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Soil mass and volume affect soil-test biological activity estimates

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Abstract

Soil-test biological activity is a key indicator for soil health assessment. Methodological details may affect accuracy and precision of this indicator. Accuracy and precision of soil-test biological activity estimates were determined for 10 replications of 10 mass and volume treatments under standard laboratory conditions using an alkali trap technique with acid titration. Five soils varying in texture and organic C and N concentrations were used to assess a gradient of soil mass and volume conditions on C mineralization following rewetting of dried soil during 0–3, 3–10, and 10–24 d of incubation at 50% water-filled pore space and 25°C. Soil type explained ≥90% of variation in C mineralization, but soil mass and volume treatments also had significant effects on estimates ($p < .001$). In sets of treatments with the same surface area exposed, increasing soil volume led to lower C mineralization across soil types. Entrapment of CO_2 in soil pores may have been the main reason for lower C mineralization, since the effect diminished over time. Treatments with 50 to 100 g of soil were nearest the median estimates of C mineralization for each soil. Precision of C mineralization estimates was greatest when soil mass was ≥50 g of soil. A consistent surface area-to-volume of ∼0.2 cm^{-1} is suggested with sufficient soil mass to account for potentially enhanced random variation of C-enriched surface soils.

1 | INTRODUCTION

Soil biological activity is an important function of soils that contributes to organic matter processing, organic C sequestration, aggregation, habitat formation, and nutrient cycling (Burns et al., 2013; Nannipieri, Greco, & Ceccanti, 1990). Historically, soil biological activity has been estimated in the field with deployment of static or dynamic chambers measuring the rate of CO_2 evolved from the soil surface as respiration (Anderson, 1982). This approach has great value in understanding net heterotrophic activity under ambient conditions in the field (Chantigny et al., 2017; Raich & Schlesinger, 1992). However, chamber measurements in the field are complicated by the fact that autotrophic respiration of plant roots is not easily separated from total CO_2 evolution (Gagnon et al., 2016; Hanson, Edwards, Garten, & Andrews, 2000). Another approach to estimate soil biological activity is to assess potential microbial activity from C mineralization by collecting soil from the field and exposing it to standard and ideal laboratory conditions (Mikha, Rice, & Milliken, 2005; Zibilske, 1994). Laboratory incubation has historically been with field-moist soil adjusted to optimum water conditions (Alvarez & Alvarez, 2000). An alternative approach is to normalize initial soil conditions by thoroughly drying, coarsely sieving, and rewetting soil to a standard and optimized moisture level prior to incubation (Franzluebbers, Haney, Honeycutt, Schomberg, & Hons, 2000). Soil handling with this latter approach is the focus of this investigation, as it pertains specifically to

estimation of soil-test biological activity as a key indicator for soil health assessment (Franzluebbers, Pershing, Crozier, Osmond, & Schroeder-Moreno, 2018b).

During past decades, a growing scientific body of literature has emerged to estimate short-term C mineralization following rewetting of dried soil (Franzluebbers, 2018), albeit with a variety of protocol variations. Nomenclature has varied depending on investigators involved, e.g. Solvita soil CO_2 burst (Woods End Laboratories, Mt. Vernon, ME), Haney soil health tool (Haney, Haney, Smith, Harmel, & White, 2018), the flush of CO_2 (Franzluebbers et al., 2000), and soil-test biological activity (Franzluebbers et al., 2018b). Variations in incubation protocol can affect absolute values of C mineralization for the same soil, although estimates may be correlated across a range of soils (Franzluebbers & Haney, 2018; Franzluebbers & Veum, 2020). Some protocol variations include length of incubation (Fine, van Es, & Schindelbeck, 2017; Franzluebbers, 1999; Haney, Hons, Sanderson, & Franzluebbers, 2001; Sainju, Senwo, Nyakatawa, Tazisong, & Reddy, 2008), soil drying temperature (Haney, Franzluebbers, Porter, Hons, & Zuberer, 2004; McGowen, Sharma, Deng, Zhang, & Warren, 2018), soil sieve preconditioning (Franzluebbers, 1999; Wade et al., 2018), water addition method and quantity (Franzluebbers & Haney, 2018; Wade et al., 2018), and CO_2 detection method (Haney, Brinton, & Evans, 2008; Sherrod, Reeder, Hunter, & Ahuja, 2012). One factor that has not been investigated thoroughly is mass and/or volume of soil during incubation. The Soil Ecology and Management Laboratory at North Carolina State University uses two small bottles of 50 g each in the same large incubation vessel (Franzluebbers et al., 2018b). Franzluebbers, Schomberg, and Endale (2007) used two subsamples of a gradient of 20 to 65 g (sieved < 4.75 mm) to target a similar CO_2 output, the Haney Soil Health Test uses 40 g of soil (sieved < 2 mm) (Haney et al., 2018), the Comprehensive Assessment of Soil Health (CASH) at Cornell University uses duplicate 20-g samples (sieved < 8 mm) (Fine et al., 2017), and recent efforts have introduced further miniaturization of some biochemical analyses with only a few grams of soil (Moore, Guillard, Geng, Morris, & Brinton, 2019) and 5 g of soil for C mineralization (Morrow, Huggins, Carpenter-Boggs, & Reganold, 2016). Variations in soil mass and volume could affect C mineralization estimates (i.e. per kilogram of soil or C) by reducing rate estimates if CO_2 is trapped in a large volume of soil matrix or by increasing random variation of estimates if soil is not uniform in small aliquots.

