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Research Letter

An Automated Laboratory Method for Measuring CO_2 Emissions from Soils

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Core Ideas

- A GC method to measure soil CO_2 emission was developed and compared with Solvita CO_2 burst method.
- A strong exponential relationship between Solvita and GC method was observed.
- CO_2 emissions from soils increased with drying temperature to a maximum at 65°C.
- The data presented highlight the narrow linear range of the Solvita method.
- The standardized procedure increased rate of analysis and reduced costs.

Abstract: A gas chromatography (GC) headspace method was developed for soil microbial respiration (CO_2 emission) analysis after a 24-h incubation of a rewetted soil. The GC method was compared with the Solvita CO_2 burst method by analyzing 18 different soils that were air-dried (22°C) or dried at 45, 65, and 105°C for 24 h before rewetting. A strong exponential relationship between the Solvita color number and GC headspace CO_2–C emission was observed. Compared with air-dried soils, drying at 65°C led to increased CO_2 emission and reduced variation among sample replicates, while drying at 105°C led to a reduction in CO_2 emission and an increase in variability. The GC method does not require sample dilution, provides data that is highly correlated to the Solvita method, and has a wider dynamic test range than the Solvita method. The developed GC method could be adapted to automation for commercial laboratory use.

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Soil microbial respiration (CO_2 emission from soil) is an important biological indicator for soil health assessment (Doran and Parkin, 1994). Respiration reflects the metabolic activity rather than abundance and diversity of the soil microbial community. Many studies have reported that soil CO_2 emission can be used as a reliable assessment of microbial activity and nutrient cycling in soils (Marumoto et al., 1982; Franzluebbers et al., 1996; Haney et al., 2004, 2008a). As such, assessments of CO_2 emission are included in recent development of soil health assessment tools such as the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2017) and the Haney soil health test (Haney, 2014).

The CASH (Moebius-Clune et al., 2017) involves assessing CO_2 emitted after rewetting of dried soils and a 4-d incubation period. This lengthy incubation period is not appealing to commercial laboratories that desire rapid sample turnaround to be competitive. The Haney soil health test (Haney, 2014) is based on the Solvita CO_2 burst test (Solvita & Woods End Laboratories), which has been adopted by some commercial laboratories. This analysis uses a 24-h incubation and a color indicator paddle instead of alkaline traps to measure CO_2 emitted from the soil. The paddles are less cumbersome to use but are costly compared with the alkaline traps. Also, it has been reported that laboratories have difficulty achieving reproducible results due to lack of standardization of the methods (Sullivan and Granatstein, 2015; Wade et al., 2018).

Direct gas chromatography (GC) headspace analysis has been used for quantifying CO_2 evolution from soils (De Jong and Schappert, 1972; Ljungholm et al., 1980; Patten et al., 1980). Past efforts, however, were performed utilizing manual injections that are not suited for automation due to the labor required for manual injection. The testing condition and results in the GC method also need to be systematically evaluated and compared with other adopted methods for method standardization and meaningful data interpretation. Therefore,

Abbreviations: GC, gas chromatography.

this study was conducted with the main goal of developing a method to assess soil microbial respiration through direct GC analysis of headspace CO_2 concentration. The use of an autosampler allows the potential for automation in commercial laboratories. We compared the developed method with the Solvita method, which has recently been adopted by some laboratories and is used to calculate the Haney Soil Health Score (Haney, 2014). In addition, we assessed the effect of soil drying temperature on CO_2 emission because most commercial laboratories use heated drying of soil samples at various temperatures to reduce drying time.

Materials and Methods

Soil samples (18) were collected from nine different sites across Oklahoma, USA, which included cultivated and noncultivated soils with diverse properties (Table 1). Soil samples were collected up to 10 cm depth with a shovel. The samples were sealed in a plastic bag and placed on ice following sampling and during transportation to preserve microbial activity and biological properties. Samples were stored in sealed bags in the dark at 4°C before use. From each bulk sample, 800 g of soil was removed and air-dried before being ground to pass through a 2-mm sieve. The sieved soil was then split into 4 × 200-g subsamples, with one subsample stored at room temperature (22°C) and the remaining further dried at 45, 65, or 105°C for 24 h. Emission of CO_2 in these soils were determined using the Solvita CO_2 burst test and the GC direct headspace method. All sample processing and analyses were conducted in triplicate.

