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Soil Carbon Changes: Comparing Flux Monitoring and Mass Balance in a Box Lysimeter Experiment

S. M. Nay* and B. T. Bormann

ABSTRACT

Direct measures of soil-surface respiration are needed to evaluate belowground biological processes, forest productivity, and ecosystem responses to global change. Although infra-red gas analyzer (IRGA) methods track reference CO_2 flows in lab studies, questions remain for extrapolating IRGA methods to field conditions. We constructed 10 box lysimeters with homogenized mixtures of sandy loam and cattle manure and kept them free of plants to create a range of CO_2 fluxes. Infra-red gas analyzer measurements, applied biweekly, were then compared to mass balance–based measures of changes in soil C over 8 mo. The CO_2 fluxes measured with IRGA were not significantly different ($P < 0.05$) from the mass balance measure in 9 of the 10 boxes. The only statistically significant difference was in the lysimeter with the highest initial C content; this box had elevated soil temperatures early in the trial, suggesting a composting effect that may have interfered with IRGA measures. Variations in the mass balance estimates were higher than expected, demonstrating how difficult establishing a true reference in field studies is. We conclude that fluxes of CO_2 from soils can be monitored with an IRGA-based chamber system in the field to produce reliable estimates of cumulative C loss. Such field measures will likely be much more variable than laboratory measures, however, and thus will require extensive sampling.

R ESPIRATION OF CO_2 from soil surfaces is one of the most important ecosystem processes. Soil respiration nearly balances net photosynthetic uptake of atmospheric CO_2 by plants, when stores of soil C are constant. With changing global climates, however, global stores of soil C—about two to three times greater than C in atmospheric CO_2—may not remain constant. For example, warmer temperatures may promote faster decomposition, and higher atmospheric CO_2 may increase photosynthesis, in turn increasing detritus production. Thus, measures of soil respiration may play an important part in unraveling how the global ecosystem will respond to changing atmospheric CO_2. Measures of soil respiration are important in many other ways as well, such as in assessing belowground biological activity; calculating C budgets and net primary production; and increasing understanding of the effects of soil disturbance, fertilization amendments, and contamination by pollutants.

Studies use different methods to measure soil respiration. Reviews of the different methodologies can be found in Schlesinger (1977), Anderson (1982), Rolston (1986), Raich and Nadelhoffer (1989), and Nakayama (1990). The accuracy of methods has long been debated in the literature. Numerous field studies have compared methodologies (de Jong et al., 1979; Edwards, 1982; Cropper et al., 1985; Freijer and Bouten, 1991; Norman et al., 1992; Rochette et al., 1992; Nakadai et al., 1993; Jensen et al., 1996; Norman et al., 1997; Rochette et al., 1997); however, these types of studies are difficult to evaluate because a reference to compare responses is lacking (Nakayama, 1990, and Nay et al., 1994).

The accuracy and precision of instantaneous flux methods relative to a known reference, under field conditions, remains untested. Establishing a reference in the field is difficult or impossible because of the difficulty in detecting soil C changes that can be attributed to respiration—because of inherent soil variability. The presence of plants further complicates C accounting.

To test an IRGA-based flux method under quasi-field conditions, we established field lysimeters without plants and with homogenized soils amended with manure that permitted us to calculate a reference C loss by mass balance. Instantaneous methods were used to monitor respiration rates and calculate long-term fluxes for comparison to mass balances.

MATERIALS AND METHODS

Box Lysimeters

To compare IRGA-based flux methods against a mass balance reference, we constructed 10 box lysimeters with homogenized soils of varying C contents (Fig. 1). The study site was located in Corvallis, Oregon. The 152- by 152- by 70-cm box lysimeters were made of plywood, lined with 0.15-mm-thick polyethylene and constrained between concrete barriers. Each box included a 10-cm-diam. drain plumbed to a catchment area. All boxes had a 10-cm layer of coarse river rock overlaid with a 10-cm layer of coarse sand. Above the coarse sand was 50 cm of soil amended with manure.