Objectives of this study were to (1) compare mean estimates of C mineralization (mg C kg^{-1} soil and g C kg^{-1} total organic C) as affected by mass and volume of soil, (2) determine the extent of variation caused by laboratory operator in titrating alkali traps, and (3) estimate random variation among replicate samples as affected by mass and volume of soil. The hypothesis was that a minimum threshold of soil mass would be needed to achieve both accurate and precise estimates of soil-test biological activity when using an alkali trap approach to CO_2 detection. Especially when using coarsely sieved soil to estimate soil biological activity, it is assumed that greater soil mass would lead to less random variation among similarly treated samples. More precise estimates of soil-test biological activity are needed to document changes in soil health condition during various stages of soil management.

Core Ideas

- Soil incubations varying in mass and volume affect C mineralization estimates.
- Coefficients of variation in C mineralization are larger with smaller soil mass.
- Robust estimates of C mineralization should be determined with ≥50 g of soil.
- Soil-test biological activity should be standardized with constant mass or volume.

2 | MATERIALS AND METHODS

2.1 | Soils and treatments

Soil was collected from two locations in Georgia and three locations in North Carolina, reflecting a diversity of soil characteristics for this methodological evaluation (Table 1). Different soils and depth of sampling yielded varying total organic C and N concentrations, which were desired to create a range of expected C mineralization. After representative field sampling, soil was dried in a forced-air oven (55°C) for ≥3 d followed by gently crushing soil and passing through a screen with 4.75-mm openings. Soil homogenization was desired to obtain representative subsamples, but some structure of the soil was desired as well to reflect naturally distributed organic matter within and among stable aggregates.

Soil was thoroughly mixed in a bucket and aliquots of 5, 10, 20, 50, 100, 200, and 500 g were scooped into handling bottles. In addition, volumetric aliquots of 23 and 74 ml were prepared with mass varying by soil type (Table 2). A total of 10 replicates were processed for each mass and volume treatment, except for NC1 (no samples for treatments of 20 g, 74 ml, 100 g, 200 g, and 500 g) and NC2 and NC3 (no samples for treatments of 100, 200 and 500 g) due to limited soil availability. Physical conditions during incubation of each soil and the 10 mass and volume treatments are described in Table 2. Mass and volume treatments of 5 g, 10 g, 20 g, and 23 ml had the least exposed soil surface

TABLE 1 Characteristics of soils evaluated for potential C mineralization

Soil property	GA1	GA2	NC1	NC2	NC3
Location	Wilkes County GA	Oglethorpe County GA	Rowan County NC	Johnston County NC	Johnston County NC
Soil series[a]	Georgeville SiL	Appling cSL	Mecklenburg CL	Norfolk LS	Wedowee SL
Sampling depth (cm)	0-10	0-10	0-10	15-30	5-15
Management	Grazed tall fescue pasture (*Schedonorus arundinaceus*)	Grazed tall fescue pasture	No-tillage corn (*Zea mays*) with poultry litter application	Unharvested switchgrass (*Panicum virgatum*)	Unharvested switchgrass
Clay (g kg^{-1})	285	158	297	208	43
Sand (g kg^{-1})	530	735	402	702	895
Soil organic C (g kg^{-1})	23.6	15.2	42.7	5.6	4.3
Total soil N (g kg^{-1})	2.10	0.95	3.87	0.33	0.26

[a]CL, clay loam; cSL, coarse sandy loam; LS, loamy sand, SL, sandy loam; SiL, silt loam.

