined with more intensive random sampling in particular areas of interest. In any case it would be necessary to know the area sampled so that scaling-up and calculation of the mass of weeds per unit area can be calculated.

Unique techniques might be required for special situations. One of these might be if the desired sampling area uniformly had either C_3 or C_4 plants present in it and the species that is considered the weed were the opposite, then bulk samples could be obtained and the delta ^{13}C determined and a ratio of C_3 to C_4 plants calculated based upon stable C isotope analyses.

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

Plant handling

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

Determination of equivalent dry-weight at 100-105 ^{o}C permits results to be expressed on dry-weight basis (Palm and Rowland, 1997). Biochemical composition varies among species, and physiological stage (Constantinides and Fownes, 1994; Heal et al., 1997) it is important to include the age or physiological stage of the material and the organs included.

ROOTS

Root sampling guidelines

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

Depth

Rooting depths of annual crops range from about 0.5 m to around 3.0 m (Borg and Grimes, 1986; Dardanelli et al., 1997; Merrill et al., 2002; Stone et al., 2002) in contrast to perennial root crops such as alfalfa (*Medicago sativa* L), which can reach depths of 6 m after several growing seasons (Borg and Grimes, 1986). However, most crops have the majority of the root biomass within the surface 60 cm, therefore, if resources are limited, roots cores should focus on the surface 60-cm (Allmaras and Nelson, 1971; Allmaras et al., 1975; Mitchell and Russell, 1971; Weaver, 1926).

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

and about 44 to 57% is in the top 10 cm (Sims et al., 1978; Jackson et al., 1996; Reeder et al., 2001).

How many cores?

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

Plant patchiness causes wide variation in root mass and distribution that occur in rangeland ecosystems (Milchunas and Lauenroth, 1989), as do differences in plant community composition associated with topography and soil type (Lauenroth and Milchunas, 1992). A

stratified sampling protocol across the factors (topography and plant species) controlling spatial patterns is required (Burke et al., 1999; Reeder, 2003).

When to sample:

As with all plant parameters, it is important to record at least the age and preferably the physiological stage at the time of sampling. Ideally, it would be best to sample at peak root biomass. However, this is not necessarily well defined for all crops. Liedgens et al. (2000) utilizing minirhizotrons reported that maximum root density occurred about 10 d after pollen shed at most positions to the plant row for corn. Wheat maximum root biomass is at anthesis (Siddique et al., 1990). Root growth of soybeans also appears to reach a maximum about seed set and begin declining after seed development starts (Mitchell and Russell, 1971). Maximum root biomass or root length density is not always available in the literature, a good first guess would be sometime between flowering and seed set. Measuring at physiological maturity would likely mean some of the belowground biomass is already been lost to decomposition. Siddique et al. (1990) reported that root-to-shoot ratio declined from 0.55 at anthesis to 0.4 at maturity, thus root measurements at maturity will underestimate total root biomass. Alfalfa is a perennial species, so root development would be expected to be considerably different than annual species; both the biomass and the chemical composition will change depending on how many years since planting, and from that stand point alfalfa may be more similar to perennial that to annual species.

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

Sampling depth and horizontal

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

Horizontal root distribution is not uniform; therefore, it is advisable to collect samples at several horizontal positions relative to the plant between two rows. For example in corn or soybean with 76 cm

row spacing, taking a probe near a plant 1-3, 12.7, 25.4 and 38.1 cm will capture some of the horizontal distribution. Three or more subplot locations within a plot are recommended.

In narrow row crops like wheat or drilled soybeans, the four horizontal positions would be next to a plant, center of inter-row, next to the next plant and the next inter-row. This strategy also can work in alfalfa, especially if it was planted with a nurse crop like wheat or oat.

To report root density (g cm^{-3}), the volume of soil sampled must be recorded. For example using a hand probe (tube inner diameter 0.75 inches) and 12 pooled probes, the volume of soil is calculated as follows:

(0.75 in /2)^2 *pi= 0.441786 in^2 0.441786 in^2*24 in= 10.60288in^3

1 in^3 =16.4cm^3 10.60288 in^3* 16.4 cm^3/in^3= 173.8872cm^3 173.8872 cm^3 *12 cores= 2087 cm^3

Root storage Store the soil cores with roots plastic bags or plastic pails, refrigerate (4 ^oC) until they can be washed, preferably within one-week. After washing and removing non-root debris. The amount of root material from 12 pooled samples will vary dramatically among crop species.

Root washing technique

Roots can be washed from the soil with hydropneumatic elutriation as described by (Smucker et al., 1982). Commercial elutriators are available from Gillison's fabrication http://www.gillisons.com/products.htm. Below is a brief low budget, low tech method for root-washing.

Equipment: 2 mm sieve (8"diameter), 0.6 mm sieve (or something similar), spray nozzle on hose, sink with soil trap, plastic buckets (ice cream pails), small containers (about 250 mL capacity), tray for final cleaning, forceps, and sample bag for roots.

Preliminary cleaning

1 Put sample in plastic bucket. Crumble sample as water is added using spray nozzle. Soak sample in water for ~30 minutes, keep sample bag under bucket is an easy way to keep track of sample number. (Sometimes water can be added directly to sample in plastic bag if sample is small enough.)

2 Hand-mix the sample and pour liquid off through bigger sieve. Add water and pour off. The sieve will trap the roots; this method obviously looses some of the fine roots.

3 Dump the entire sample into sieve and wash with nozzle.

4 Place well cleaned big clumps of roots into small containers with number written on outside to keep track of the sample.

5 Wash out as much soil as possible from the bigger sieve.

6 Dump the sample from sieve back into bucket, hand mix and try to get roots out. Add water; pour liquid into sieve repeatedly until no more roots seen in sample.

7 Dispose of soil left in bucket.

8 Wash sample from bigger sieve into bucket.

9 Pour roots from bucket into smaller sieve and from smaller sieve into small container (with number from step 4).

10 Keep sample in water in small container at 4 ^oC until final cleaning.

Final cleaning

1 Pour sample onto blue tray (10"x13"x1"deep) or Pyrex crystallizing dish with plenty of water. The idea is to be able to see the roots and pick them out of the debris.

2 Pick out roots and place on white lab towel

3 Blot roots and take wet weight into spreadsheet

4 Place roots into numbered bag and store in freezer until ready for freeze drying.

5 Freeze dry and take dry weight. (If you do not have a freeze drier, dry at 45 ^oC). The low temperature assumes there will be analysis beyond dry weight. If there is enough root biomass,

determine ash-free biomass of a small sample.

6 Determine dry weight by drying a subsample at 105°C. If no chemical analysis is to be completed, the entire sample can be dried at 105°C.

7 Determine ash-free weight at 550 to 600°C Details for determining ash-free biomass can be found at http://www.eere.energy.gov/biomass/analytical_procedures.html. (NREL, 2005).

Optional Plant quality assessment

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

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

Analytical methods

There are several approaches for characterizing residue quality. One is to use a sequential extraction scheme (Figure 1). Sequential extraction allows isolation of more specific components with a limited amount of plant material; however, it is time consuming, expensive, and has more potential for experimental error (Palm and Rowland, 1997). A second method is to do separate extractions of a limited number of components (Figure 2). For example, lignin could be extracted without first extracting starch. Separate extraction tends to reduce the experimental error (Palm and Rowland, 1997). Another method of assessing plant quality is quantify neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest and Wine, 1968; Van Soest et al., 1991), which is a common method for determining digestibility of forage crops. Protocols for determining extractives, starch, total carbohydrate by HPLC, acid-soluble lignin, acid-insoluble lignin and ash have been developed by the National Renewable Energy Laboratory (NREL) at Golden, CO, and have been accepted by the ASTM as ASTM standard test methods. These methods are available at the NREL website: http://www.eere.energy.gov/biomass/analytical_procedures.html as standard biomass analytical procedures (Table 1). The methods at the NREL site have the advantage of being very detailed, complete with background references, step-by-step protocols and sample calculation. Currently, the methods can be downloaded free of charge. In addition this web site has a biomass feedstock composition and property database, which has information on agricultural residues, wood, herbaceous energy crops and other potential biofuel sources.

