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Comparison of two alkali trap methods for measuring the flush of CO_2

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Abstract

Soil biological activity is a key feature of healthy soil. The flush of CO_2 during the first few days after rewetting of a dried soil is a rapid indicator of soil biological health, but variations in approach require testing and calibration. A 3-d incubation method (25°C, 50% water-filled pore space, acid titration) was compared with a 4-d incubation method (~20°C, capillary wetted, electrical conductivity) from two long-term field experiments in Missouri (silt loam soils) and North Carolina (sandy loam and loamy sand soils). The two methods were related ($p < .001$) to each other ($r^2 = .93$, $n = 211$ for Missouri soils and $r^2 = .68$, $n = 126$ for North Carolina soils), but results differed in absolute value in an unexpected manner. Differences in incubation time (3 vs. 4 d), temperature (~20 vs. 25°C), and water delivery (50% water-filled pore space vs. capillary wetting) were major factors affecting relationships between methods. Time and temperature were predictable and scalable factors, but water delivery approach likely caused random variations specific to soil type. Both methods were able to discern depth stratification of soil biological activity, but subtle differences due to landscape position and soil texture were detected only with the 3-d method. We suggest that greater standardization of soil biological activity protocols based on key factors of soil moisture, temperature, and time of incubation be adopted to improve reliability and value to stakeholders.

1 | INTRODUCTION

Soil biological activity as determined by the flush of CO_2 is a key component of soil health assessments, as it indicates soil nutrient cycling, relates to soil structural changes, informs the potential for soil biodiversity development, and parallels changes in soil organic C and N sequestration (Doran, Coleman, Bezdicek, & Stewart, 1994; Franzluebbers, Pershing, Crozier, Osmond, & Schroeder-Moreno, 2018a). How this important attribute is measured has been a continuing evolution over the past century, with rapid developments during the past couple of decades. Waksman and Starkey (1924) recommended a method that determined CO_2 evolution during 14 d of incubation from 1 kg of fresh soil. In contrast, Lebedjantzev (1924) suggested that air-drying soil was an important step to increase the fertility of soil. Successive drying and rewetting soil led to repeated flushes of CO_2 that were considered of biological origin (Birch, 1958). The magnitude of N mineralized following rewetting of dried soil was shown to be a function of the amount of CO_2 evolved (Birch, 1960).

Abbreviations: CASH, Comprehensive Assessment of Soil Health; SEM, Soil Ecology and Management.

As an exploratory practice of evaluating soil microbial biomass and N mineralization potential in modern farming systems, Franzluebbers, Haney, Hons, and Zuberer (1996) determined the flush of CO_2 following rewetting of dried soil during 1 d of incubation. With more experience, the flush of CO_2 following rewetting of dried soil was proposed and standardized with conditions of 3-d incubation, 50% water-filled pore space, and 25°C (Franzluebbers, Haney, Honeycutt, Schomberg, & Hons, 2000). Using this approach, the flush of CO_2 as a short-term estimate of soil biological activity, was shown to be sensitive to derivation of soils from diverse climatic conditions (Franzluebbers et al., 2001), long-term tillage management (Franzluebbers, Schomberg, & Endale, 2007), crop rotation (Franzluebbers & Stuedemann, 2008), soil N nutritional status (Franzluebbers & Pershing, 2018), history of organic amendment (Jangid et al., 2008), long-term land use (Jangid et al., 2010), pasture management (Franzluebbers & Stuedemann, 2003), soil aggregate disruption (Franzluebbers & Arshad, 1997), and soil depth (Franzluebbers & Stuedemann, 2015). A quick, colorimetric determination of CO_2 evolution was adopted with use of a gel paddle (Haney, Brinton, & Evans, 2008) and further simplicity developed with rewetting by capillarity through perforations on the bottom of incubation vessels (Haney & Haney, 2010). These rapid determination procedures piqued the interest of many researchers and stakeholders in the soil health community (Mitchell et al., 2017).