TABLE 2 Physical conditions during incubation of soil

		Soil mass and volume treatment									
Property	Soil[a]	5 g	10 g	20 g	23 mL	50 g	74 mL	100 g[b]	100 g	200 g	500 g
Surface area-to-volume ratio (cm^{-1})	GA1	0.91	0.50	0.27	0.22	0.22	0.14	0.22	0.63	0.32	0.14
	GA2	1.00	0.56	0.29	0.22	0.25	0.14	0.25	0.67	0.37	0.15
	NC1	0.83	0.46	ND	0.22	0.18	ND	0.18	ND	ND	ND
	NC2	1.11	0.59	0.33	0.22	0.27	0.14	0.27	ND	ND	ND
	NC3	1.43	0.71	0.37	0.22	0.29	0.14	0.29	ND	ND	ND
Soil mass (g)	GA1	5	10	20	26	50	86	100	100	200	500
	GA2	5	10	20	30	50	93	100	100	200	500
	NC1	5	10	ND	25	50	ND	100	ND	ND	ND
	NC2	5	10	20	32	50	100	100	ND	ND	ND
	NC3	5	10	20	36	50	112	100	ND	ND	ND
Gravimetric water content (g kg^{-1})	GA1	260	250	250	261	261	242	261	251	251	251
	GA2	200	200	205	201	211	207	211	204	204	204
	NC1	280	280	ND	278	308	ND	308	ND	ND	ND
	NC2	179	179	179	179	161	179	161	ND	ND	ND
	NC3	140	140	135	132	146	140	146	ND	ND	ND
Bulk density (Mg m^{-3})	GA1	ND	ND	ND	1.11	1.11	1.16	1.11	ND	ND	ND
	GA2	ND	ND	ND	1.28	1.25	1.27	1.25	ND	ND	ND
	NC1	ND	ND	ND	1.07	1.01	ND	1.01	ND	ND	ND
	NC2	ND	ND	ND	1.36	1.43	1.36	1.43	ND	ND	ND
	NC3	ND	ND	ND	1.56	1.50	1.52	1.50	ND	ND	ND

[a]GA1, Georgeville silt loam; GA2, Appling coarse sandy loam; NC1, Mecklenburg clay loam; NC2, Norfolk loamy sand; NC3, Wedowee sandy loam; ND, not determined
[b]Composed of two 50-g aliquots in separate bottles, but incubated together in same canning jar.

area of 5.3 cm^2 and mass treatments of 100 g, 200 g, and 500 g had the most exposed soil surface area of 45.4 cm^2. Intermediate levels of exposed soil had surface areas of 6.6 cm^2 for the 50-g treatment, 13.2 cm^2 for the 100-g* treatment (composed of two 50-g aliquots in separate bottles, but incubated together in same canning jar), and 14.5 cm^2 for 74-ml treatment.

2.2 | Soil analyses

Soil was wetted to ~50% water-filled pore space for each of the soil mass and volume treatments with a pipette delivering water to the top of each soil sample. Uniform water distribution throughout the sample was assumed due to strong capillary action in all soils. Gravimetric water content for each soil

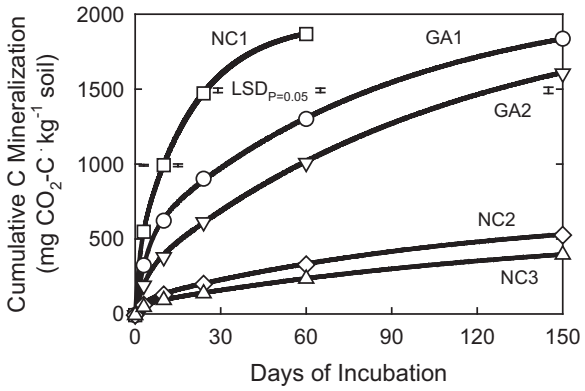

FIGURE 1 Cumulative C mineralization as affected by soil (GA1, Georgeville silt loam; GA2, Appling coarse sandy loam; NC1, Mecklenburg clay loam; NC2, Norfolk loamy sand; NC3, Wedowee sandy loam)