Literature cited

Allen, S.E., (ed.) 1989. Chemical Analysis of Ecological Material 2nd edition, pp. 1-368. Blackwell, Oxford, UK.

Allmaras, R.R., and W.W. Nelson. 1971. Corn (*Zea mays* L.) root configuration as influenced by some row-interrow variants of tillage and straw mulch management. Soil Sci. Soc. Am. Proc. 35:974-980.

Allmaras, R.R., W.W. Nelson, and W.B. Voorhees. 1975. Soybean and corn rooting in Southwestern Minnesota: Root distribution and related water inflow. Soil Sci. Soc. Am. J. 39:771-777.

Ambus, P., E.S. Jensen, and G.P. Robertson. 2001. Nitrous oxide and N-leaching losses from agricultural soil: Influence of crop residue particle size, quality and placement. Phyton-Annales REI Botanicae 41:7-15.

Anderson, J.M., and J.S.I. Ingram. 1993. Tropical Soil Biology and Fertility: A Handbook of Methods. 2nd ed. CAB International, Wallingford, UK.

Baggs, E.M., R.M. Rees, K. Castle, A. Scott, K.A. Smith, and A.J.A. Vinten. 2002. Nitrous oxide release from soils receiving N-rich crop residues and paper mill sludge in eastern Scotland. Agric. Ecosyst. Environ. 90:109-123.

Ball, B.C., J.P. Parker, and A. Scott. 1999. Soil and residue management effects on cropping conditions and nitrous oxide fluxes under controlled traffic in Scotland 2. Nitrous oxide, soil N status and weather. Soil Tillage Res. 52:191-201.

Borg, H., and D.W. Grimes. 1986. Depth development of roots with time: An empirical description. Trans ASAE 29:194-197.

Burke, I.C., W.K. Lauenroth, R. Riggle, P. Brannen, B. Madigan and S. Beard. 1999. Spatial variability of soil properties in the shortgrass steppe: the relative importance of topography, grazing, microsite and plant species in controlling spatial patterns. Ecosystems 2:422-438.

Constantinides, M., and J.H. Fownes. 1994. Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biol. Biochem. 26:49-55.

Coupland, R.T. 1992. Mixed prairie. *In* Robert T. Coupland (ed), *Natural Grasslands, Introduction and Western Hemisphere, Ecoystems of the World 8a*, Elsevier, Amsterdam, pp. 151-182.

Dardanelli, J.L., O.A. Bachmeier, R. Sereno, and R. Gil. 1997. Rooting depth and soil water extraction patterns of different crops in a silty loam Haplustoll. Field Crops Res. 54:29 38. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-358.

Franck, W.M., B.A. Hungate, F.S. Chapin, and C.B. Field. 1997. Decomposition of litter produced under elevated CO_2 - dependence on plant species and nutrient supply. Biogeochemistry 36:223-237.

Gorissen, A., J.H. van Ginkel, J.J.B. Keurentjes, and J.A. Van Veen. 1995. Grass root decomposition is retarded when grass has been grown under elevated CO_2. Soil Biol. Biochem. 27:117-120.

Heal, O.W., J.M. Anderson, and M.J. Swift. 1997. Plant litter quality and decomposition: An historical overview., p. 47-66, *In* G. Cadish and K. E. Giller, eds. Driven by Nature, Plant Litter Quality and Decomposition. CAB International, Wallingford.

Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissue. Crop Sci. 33:1306-1311. Jackson, R.B., J. Canadell, J.R. Ehleringer, H.A. Mooney, O.E. Sala and E.D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108:389-411.

Lauenroth, W.K., and D.G. Milchunas. 1992. Short grass steppe. *In* R.T. Coupland (ed), Natural Grasslands, Introduction and Western Hemisphere, Ecosystems of the World 8a, Elsevier, Amsterdam, pp. 183-226.

Lauenroth, W.K., and W.C. Whitman. 1977. Dynamics of dry matter production in a mixed grass prairie in western North Dakota. Oecologia 27:339-351.

Liedgens, M., A. Soldati, P. Stamp, and W. Richner. 2000. Root development of maize (*Zea mays* L.) as observed with minirhizotrons in lysimeters. Crop Sci. 40:1665-1672.

Martens, D.A., and W.T.J. Frankenberger. 1991. Determination of saccharides in biological materials by high-performance anion-exchange chromatography with pulsed amperometric detection. J. Chromatogr. 546:297-309.

Martens, D.A., and K.L. Loeffelmann. 2002. Improved accounting of carbohydrate carbon from plant and soils. Soil Biol. Biochem. 34:1393-1399.

Merrill, S.D., D.L. Tanaka, and J.D. Hanson. 2002. Root length growth of eight crop species in Haplustoll soils. Soil Sci. Soc. Am. J. 66:913-923.
Milchunas, D.G., and W.K. Lauenroth. 2000. Belowground primary production by carbon isotope decay and long-term root biomass dynamics: Ecosystems 4:139-150.
Milchunas, D.G., and W.K. Lauenroth. 1989. Three-dimensional distribution of plant biomass in relation to grazing and topography in the short grass steppe. Oikos 55:82-86.
Mitchell, R.L., and W.J. Russell. 1971. Root development and rooting patterns of soybean (*Glycine max* (L.) Merrill) evaluated under field conditions. Agron. J. 63:313-316.
NREL. 1996. Determination of carbohydrates in biomass by high performance liquid chromatography LAP-002. Standard biomass analytical methods, NREL Laboratory Analytical Procedures, Vol. 2002. National Renewable Energy Laboratory.
NREL. 2005. Determination of Ash in Biomass LAP-005. Standard biomass analytical methods, NREL laboratory analytical procedures [Online]. Available by US-Department of Energy, National Renewable Energy Laboratory http://www.eere.energy.gov/biomass/analytical_procedures.html (posted 10/14/2005).
Palm, C.A., and A.P. Rowland. 1997. A minimum dataset for characterization of plant quality for decomposition, p. 379-392, *In* G. Cadisch and K. E. Giller, eds. Driven by Nature: Plant litter quality and decomposition. CAB International, Cambridge, UK.
Reeder, J.D. 2003. Overcoming spatial variation in measuring soil carbon stocks and sequestration potential of native rangelands in the western U.S. *In* C.A.S. Smith (ed.) Soil Organic Carbon and Agriculture: Developing Indicators for Policy Analyses. Proceedings of the OECD Expert Meeting on Soil Organic Carbon Indicators for Agricultural land. October 15-18, 2002. Ottawa Canada. pp. 193-200.
Reeder, J.D., C.D. Franks and D.G. Milchunas. 2001. Root biomass and microbial processes. *In* R.F. Follett, J.M. Kimble and R. Lal (eds) *The Potential of U.S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect.* Lewis Publishers. pp. 139-166.
Shelp, M.L., E.G. Beauchamp, and G.W. Thurtell. 2000. Nitrous oxide emissions from soil amended with glucose, alfalfa, or corn residues. Commun. Soil Sci. Plant Anal. 31:877 892. Siddique, K.H.M., R.K. Belford, and D. Tennant. 1990. Root:shoot ratios of old and modern, tall and semi-dwarf wheats in a mediterranean environment. Plant Soil 121:89-98.
Sims, P.L., J.S. Singh and W.K. Lauenroth. 1978. The structure and function of ten western North American Grasslands. I. Abiotic and vegetational characteristics. J. Ecol. 66: 251-285.
Smucker, A.J.M., S.L. McBurney, and A.K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by hydropneumatic elutriation systems. Agron. J. 74:500 503.
Stone, L.R., D.E. Goodrum, A.J. Schlegel, M.N. Jaafar, and A.H. Khan. 2002. Water depletion depth of grain sorghum and sunflower in the Central High Plains. Agron. J. 94:936-943.
Taylor, H.M. 1986. Methods of studying root systems in the field. Hortscience 21:952-956.
Van Soest, P.J., and R.H. Wine. 1968. Determination of lignin and cellulose in acid-detergent fiber with premanganate. J. Official Assoc. Anal. Chem. 51:780-785.
Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implication in dairy cattle. J. Dairy Sci. 74:3583-3597.
Waterman, P., and S. Mole. 1994. Analysis of phenolic plant metabolites Blackwell Scientific Publications, London, UK.
Weaver, J.E. 1926. Root development of field crops McGraw-Hill, New York.
Table 1. A partial list of protocols available at NREL for characterizing residue quality.

