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Soil properties differently influence estimates of soil CO_2 efflux from three chamber-based measurement systems

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Abstract. Soil CO_2 efflux is a major component of net ecosystem productivity (NEP) of forest systems. Combining data from multiple researchers for larger-scale modeling and assessment will only be valid if their methodologies provide directly comparable results. We conducted a series of laboratory and field tests to assess the presence and magnitude of soil CO_2 efflux measurement system × environment interactions. Laboratory comparisons were made with a dynamic, steady-state CO_2 flux generation apparatus, wherein gas diffusion drove flux without creating pressure differentials through three artificial soil media of varying air-filled porosity. Under these conditions, two closed systems (Li-6400-09 and SRC-1) exhibited errors that were dependent on physical properties of the artificial media. The open system (ACES) underestimated CO_2 flux. However, unlike the two other systems, the ACES results could be corrected with a single calibration equation that was unaffected by physical differences in artificial media. Both scale and rank changes occurred among the measurement systems across four sites. Our work clearly shows that soil CO_2 efflux measurement system × environment interactions do occur and can substantially impact estimates of soil CO_2 efflux. Until reliable calibration techniques are developed and applied, such interactions make direct comparison of published rates, and C budgets estimated using such rates, difficult.

Introduction

Soil CO_2 efflux is a major component of net ecosystem productivity (NEP) of forest systems and so its behavior over time greatly impacts C sequestration. Advances in technology and commercialization of measurement techniques have led to the publication of numerous manuscripts that describe environmental controls, management effects and ecological implications of carbon flux from soil. Despite the growing popularity of eddy covariance systems, chambers are the most direct means to determine carbon flux from the soil surface or forest floor (through litter) (Davidson et al. 2002). They allow greater flexibility in selecting sample locations, separating closely spaced treatments or isolating specific ecosystem components. Many chamber-based techniques exist for estimating soil CO_2 efflux, each with their own advantages and limitations.

Numerous technical comparisons of soil CO_2 efflux measurement systems have been documented under field and/or laboratory conditions. While many findings are specific to the design of the particular experiments, there are generalities that can be derived from the literature. Previous work has shown that static alkali absorption methods (closed chamber) give lower estimates of moderate to high soil CO_2 efflux rates relative to dynamic methods (Cropper et al. 1985; Rochette et al. 1992; Jensen et al. 1996; Norman et al. 1997; Pongracic et al. 1997; Rochette et al. 1997) and may overestimate at low flux rates (Nay et al. 1994; King and Harrison 2002). Closed chamber techniques (both static and dynamic) are the most common means of measuring soil gas flux, but within this category large differences in performance have been observed (Norman et al. 1997; Le Dantec et al. 1999; Janssens et al. 2000). Dynamic systems typically use infrared analyzers to monitor CO_2 accumulation *in situ*, permitting faster measurement than static systems. Fewer studies have compared closed chamber techniques to open (flow-through) chambers. Norman et al. (1997) reported that several closed systems gave lower efflux rates than an open chamber under field conditions, while Widen and Lindroth (2003) found the opposite relationship with an efflux generating apparatus.

Field comparisons made between measurement systems may be as simple as several measurements made at one site for several days (Pumpanen et al. 2003) or a time series at a particular site (e.g. de Jong et al. 1979; Cropper et al. 1985; Freijer and Bouten 1991; Le Dantec et al. 1999; Janssens et al. 2000; Yim et al. 2002). However, it is critical to assess if measurement system × site interactions exist. Rayment (2000) hypothesized that closed chambers are susceptible to errors that vary with soil conditions, namely air-filled porosity of soil. This theory is supported by experimental results presented by Conen and Smith (2000) and Butnor and Johnsen (2004). To quantify C budgets across sites with different soil properties, calibration of measurement systems to provide true flux rates is required. Correction equations derived from field comparisons can be of considerable value, but they are site specific and do not reveal which technique is the most accurate (Janssens et al. 2000) or provide a true calibration.

Comparing techniques under standard laboratory conditions are a useful means to identify sources of error among different measurement systems and provide calibration equations, while limiting spatial and temporal heterogeneity. The intent is to create a uniform CO_2 flux whose magnitude can be quantitatively determined. Bekku et al. (1997) used soil microbes to generate CO_2 in a vermiculite medium; however their means for determining instantaneous surface efflux was limited. Total efflux was calculated by determining the mass of glucose substrate respired after a ten day incubation. Kawbe et al. (2002) injected CO_2 into soil megacosms filled with field soils to enhance CO_2 gradients, but needed to account for microbial contributions to surface efflux, which were operating independently of gas injections. Flux generating systems described by Nay et al. (1994), Widen and Lindroth (2003), and Butnor and Johnsen (2004) create fluxes through artificial soils in a manner that is easily quantified and are driven by gas diffusion processes, without producing pressure gradients. Since the amount of air-filled porosity in soil has been shown to affect the accuracy of closed techniques (Conen and Smith 2000; Rayment

2000; Butnor and Johnsen 2004), a calibration system should have the potential to use soils of different air volume or be able to moderate air content with water (Widen and Lindroth 2003). Few studies relating laboratory findings to field results are available using current techniques.