Box lysimeter soils were created by combining a sandy loam–loamy sand (USDA textural classification) and dewa-

Abbreviations: IRGA, infra-red gas analyzer; LOI, loss on ignition; TOC, total organic carbon.
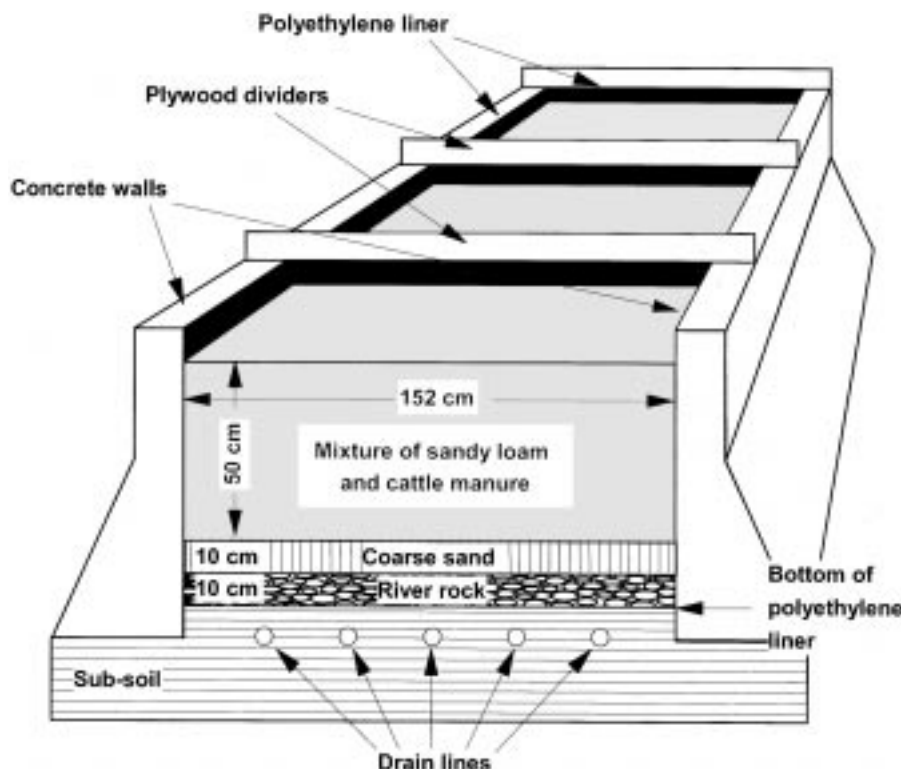
Fig. 1. Box lysimeters were made between cement barriers with various soil and manure mixtures. Lysimeters were lined and drainage was monitored.

tered cattle manure. The manure was 1 mo old at the time of mixing with the mineral soil. Both mineral soil and manure were passed through a 2.5-cm sieve to remove rocks and break up large aggregate material. The soil materials were mixed repeatedly with a front-end loader. Soil and manure were combined to create a starting soil with organic matter contents ranging from 0.05 to 0.28 kg kg^{-1}. Each box was filled with about 60 cm of the soil mixture and allowed to sit for at least 5 d, after which the excess soil above 50 cm was removed. To prevent additions of C by photosynthesis, boxes were kept free of plants throughout the experiment.

A sampling area, 137 by 137 cm, subdivided into 36 subsampling cells identified locations for various sampling activities including soil coring, CO_2 flux, and moisture measurements. Soil moisture was measured with TDR (IRAM's Soil Moisture Analyzer, CPN Corp., Martenez, CA) with 30-cm probes. Incompatible sampling activities were avoided; for example, cells that had initial soil coring were excluded from CO_2 flux monitoring and further soil sampling.

Soil mixtures were made in early May and allowed to set for 10 d. Initial soil cores were then extracted to delineate the beginning of the monitoring experiment. Final soil cores were extracted in January, 8 mo after the initial soil cores.