Component	NREL Link: protocol http://www.eere.energy.gov/biomass/analytical_procedures.html
Total solids (biomass)	LAP-001
Extractives	LAP-010
Starch	LAP-016
Carbohydrates	LAP-002
Acid-insoluble	LAP-003

lignin
Acid-soluble LAP-004
lignin
Ash LAP-005

Table 2. Additional methods for characterizing residue quality.

Component Citation
Nonstructural Carbohydrates (Hendrix, 1993; Martens and Frankenberger, 1991)
Soluble sugars (Dubois et al., 1956)
Soluble C and N (Anderson and Ingram, 1993)
Soluble phenolics (Waterman and Mole, 1994)

Alkaline extractable phenolics (Martens and Loeffelmann, 2002)

Neutral and acid digestible fiber (Van Soest and Wine, 1968; Van Soest et al., 1991)

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Figure 1. Schematic of separate extraction for plant residue quality parameters

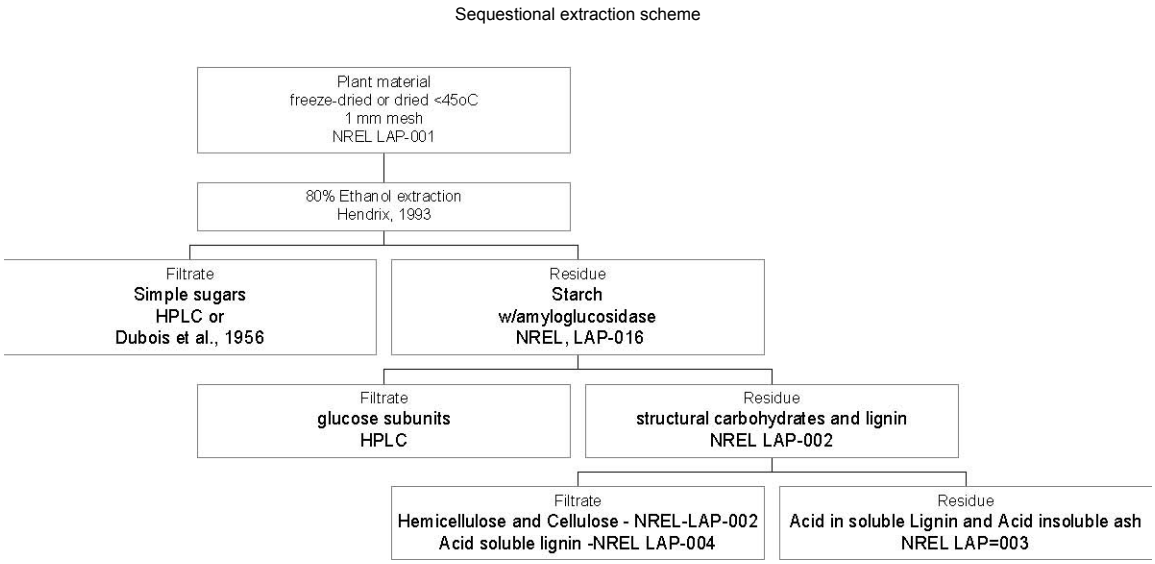
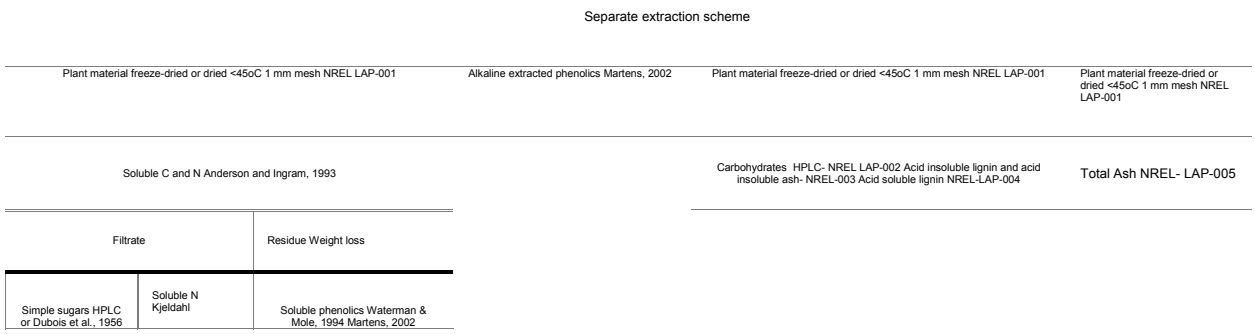


Figure 2. Schematic of separate extraction for plant residue quality parameters



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III. Trace gas sampling guidelines

GRACEnet
Chamber-based Trace Gas Flux Measurement Protocol
www.GRACEnet.usda.gov April 24, 2003

Trace Gas Protocol Development Committee: Tim Parkin, Arvin Mosier, Jeff Smith, Rod Venterea, Don Reicosky, Greg McCarty, Geoffrey Doyle, John Baker

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 (e.g., Minnesota, Iowa).

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.

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. -Minimize anchors or collars height to reduce 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:

Chambers have two parts; 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 were made of 20 cm (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 perturbations (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 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, and 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 emission 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 deploy chambers 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 two 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.

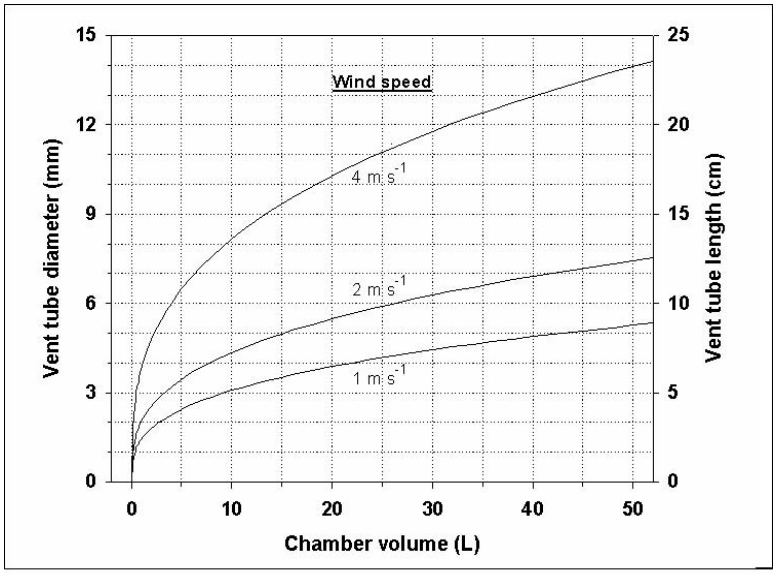


Figure 1. Optimum vent tube diameter and length for selected wind speeds and enclosure volumes as described by Hutchinson and Mosier (1981).

r
m

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 react with CH_4. However, others report no problems with coated red 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 D2.