type was calculated at 50% water-filled pore space from the average of all 50-g subsamples (n = 30 for each soil type) and used to inform water additions for all other mass and volume treatments. Soil was incubated at 25°C in a sealed canning jar in the presence of an alkali trap. For small soil mass and volume treatments (i.e. 5-g, 10-g, 20-g, and 23-ml treatments in 13-mm diameter plastic bottles), 0.5-L canning jars were used and alkali traps were either 5 or 10 ml of 0.1 M NaOH. For medium soil mass and volume treatments (i.e. 50-g [one 25-mm diameter glass bottle], 74-ml [one 45-mm diameter glass bottle], and 100-g* [two 25-mm diameter glass bottles]), 1-L canning jars were used and alkali traps were 10 ml of 1 M NaOH. For large soil mass and volume treatments (i.e. 100-g, 200-g, and 500-g treatments placed directly in the bottom of canning jar), 1-L canning jars were used and alkali traps were 10 or 30 ml of 1 M NaOH (placed on top of soil; covering 14% of soil surface). Volume and concentration of alkali varied among treatments so that optimum titration could be performed within the vessel containing alkali. Unreacted alkali was 67 ± 25% across all samples (n = 1216). At the end of 3 d of incubation, alkali traps were removed and replaced with a fresh container for further incubation to 10 and 24 d sequentially. Additionally, soil mass and volume treatments of 74 ml and 100 g* were incubated further to 60 d and the 100-g* treatment incubated to 150 d following removal of one of the bottles of soil and alkali trap replacement at 60 d.

Ten replicates of each soil mass and volume treatment were titrated by two different operators (5 replicates each) to explore if and how large operator bias might be. Determination of evolved CO_2-C was by acid titration with vigorous stirring to neutralize remaining alkali to a phenolphthalein endpoint following precipitation of carbonate with excess 1.5 M $BaCl_2$.

2.3 | Statistical analyses

Statistical analyses of soil C mineralization data were conducted with the general linear model of SAS v. 9.4 (SAS Institute, Cary, NC) using a completely randomized design with factorial arrangement of 5 soil types by 10 soil mass and volume treatments by 2 titration operators by 5 replications. Coefficient of variation among the 10 replications of a treatment (after accounting for titration operator variation) was used to characterize unexplained variation along a gradient of soil mass and volume treatments. Significance was declared at $p \leq .05$. Linear regression with SigmaPlot v. 14 (Systat Software, San Jose, CA) was used to assess strength of association among response variables.

3 | RESULTS AND DISCUSSION

3.1 | Carbon mineralization among soils and incubation periods

The flush of CO_2 following rewetting of dried soil across all soil types and treatments averaged 237 mg CO_2-C kg^{-1} soil (0–3 d) and the middle 50% of observations were in the range of 67 to 357 mg CO_2-C kg^{-1} soil (0–3 d). The five soil types provided a reasonable diversity of both physical and biochemical conditions, similar to that of other field assessments in the region (Franzluebbers, Pehim-Limbu, & Poore, 2018a; Franzluebbers et al., 2018b). Continuation of incubation resulted in cumulative C mineralization that had 25 and 75% quartile limits of 128 to 667 mg CO_2-C kg^{-1} soil (0–10 d), 190 to 935 mg CO_2-C kg^{-1} soil (0–24 d), 249 to 1202 mg CO_2-C kg^{-1} soil (0–60 d), and 474 to 1743 mg CO_2-C kg^{-1} soil (0–150 d). Normalized per unit of total organic C on a daily basis, C mineralization rate across soils and treatments was 4.6 ± 0.6, 1.7 ± 0.3, 0.9 ± 0.3, 0.4 ± 0.1, and 0.4 ± 0.1 g CO_2-C kg^{-1} total organic C d^{-1} at 0–3, 3–10, 10–24, 24–60, and 60–150 d, respectively. Actual rates and how they declined with time were similar to those reported for a diversity of soil textures in Georgia (Franzluebbers, 1999). Relative differences among soils that occurred immediately at 3 d of incubation continued throughout the entire incubation (Figure 1).