Currently, a handful of variations of the flush of CO_2 following rewetting of dried soil are available to rapidly determine soil biological activity. Some are focused solely on commercial application and others on research, with some variations in between. The Cornell Comprehensive Assessment of Soil Health (CASH) is a commercial soil test that utilizes a version of soil respiration that has not been thoroughly tested or compared with other soil biological activity approaches. We wanted to compare the relative relationship between the CASH respiration method and a standard research method used in the Soil Ecology and Management (SEM) lab (Franzluebbers, 2018a; Franzluebbers et al., 2000), which shares similarity in multiple-day incubation with alkali trap to detect CO_2 evolution, but has differences in other features. Other common CO_2 detection approaches include a commercially available kit with gel paddle (Brinton & Vallotton, 2019; Haney et al., 2008), infrared gas analyzer (Sherrod, Reeder, Hunter, & Ahuja, 2012), and gas chromatograph (McGowen, Sharma, Deng, Zhang, & Warren, 2018).

Core Ideas

- Deviations in standard operating protocol cause undesired variations in the flush of CO_2.
- Temperature and soil water content must be carefully controlled in respiration analyses.
- A standard approach to measuring the flush of CO_2 is available and should be deployed.

2 | MATERIALS AND METHODS

Two long-term field experiments were sampled in this evaluation. In April 2016, soil from the Goodwater Creek Experimental Watershed in the Central Mississippi River Basin of Missouri (39° 14′ N, 92° 7′ W) was sampled at depths of 0–5 and 5–15 cm from four cores (3.1-cm diameter) pooled within 1 m of historical geo-referenced points. Management systems were initiated in 1991 with a few changes for some treatments during the course of the study (Veum et al., 2015). Twelve treatments replicated three times were sampled at three different landscape positions (summit, backslope, and toeslope; Table 1). Soils were classified as Adco silt loam (fine, smectitic, mesic Vertic Albaqualfs) in summit position and Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) in backslope and toeslope positions. A total of 216 samples were collected and analyzed for this study. Samples were homogenized in field-moist condition by passing through a screen with 10-mm openings, air-dried, and then sieved to pass 2-mm openings prior to analyses.

In February 2018, soil from the Farming Systems Research Unit at the Center for Environmental Farming Systems in the Eastern Coastal Plain region of North Carolina (35° 22′ N, 78° 2′ W) was sampled at depths of 0–6, 6–12, and 12–20 cm from five cores (4-cm diameter) pooled from historical geo-referenced points within a plot. Soils in this floodplain of the Neuse River were mapped as Johns sandy loam (fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Aquic Hapludults), Kalmia loamy sand (fine-loamy over sandy or sandy-skeletal, siliceous, semiactive, thermic Typic Hapludults), Lumbee sandy loam (fine-loamy over sandy or sandy-skeletal, siliceous, subactive, thermic Typic Endoaquults), Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults), and Wickham loamy sand (fine-loamy, mixed, semiactive, thermic Typic Hapludults). Fourteen treatments were replicated three times (Table 1). Treatments were classified into five primary groups, including (i) conventional grain cropping, (ii) organic grain cropping, (iii) integrated crop-livestock system, (iv) plantation forestry, and (v) old-field succession. A total of 126 samples were collected and analyzed for this study. Samples were dried in an oven at 55°C for 3 d and homogenized by gently crushing with a pestle over a screen with 4.75-mm openings and thorough mixing. Stones and organic material not passing the sieve were discarded (<1% of soil). Sand concentration was 649 ± 146 g kg^{-1}.

The flush of CO_2 was determined by alkali absorption using (i) a 3-d incubation and acid titration to a

TABLE 1 Treatments sampled at the Goodwater Creek Experimental Watershed in the Central Mississippi River Basin of Missouri and at the Farming Systems Research Unit of the Center for Environmental Farming Systems in Goldsboro, NC