Samples for the Solvita CO_2 burst test were prepared as outlined by Woods End Laboratories (2013). Forty grams of dried soil was taken in a 50-mL Solvita beaker, which was perforated at the bottom. The beaker was then placed in a 200-mL jar before being rewetted. Initially, the procedure outlined in the soil CO_2 emission test official Solvita guideline manual (Woods End Laboratories, 2013) was followed. In this procedure, 20 mL of deionized water was dispensed to the bottom of the glass jars supplied and allowed to move into 50-mL plastic beakers through perforations in the bottom of the beaker and wet the soil sample via capillary action. However, some soils became saturated and excessively wet while others remained relatively dry. Therefore, in an effort to modify the method, 10 mL of water was applied directly to the soil for all the Solvita analyses presented in the comparison to the GC direct headspace method. After 24-h incubation at 22°C, the paddles were removed from the jars one at a time and analyzed using the Digital color reader version 700.2. Both paddle color and the CO_2 emission were recorded from the paddle reader. The CO_2–C emissions given by the reader in CO_2–Low mode and Alt mode are reported.

Each sample was also analyzed using a Varian 450-Gas Chromatogram with a thermoconductivity detector to measure CO_2 in the headspace after 24-h incubation. Briefly, 5 g of soil was placed in a 20-mL glass vial and rewetted with 1.25 mL of deionized water before being sealed with a gray butyl septa and metal collar. The same moisture ratio (4:1 soil/water by weight) was used for the GC method as was used for the Solvita. Once sealed, the samples were incubated at room temperature (22°C) for 24 h. Water was added to eight vials per hour during an 8-h period, and the GC analysis was initiated the following day at 24 h after the first sample was wetted. This was done because the GC method used for this analysis required 7.5 min per sample; therefore, only eight samples were run in 1 h. This allowed only 24-h incubation for the subsequent samples.

The GC was configured with a 1041 on-column injector set at 130°C, as well as sample valve, which reduced the sample volume to 250 μL before introduction to a HayeSept

Table 1. Location, texture, tillage management, moisture, and chemical properties of soil samples. Chemical properties included N, P, K, pH, and organic C (OC).

Sample ID	Location	Tillage†	pH	N	P	K	Gravimetric moisture	OC	Texture class
				ppm			%	%	
1	Chickasha	NT	7.5	10	119.5	329	12.3	3.3	Silt loam
2	Chickasha	CT	5.2	19.5	75.5	155.5	7.5	1.04	Loam
3	Altus	NT	7.8	2	104	842	12.2	2.6	Clay loam
4	Altus	CT	8	36.5	32.5	382.5	8.5	0.7	Clay loam
5	Tipton	NT	7.5	9	73.5	559.5	9.7	2.1	Loam
6	Tipton	CT	8.3	6.5	26.5	388.5	11.8	0.6	Loam
7	Idabel	NT	5.5	1.5	18	85	14.2	1.71	Sandy loam
8	Idabel	CT	7.8	3.5	38.5	106.5	16.5	0.9	Silt loam
9	Bessie	NT	8.1	0.5	11.5	243.5	5.9	2.1	Silt loam
10	Bessie	CT	7.7	1	12.5	441	11.4	1.1	Clay loam
11	Goodwell	NT	8.3	0.5	24.5	623	11.3	0.51	Clay loam
12	Goodwell	CT	8.3	16	51.5	765.5	16.9	0.6	Clay loam
13	Stillwater	NT	5.6	21	20.5	233	29.7	4.29	Loam
14	Stillwater	CT	6.5	14	94.5	240	10.5	1.69	Loam
15	Perkins	NT	6.1	8.5	35.5	310.5	16.9	1.78	Loam
16	Perkins	CT	6.2	11.5	25.5	165.5	7.7	0.82	Sandy loam
17	Lahoma	NT	6.8	7.5	37.5	565.5	23.2	2.43	Silt loam
18	Lahoma	CT	6.2	20	33.5	334.5	9	1	Loam

† CT, conventional till; NT, no-till.