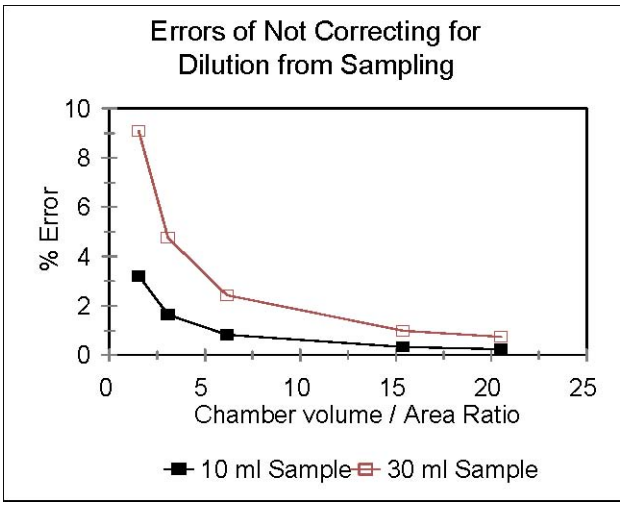


Figure 2. Percentage underestimation of flux rate due to headspace dilution as a result of sampling presented as a function of chamber geometry and gas sample size.

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 are run in sequence (e.g. t_o, t_1, t_2,) rather than segregating all the samples by time (e.g. all samples run together) and 2) standards are 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 [e.g., 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}.

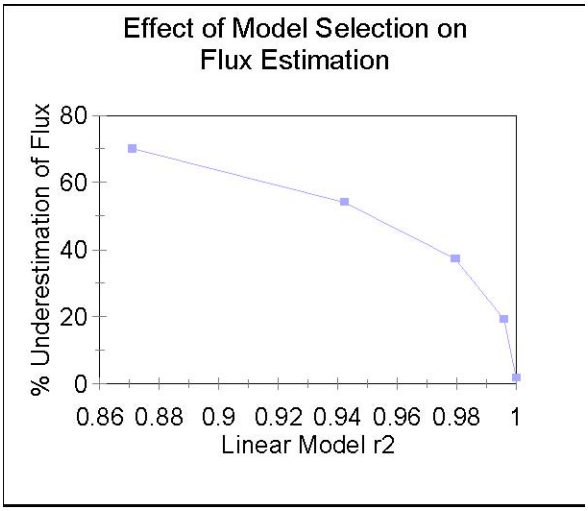
Fig. 3. Percentage underestimation of flux from linear regression as compared to non-linear analysis from Eq. 1

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 accommodate more than three 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 relationship between altitude and atmospheric pressure model.



Simulation data shows that even slight deviations from linearity can have a dramatic influence on the calculated flux (Fig. 3).

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)

$$PV = nRT$$

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

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

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} K^{-1} is used, units of P, V, n and T have corresponding units of Atm, Liters, Moles, uMol/Mol and °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.

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

Sample calculation to convert uL gas to uMol.

(Note: conversion from °C to °K by adding 273)

For example, at an altitude of 1000 ft., and at an air temperature of 20°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 °K=(273+ °C).

Noisy Data

The change in chamber headspace trace gas concentration over time typically will be linear or curvilinear. In these situations linear regression or the non-linear diffusion based models can be used to calculate the flux. However, 'concentration with time' data are often noisy and time course data are obtained (Anthony et al., 1995). Determination of a flux from noisy data often requires investigator judgment. Several possibilities exist for flux estimation from noisy data including: 1) linear regression using all the points, 2) with 3 points, calculate 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 judgment 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 to 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.

1 Immediately after these vials are prepared, run 20 of these samples.

2 After one-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.

3 After one-week of storage, run the final 20 vials from the test set along with 20 vials freshly prepared.

4 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.

Tim Parkin USDA-ARS-
MWA National Soil Tilth Lab
2150 Pammel Dr. Ames, IA
50014
(515) 294-6888
parkin@nstl.gov

Rod Venterea USDA-ARS-MWA Soil & Water Management Unit 439 Borlaug Hall 1991 Upper Burford Circle University of Minnesota St. Paul, MN 55108 - 6028
(612) 624-7842
venterea@soils.umn.edu

Greg McCarty USDA-ARS-BA Environmental Quality Laboratory Bldg 007 Room 202 BARC-West Beltsville, MD 20705
(301) 504-7401
mccartyg@ba.ars.usda.gov

Jeff Smith USDA-ARS-PWA 215
Johnson Hall Washington State
University Pullman, WA 99164-
6421
jlsmith@mail.wsu.edu

Literature cited

Hutchinson, G.L. and G.P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. *In* L. Harper et al. (eds) Agricultural Ecosystem Effects on Trace Gases and Global Climate Change, pp. *63-78.* ASA Special Publication 55, ASA, CSSA, SSSA, Madison, WI.

Hutchinson, G.L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45:311-316.

Livingston, G.P. and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. *In*. P.A. Matson and R.C. Harriss (eds.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Methods in Ecology.pp 14-51. Blackwell Science Cambridge University Press, London.

Mosier, A. 1989. Chamber and isotope techniques. *In* M.O. Andreae and D.S. Schimel (eds.), Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere, pp.175-188. Report of the Dahlem Workshop, Berlin, Feb 19-24, 1989. John Wiley and Sons, New York.

Mosier, A.R. Gas flux measurement techniques with special reference to techniques suitable for measurements over large ecologically uniform areas. *In* Soils and the Greenhouse Effect. Bouwman, A.F. (ed.). John Wiley & Sons Ltd. pp. 289-301. 1990.

Other Useful References

Anthony, W.H., G.OL. Hutchinson, and G.P. Livingston. 1995. Chamber measurements of soil-atmosphere gas exchange: Linear vs. diffusion-based flux models. Soil Sci. Soc. Am. J. 59:1308-1310.

Arnold, S., T.B. Parkin, J.W. Doran, and A.R. Mosier. 2001. Automated gas sampling system for laboratory analysis of CH_4 and N_2O. Commun. Soil Sci. Plant Anal. 32:2795-2807.

Aulakh, M.S., Doran, J.W. and Mosier, A.R. 1991. Field evaluation of four methods for measuring denitrification. Soil Sci. Soc. Am. J. 55:1332-1338.

Blackmer, A.M., S.G. Robbins, and J.M. Bremner. 1982. Diurnal variability in rate of emission of nitrous oxide from soils. Soil Sci. Soc. Am. J. 46:937-942.

Bronson, K.F. and A.R. Mosier. 1993. Nitrous oxide emissions and methane consumption in wheat and corn-cropped systems in Northeastern Colorado. *In* L. Harper et al. (eds) *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change, pp. 133 144.* ASA Special Publication 55, ASA, CSSA, SSSA, Madison, WI.

Brooks, P.D. D.J. Herman, G.J. Atkins, S.J. Prosser, and A. Barrie. 1993. Rapid, isotopic analysis of soil gases at atmospheric concentrations. *In* L. A. Harper, A. Mosier, J.M. Duxbury, and D.E.

Rolston (eds.) Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. ASA Special Publication #55. ASA,CSSA, SSSA, Madison, WI.

Chan, A.S.K., Prueger, J. and Parkin, T.B. 1998. Comparison of closed-chamber and bowenratio methods for determining methane flux from peatland surfaces. J. Environ. Qual. 27:232-239. 1998.

Chan, A.S.K and Parkin, T.B. 2000. Evaluation of potential inhibitors of methanogenesis and methane oxidation in a landfill cover soil. Soil Biol. Biochem. 32:1581-1590. 2000. Chan, A.S.K, and T.B. Parkin. 2001. Effect of land use on methane flux from soil. J. Environ. Qual. 30:786-797. Chan, A.S.K, and T.B. Parkin. 2001. Methane oxidation and production activity in soils from natural and agricultural ecosystems. J. Environ Qual. 30:1896-1903. Chang, C., H.H. Janzen, C.M. Cho, and E.M. Nakonechny. 1998. Nitrous oxide emission through plants. Soil Sci. Soc. Am. J. 62:35-38.