We compared the performance of three soil CO_2 efflux measurement systems under laboratory and field conditions. Laboratory comparisons were made between two commercially available, dynamic closed chambers and one automated, multi-port open chamber system using the CO_2 flux apparatus described by Butnor and Johnsen (2004) on three dry, artificial soils with different air-filled porosity. The soil CO_2 efflux measurement techniques were also compared on four field sites in North Carolina, which varied in physical properties, including air-filled porosity. We evaluated how well trends observed in the laboratory under controlled conditions related to measures made in the field. Using these data, we also calculated sample size requirements for detecting treatment differences of varying magnitude, for each the three measurement systems studied.

Methods

Soil CO_2 measurement systems

Li-Cor soil CO_2 flux chamber

A Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, Nebraska) with a soil CO_2 flux chamber (Li-6400-09) was configured to the manufacturer's specifications to measure soil CO_2 efflux. The Li-6400-09 uses a dynamic closed technique to measure CO_2 efflux and has a diameter of 9.55 cm allowing 71.6 cm^2 of soil area to be measured. The chamber is shallowly inserted into the soil and the CO_2 concentration of the chamber headspace is drawn down to just below ambient. The user selects the range for CO_2 accumulation (Δ CO_2) during the measurement cycle and flux is measured as CO_2 accumulates within the chamber. Preliminary experimentation showed that the Li-6400-09 was sensitive to Δ CO_2 settings, having a setting that was too low usually lead to the measurement being lower, compared to a subsequent measure with a higher Δ CO_2. We used the following Δ CO_2 settings as a rough rule of thumb: 0–2 μmol m^2 s^{-1} $\Delta = 10$ μmol mol^{-1}, 2–4 μmol m^2 s^{-1} $\Delta = 20$ μmol mol^{-1}, 4–6 μmol m^2 s^{-1} $\Delta = 30$ μmol mol^{-1}, 6–10 μmol m^2 s^{-1} $\Delta = 40$ μmol mol^{-1}, 10–14 μmol m^2 s^{-1} $\Delta = 50$ μmol mol^{-1}. The system is programmed to allow several measurement cycles, enhancing system precision. The Li-6400-09 allows the user to determine the insertion depth, thus varying of the system volume. System volume at zero insertion depth is 991 cm^3, we used an insertion depth of 2 cm resulting in a volume of 847.8 cm^3. The IRGAs are located within the structure of the soil chamber for rapid detection of CO_2 changes. The soil chamber is pressure equilibrated and does not produce pressure artifacts that would bias measures (Takle et al. 2003). Air mixing within the chamber is achieved with low volume flows through manifolds without the use of fans.

PP Systems SRC-1 soil respiration chamber
A PP Systems SRC-1 soil respiration chamber (PP Systems, Amesbury, MA) was attached to an environmental gas monitor (EGM-3) to measure soil CO_2 flux. The SRC-1 has a diameter of 10 cm, which can effectively measure 78.5 cm^2 of substrate surface with a chamber height of 15 cm. The SRC-1 uses a dynamic closed technique to measure CO_2 fluxes. The user is prompted to lift the chamber and a fan is activated to clear the contents of the chamber, allowing the system to be filled with air of ambient CO_2 concentration. During the measurement period, continuous closed loop sampling of the CO_2 concentration is accomplished by the EGM-3. The manufacturers suggested measurement protocol was followed. The hardware and software configuration we used operated a fan at slow speeds while the sample was being collected. There was no option to control fan speed as delivered from PP Systems.

Automated Carbon Efflux System
The Automated Carbon Efflux System (ACES) (US Patent 6,692,970) is a chamber-based, multi-port respiration measurement system that is similar in concept to Maier et al. (1998) and Maier and Kress (2000) and was developed at USDA Forest Service, Southern Research Station Laboratory in Research Triangle Park, NC by J.R. Butnor, C.A. Maier and K.H. Johnsen. The system is comprised of: (1) control unit that controls logic, gas flow and gas analysis, (2) soil measurement chambers, (3) tubing and thermocouple wire connecting the chambers to the rest of the system (15 m extensions yielding a 30 m diameter sampling area), (4) ballast tank which moderates fluctuations in CO_2 concentration of reference air, (5) an exhaust pump which provides fresh air to chambers that are not actively being sampled. The ACES sequentially measures CO_2 fluxes from 15 soil chambers using an open measurement technique. The circular soil chambers are constructed of 25 cm diameter PVC pipe (491 cm^2, 10 cm height) and equipped with thermocouples to measure air and soil temperature. The chamber is covered with clear Lexan™ (DuPont Corp., Wilmington, DE) and is equipped with two pressure equilibration ports to ensure that minute differences in chamber pressure do not compromise the quality of the soil CO_2 efflux measurement (Fang and Moncrieff 1996). Within the measurement chamber there are two diffuser rings which line the inner circumference. They are constructed from 0.6 cm inner diameter tubing, perforated with small holes. One diffuser delivers reference air to the chamber, while the other pulls sample gas to the analyzer.

Reference air is collected on site from a 135 l ballast tank. The ballast tank serves to dampen large CO_2 fluctuations in ambient air. Reference air is drawn from the ballast tank and delivered to the measurement chamber via a circular diffuser ring located near the soil surface. Air is drawn from the chamber through a second diffuser ring located near the top of the chamber. The diffuser rings ensure adequate air mixing, alleviating the need for a fan. Chamber air is drawn at a lower flow rate, approximately 10% less than the reference gas, ensuring that all leaks are outward. Two pressure equilibration ports eliminate positive chamber pressures caused by the disparate flow rates. The CO_2 concentration of the reference and chamber air is measured sequentially on a 30 s cycle with an infrared gas analyzer (PP Systems, EGM series). Gas flow is monitored and recorded by a digital mass flow meter (model 822, Sierra Instruments, Monterey, CA) and controlled by adjusting the voltage to a 12 V DC pump (Brailsford Inc., Rye, NY). Flow rate can be adjusted from 0 to 3 lpm, though the usual setting for the standard soil chamber is 2 lpm.