3.2 | Sources of variation

Soil type explained ≥90% of total variation in C mineralization when expressed per unit soil mass (data not shown). However, C mineralization per unit of total organic C was most affected by mass and volume treatment (12-58% of total variation) and the least by laboratory operator (0–26% of total variation) (Table 3). Although the effect of soil type

TABLE 3 Percentage of variation explained by different sources affecting C mineralization (mg CO_2-C g^{-1} total organic C) during different incubation periods

Source of variation	0–3 d	3–10 d	10–24 d	24–60 d	60–150 d
Soil type	10.6***	17.3***	17.4***	10.1***	46.7***
Mass and volume treatment	35.0***	16.3***	12.4***	58.4***	ND[a]
Soil type x mass/volume treatment	12.5***	9.7***	12.6***	2.4**	ND
Laboratory operator	0.1	0.1	0.8*	6.6***	25.8***
Random	41.8	56.7	56.9	22.5	27.5

[a]ND, not determined.
*Significant at $p < .05$,
**Significant at $p < .01$,
***Significant at $p < .001$.

was normalized per unit of total organic C, it still explained 10–47% of variation in C mineralization, possibly due to soil textural differences and allocation of organic C within aggregates and particulate organic matter. Laboratory operator was not a significant source of variation during 0–3 d and 3–10 d incubation periods, but became an increasingly larger proportion of total variation with longer incubation periods. Part of this greater proportion of variation from operator was simply due to fewer treatment comparisons that were available after 24 d, and therefore, greater relative variation could be attributed to remaining factors of soil type and laboratory operator. The effect of laboratory operator was significant in 11 of 39 pair-wise comparisons during 0–3 d of incubation, in 7 of 39 comparisons during 3–10 d of incubation, in 4 of 34 comparisons during 10–24 d of incubation, in 4 of 9 comparisons during 24–60 d of incubation, and in 4 of 4 comparisons during 60–150 d of incubation. Power to detect significant differences was high with 10 replications for each pair. Coefficient of variation in these pair-wise comparisons was as low as 6 ± 4% in the Georgeville silt loam to as high as 25 ± 15% in the Norfolk loamy sand. Overall, operator variation was considered low and not a large factor in affecting estimates of C mineralization, particularly for the 0–3 d period used to estimate soil-test biological activity.

Soil mass and volume treatment was a significant source of variation at all incubation periods (Table 3). At 0–3 d of incubation (i.e. the period defined for soil-test biological activity), C mineralization responded to soil mass and volume treatment in a similar pattern across soils (Figure 2). For example in treatments with common surface area of 5.3 cm^2 (i.e. 5 g, 10 g, 20 g, and 23 ml), increasing soil mass and volume led to significantly lower estimates of soil-test biological activity in all soils. Across all soil types, C mineralization declined 17 mg CO_2-C g^{-1} total organic C for every 10 g increase in soil mass ($p < .001$). In treatments with 45.4 cm^2 surface area (i.e. 100 g, 200 g, and 500 g), a similar decline in C mineralization occurred with increasing soil mass and volume ($p = 0.01$), but the decline was not nearly as dramatic at 2 mg CO_2-C g^{-1} total organic C for every 10 g increase in soil mass.

It seems plausible that CO_2 produced at the same rate from the same soil type was being held longer in soil pores when soil depth was greater. If this were true, then longer incubation periods should have diminished this effect. Carbon mineralization in treatments with 5.3 cm^2 of surface area tended to decline with time and increasing soil mass and volume, but was only significant initially [17, 8, and 11 mg CO_2-C g^{-1} total organic C for every 10 g increase in soil mass ($p < .001$ for 0–3 d, $p = 0.07$ for 3–10 d, and $p = 0.18$ for 10–24 d), respectively]. In treatments with 14.5 cm^2 of surface area, the decline in C mineralization with increasing soil mass and volume was significant at all periods, but very small [1.6, 1.4, and 1.2 mg CO_2-C g^{-1} total organic C for every 10 g increase in soil mass ($p = 0.004$ for 0–3 d, $p = 0.005$ for 3–10 d, and $p = 0.05$ for 10–24 d), respectively]. Therefore, the dampening effect of greater soil mass and volume may not have only been mostly from physical entrapment of CO_2 in soil pore spaces, but other unidentified factors may have also been important.