Chen, X., P. Boeckx, S. Shen, and O. Van Cleemput. 1999. Emission of N_2O from rye grass (Lolium perenne L.). Biol. Fertil. Soils 28:393-396.

Delgado, J.A., Mosier, A. R., Follett, R.H., Westfall, D., Klemedtsson, L.K. and Vermeulen, J. 1996. Effect of N management on N_2O and CH_4 fluxes and N recovery in an irrigated mountain meadow. Nutr. Cycling Agroecosystems. 46:127-134.

Delgado, J.A. and Mosier, A.R. 1996. Mitigation alternatives to decrease nitrous oxide emissions and urea-nitrogen loss and their effect on methane flux. J. Environ. Qual. 25:1105-1111.

Denmead, O.T. 1979. Chamber systems for measuring nitrous oxide emissions from soils in the field. Soil Sci. Soc. Am. J. 43:89-95.

Epstein, H.E., Burke, I.C., Mosier, A.R. and Hutchinson, G.L. 1998. Plant species effects on trace gas fluxes in the shortgrass steppe. Biogeochemistry. 42:145-168.

Folorunso, O.A. and D.E. Rolston. 1984. Spatial variability of field measured denitrification gas fluxes. Soil Sci. Soc. Am. J. 48:1214-1219.

Gilbert, R.O. 1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold Co., Inc., New York.

Halvorson, J. J., J. L. Smith and R. I. Papendick. 1997. Soil quality: Issues of scale. J. Soil Water Conserv. 25:26-30.

Healy, R. W., R. G. Striegl, T. F. Rusell, G. L. Hutchinson, and G. P. Livingston, 1996. Numerical evaluation of static- chamber measurements of soil -atmosphere gas exchange: Identification of physical processes, Soil Sci. Soc. Am. J. 60:740-747.

Hutchinson and Livingston (Gas Flux chapter in new Methods of Soil Analysis - Physical Methods book -

Hutchinson, G., G. Livingston, R. Healy, and R. Striegl. 2000. Chamber measurement of surface-atmosphere trace gas exchange: Numerical evaluation of dependence on soil, interfacial layer, and source/sink properties, J. Geophys. Res.-Atmos. *105*(D7), 88658875.

Hutchinson, G.L. and Mosier, A.R. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45:311-316.

IAEA. 1992. International Atomic Energy Agency Manual on measurement of methane and nitrous oxide emissions from agriculture. IAEA-TECDOC-674, 91 p.

Jury, W.A., Letey, J., and Collins, T. 1982. Analysis of chamber methods used for measuring nitrous oxide production in the field. Soil Sci. Soc. Am. J. 46:250-256.

Keerthisinghe, D.G., X.J. Lin, Q.X. Lou, and A.R. Mosier. 1996. Effect of encapsulated calcium carbide and urea application methods on denitrification and N loss from flooded rice. Fert. Res. 45:31-36.

Kessavalou, A., Mosier, A.R., Doran, J.W., Drijber, R.A., Lyon, D.J. and Heinemeyer, O. 1998. Fluxes of CO_2, N_2O and CH_4 in grass sod and winter wheat-fallow tillage management. J. Environ. Qual. 27:1094-1104.

Kessavalou, A., J.W. Doran, A.R. Mosier, and R.A. Drijber 1998.. CO_2, N_2O and CH_4 fluxes following tillage and wetting in a winter wheat-fallow tillage management system. J. Environ. Qual. 27:1105-1116.

Livingston, G.P., and G.L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. *In*. P.A. Matson and R.C. Harriss (eds.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Methods in Ecology. Blackwell Science Cambridge University Press. pp 14-51.

Lapitan, R.L., Wanninkhof, R. and Mosier, A.R. 1999. Methods for stable gas flux determination in aquatic and terrestrial systems. *In* A.F. Bouwman (ed.) Approaches to Scaling of Trace Gas Fluxes in Ecosystems. Developments in Atmospheric Science 24. Elsevier, Amsterdam. pp. 27-66.

Massman, W.J., Sommerfeld, R.A., Mosier, A.R., Zeller, K.F., Hehn, T.J. and Rochelle, S.G. 1997. A model investigation of turbulence-driven pressure pumping effects on the rate of diffusion of CO_2, N_2O and CH_4 through layered snowpacks. J. Geophys. Res.-Atm. 102:18851-18863.

Martin, R.E., Scholes, M.C., Mosier, A.R., Ojima, D.S., Holland, E.A. and Parton, W.J. 1998. Controls on annual emissions of nitric oxide from soils of the Colorado shortgrass steppe. Global Biogeochem. Cycles. 12:81-91.

Matthias, A.D., A.M. Blackmer, and J.M. Bremner. 1980. A simple chamber technique for field measurement of emissions of nitrous oxide from soils. J. Environ. Qual. 9:251-256.

Mosier, A.R. Chamber and isotopic techniques. 1989. *In* Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere. Dahlem Konferenzen. Andreae, M.O. and Schimel, D.S. (eds.). Chichester: John Wiley & Sons Ltd. pp. 175-187.

Mosier, A. 1989. Chamber and isotope techniques. *In* M.O. Andreae and D.S. Schimel (eds.). Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere, pp.175-188. Report of the Dahlem Workshop, Berlin, Feb 19-24, 1989. John Wiley and Sons, New York.

Mosier, A.R. 1990. Gas flux measurement techniques with special reference to techniques suitable for measurements over large ecologically uniform areas. *In* Soils and the Greenhouse Effect. Bouwman, A.F. (ed.). John Wiley & Sons Ltd. pp. 289-301.

Mosier, A.R., and J.A. Delgado. 1997. Methane and nitrous oxide fluxes in grasslands in western Puerto Rico. Chemosphere 35:2059-2082.

Mosier, A.R., J.A. Delgado, and M. Keller. 1998. Methane and Nitrous oxide fluxes in an acid oxisol in western Puerto Rico: Impact of tillage, liming, and fertilization. J. Soil Biol. Biochem. 30:2087-2098.

Mosier, A.R., J.A. Delgado, V.L. Cochran, D.W Valentine, and W.J. Parton. 1997. Impact of agriculture on soil consumption of atmospheric CH_4 and a comparison of CH_4 and N_2O flux in subarctic, temperate and tropical grasslands. Nutr. Cycling Agroecosystems. 49:71-83.

Mosier, A.R, J.M. Duxbury, J.R. Freney, O. Heinemeyer, and K. Minami. 1998. Mitigating agricultural emissions of methane. Climatic Change. 40:39-80.

Mosier, A.R, J.M. Duxbury, J.R. Freney, O. Heinemeyer, and K. Minami. . 1996. Nitrous oxide emissions from agricultural fields: Assessment, measurement and mitigation. Plant Soil.181:95-108.

Mosier, A.R., and G.L. Hutchinson. 1981. Nitrous oxide emissions from cropped fields. J. Environ. Qual. 10:169-173.

Mosier, A.R., G.L. Hutchinson, B.R. Sabey, and J. Baxter. 1982. Nitrous oxide emissions from barley plots treated with ammonium nitrate or sewage sludge. J. Environ. Qual. 11:78 81.

Mosier, A.R. and O. Heinemeyer. 1985. Current methods used to estimate N_2O and N_2 emissions from field soils. *In* Denitrification in the Nitrogen Cycle. Plenum Publishing Corp. Golterman, H.L. (ed.). pp. 79-99.