When operated automatically, fifteen sample chambers and one null calibration chamber were measured sequentially for 10 min each, allowing a complete run every 2 h and 40 min or nine complete runs per day. When not being actively sampled, the other 15 chambers were refreshed with reference air (from the ballast tank) to prevent any buildup of CO_2 in the chambers. For laboratory experimentation the ACES was manually operated using only one of the measurement ports. When field studies were conducted, the system was connected to all 15 sample chambers and allowed to operate automatically. The ACES uses an empirically derived calibration correction that corrects system underestimates that vary with flux magnitude (0–25% correction). For the purpose of this experiment, both the raw flux data and data that were adjusted with the real-time correction (ACESadj) will be presented.

The findings derived from the custom-designed ACES, are applicable to other open chamber systems. The ACES requires a correction equation to compensate for resistance to diffusion caused by accumulation of CO_2 in the chamber headspace (e.g. at a flow rate of 2 lpm, a differential of 139 μmol mol^{-1} CO_2 above ambient, yields an efflux rate of 5 μmol m^{-2} s^{-1}). Open chambers that minimize this gas accumulation by higher gas turnover or greater chamber volume to soil surface area would require smaller corrections, but would be expected to perform similarly.

Laboratory experiment

To directly compare the performance of the SRC-1, Li-6400-09 and ACES, CO_2 fluxes were generated with a dynamic efflux apparatus described by Butnor and Johnsen (2004). The design of the apparatus was based on that of Nay et al. (1994) and involves supporting an artificial soil above an air-filled footspace, wherein the concentration of CO_2 in air can be manipulated. The footspace was not pressurized, so that only the diffusion gradient between footspace and the outside atmosphere drives the CO_2 efflux. The apparatus was constructed using a rectangular Nalgene container (91 cm $L \times$ 51 cm $W \times$ 51 cm H). A frame supported the soil medium (upper 20 cm; 0.093 m^3) above the air-filled space (lower 31 cm; 0.144 m^3). The footspace was equipped with five small electric fans to mix the air, six 1/4 in. (6.4 mm) ports and four 3/8 in. (9.5 mm) ports for pressure equilibration. During normal operation, two or three fans were used to achieve optimum mixing. Pressure in the footspace was monitored with a digital manometer (model MA2-005P, Modus Instruments Inc., Northboro, MA). To select the minimum number of open ports needed to achieve equilibrium with atmospheric pressure, all ports were closed and then sequentially opened until the pressure differential with the atmosphere fell below detectable limits (+0.1 Pa). CO_2 concentration in the footspace was monitored with an infrared gas analyzer (EGM-2, PP Systems, Amesbury, MA) scaled 0–10,000 μmol CO_2 mol^{-1}, configured to sample footspace air in a closed loop. The CO_2 concentration at the soil surface was monitored with an EGM-2 scaled 0–2000 μmol mol^{-1}. A Campbell data logger Model 21 X (Campbell Scientific Inc., Logan, UT) was programmed to collect data from the two IRGAs, calculate real-time CO_2 efflux from the soil medium surface and maintain a pre-selected rate of efflux by injecting 2% by volume CO_2 in air into the footspace via a Sierra sidetrak mass flow controller Model 840L (Sierra Instruments, Inc., Monterey, CA) as needed.

Fluxes were generated through three artificial media: fine sand, landscaping pebbles, and a 50/50 mix of fine sand and pebbles (mixed). The mixed soil had the lowest air content (26.4%) and CO_2 diffusion coefficient, while the pebble medium had the greatest (46.3%). The sand medium had properties that fell between the other types (38.0% air content). Physical properties of soil media are presented in detail by Butnor and Johnsen (2004). The flux apparatus was filled with one of the three soil media and prepared to generate CO_2 fluxes. A series of flux rates were created to cover the range obtainable for each media type. This range was governed by the diffusion coefficient of each medium and the upper limit of the IRGA (10,000 μmol CO_2 mol^{-1} air) that monitored footspace CO_2 concentration. It usually took 60–80 min to ensure system equilibrium after a 1 μmol m^{-2} s^{-1} change in efflux. Since the ACES could be operated in continuous mode, it was used to monitor surface flux and verify that efflux rate had stabilized (5% coefficient of variation over a 20 min period). We measured as many rates as possible in a 2–3 day period, then refitted the apparatus for another diffusive medium. The ACES chamber was left in place during the efflux sampling on each media, since it was designed for continuous monitoring of CO_2 fluxes from soil while both the SRC-1 and the Li-6400-09 were lifted after each measurement as would be done in the field. Linear regression was used to describe the relation between generated CO_2 efflux and efflux measured by each technique.

Field experiment

The three measurement systems were used to analyze soil CO_2 efflux on four field sites in June 2001. The sites are described below:

SETRES

The Southeast tree research and education site (SETRES) is located in the Carolina Sand Hills, 17 km north of Laurinburg NC. SETRES is 18 year-old plantation of loblolly pine situated in the Wakula soil series, typified by excessively drained sandy soil with occasional clay lenses (sandy silicious thermic psammentic hapludult). Measurements were taken on a bare soil, all litter had been removed.