Missouri treatments	North Carolina treatments
1. Mulch-till cropping system a. Corn (*Zea mays*)–soybean (*Glycine max*) rotation 2. No-till and mulch-till cropping systems a. No-till corn–mulch-till soybean rotation b. No-till corn–mulch-till soybean rotation with targeted switchgrass (*Panicum virgatum*) buffers 3. No-till cropping systems a. Corn–soybean rotation b. Corn–soybean rotation with targeted switchgrass buffers c. Corn–soybean–wheat (*Triticum aestivum*) rotation with mixed cover crops (all three rotation phases present each year) 4. Perennial grass systems a. Cool-season conservation reserve: Creeping meadow foxtail (*Alopecurus arundinaceus*), smooth brome (*Bromus inermis*), orchardgrass (*Dactylis glomerata*), Canada wildrye (*Elymus Canadensis*), Virginia wildrye (*Elymus virginicus*), *Lespedeza* sp., bird's-foot trefoil (*Lotus corniculatus*), alfalfa (*Medicago sativa*), timothy (*Phleum pretense*), and triticale (x *Triticosecale*) b. Warm-season conservation reserve: big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), indiangrass (*Sorghastrum nutans*), composite dropseed (*Sporobulus compositus*), and eastern gamagrass (*Tripsacum dactyloides*) c. Cool-season and warm-season mixed hay d. Switchgrass hay	1. Conventional grain cropping a. 3-yr rotation: corn–wheat and soybean–sorghum (*Sorghum bicolor*)] with conventional tillage b. 3-yr rotation (corn–wheat and soybean–sorghum) with no tillage 2. Organic grain cropping a. 6-yr grain (corn–wheat and soybean–sorghum)– cool-season hay [tall fescue (*Lolium arundinaceum*) + red clover (*Trifolium pratense*)] rotation b. 3-yr rotation with green fallow [corn–wheat and soybean–sudangrass (*Sorghum x drumondii*)] c. 3-yr grain rotation [corn–wheat and soybean–sunflower (*Helianthus annuus*)] with conventional tillage d. 3-yr rotation (corn–wheat and soybean–sunflower) with minimum tillage 3. Integrated crop-livestock system a. 6-yr crop (corn–wheat and soybean–sorghum)– cool-season hay (tall fescue + red clover) rotation b. 12-yr crop (corn–wheat and soybean-sorghum-corn-wheat and soybean-sorghum)– warm-season pasture (big bluestem, eastern gamagrass, indiangrass, switchgrass) rotation (both rotation phases present simultaneously) 4. Plantation forestry a. Longleaf pine (*Pinus palustris*) b. Bald cypress (*Taxodium distichum*) c. Green ash (*Fraxinus pennsylvanica*) d. Black walnut (*Juglans nigra*) 5. Old-field (agriculture abandonment)

TABLE 2 Characteristics of two approaches to measuring the flush of CO_2 following rewetting of dried soil, as deployed at the NC State University Soil Ecology and Management laboratory (SEM Method) and the Comprehensive Assessment of Soil Health at Cornell University (CASH Method) as deployed by the USDA-ARS Soil Health Research Lab in Columbia, MO

Component	SEM method	CASH method	CASH modifications in ARS Columbia lab
Soil processing	Dried 55°C for ≥3 d, sieved < 4.75 mm	Air dried, sieved < 8 mm	Sieved field-moist < 10 mm, then air-dried and sieved again < 2 mm
Soil weight	Two 50-g subsamples in same incubation jar	20 g	20 g, duplicate analyses
Water delivery	50% water-filled pore space	Capillary from bottom up to 0.375 g water g^{-1} soil	No change
Incubation	3 d at 25 ± 0.5°C in 1-L jar	4 d at room temperature in 0.5-L jar	No change, but room temperature of 20°C
CO_2 detection	Acid titration of 1 M NaOH trap to phenolphthalein endpoint following rapid stirring with $BaCl_2$ addition	Electrical conductivity of 0.5 M KOH trap	No change

phenolphthalein endpoint, as routinely determined in the SEM lab (Franzluebbers & Stuedemann, 2008; Franzluebbers et al., 2018a) and (ii) a 4-d incubation and electrical conductivity change of the alkali solution, as determined typically for CASH, but as deployed in this study in the ARS Columbia lab (Moebius-Clune et al., 2016; Schindelbeck, Moebius-Clune, Moebius-Clune, Kurtz, & van Es, 2016). Several differences other than incubation time and CO_2 detection were inherent in these two approaches (Table 2). The two approaches were labeled the SEM Method and the CASH Method throughout

to avoid unfairly differentiating on several potentially influential factors.