Q chromatography column in an oven set at 50°C before entering a thermoconductivity detector with a heat zone set at 120°C, filament temperature of 150°C, and current of 137 mA. The system utilized a carrier gas of helium at a pressure of 126 KPa with a flow rate of 30 mL min^{-1} through the column. Peak detection was conducted at a retention time of 3.67 min with a peak width of 0.3 and threshold of 10.

Before sample analysis, the GC was calibrated by flushing a sealed, empty vial with standardized gas to ambient pressure by inserting a needle into the septa to allow for the escape of gas. Then, a double-ended needle was inserted into the vial and a septum inserted into the outlet of a regulator with the outlet pressure set at 6.9 kPa. The standard vials were flushed for 60 s before being removed from the double needles on the regulator. The flush needle was then removed promptly. Standard gas cylinders containing certified concentrations of CO_2 at 984, 20,420, and 40,270 μL L^{-1} (AirGas USA, LLC) were used. All standardization vials were kept at atmospheric pressure to represent the incubation condition. Therefore, when head space air (5 mL) was extracted with the Combipal (Sigma-Aldrich) auto sampler, the same negative pressure would be exerted. To minimize the effects of ambient air CO_2 during sample preparation, an ambient air check was taken with each set of samples and analyzed by the GC at the end of each sample set.

The mass of CO_2–C (m) in the headspace of vials was calculated using the following equation:

$$m = PVM/RT$$

where P is the atmospheric pressure of 0.97 atm at 300 m asl, V is the volume of CO_2 in the headspace, which is calculated by multiplying the measured concentration in ppmv by the headspace volume (headspace volume of 0.015 L was used for the soil samples and a headspace volume of 0.020 L was used for the empty vials), M is the molar mass of C (12.01 g mol^{-1}), R is the gas constant (0.08206 L atm mol^{-1} K^{-1}), and T is the temperature in Kelvin (295 K).

The mass of CO_2–C emitted from the soil was calculated based on the difference between the headspace above the soil and the headspace in the ambient vial. The CO_2–C emitted was then divided by the mass of soil to calculate the CO_2–C emitted in a 24-h period per unit mass of dry soil.

For additional analysis (Table 1), soil samples were also submitted to the Soil, Water and Forage Analytical Laboratory at Oklahoma State University. In this analysis, pH was measured using a glass pH electrode in a solid/solution ratio of 1:1 (Sikora, 2006), nitrate extracted with a 0.008 M calcium phosphate extracting solution and quantified by the cadmium-reduction method (Lachat, 1994), P and K content measured using Mehlich-III solution (Mehlich, 1984), before being analyzed using the inductively coupled argon plasma analyzer (ICP–AES; Spectro Ciros), and organic matter content measured using a TrueSpec CN analyzer (LECO, Inc.). Samples with a pH >7.2 were analyzed for inorganic C content using the Pressure Calcimeter method proposed by Sherrod et al. (2002). The calculated inorganic C was then subtracted from the calculated total C to determine actual organic C content for these soils. Samples were also analyzed for soil moisture by drying a 20-g sample overnight at 105°C and active C using the $KMnO_4$ extraction method outlined by Weil et al. (2003).

Analysis of variance (ANOVA) comparing CO_2–C emission values from the Solvita and GC methods and for different drying temperatures was conducted using PROC MIXED in SAS (SAS Institute, 2008). All indicators analyzed were considered to be significant at the $P < 0.05$ level to account for variability. The coefficient of variation (CV) was calculated for triplicate analyses of each sample at each drying temperature using the two different methods. The average of these CVs was then determined to evaluate the analytical variability of the two methods across field samples and the drying times used.