Soil-test biological activity in mass and volume treatments with intermediate surface area (i.e. 6.6 to 14.5 cm^2) averaged the same as in treatments with lower and greater surface area, across soils. However, soil-test biological activity was 5% greater ($p < .001$) with intermediate surface area than with other treatments in the Georgeville silt loam, 7% lower ($p < .001$) with intermediate surface area than with other treatments in the Appling coarse sandy loam, and no different ($p > .05$) among these comparisons in the other three soils (Figure 2). Surface area-to-volume ratio in these three intermediate mass and volume treatments varied from 0.14 to 0.29 cm^{-1}, and was the same for the 50 g and 100 g* treatments because there were duplicate aliquots of the same sized container in the 100 g* treatment. Lower surface area-to-volume ratio in the 74-ml treatment than both one 50-g aliquot and two 50-g aliquots led to 9% lower ($p < .001$) soil-test biological activity in the Georgeville silt loam, but this difference in surface area-to-volume ratio had no significant effect in the other four soils. Soil-test biological activity was 6% lower ($p = 0.05$) and 18% lower ($p = 0.01$) from two 50-g aliquots of soil than from one 50-g aliquot of soil in the Appling coarse

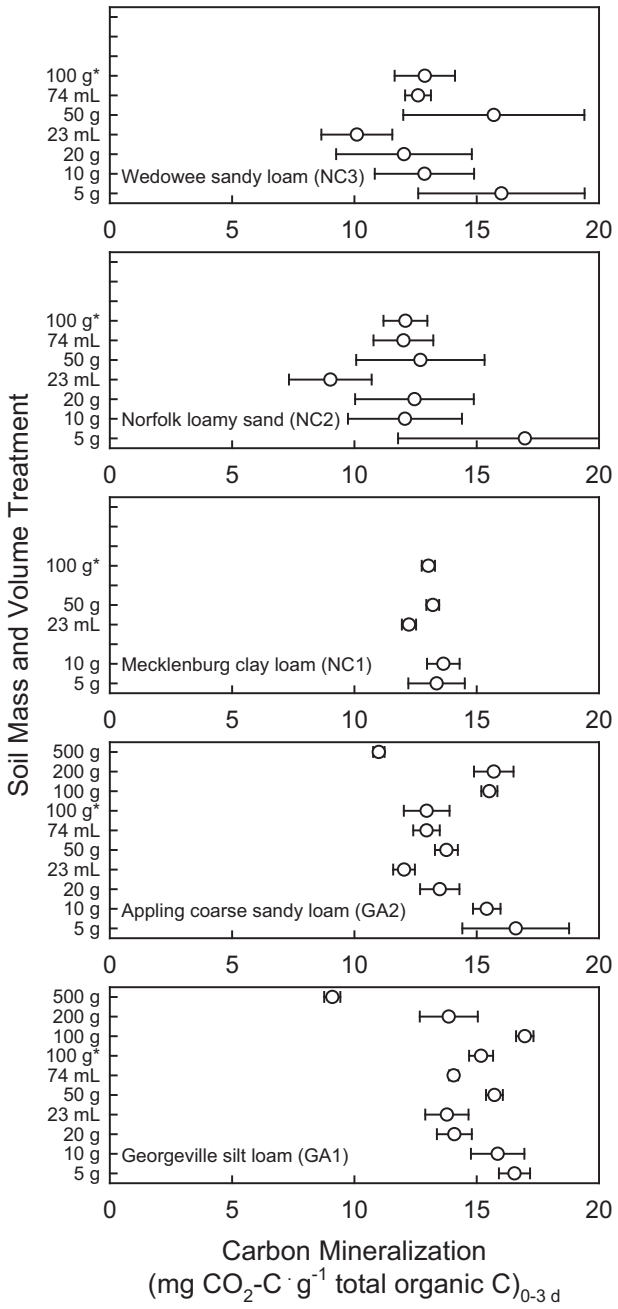

FIGURE 2 Soil-test biological activity (i.e. C mineralization during 0–3 d period) as affected by soil mass/volume treatments in five different soil types

sandy loam and Wedowee sandy loam, respectively, but not different between treatments in the other three soils. Significant differences among these treatments were even less prevalent when considering C mineralization during 3-10-d and 10-24-d periods (data not shown). Except for the difference in C mineralization during both the 0-3-d and 3-10-d periods between intermediate and other surface area conditions in the Georgeville silt loam, no other differences were observed at 3-10-d and 10-24-d periods. Overall, significant differences in C mineralization in the intermediate surface area treatments were small, variable, and infrequent.