For relative comparison to the flush of CO_2 on North Carolina soils, we also determined cumulative C mineralization during 24 d (continuation of SEM Method with alkali trap replaced at 3, 10, and 24 d), net N mineralization during 24 d of aerobic incubation (ammonium N and nitrate N determined on segmented-flow colorimeter from filtered extract [10 g soil:20 mL 2 M KCl] of dried [55°C] and sieved [2 mm] soil at 0 and 24 d of incubation), soil microbial biomass C (chloroform fumigation-incubation without subtraction of control), and total organic C concentration (dry combustion), as described in Franzluebbers et al. (2018). On Missouri soils, associations were made between the flush of CO_2 and soil organic C, total soil N, β-glucosidase (Eivazi & Tabatabai, 1988), permanganate oxidizable C (Weil, Islam, Stine, Gruver, & Samson-Liebig, 2003), and aggregate stability of the 1–2-mm size fraction.

Statistical evaluation of the two methods was made by (i) paired *t*-test across all observations, (ii) comparing slope, intercept, and coefficient of determination across all values within each of the two long-term experiments, (iii) correlation with other biochemically relevant soil properties, and (iv) statistical significance of fixed effects from *F*-values following standard analysis of variation of treatment structure in the two experiments. Alpha level was set at .01 to declare statistical significance. In the Missouri experiment, 3-factor analysis of variance for management, landscape position, and soil depth was conducted as a randomized complete block design (3 blocks) using SAS v. 9.4 (SAS Institute, Cary, NC). More details of the experimental design can be found in Veum et al. (2015). In the North Carolina experiment, 2-factor analysis of variance for management and soil depth was conducted as a randomized complete block design (3 blocks) using SAS. Blocks in the North Carolina experiment were delineated in the original design by soil textural differences.

3 | RESULTS AND DISCUSSION

The two long-term experiments provided a range of soil conditions within each study, but also a diversity of soil conditions across studies. Soils from Missouri were mostly silt loam and soils from North Carolina were sandy loam and loamy sand. Treatments in both studies contained a gradient of disturbance due to tillage, as well as diversity of plant inputs from farming systems (e.g., grain cropping and forage management variables in Missouri and grain cropping, forage management, timber production, and natural succession in North Carolina) (Table 1).

Across all samples ($n = 337$ with 5 missing from original total of 342), the flush of CO_2 was significantly lower with CASH Method than SEM Method (158 vs. 168 mg CO_2–C kg^{-1} soil, $p < .001$ from paired *t*-test). When the differences between CASH Method and SEM Method were rank sorted, the lower one-third of differences had flush of CO_2 of 253 ± 14 mg CO_2–C kg^{-1} soil (mean ± standard error), the middle one-third had flush of CO_2 of 144 ± 8 mg CO_2–C kg^{-1} soil, and the upper one-third had flush of CO_2 of 107 ± 9 mg CO_2–C kg^{-1} soil. Relative to the SEM Method, these results suggested that the CASH Method tended to overestimate at low flush of CO_2 (43 ± 8%) and underestimate at high flush of CO_2 (−21 ± 1%).

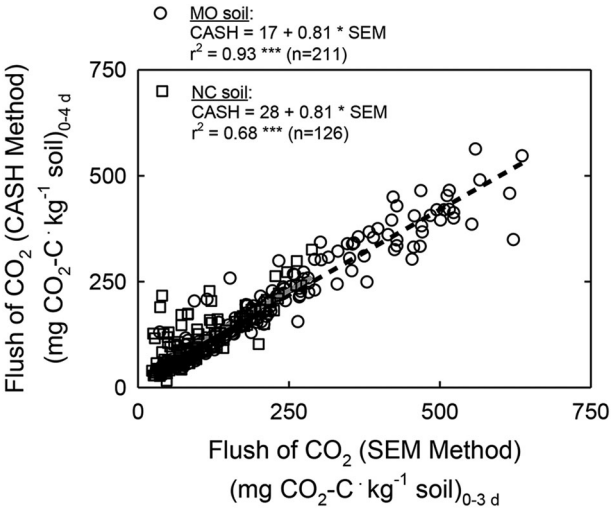

FIGURE 1 Association of the flush of CO_2 following rewetting of dried soil using two methods, Comprehensive Assessment of Soil Health Method (CASH) and Soil Ecology and Management Method (SEM), on two different long-term experiments in Missouri (MO) and North Carolina (NC). *** indicates significance at $P < .001$

Across all samples in Missouri, the association between SEM Method and CASH Method was very strongly linear within the range of 36–636 mg CO_2–C kg^{-1} soil (Figure 1). However, the intercept was significantly greater than zero (17 ± 4 mg kg^{-1}) and the slope was significantly less than one (0.81 ± 0.02 kg kg^{-1}). Across all samples in North Carolina, association between SEM Method and CASH Method occurred within a smaller range (22–288 mg CO_2–C kg^{-1} soil) and similar slope of regression, but the intercept was farther from zero (28 ± 6 mg kg^{-1}) than in the Missouri dataset.