Mosier, A.R., W.D. Guenzi, and E.E. Schweizer. 1986. Soil losses of N_2 and N_2O from irrigated crops in northeastern Colorado. Soil Sci. Soc. Am. J. 50:355-348.

Mosier, A.R., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. Van Cleemput. 1998.Closing the global atmospheric N_2O budget: Nitrous oxide emissions through the agricultural nitrogen cycle. Nutr. Cycling Agroecosystems. 52:225-248.

Mosier, A.R., W.J. Parton, D.W. Valentine, D.S. Ojima, D.S. Schimel, and J.A. Delgado. 1996. CH_4 and N_2O fluxes in the Colorado shortgrass steppe: I. Impact of landscape and nitrogen addition. Global Biogeochem. Cycl. 10:387-399.

Mosier, A.R., W.J. Parton, D.W. Valentine, D.S. Ojima, D.S Schimel. and O Heinemeyer. 1997. CH_4 and N_2O fluxes in the Colorado shortgrass steppe: 2. Long-term impact of land use change. Global Biogeochem. Cycles. 11:29-42.

Mosier, A.R., M. Stillwell, W.J. Parton, and R.G. Woodmansee. 1981. Nitrous oxide emissions from a native shortgrass prairie. Soil Sci. Soc. Am. J. 45:617-619.

Mosier, A.R., D.S. Schimel, D.W. Valentine, K.F. Bronson, and W.J. Parton. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature. 350:330

332. Mummey, D. L., J.L. Smith and H. Bolton, Jr. 1994. Nitrous oxide flux from a shrub-steppe ecosystem: Sources and regulation. Soil Biol. Biochem. 26:279-286. Mummey, D. L., J.L. Smith and H. Bolton, Jr. 1997. Small-scale spatial and temporal variability of N_2O flux from a shrub-steppe ecosystem. Soil Biol. Biochem. 29:1699-1706. Parkin, T.B. 1987. Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. 51:1194-1199.

Parkin, T. B., S.T Chester, J.L. Starr, J.J. Meisinger, and J.A. Robinson. 1988. Evaluation of statistical estimation methods for log-normally distributed variables. Soil Sci. Soc. Am. J. 52:323-329.

Parkin, T.B. and J.A. Robinson. 1992. Analysis of Lognormal Data. *In* B. A. Stewart (ed.), Advances in Soil Science, 20:193-235, Springer-Verlag, New York.

Parkin, T.B. 1993. Evaluation of statistical methods for determining differences between samples from lognormal populations. Agron. J. 85:747-753.

Pedersen, A.R., S.O. Petersen, and F.P. Vinther. 2001. Stochastic diffusion model for estimating trace gas emissions with static chambers. Soil Sci. Soc. Am. J. 65:49-58.

Rolston, D.E. 1986. Gas Flux. *In* A. Klute (ed), Methods of Soil Analysis, Part 1., Physical and Mineralogical Methods. Pp. 1103-1119. Agronomy Monograph no. 9 (2nd edition), Soil Sci. Soc. Am. Madison, WI.

Smart, D.R., and A.J. Bloom. 2001. Wheat leaves emit nitrous oxide during nitrate assimilation. Proc. Natl. Acad. Sci. U.S.A. 98:7875-7878.

Smith, K., H. Clayton, I. McTaggart, P. Thomson, J. Arah, and A. Scott. 1995. The measurement of nitrous oxide emissions from soil by using chambers, Philos. Trans. R. Soc. London, 351:327-338.

Sommerfeld, R.A., R.C. Musselman, J.O. Reuss, and A.R. Mosier. 1991. Preliminary measurements of CO_2 in melting snow. Geophys. Res. Let. 18:1225-1228.

Sommerfeld, R.A., A.R. Mosier, and R.C. Musselman. 1993. CO_2, CH_4 and N_2O flux through a Wyoming snowpack and implications for global budgets. Nature. 361:140-142.

Sommerfeld, R.A, W.J. Massman, R.C. Musselman, and A.R. Mosier. 1996. Diffusional flux of CO_2 through snow: Spatial and temporal variability among alpine-subalpine sites. Global Biogeochem. Cycl. 10:473-482.

Appendix III A: 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).

1 Remove 10 ml gas sample.

1 Inject sample into vial.

1 Flush syringe with Air 2x.

1 Place chamber on anchor #2.

1 Remove 10 ml gas sample.

1 Inject sample into vial.

1 Flush syringe with air 2x.

 b. Move to next pair of chambers in plot.

1 Record time on stop watch.

1 Place chamber 3 on anchor.

1 Remove 10 ml gas sample.

1 Inject into vial.

1 Flush syringe with Air 2x.
1 Place chamber 4 on anchor.
1 Remove 10 ml gas sample.
1 Inject into vial.
1 Flush syringe with air 2x.
 c. Move to next plot.
1 Record Temperatures.
1 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.
1 Remove all chambers, Move to next set of 12 anchors. Repeat steps 1-5.
2 When all plots have been done, one person collects all chambers and place in truck while other person takes soil moisture readings in each plot (4 measurements/plot).

Appendix III B: 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 four 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.

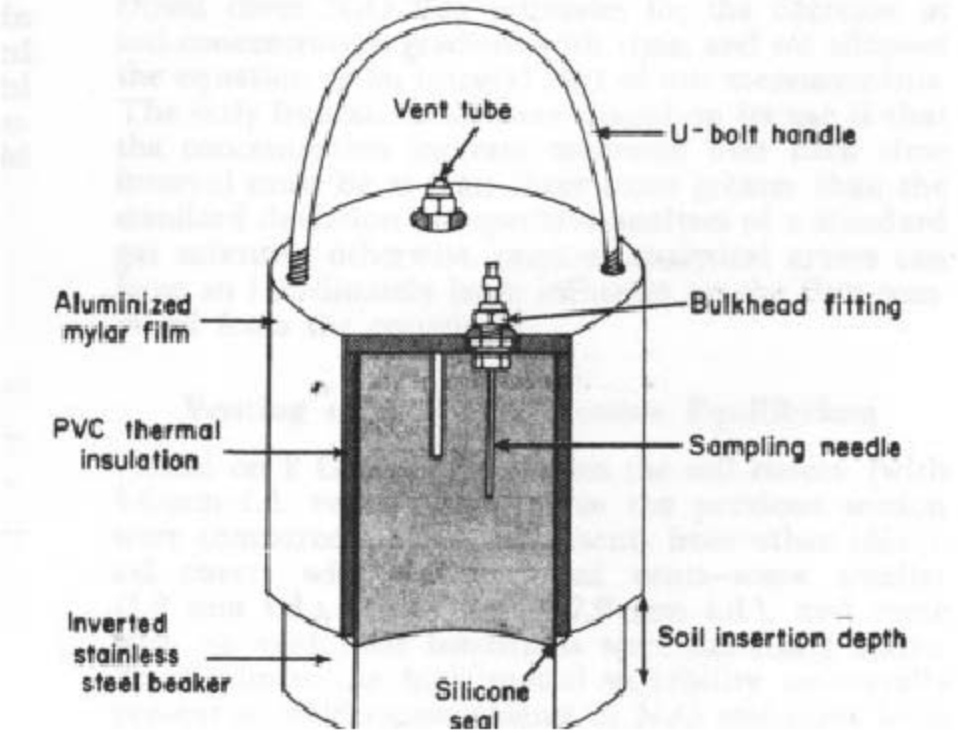
Syringes: Beckton-Dickenson (obtained from most laboratory supply companies) *Syringe stopcocks:* (ColeParmer # A-30600-000: Qosina, #99705 or #99717).

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) 5639405, 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.