Reference Measures by Mass Balance

We used mass balance to calculate reference of C loss, as CO_2, to the atmosphere. This analysis considered C dissolved in rainfall and drainage, and changes in soil C. Outflow of water draining the ecosystems was measured in 5 of 10 boxes. Outflow during the first month was not examined for C content, however, this outflow was no more than 10 L m^{-2}. No outflow was observed between mid-June and mid-October. From mid-October until the end of the experiment, outflow water was collected following rainfall events. Carbon loss as dissolved and suspended organic C or total organic C (TOC)

was determined through analysis in a C analyzer (Dohrmann Carbon Analyzer, Model DC-80). Inorganic C was purged from the water samples through acidification (0.5 mL 4 *M* H_2SO_4 in 50 mL H_2O) before the analysis. The C in soil water represented about 0.1% of the changes in soil C. Inputs in rainfall were not measured because HCO^{-3} and organic acids are not believed to be significant inputs. Because of these measurements and assumptions, inputs and outputs were not included in mass balance calculations.

Change in storage of soil C was assessed in each box by comparing 10 soil cores extracted at the beginning and 10 at the end of the experiment. Cores were 5 cm in diameter and extended to the bottom of each soil. Locations for soil coring were chosen at random. Core holes were filled with like soil. Soil cores were used to determine a ratio of C to mineral soil mass.

The mineral mass of the soil in each box was estimated by careful excavation of quantitative monolith samples. Three soil monolith samples—14.8 by 14.8 cm by depth of soil—were extracted from each of the boxes at the end of the experiment. Changes in C mass of the boxes was then calculated by combining changes in C to mineral mass from soil cores with bulk mineral-soil estimates. Soil samples were oven dried at 70°C for a minimum of 72 h, then ground with a disc pulverizer (Bico Inc., Burbank, CA) to <425 μm (40 mesh).

Loss on ignition (LOI) was used to determine organic matter content by mass loss 1 to 5 g of ground soil after baking at 450°C for 4 h. A portion of the soil-core samples was ground to <250 m (60 mesh). The finer ground samples were then analyzed in a C, N, and S analyzer (NA1500 Series 2, Carlo-Erba Instruments, Milan, Italy) to determine C to organic matter (OM) ratios. Initial core samples had a mean C:OM of 0.528 (SE = 0.018), and the final core samples had a mean C:OM of 0.489 (SE = 0.016). These values were used to calculate C mass from LOI measures. Carbon/N ratios were approximately 25 in the beginning and 19 at end.
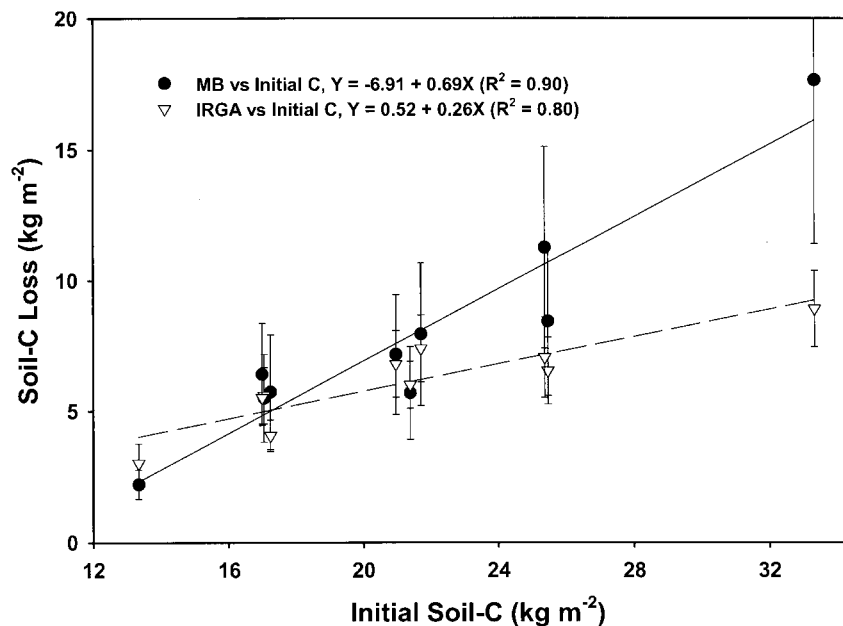
Fig. 2. Mass balance (MB) and infra-red gas analyzer (IRGA) estimates of C loss over 244 d vs. initial soil-C. Error bars are 95% confidence intervals.