Results and Discussion

Table 2 shows the average CO_2–C emitted as determined by the GC headspace method and the Solvita method with digital color reader set on the CO_2–Low mode. Soil CO_2–C emission increased with increasing drying temperature, with the highest found in soils dried at 65°C, and was significantly lower in soils dried at 105°C (Table 2). Also, the 45 and 65°C drying temperatures resulted in the lowest average CV calculated from analytical replicates. The average CV within the analytical replicates with the Solvita and GC methods after the 65°C treatment were 4 and 5%, respectively. The average CV for samples dried at 105°C was 36 and 27% for the Solvita and GC methods. Haney et al. (2004) reported that CO_2 emissions after 40 and 60°C dried samples were highly correlated to that from moist samples but that drying at 100°C produced unreliable results. In fact, the variation was so high that these samples were removed from the subsequent regression analyses. These data suggest that 65°C will be optimum for use in commercial laboratories as it optimized drying time without providing CO_2–C emission values proportional to air-dried sample with reduced variability. Table 2 also shows that the Solvita method using the CO_2–Low setting on the digital color reader generates values that are as much as six times greater than those measured with the GC method.

The relationship between the CO_2–C emission measured by the GC headspace method and the Solvita color numbers for samples dried at 22, 45, and 65°C was evaluated (Fig. 1). It is clear that the Solvita color number becomes saturated at a value around 5.45. In fact, Woods End Laboratories (2016) states that the greatest accuracy for the method is below a Solvita color number of 4, and Solvita (2017) states that

Table 2. The average soil microbial respiration as CO_2–C evolved from both the Solvita CO_2 burst test and gas chromatography (GC) analysis of samples dried at 22, 45, 65, and 105°C.

Temperature	Solvita burst	GC	Solvita burst	GC
	mg CO_2–C kg^{-1}		% CV	
22°C	89.3b†	13.8b	12	15
45°C	116.7a	24.3a	6	7
65°C	140.2a	35.9a	4	5
105°C	30.6c	7.1b	36	27

† Different lowercase letters next to each number indicate significant differences within a column.

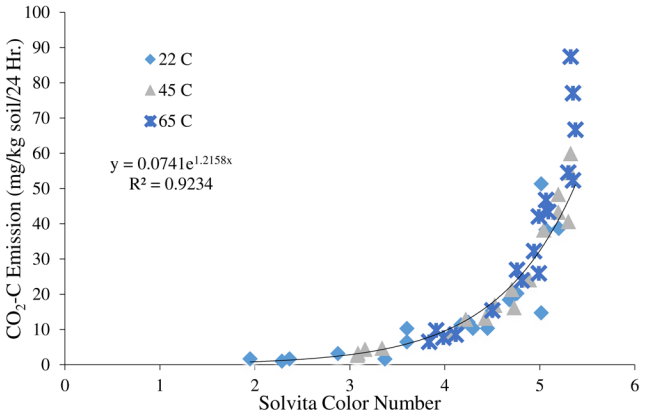

Fig. 1. The relationship between the CO_2–C emission measured by the gas chromatography headspace method and the Solvita numbers measured for samples dried at 22, 45, and 65°C.

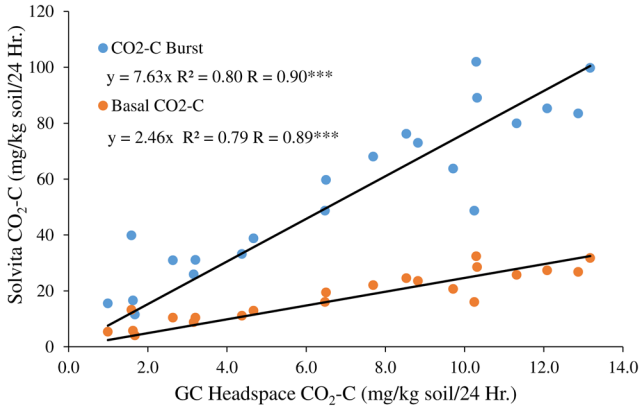

Fig. 2. The relationship between CO_2–C as determined from calibration curves for the CO_2–C Burst as described by Woods End Laboratories (2013) for Solvita color numbers <4.5 and the gas chromatography (GC) headspace method.

samples with Solvita color numbers >4.5 should be diluted before testing. Unfortunately, 31 of the 54 tested samples presented in Fig. 1 produced Solvita color numbers >4.5. With increasing drying temperature, the portion of samples above this threshold increased to 78% for samples dried at 65°C.