RTP

The Research Triangle Park (RTP) site is located at the Southern Research Station, Forest Sciences Laboratory in Research Triangle Park. The RTP site is best described as the Cecil soil series. The measurement site was located in a 35–40 year old loblolly pine stand. The soils were particularly dense and difficult to penetrate. Prior to sampling, all leaf litter was removed.

Duke Pine

The Duke Pine site is located in the Duke University Forest near Chapel Hill, NC. This plot is commonly known as the 'reference' to the nearby free air carbon enrichment (FACE) prototype experiment (Oren et al. 1998). The soils are classified as an Enon silt loam, a low fertility Hapludalf. The measurement area was located in a stand of 20 year-old loblolly pine. All measurements were made with leaf litter left intact on the soil surface.

Duke Hardwood

The Duke Hardwood site is a stand of 60–80 year-old mixed hardwood located in the Duke University Forest, approximately 1 km from the Duke Pine site and is described in detail by Pataki and Oren (2003). The soils are classified as an Iredell gravelly loam and have a well developed organic surface layer. All measurements were made with the leaf litter intact on the soil surface.

The physical properties of soil at each site are presented in Table 1. Total porosity in upper 10 cm of soil ranged from 35 to 61%. Air-filled porosity was calculated by deducting the pore volume that was occupied by water. Air-filled porosity, which better represents the soil volume available for gaseous

Table 1. Physical properties of field soils used for comparing soil respiration measurement techniques.

	Sand %	Clay %	Silt %	B.D.[a]	Porosity[b]	Moisture content[b]	Air-filled porosity[b]
RTP site	61.9	10.4	27.7	1.43	0.35	0.30	0.05
Duke Pine site	52.8	12.2	35.1	1.33	0.54	0.32	0.22
Duke Hardwood site	46.2	14.6	39.2	0.97	0.61	0.36	0.26
SETRES	85.1	4.8	10.1	1.20	0.47	0.08	0.39

Each observation is the average value of five cores (5 cm diameter) to a depth of 10 cm.
[a] $Mg\ m^{-3}$.
[b] m^3/m^{-3}.

diffusion, ranged from 5.0% at RTP to 39.4% at SETRES. Leaf litter was removed 48 h prior to sampling at RTP and SETRES, no measurement of preexisting litter was made. Litter was left intact on the forest floor at the Duke Forest sites. Average litter depth was 2.8 cm at the Duke Pine site and 3.7 cm at the Duke Hardwood site.

Since each chamber type commanded a different soil surface area it was difficult to make direct comparisons across measurement systems. Spatial heterogeneity also introduced variation between adjacent measurement locations. Our approach was to make measurements at 15 locations per site, installing ACES chambers adjacent to locations where the SRC-1 and the Li-6400-09 would be used on the soil surface. The average soil CO_2 efflux values and their coefficients of variation were used to compare results across sites. For every measurement technique/site combination the sample size necessary for several levels of precision and confidence was calculated (Folorunso and Rolston 1984). Twenty four hours before sampling, measurement locations were pierced (with soil chambers), to allow the chambers to be easily inserted with minimal soil disturbance when sampling the following day. The three sample locations (ACES, Li-6400-09, SRC-1) were located within 20 cm of each other. At each site the ACES system with 15 automated chambers was setup and allowed to run for at least one full cycle (2 h 40 min) prior to any comparisons. The ACES system usually requires 10 min to sample each measurement chamber, once the chamber finished actively sampling, measurements were made with the portable closed systems.

Results

Laboratory experiment

Generating CO_2 fluxes through media of varying physical properties revealed differences in accuracy across the measurement techniques. ACESadj, Li-6400-09, and SRC-1 produced similar CO_2 efflux measures on the mixed soil (Figure 1) and their deviation from a 1:1 relationship with generated efflux was small (Table 2). ACESadj was in close agreement with generated flux across all media types (Figure 1). Its slope deviation from a 1:1 relationship varied from −4 to +2% (Table 2). Raw ACES data underestimated CO_2 efflux, but exhibited very little variation across media types. The Li-6400-09 underestimated flux rates on the more porous materials, though the difference between sand and pebble media was small (Figure 1 and Table 2). The SRC-1 underestimated flux rates on sand. Using the pebble medium where the air content and porosity of the substrate was higher, the system radically overestimated flux rates, providing values nearly double what was expected (Figure 1). The data collected from all three measurement techniques were readily fitted to linear regression equations (R^2 0.93–0.99), though the SRC-1 exhibited the greatest variability (Table 2). Due to the linear relationship between observed