Monitoring Flux of Carbon Dioxide from Soil Surfaces

Instantaneous flux measures were made with an IRGA (LI-6200, Li-Cor Inc., Lincoln, NE) attached to a dynamic chamber, following the methods of Norman et al. (1992) and Nay et al. (1994), where the flux was determined with an IRGA within 60 s of placing the chamber on the soil surface. Chambers were made of PVC, 21 cm in diameter and 20-cm deep. A larger chamber (60 cm deep) was used in initial measurements to accommodate high rates in freshly mixed soils. Collars place in the soil—often used in other studies—were found unnecessary with these soil surfaces. Chambers were inserted into the soil approximately 0.5 cm. Flow rates through the IRGA and chamber were approximately 0.027 L s^{-1}. Flux rates were determined using a linear interpolation of the changes in CO_2 concentration with time. Chamber CO_2 concentrations at time of rate estimates were generally 5 to 25 μL L^{-1} higher than ambient dependent on flux rates.

Five randomly assigned cells were repeatedly monitored about every 14 d for 8 mo using the IRGA-flux method. All flux monitoring was done between 0800 and 1600 h. Three sets of diel measurements were done to determine 24 h temporal patterns. Total CO_2 flux was calculated by integrating the data from each sample cell over the course of the experiment.

Statistics

Change in soil C was calculated by difference in the mean C content per m^2 at the beginning and end of the experiment. Statistical bootstraping methods (Efron and Tibshirani, 1986) were used to calculate a 95% confidence interval for C loss in each box. The C contents per soil area (monolith samples-LOI), C contents per mineral soil (soil cores-LOI) and C analysis of organic matter data and were randomly sampled and multiplied together. One thousand iterations of this process were done to obtain a distribution for the products of these calculations.

Estimates of C loss by IRGA flux monitoring were determined by integrating the CO_2 flux rate data from each of the five regularly monitored sample locations per box. The 95%

confidence intervals for CO_2 fluxes were then based on the variation of five integration values. Ninety-five percent confidence intervals from mass balance and the IRGA monitoring data were then compared. Additionally a comparison of regression lines of C loss vs. initial C content for both methodologies was also done.

RESULTS

Carbon Loss Based on a Mass Balance as a Reference

Carbon losses in drainage were <0.14%, leaving soil-C changes as the only elements in the mass balance. Soil-C losses ranged from 2.2 to 17.7 kg C m^{-2} over the 244 d of the experiment (Fig. 2). Mass loss as a percentage of the initial contents ranged from 17 to 53%, with the lowest percent loss recorded from the box containing the lowest initial C (13.4 kg m^{-2}) and the highest percent loss occurring in the box with the highest initial C (33.3 kg m^{-2}). The amounts and pattern of higher decomposition rates with higher amendments corresponds to rates reported by Sommerfeldt et al. (1988) in a manure amendment experiment. Gregorich et al. (1998) made measurements of soil respiration in agricultural fields with cattle manure added and showed proportionally less decomposition with additional manure; however, the maximum quantity added in their experiment was only about 40% of our smallest amendment.