Greater incubation time of the CASH Method (4 d) than the SEM Method (3 d) was expected to yield a larger value of C mineralization for the same soil sample. In fact, using the SEM Method along with further incubation to 24 d (i.e., cumulative C mineralization during 24 d) resulted in a predicted flush of CO_2 during 4 d that was 1.15–1.21 times greater than that at 3 d (Figure 2). This calculation contrasted dramatically with the observed slope value for the 4-d CASH Method of 0.81 times that of the 3-d SEM Method in both datasets. Therefore, other differences in approaches were likely counteracting this length of time difference.

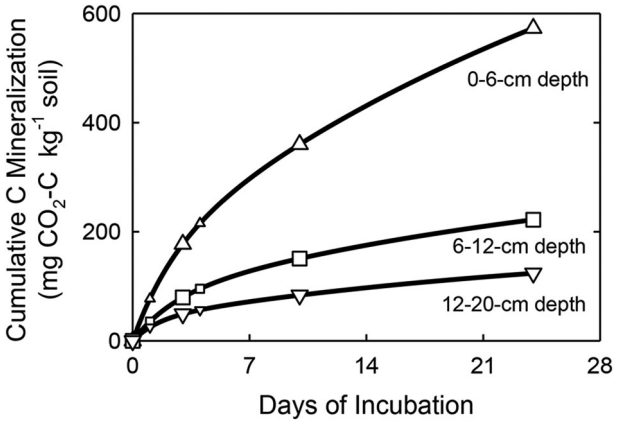

FIGURE 2 Cumulative C mineralization following rewetting of dried soil using the Soil Ecology and Management Method from measured values (3, 10, and 24 d) across depths (large symbols) in a long-term experiment in North Carolina. Nonlinear regressions were fitted to temporal means and small symbols indicate predictions at 1 and 4 d of incubation based on regressions. Values at 1 d were 77, 35, and 25 mg kg^{-1} at 0–6, 6–12, and 12–24 cm depths, respectively, at 3 d were 177, 79, and 49 mg kg^{-1}, respectively, and at 4 d were 215, 95, and 57 mg kg^{-1}, respectively

One key difference was the temperature of incubation. The SEM Method maintains temperature in a cabinet at 25 ± 0.5°C, while the CASH Method states incubation at room temperature. The ARS Columbia protocol considers room temperature as 20°C. Unfortunately, "room temperature" might vary from season to season within a location, as well as among different locations. Temperature is a major controlling factor in biological activity. This can be expressed as the Q_{10} factor, in which activity is generally expected to double with a 10°C increase in temperature (Ellert & Bettany, 1992). If incubation temperature is set at 25°C as a standard method and expected flush of CO_2 is 150 mg CO_2–C kg^{-1} soil 3 d^{-1}, but the temperature reaches only 22°C (intentionally as part of protocol or unintentionally as malfunction), then the flush of CO_2 would be expected to be 106 mg CO_2–C kg^{-1} soil 3 d^{-1} with $Q_{10} = 2$. This 3°C temperature difference would yield a value only 81% of expected, while temperature of 20°C would yield an estimate of 71% of expected. Therefore, the regression slope of 0.81 kg kg^{-1} between CASH Method and SEM Method in Figure 1 could be largely attributable to difference in incubation temperature. Temperature of incubation must be controlled within a lab, and standardized across labs, to avoid the need for unique calibrations and possible interactions with other factors.