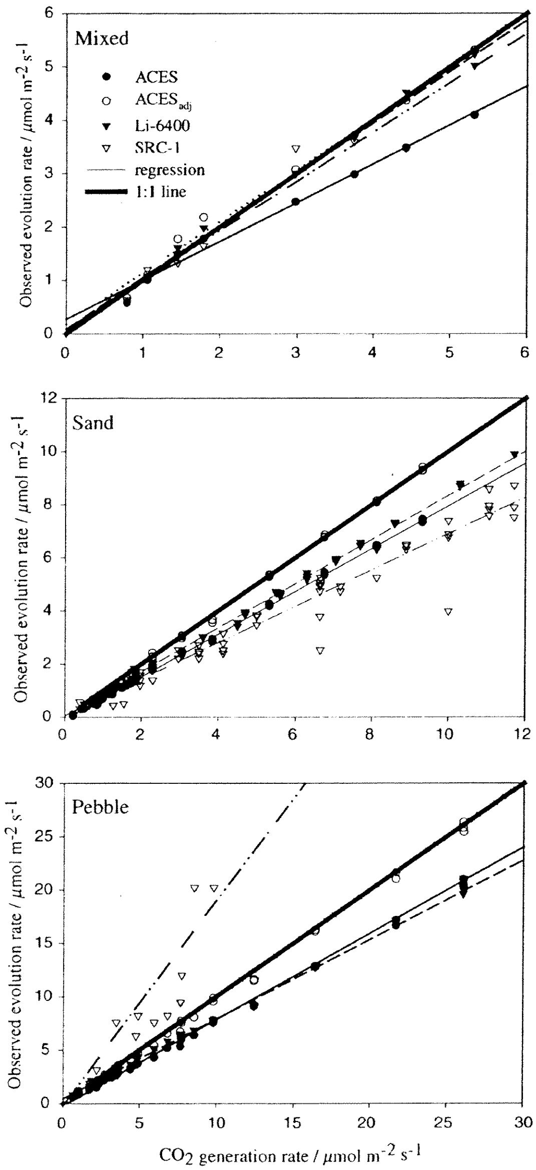

Figure 1. Comparison of CO_2 flux generated through three artificial soil media, and measurements made with the ACES (raw data and adj), the Li-6400-09 and the SRC-1. The solid line represents a 1:1 ratio and the expected value. Linear regression equations and R^2 values are presented in Table 2.

Table 2. Linear regression equations fitting measured efflux to generated efflux for each media/measurement technique combination.

Media	Method	Measured regression		R^2	Correction regression	
		Y_o	a		Y_o	a
Mixed	ACES	0.27	0.73	0.99	−0.27	1.37
	ACESadj	0.17	0.96	0.99	−0.17	1.04
	SRC-1	0.08	0.92	0.94	−0.08	1.08
	Li-6400-09	0.09	0.96	0.99	−0.09	1.04
Sand	ACES	−0.09	0.80	0.99	0.09	1.24
	ACESadj	−0.18	1.02	0.99	0.18	0.98
	SRC-1	0.05	0.68	0.94	−0.05	1.46
	Li-6400-09	0.05	0.83	0.98	−0.05	1.21
Pebble	ACES	−0.23	0.81	0.99	0.23	1.24
	ACESadj	−0.31	1.02	0.99	0.31	0.98
	SRC-1	−0.20	1.92	0.93	0.20	0.52
	Li-6400-09	0.45	0.74	0.99	−0.45	1.34

The measured efflux can be brought into agreement with the generated efflux by employing the linear correction equation.

and expected data, linear correction equations can be employed to bring measured efflux into agreement with generated efflux for each artificial soil (Table 2).

Field experiment

To facilitate comparison between systems with different chamber sizes, mean CO_2 efflux measures ($\pm$standard deviation) collected with the three techniques are presented for each site (Figure 2). Based on $n = 15$ samples, there was no significant difference in mean flux rates between systems on any site. All three techniques gave similar results at the RTP site (Figure 2). The Li-6400-09 and ACESadj measures were very similar at the Duke Hardwood site, however measurements made with the SRC-1 configuration were substantially higher. Observations at SETRES are quite different than the Piedmont sites; the ACESadj values gave higher readings than the other two techniques.

Spatial heterogeneity in soil CO_2 efflux rates is primarily a site characteristic, but quantifying this variability for a particular measurement technique is necessary for selecting an appropriate sample design. Calculating the necessary sample size to achieve a specific level of confidence based on one period of field sampling provides some insight into the variability inherent in each technique. For each technique/site combination, mean soil CO_2 efflux values with coefficient of variation are presented in Table 3 and sample size estimates are presented in Table 4(a–d). The Duke Hardwood and Pine sites were measured with forest floor litter intact and these possessed the greatest variability. The SRC-1 measurements were more variable than the other systems on all of the sites.

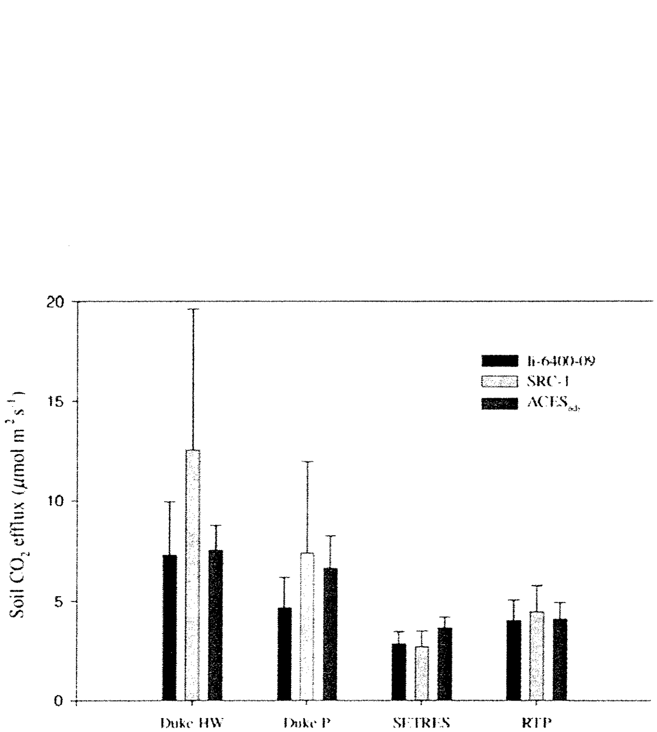

Figure 2. Mean soil CO_2 flux (n = 15, ±s.d.) measured at four field sites in central North Carolina.