Carbon Loss Based on Infra-Red Gas Analyzer–Based Flux Monitoring

Carbon dioxide fluxes were very high initially and declined over the course of the experiment as expected (Fig. 3). Final fluxes were only about 5% of initial fluxes. Total C loss, based on integration of the flux rate monitoring, ranged from 3.0 to 8.9 kg m^{-2}. Rates observed
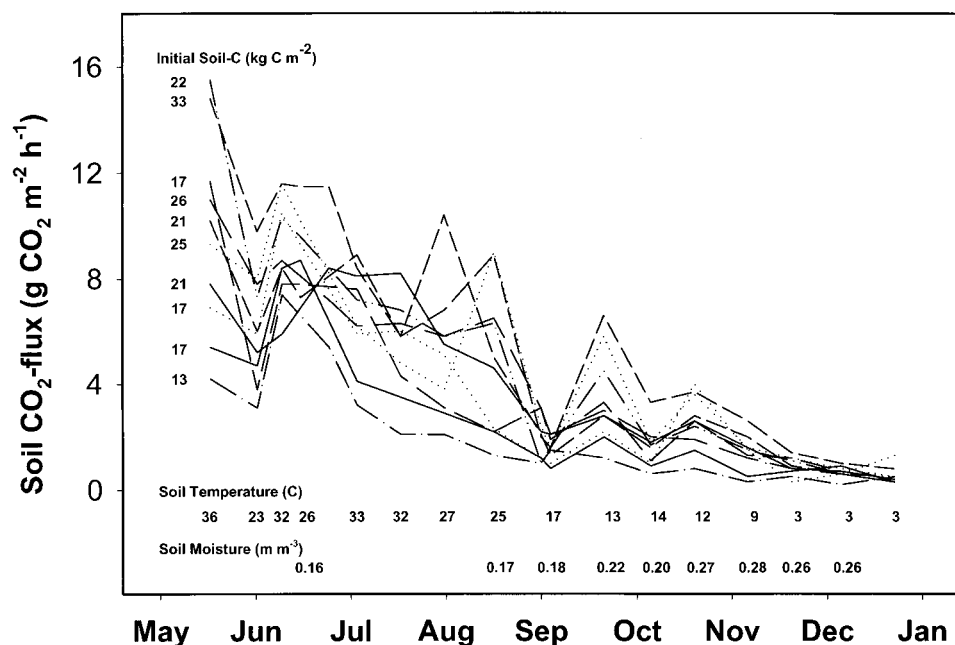
Fig. 3. The CO₂ flux from box lysimeters measured by infra-red gas analyzer over the course of the experiment. Numbers to the left of lines are initial soil C content (kg m⁻²). Mean soil temperature and moisture by monitoring event are also shown.

in this experiment were high compared to typical soils amended with manure. The highest fluxes were from boxes with the highest initial C content. Fluctuations in flux appeared to correspond to changes in soil temperature. Rain was minimal until late September. In the beginning soil temperatures ranged from 32 to 39°C, with the higher temperatures corresponding to higher initial C content.

Mass Balance Compared to Infra-Red Gas Analyzer–Based Flux Monitoring

Mass balance and flux monitoring estimates were not significantly different ($P < 0.05$) in 9 of the 10 box lysimeters. Estimates were most similar in the boxes with lower initial-C contents (Fig. 2). The significant difference observed in the box with the largest initial-C content was unexpected and is discussed later. A comparison of regression lines for the two methods showed a significant difference ($P < 0.01$) in the slopes of regression lines for estimates of soil-C loss as a function of initial soil-C, with mass balance having a slope of 0.69, whereas IRGA estimates had a slope of 0.26. The leverage of the high initial soil-C data largely accounts for the differences in slope (Fig. 2). A second unexpected result was that the confidence intervals for the mass balance means were nearly twice the confidence intervals for flux-monitoring means. The lower variation in flux-monitoring means may be due to larger sampling area of the dynamic chamber. Sampling by the dynamic chamber covered an area 8.6 times as large as the soil cores and 2.7 times as large as the monolith samples. Several conclusions are drawn from these data: an IRGA-based flux-monitoring method can produce estimates similar to mass balance estimates, except possibly at very high rates of C loss: and mass balance estimates

do not necessarily provide a low-uncertainty reference, as was expected.

Uncertainties in Mass Balance Estimates

Mass balance calculations for the box lysimeters used the following variable: bulk soil per unit area; and the following ratios: mineral matter to bulk soil, organic matter to mineral soil, and C to organic matter. Randomly chosen samples from each component of the mass balance equation were repeatedly selected in a bootstrapping technique (Efron and Tibshirani, 1986) to generate an estimate of the change in soil C and the 95% confidence interval of that estimate (Fig. 2). Mean ranges and coefficients of variation (CV) can be found in Table 1.

The component with the highest variation for calculating mass balance was in the LOI. The higher variations tended to correspond to boxes with higher organic matter content. Apparently our box lysimeter soils were less homogenized than we thought, the higher the soil C, the less homogeneous.

Uncertainties in Flux-Monitoring Estimates

Variation in cumulative C loss by IRGA-based flux monitoring was about half that of our presumed reference based on mass balance with CVs ranging from 6 to 22%. The CVs for any monitoring event of a box ($n = 5$) ranged from 4 to 134%.