Another factor that may have caused discrepancy between methods was water/air balance that could have influenced microbial activity. The CASH Method allows excess water (maximum of 0.375 g water g^{-1} soil) from capillary action at the base of the sample to rewet soil, while the SEM Method calculates water needed to achieve 50% water-filled pore space and is delivered with a pipette from the top. Greater water-filled pore space with capillary uptake from the bottom compared with controlled delivery from the top has been shown to reduce the flush of CO_2 by as much as 30%, but the effect appears to be dependent on soil texture (Franzluebbers & Haney, 2018). The difference in water-filled pore space between methods had an even greater negative effect on net N mineralization when incubated for 24 d (Franzluebbers & Haney, 2018). In California soils, capillary wetting of soil for determining the flush of CO_2 during 1 d of incubation led to only 45% of the flush of CO_2 compared with wetting to 50% water-holding capacity (Wade et al., 2018). Although capillary wetting simplifies the procedure, it has a negative and variable effect on the flush of CO_2. If the effect were consistent across soils, then the issue would not be so critical. Soil water conditions during incubation must be controlled within a lab, and preferably standardized across labs, to avoid the need for unique calibrations and possible interactions with other factors.

Other differences in methods were considered minor, but may have also contributed to inherent bias and/or random variation. How soil was dried and sieved may have altered the flush of CO_2. In a soil textural gradient in Georgia, reducing sieve opening along a gradient from 7.9 to 0.5 mm to homogenize dried soil generally resulted in greater flush of CO_2 during 3 d following rewetting (Franzluebbers, 1999). In that study, the flush of CO_2 was statistically similar when soil was sieved <7.9 mm and <4.75 mm, but ~50% greater when soil was sieved <0.5 mm. However, in an evaluation of a dozen soil types in North Carolina, Pennsylvania, and Virginia, the flush of CO_2 was not affected by sieving of dried soil to <4.75, 2, and 0.5 mm (Franzluebbers & Haney, 2018). The issue of sieve size can be important for reasons other than simply the magnitude of response. Larger aggregate size leads to better air-water relations during incubation and preservation of macro-aggregate-protected organic C (Elliott, 1986; Franzluebbers & Arshad, 1997), but potentially more random variation in soil properties when subsample weight is limited. The ARS Columbia modification of the CASH Method used duplicate 20-g portions of soil to overcome some inherent variability. The SEM Method used two 50-g portions of soil to overcome larger variability associated with 4.75-mm sieved rather than 2-mm sieved soil. Median coefficient of variation between duplicate 20-g portions of soil using the CASH Method was 3.1% for Missouri soils that had been sieved <2 mm, but was 11.4% for North Carolina soils that had been sieved <4.75 mm.

In soils from North Carolina, the flush of CO_2 by SEM Method always had greater strength of association with other soil biochemical properties than the flush of CO_2 by CASH Method (r^2 = .79 ± .07 and .52 ± .08, respectively). In soils from Missouri, strength of association with other soil properties was similar between SEM Method and CASH Method

(r^2 = .67 ± .19 and .69 ± .19, respectively). The looser relationship between CASH Method and other biochemical properties in North Carolina soils may have been a function of soil texture, in which soils were predominantly coarse-textured as compared with fine-textured soils in Missouri. Greater water content with capillary wetting (as used in the CASH Method) has been shown to adversely affect the flush of CO_2 in coarse-textured soil (Franzluebbers & Haney, 2018), and this texture-induced effect may have caused greater variation as well.

Mean flush of CO_2 values were equally discriminating of soil depth using the two methods in Missouri and North Carolina (Table 3). The flush of CO_2 using both methods showed strong stratification with depth, which is a common occurrence in conservation agricultural systems (Franzluebbers, 2002; Franzluebbers & Stuedemann, 2008; Franzluebbers et al., 2007).

The effectiveness of each method to detect known sources of edaphic and management variation within each long-term experiment was assessed (Table 4). In the Missouri experiment, depth was the largest effect, followed by management treatment and the interaction of depth × management. Both methods showed similar ability to distinguish these effects. A difference in detection between methods occurred for landscape position and its interactions with other variables. The SEM Method was sensitive to all of these landscape position effects, while the CASH Method did not exhibit significance for landscape position.

In the North Carolina experiment, the depth effect was again the largest source of variation with both flush of CO_2 methods. The SEM Method was more sensitive to replication and management effects than was the CASH Method, which was not significantly affected by these sources of variation. Therefore, as a broad indicator of soil biological activity, both methods appeared to be capable of detecting major management zone changes, but finer-scale ecosystem differences based on landscape position (e.g., in the Missouri experiment), soil type (e.g., replicate blocks in the North Carolina experiment that were delineated by soil textural differences), and management diversity (e.g., in the North Carolina experiment) were more detectable with the SEM Method than the CASH Method.