To compare the CO_2–C emission measured using the GC headspace method to that measured with the Solvita method, we used all samples with a Solvita number <4.5 except for those samples heated at 105°C. The digital color reader is precalibrated with values reported as ppm CO_2–C burst (CO_2–Low mode) and as basal CO_2–C (Alt mode) emission (mg kg^{-1} d^{-1}) as described by Woods End Laboratories (2013). The CO_2–C burst method produced values that are 7.6 times greater than the GC method, and the basal CO_2–C method produced results that are 2.4 times greater as determined by the slopes presented in Fig. 2.

Haney et al. (2008b) pointed out that the Solvita gel system absorbs an amount of CO_2 that is proportional to the amount emitted from the soil. In fact, it is proportional to the concentration in the headspace, which would be influenced by the headspace volume as well as the rate of CO_2 emission. According to Wade et al. (2018), the sample preparation methods, including sieving, soil moisture, incubation, rewetting, and so on, could also be the potential sources of variation during repetition of mineralizable C measurements. This suggests that the Solvita color number and its relationship with the CO_2–C emission as measured by a reference method could be influenced by the experimental conditions used to calibrate the system such as soil drying temperature, incubation temperature, and duration of incubation. This, for example, likely explains why the CO_2–C burst values resulting from the Solvita system are three times larger than values reported as basal CO_2–C emission per day. Furthermore, a proficiency analysis conducted by Woods End Laboratories (Brinton, 2016) suggested that the soil wetting procedure influenced the CO_2–C emission. Specifically, this analysis indicated that using a procedure by which soils were wetted to 50% water filled pore space had CO_2–C emissions as much as three times of those wetted by the standard capillary wetting method initially used. In a recent study, Wade et al. (2018) reported similar results, where soils wetted to 50% water holding capacity from the top resulted in nearly more than double CO_2–C emissions than from soils wetted from the bottom through capillary action. Wade et al. (2018) concluded that a possible reason for this behavior of mineralization is that wetting from the bottom resulted in slower and uneven distribution of water among the pores, whereas wetting from top resulted in wetting of all the pores followed by draining in relatively shorter time. On the other hand, recent studies on C mineralization conducted by Sherrod et al. (2012) and Haney et al. (2008b) reported stronger correlation between infrared gas analysis, GC, and alkali methods (R^2 > 0.90) than Solvita gel paddles with infrared gas analysis (R^2 = 0.79) (Haney et al., 2008b) and alkali traps (R^2 = 0.84) (Haney et al., 2008a). Therefore, it is challenging to identify reasons that led to the observed discrepancy between the Solvita CO_2–C 24-h burst values and the GC headspace CO_2–C values presented in Fig. 2 without more information on the conditions under which the color paddles were calibrated. Nevertheless, data obtained from the two methods are highly correlated (Fig. 1 and 2).

Summary

Emission of CO_2 after rewetting of a soil was highest at soil drying temperatures of 65°C and declined at drying temperature of 105°C. Furthermore, heated drying of the soils led to lower variation among analytical replicates compared with the air-dried soils. In fact, this study supports the use of 65°C drying temperature to optimize drying times while providing values that are proportional to air dried samples but with reduced variation.

These data highlight the limitations of the narrow linear range of the Solvita test. Approximately 78% of the samples after heating at 65°C were above the reported linear threshold for the Solvita method. The CO_2–C emissions measured by the GC headspace method were highly correlated with Solvita color number following an exponential function. Furthermore, within the analytical range reported for the Solvita method, the CO_2–C emission measured by the GC headspace method was linearly related to the CO_2–C emission values from the precalibrated digital color reader.

However, the CO_2–C burst and basal CO_2–C were 7.6 and 2.5 times the values measured with the GC method, respectively.

The GC headspace method along with the standardized procedure outlined above provides an approach to reduce costs and increase rate of analysis for commercial and research laboratories. This can be achieved through automation as well as reduction in the number of samples requiring dilution after heated drying. Currently, CO_2 burst simply serves a relative metric with no calibration to crop productivity or environmental outcome. However, the high throughput method developed will expedite our effort to better understand the impact of management and soil properties on CO_2–C burst and facilitate decision making in management and/or conservation compliance based on this soil property. However, the initial cost of purchasing a GC system should be considered against the number of samples to be analyzed; while the initial cost is high, its per sample cost can be low if depreciated over tens of thousands of samples.

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