Variation was primarily spatial and not temporal. Three separate diel monitoring tests (one done in September and two done in October) showed no difference in time of measurement. For the diurnal test done in Sept. the means of a single box ranged from 1.3 to 1.7 g CO_2 m⁻² h⁻¹ over 24 h, when measured every hour. In contrast, when the same data were analyzed by spatial location, means ranged from 0.9 to 2.9 g CO_2 m⁻² h⁻¹.

Table 1. Sources of variation in mass balance estimates.

Sources of variation	Means	CV
		%
Bulk soil/area, kg m^{-2}	191–489	1.0–4.9
Mineral matter/bulk soil, kg kg^{-1}	0.8–1.0	0.3–3.4
Initial organic/mineral, kg kg^{-1}	0.06–0.39	7.6–21.4
Final organic/mineral, kg kg^{-1}	0.05–0.20	4.3–30.1
Initial C/organic, kg kg^{-1}	0.53	7.9
Final C/organic, kg kg^{-1}	0.49	7.3

We do not believe that our sampling during daylight hours introduced a bias in our monitoring of soil respiration. The diel experiments confirm no diel pattern. Soil temperatures for the first 6 wk of the experiment were higher than ambient soil temperatures by up to 9°C. Thus, we believe that heat was being generated from within our boxes by the composting of the manure. The IRGA-based method provided repeatable results, even though spatial variation was high.

DISCUSSION

Monitoring fluxes of CO_2 with an IRGA-based chamber system can produce cumulative estimates of C loss that are similar to those from mass balance estimates, except perhaps at very high rates of C loss. We make this assertion for a homogenized soil, kept free of plants, and in an open field. Variation in this quasi-field study, however, was higher than expected. Most notably, variations in mass balance estimates were high, leading us to conclude that a mass balance reference is difficult to produce in greenhouse or field studies.

Mass balance and CO_2 flux did not match in the box with the highest initial soil C. One possible explanation is inadequate monitoring of the flux at the very beginning of the experiment when rates were the highest. The first measurements of CO_2 flux took place 9 d after the beginning of the experiment and, on average, accounted for about 15% of the total C loss for the entire experiment. Rates then declined rapidly with the following eight measurements each representing, on average, 7 to 8% of the total. Thus, the integration value calculated for the first measurement period, likely underrepresented the actual soil-CO_2 flux in the beginning.

Hutchinson and Livingston (1993) and Healy et al. (1996) have suggested that the soil CO_2 concentrations can quickly be altered with deployment of chambers to the soil surface. Comparison of instantaneous IRGA measurements to a reference system of a highly porous material (Nay et al., 1994) produced a 15% underestimate that could possibly be accounted for by this type of error. We would expect that any bias caused by this effect would be less in less porous soils and consistent relative to rates.

Inherent spatial variation and other technical problems may make field studies with the IRGA difficult. Although our soils were not completely homogenized, variation relative to rates in the field is expected to be higher, suggesting that extensive sampling would be needed. The rates we observed in the beginning of this experiment (4–16 g CO_2 m^{-2} h^{-1}), though, are not likely to be encountered in most field situations. Soil surface

CO_2 flux rates for temperate forests tend to be <1 g CO_2 m^{-2} h^{-1} (Ewel et al., 1987; Hanson et al., 1993; Pinol et al., 1995; Davidson et al., 1998). Russel and Voroney (1998) report rates up to 1.4 g CO_2 m^{-2} h^{-1} for a northern boreal forest. All these studies use similar IRGA and chamber methods. The spatial variation we observed, though, is consistent with that reported by Griffin et al. (1996) for a mesocosm experiment with 150 measurements on a surface area about twice that of our box lysimeters. The high variability that we report also corresponds with that reported by Rochette et al. (1991). They concluded that spatial patterns of variability in soil-CO_2 flux for an agricultural soil appeared at distances less than 0.15 m. Measurements of CO_2 flux from soils with an IRGA and a dynamic chamber should yield good quantitative results, provided that adequate attention is given to the difficult challenge of obtaining sufficient samples in field studies.

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