Taken together, the flush of CO_2 results of this study suggest relative agreement between the CASH and SEM methods on a broad level, but not at a finer level nor on an absolute basis necessary to utilize either method interchangeably when interpreting subtle effects of management on soil biological

TABLE 3 Analysis of soil depth effects in two long-term studies in Missouri and North Carolina as determined by two flush of CO_2 methods, Comprehensive Assessment of Soil Health (CASH) and Soil Ecology and Management (SEM)

Missouri soil			North Carolina soil		
Soil depth	SEM method	CASH method	Soil depth	SEM method	CASH method
cm	mg CO_2–C kg^{-1} soil		cm	mg CO_2–C kg^{-1} soil	
0–5	315	278	0–6	177	170
5–15	102	92	6–12	79	104
LSD[a] $_{(P = .01)}$	14	11	12–20	49	56
			LSD $_{(P = .01)}$	15	25

[a]LSD, least significant difference.

TABLE 4 Analysis of variance in two long-term studies in Missouri and North Carolina as determined by two flush of CO_2 methods, Comprehensive Assessment of Soil Health (CASH) and Soil Ecology and Management (SEM)

Missouri soil				North Carolina soil			
Source of variation	*df*	F-value SEM method	F-value CASH method	Source of variation	*df*	F-value SEM method	F-value CASH method
Rep	2	2.1	1.1	Rep	2	17.4***	2.0
Depth (D)	1	1651.8***	1820.0***	D	2	212.4***	60.7***
Management (M)	11	62.5***	67.4***	M	13	4.0***	1.3
D × M	11	29.9***	22.9***	D × M	26	1.4	0.6
Position (P)	2	5.0**	1.4				
D × P	2	3.3*	0.5				
M × P	22	3.8***	2.1**				
D × M × P	22	2.7***	0.9				

*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

activity. Numeric outputs of soil health indicators are important for making association with key soil forms and functions. Methods that differ in absolute value and association with key soil functions should only be considered in isolation and not be interchangeably integrated between research and commercial application, as this causes confusion and mistrust among all approaches, whether validated or not. As described in a review of short-term C mineralization, the flush of CO_2 using the SEM Method has had more than a dozen years of research results and use of consistent approach (Franzluebbers, 2018a). The SEM Method has consistently shown high relevance to a variety of other soil biochemical attributes determined in the laboratory, including cumulative C mineralization, basal soil respiration, microbial biomass C, net N mineralization, aggregation, and soil C sequestration (Franzluebbers, 2018a). More prominently, as an expression of field-relevant soil functioning, recent evaluations of soil-test biological activity (i.e., the flush of CO_2 during 3 d) with the SEM Method have shown great potential to estimate soil N availability in corn grain and/or silage production (Franzluebbers, 2018b) and fall-stockpiled tall fescue production (Franzluebbers, Pehim-Limbu, & Poore, 2018b). These field studies suggest there is an economic effect of soil-health building practices that can be measured and interpreted from a standard test of soil biological activity using the flush of CO_2.

Further method comparisons are warranted, but the use of unlimited capillary wetting has to be seriously questioned as to its value in commercial soil testing, since it has been repeatedly shown to hinder C and N mineralization in some soil types (Franzluebbers & Haney, 2018; Wade et al., 2018).

4 | CONCLUSIONS

The two methods of rapidly determining soil biological activity (i.e., as the flush of CO_2) were highly related with each other and either method was able to discern overwhelming differences in depth stratification in long-term experiments in Missouri and North Carolina. However, several key method details were entangled in confounding effects that led to differences in absolute value between methods, as well as sensitivity to edaphic and management factors. Primary among these methodological differences were length of incubation time, temperature, and water delivery (resulting in differences in water-filled porosity during incubation). Unlimited capillary wetting has to be seriously questioned as to its value in soil biological testing, since it has been repeatedly shown to hinder the flush of CO_2 in coarse-textured soils. Any method that alters numeric outputs from the same sample will lead to confusion and mistrust among researchers and commercial clientele. Therefore, we recommend that greater standardization of soil biological activity methodology be pursued and/or adopted to ensure that stakeholders interested in understanding effects of their management on soil health and ecological implications be presented with consistent and meaningful results and interpretations.

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