Table 3. Mean soil respiration (±c.v.) measured in the field and transformed using the equation described by Conen and Smith (2000) to correct closed chambers.

	Li-6400-09	SRC-1	ACESadj
(A) Duke Forest Hardwood			
Measured soil respiration (c.v.)[a]	7.30 (36%)	12.54 (56%)	8.84 (24%)
Conen and Smith (2000)[b]	8.42	14.06	na
(B) Duke Forest Pine			
Measured soil respiration	4.66 (33%)	7.42 (61%)	4.30 (38%)
Conen and Smith (2000)	5.28	8.21	na
(C) SETRES[c]			
Measured soil respiration	2.85 (21%)	2.72 (29%)	3.65 (15%)
Conen and Smith (2000)	3.53	3.23	na
(D) RTP[c]			
Measured soil respiration	4.04 (25%)	4.47 (29%)	4.10 (20%)
Conen and Smith (2000)	4.16	4.56	na

Results are based on a population of 15 measures at sites: (A) Duke Hardwood, (B) Duke Pine, (C) SETRES and (D) RTP in June 2001.
[a]μmol m^{-2} s^{-1} CO_2.
[b]Calculated with an assumed depth of 30 cm.
[c]Litter removed.

Integrating laboratory and field experiments

In the laboratory experiment, differences between ACESadj and the generated efflux were negligible (Figure 1). Applying linear laboratory calibrations from the three artificial media to uncorrected ACES field data yielded

Table 4. Number of soil respiration measurements required to achieve a specific level of precision (± 10% of the population mean) and four confidence intervals (80–99%) based on a population of 15 measures at four sites: (A) Duke Hardwood, (B) Duke Pine, (C) SETRES and (D) RTP in June 2001.

Interval about the mean	C.I.	Li-6400-09	SRC-1	ACESadj
(A) Duke Forest Hardwood site				
± 10	0.99	115	283	23
	0.95	60	148	12
	0.9	40	99	8
	0.8	24	58	5
(B) Duke Forest Pine site				
± 10	0.99	96	330	119
	0.95	50	172	62
	0.9	34	115	41
	0.8	20	68	24
(C) SETRES[a]				
± 10	0.99	41	72	19
	0.95	21	38	10
	0.9	14	25	7
	0.8	8	15	4
(D) RTP, NC[a]				
± 10	0.99	56	76	36
	0.95	29	40	19
	0.9	20	27	13
	0.8	12	16	7

[a]Litter removed.

similar results regardless of site (Table 3). The maximum difference across all three correction equations derived from laboratory findings were as follows when applied to the mean soil CO_2 efflux rate at each site: (A) Duke Hardwood 2%, (B) Duke Pine 6%, (C) SETRES 7%, and (D) RTP 6%. The ACES appears to be insensitive to soil–air volume induced errors. Since there are no independent means to determine actual CO_2 flux in the field, the ACESadj data were assumed to be the closest measure available for further consideration of the closed techniques, based on laboratory findings (Figure 1 and Table 2). Based on this assumption, the trend for the closed chambers to underestimate efflux as porosity increases as reported in the laboratory were also observed in the field experiment. The ACES gave the highest readings at SETRES, a sandy site, which corresponds with the data in Figure 1 (sand). Since the closed chambers are thought to be influenced by soil porosity, direct application of linear corrections derived on laboratory soils would not be expected to match field soils with different physical properties.

Conen and Smith (2000) introduced a correction equation that can be used to predict closed chamber underestimates based on the ratio of soil–air to chamber volume. However, there is some difficulty in applying this equation; it requires knowledge of the depth of soil, whose volume is directly affecting the closed chamber. For the laboratory apparatus, we used the depth of medium (20 cm), for the field experiment a depth of 30 cm was assumed. This is depth corresponds with the shallow rooting of pine found on many piedmont sites, but it is an arbitrary value being used for the sake of comparison and discussion. Values of soil–air volume (porosity) used in Conen and Smith's (2000) equation for each field site are listed in Table 1. The gas flux measured in the laboratory with the Li-6400-09 closely matched the predicted value on the sand and pebble substrates (Figure 3). A 10% underestimate was predicted on the mixed medium, but only 3% was observed. In the laboratory the SRC-1 underestimate matched the predicted value on the mixed medium, then deviated sharply from expected on the other media types. Using the aforementioned assumptions in the field, measures made with the Li-6400-09 closely matched the predicted value on 3 of 4 sites (Figure 3). The equation was less successful at predicting differences between observed and expected values with the SRC-1 (Figure 3).