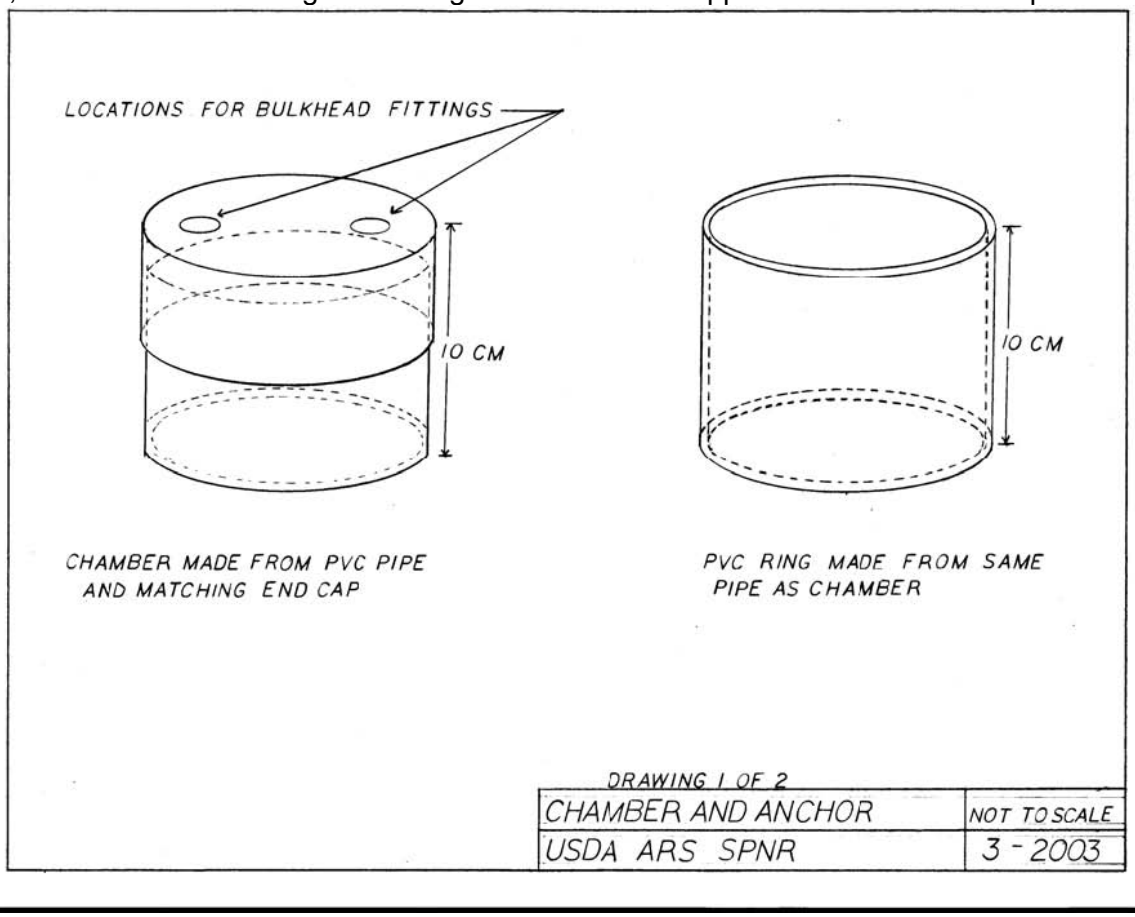
PVC soil anchor and chamber used by Mosier. Rectangular chambers used by Mosier

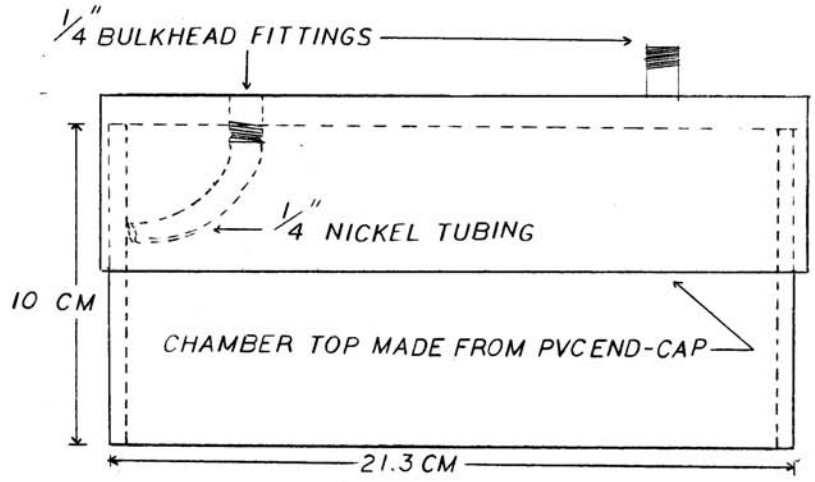
Example of temporary/portable chamber used by Parkin. Chamber has an attached polethylene skirt held in place on the soil surface with a length of chain. As shown, the chamber is monitoring soil CO_2 flux by recirculating gas through an infrared analyzer. Gas samples can be withdrawn through septum in top of chamber for N_2 and CH4 analyses.

Round PVC chamber description:

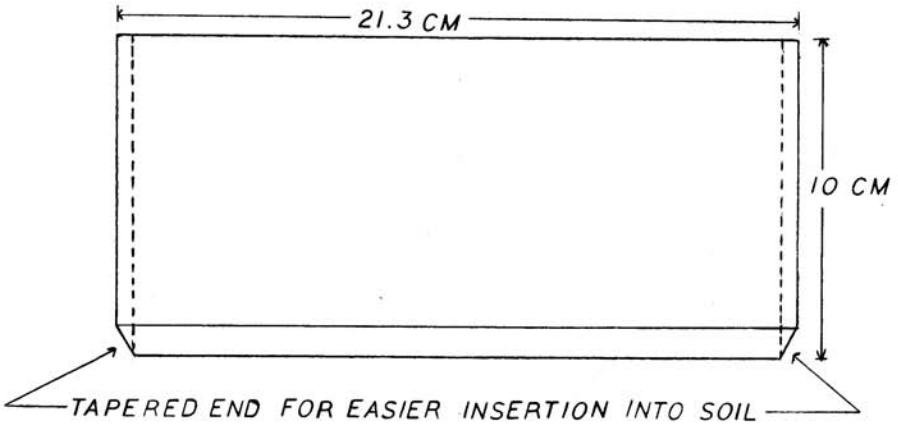
Anchor: Made from PVC pipe, 15 – 30 cm diameter. It can be tapered on the bottom for easier insertion into the soil. We typically insert the anchor 8-9 cm into the soil. The chamber can fit onto the anchor, flush (resting on the anchor), inserted into the anchor, or if an end cap is used, fit over the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