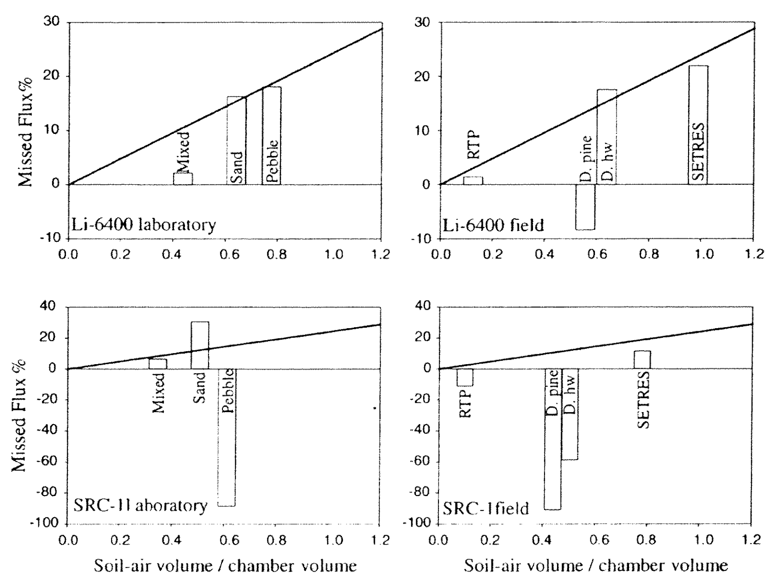

Figure 3. Comparison of flux underestimation (missed flux) by closed chamber techniques with the ratio of soil–air volume and measurement chamber volume. Missed flux is the % underestimation relative to the generated flux 1 − (observed/expected) × 100. Soil–air volume was directly measured in the laboratory apparatus for each soil medium. For the field results, air-filled porosity was measured at the time of respiration sampling and an assumed soil depth of 30 cm was used to calculate soil volume for comparison. In the field experiment there is no definitive value of missed flux. The solid line represents the relationship predicted by Conen and Smith (2000).

Discussion

Physical heterogeneity, tortuosity of the diffusion pathway, soil–air content, soil water status, pressure differentials and boundary layer resistance can all influence chamber-based soil CO_2 efflux measures (Kimball and Lemon 1971; Freijer and Bouton 1991; Rayment and Jarvis 1997; Fang and Moncrieff 1998; Le Dantec et al. 1999; Conen and Smith 2000; Rayment 2000; Welles et al. 2001; Butnor and Johnsen 2004). Use of artificial soil media in the efflux apparatus greatly reduced physical heterogeneity and permitted simpler comparison among the three systems. Porosities achieved with the artificial media (0.26–0.43) in this experiment were within the range commonly observed in the field (0.15–0.60), but are not as small as those found on poorly drained soils (Gliński and Stępniewski 1985). Porosity in the laboratory apparatus could have been further reduced by adding water; however our steady-state system requires extremely precise determination of gas diffusivity to calculate efflux. Unlike other gas flux generators, the air-filled footspace is held at a constant concentration and flux is calculated using Fick's law, hence it was undesirable to deal with variable hydration in the media profile.

In the laboratory, use of the least porous artificial soil medium (mixed) elicited small underestimates, while responses using the other two media exhibited larger errors dependent on measurement technique. The Li-6400-09 followed the underestimation prediction described by Conen and Smith (2000) closely (Figure 3). An earlier version of the Li-Cor closed chamber (Li-6000-09) was shown to measure 7% lower than a custom designed; pressure equilibrated open system in a black spruce forest (Norman et al. 1997). The SRC-1 followed this trend on the mixed and the sand media. Instead of underestimating on the most porous medium (pebble), the SRC-1 drastically overestimated efflux rates. It seems the SRC-1 was no longer following the soil–air volume hypothesis (Conen and Smith 2000; Rayment 2000) and the observed bias was caused by a different mechanism, possibly excessive turbulence from its internal fan. Despite seemingly small differences in air content 38% (sand) vs. 46% (pebble) the affect on CO_2 efflux values was marked. Measurement chambers need to be well mixed, however increasing wind speed within a chamber has been shown to elevate measured values (Hanson et al. 1993). Le Dantec et al. (1999) demonstrated that the turbulence generated by the internal fan was causing the SRC-1 to give higher values than the Li-6400-09. The authors found this on field soils under forest cover (1.49 adjustment factor) and in the laboratory on reconstructed forest soils (1.30 adjustment factor).

The uncorrected ACES consistently underestimated true flux rates. The ACESadj was relatively insensitive to differences in artificial soil media and displayed a high degree of accuracy with respect to the generated efflux under laboratory conditions. The standard calibration used for ACESadj was determined in 1998 using a simplified apparatus very similar to Butnor and Johnsen (2004), using aquarium gravel with an air content of 43%. The ACES correction used in ACESadj is essential to the operation of the system and has been utilized since its inception. While the ACES correction was empirically derived, its basis can be theoretically explained. The bulk of the underestimate is due to increased in headspace CO_2 causing a predictable negative feedback on gas flux (Rochette et al. 1997) and flow/pressure loss over the length of tubing causing flow meters to read a slightly higher at the system than is measured at the end of 50 ft of (1/16th in. inner diameter) tubing. Open systems function best if the gas analyzer is directly measuring concentration within the chamber headspace and Δ CO_2 is kept to a minimum. Increases in headspace CO_2 concentration can dampen the exchange between the soil–air by reducing the driving force between chamber and soil, thereby favoring diffusion through alternate pathways. The ACES has a maximum flow rate of 3 lpm, which enters the 5.5 l chamber via a diffuser ring eliminating the need for a fan. This flow rate and chamber design does not appear to elicit any turbulence-based error as seen with the SRC-1. The trade-off between allowing some accumulation of headspace CO_2, that is easily corrected, is preferable to spuriously high CO_2 flux measurements associated with turbulence. The low flow rates utilized with the ACES may better simulate low-turbulence environments like that found in a closed canopy forests, but are much less variable and lower than often found during early stand establishment or windy intervals.

The site comparisons were needed to test laboratory findings under more complex and realistic conditions. While relative differences that paralleled laboratory results were observed, there was no independent determination of absolute flux. Based on laboratory findings (Figure 1 and Table 2), the ACES appears insensitive to soil–air volume induced errors and the ACESadj data can be assumed to be the closest measure available for further consideration of the closed techniques. Sites where litter was removed *a priori* (SETRES and RTP) had the closest agreement with laboratory results. On the RTP site, which had the least porous soil, results from all three measurement systems techniques were similar.

The opposite was true on the coarse sandy soil at SETRES where, as predicted by Rayment (2000) and Conen and Smith (2000), the two closed systems both indicated lower soil CO_2 efflux rates relative to ACESadj. The SRC-1 did not display the overestimation response seen in the laboratory using the coarse artificial soil media, indicating the internal fan had not reached the threshold where it interfered with the soil–air volume underestimation. Soil CO_2 efflux measures on sites with litter left intact exhibited greater variability regardless of technique, the SRC-1 consistently exhibited the highest variability suggesting a systemic problem. At Duke Hardwood, ACESadj and the Li-6400-09 gave similar CO_2 efflux estimates, though the SRC-1 estimates were 73% greater than ACESadj. The litter at the Duke Hardwood site was particularly light and friable. In this case, the internal fan used in the SRC-1, as configured in this study, was probably dislodging CO_2 within the litter layer resulting in higher instantaneous measures of soil CO_2 efflux. Litter air-volume was not quantified in this study, depending on litter structure and degree of decomposition, the litter porosity would be expected to be substantially greater than soil porosity.

Evidence from the laboratory experiment demonstrated that data collected with the open-designed ACES is not dependent upon soil porosity or diffusivity to CO_2. From these results we infer the additional complexity of sampling through litter is not eliciting any major effects on ACES derived measures under low-turbulence conditions.

Despite differential responses, the relationship between soil CO_2 efflux measured with each system and the generated efflux was linear on each soil type. Thus, simple linear corrections can be applied to compensate for deviations from based on results using the artificial soils. The difficulty in utilizing these equations for calibrating field data lies in selecting the appropriate correction for the two closed systems where the complexities of litter structure may confound laboratory predictions. The equation for predicting closed chamber flux underestimates (Conen and Smith 2000), is a useful diagnostic tool. The Li-6400-09's close adherence to the model in both the laboratory and field experiments indicates it is a properly functioning closed system, whose errors are directly dependent upon its closed design and not unduly affected by other forms of chamber-induced bias. It shows that this system is operating in a predictable fashion, lending its self to corrections if necessary. The SRC-1 did not closely follow model predictions in either the laboratory or field, indicating that there are other sources of error than would be expected from the closed design alone. While the equation of Conen and Smith (2000) is useful for this type of inquiry, it would be difficult to apply to retroactively correct past datasets without additional information pertaining to soil–air volume, which is constantly changing with soil moisture. Rayment (2000) devised a means of determining the depth of soil whose volume is impacting closed measurement chambers, however it involves using an open-designed chamber, defeating the purpose of independently correcting closed chamber errors. Also, field-derived corrections to bring different techniques into agreement (e.g. Norman et al. 1997), have value for a particular site and field conditions, but they may not hold for other sites.

The field data were also useful for assessing variability within site/system combinations and for predicting sample sizes required for statistically detecting treatment variation (Table 4). The ACES was expected to exhibit lower variation due to relative chamber size (six times larger than the other chambers), making the contrast between the Li-6400-09 and the SRC-1 more instructive. On all sites, the SRC-1 displayed the most variability among sample estimates and so requires the highest sample sizes to achieve statistical power to detect treatment variation. On all but the Duke pine site, the ACES displayed the lowest variability and so required the lowest sample sizes where instantaneous measurements are made. It is important to note that as an automated multi-port system, the ACES was designed to capture both spatial and temporal variation (Butnor et al. 2003).

Our findings support the hypothesis that closed chambers underestimate soil CO_2 efflux. While specific chamber characteristics can modify this response (i.e. SRC-1 reading high on Duke Hardwood site and pebble media), the underlying concept that air-filled porosity alters the effective chamber volume yielding underestimates as noted by Conen and Smith (2000) and Rayment (2000) is supported. This being stated there are several practical and technical considerations that may seem to favor the use of closed systems over open systems. Closed chambers are less expensive and portable, they do not require the same level of flow control precision, nor do they require ballast tanks for reference air supply. Closed systems can make rapid measures; both the SRC-1 and the Li-6400-09 required less than 50% of the time required for the ACES to make a measurement. Gas concentration in closed chambers can be analyzed with portable gas chromatographs, allowing concurrent measures of trace gas evolution. If the use of a closed system is necessary, a well designed chamber that avoids common errors attributed to pressurization and excess turbulence may be further improved by increasing the chamber height (Conen and Smith 2000). Increasing the volume to soil surface area ratio will reduce the influence of soil porosity on measurement accuracy. Special consideration is still necessary on highly porous media. Our findings are even more relevant when litter conditions are considered. Litter porosity can be significantly higher than soil porosity; sites with deep forest floor litter or thick layers of dry organic matter would likely provide the most difficult situation to use a closed chamber. We predict that the closed systems will be more accurate on soils with low porosity.

The intention of this work was not to provide a 'report card' style grade for specific systems, but to highlight areas of concern and consideration in the measurement of soil CO_2 efflux. Our work clearly shows that soil CO_2 efflux measurement system × environment interactions do occur and can substantially impact estimates of soil CO_2 efflux. Until reliable calibration techniques are developed and applied, such interactions make direct comparison of published rates, and C budgets estimated using such rates, difficult.

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