- 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



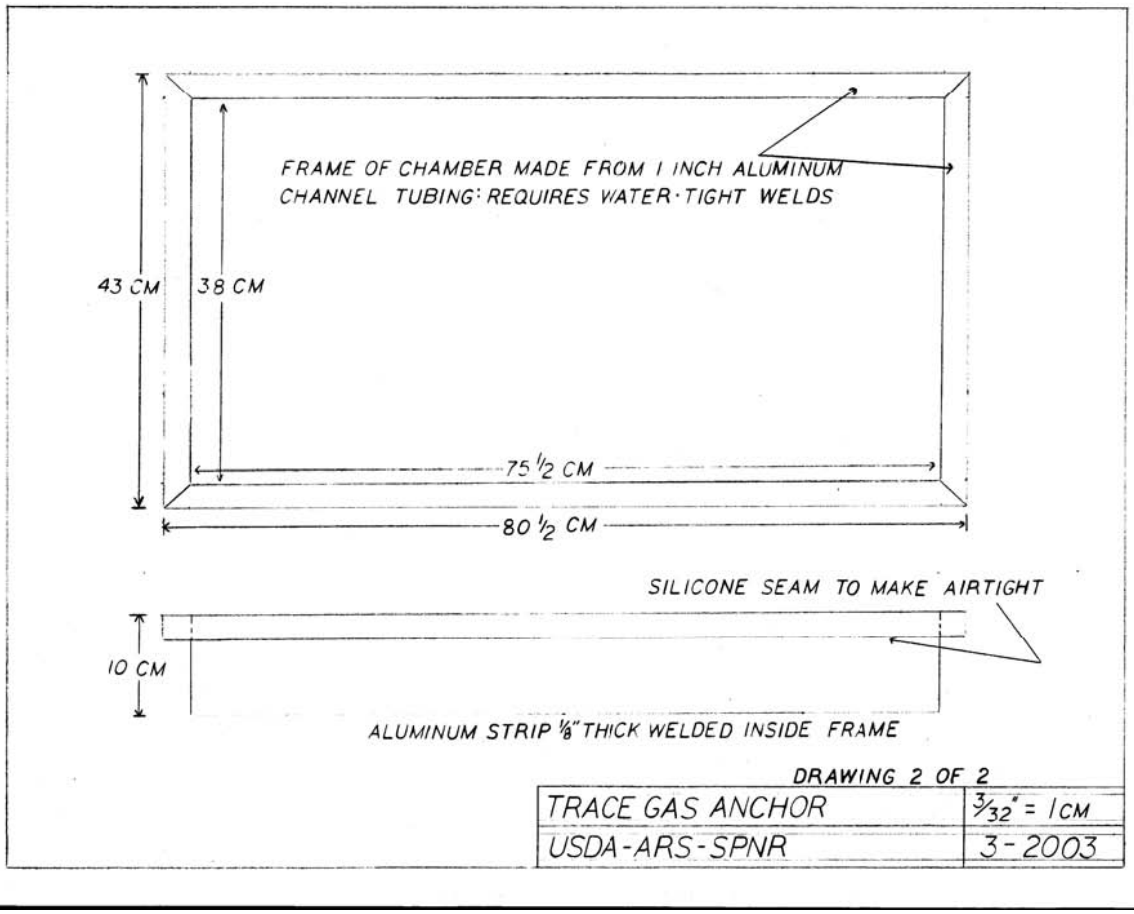
DRAWING 2 OF 2

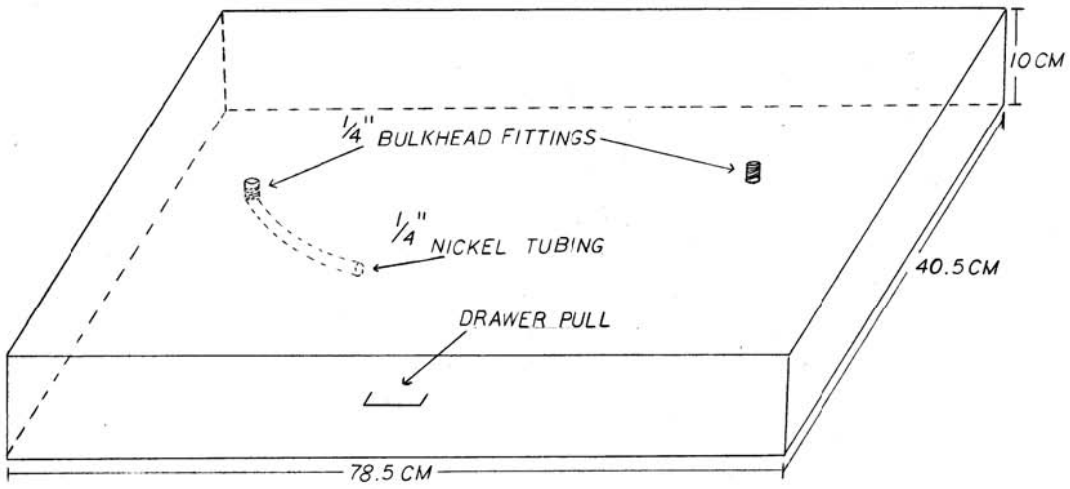
PVC CHAMBER AND RING SET	1/4" = 1 CM
USDA-ARS-SPNR	3 - 2003

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

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

Chamber: Made from sheet aluminum to desired dimensions. Two holes, to accommodate Swagelock 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

IV. Micrometeorological measurements

Steering committee: John Baker, Bruce Kimball (*Who else in on this committee?)*

Weather and climate data sets for all GRACENET locations will be necessary, both for interpreting other measured field data and for the added value obtained through modeling of C processes. It is important to distinguish between weather and climate data. Climatic data are needed for general site characterization and for generating long-term simulated weather variables for modeling. In general, proximity is not as critical as the quality of the data and the length of the record. The nearest weather station for which data are archived at the National Climatic Data Center should be sufficient. Standardized methodology (e.g., Easterling et al, 1996) should be used to extract and develop climatic data that are used for GRACE.net purposes.

Current weather data, needed in conjunction with specific field experiments, must be measured as proximally as possible. Ideally, all research locations will have weather stations on site, or at least sufficiently close that the data will be sufficiently representative. This criterion is inexact, and varies for different weather variables; as a general guideline it is desirable to have a basic agricultural weather station (Hubbard and Hollinger, 2005) within 2 km of each field research site. In this context precipitation is the most critical parameter. If the nearest weather station is more than 1-2 km distant, it is recommended that a rain gauge be installed on site.

The suggested minimum data set for weather should include the following:

Daily weather

- Air temperature maximum ($^{\circ}$C)
- Air temperature minimum ($^{\circ}$C)
- Average dew point ($^{\circ}$C)
- Daily total precipitation (mm)
- Daily total solar radiation (MJ/m^2)
- Average daily wind speed (m/s)
- Average daily 10-cm soil temperature ($^{\circ}$C)

Optional data, that are desirable for many purposes but not deemed absolutely necessary, include the following:

- Wind direction (degrees from north)
- Pan evaporation (mm)
- N deposition, wet and dry
- Net radiation (MJ/m^2)
- Rainfall intensity (mm/hr)
- Soil heat flux (MJ/m^2)
- Soil temperature (($^{\circ}$C)
- Soil water content profile (m^3/m^3)
- Snow depth (mm)

In addition, for detailed mechanistic modeling it may be necessary for some sites to collect weather data with higher temporal resolution, e.g.-30 minute or hourly. These sets would typically include:

- Air temperature
- Relative humidity (%) or dew point ($^{\circ}$c)
- Wind speed (m/s)
- Solar radiation (w/m^2)

- Net radiation (w/m^2)
- Precipitation (mm)
- Canopy temperature ($^{\circ}C$)
- PAR, incoming ($\mu mol\ m^{-2}\ s^{-1}$)
- PAR, reflected ($\mu mol\ m^{-2}\ s^{-1}$)
- Soil heat flux (W/m^2)

Details regarding the proper measurement of all variables can be found in Hatfield and Baker (2005). Measurement heights and instrument type should be reported for all measurements.

Climate data

Climate data are expected to include the following:

- Mean monthly air temperatures ($^{\circ}C$)
- Annual mean maximum and minimum air temperature ($^{\circ}C$)
- Total monthly and annual precipitation (mm)
- Annual snowfall (mm)

Literature cited:

Easterling, D.R., T.C. Peterson, and T.R. Karl. 1996. On the development and use of homogenized climate datasets. J. Climate 9(6):1429-1434.

Hatfield, J.L., and J.M. Baker (eds). 2005. Micrometeorology in Agricultural Systems. Agronomy Monograph no. 47. American Society of Agronomy, Madison, WI. 584 pp.

Hubbard, K.G., and S.E. Hollinger. 2005. Standard meteorological measurements. *In* Hatfield, J.L., and J.M. Baker (eds). 2005. Micrometeorology in Agricultural Systems. Agronomy Monograph no. 47. American Society of Agronomy, Madison, WI. 584 pp.