Across all soil mass and volume treatments, soil-test biological activity had a median value of 54, 66, 211, 355, and 554 mg CO_2-C kg^{-1} soil 3 d^{-1} in the Wedowee sandy loam, Norfolk loamy sand, Appling coarse sandy loam, Georgeville silt loam, and Mecklenburg clay loam, respectively. These values were in close association with soil organic C ($r^2 = 0.99$, $p < .001$) and total soil N ($r^2 = 0.98$, $p = 0.001$). Mass and volume treatments that had the most observations within ± 5% of the median value were the 74-ml treatment (surface area-to-volume ratio of 0.14 cm^{-1}) in the Wedowee sandy loam, the 100-g* treatment (0.27 cm^{-1}) in the Norfolk loamy sand, the 50-g treatment (0.25 cm^{-1}) in the Appling coarse sandy loam, the 100-g* treatment (0.22 cm^{-1}) in the Georgeville silt loam, and the 100-g* treatment (0.18 cm^{-1}) in the Mecklenburg clay loam. Similarly across all soils, mass and volume treatments that had the most observations within ± 10% of the median were the three intermediate treatments (50 g, 74 mL, and 100 g*).

Regression analysis was used to test whether variations in soil surface area, depth, mass, or water content had greater influence on mean C mineralization estimates. Considering the five soils as replicate blocks, soil mass was the single most important factor influencing C mineralization rate across mass and volume treatments at 0–3, 3–10, and 10–24 d periods ($p < .001$). Greater soil mass led to lower C mineralization rate. However, if the treatments with largest soil mass were removed from the regression (i.e. 100, 200, and 500 g), the effect of soil mass on C mineralization was not significant at 0–3 and 10–24 d of incubation and the effect at 3–10 d of incubation was diminished ($p = 0.02$). Surface area-to-volume ratio also negatively influenced C mineralization at 0–3, 3–10, and 10–24 d of incubation ($p < .001$). In multiple regression, C mineralization increased with increasing surface area ($p = 0.004$) and declined with interaction of surface area and soil mass ($p < .001$) at 0–3 d.

3.3 | Precision estimates

Inherent variability in C mineralization was also explored by calculating coefficient of variation among the 10 replications (after accounting for laboratory operator) within each mass and volume treatment for each soil type. Pooled across three incubation periods of 0–3, 3–10, and 10–24 d, coefficient of variation was non-linearly related to soil mass (Figure 3). Low soil mass treatments had greater inherent variability than higher soil mass treatments. Across all incubation periods, coefficient of variation was minimized at 7.7% whenever soil mass was ≥76 g (53 g if coefficient of variation was set at 1.05 times this threshold). When removing from analysis the three treatments with soil placed directly in the bottom of the

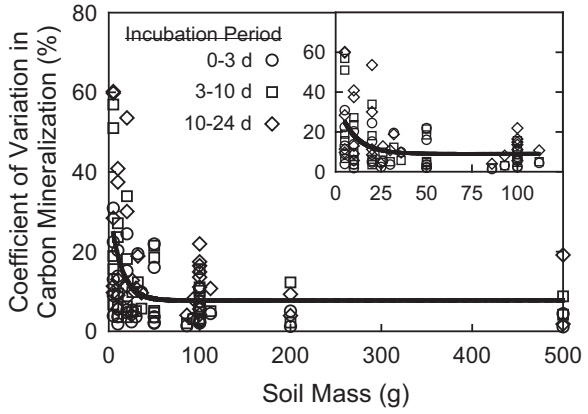

FIGURE 3 Coefficient of variation in C mineralization during three different incubation periods as affected by soil mass

canning jar (i.e. 100, 200, and 500 g) (as shown in Figure 3 inset), a similar response occurred with minimal coefficient of variation at 8.9% when soil mass was ≥72 g (38 g if coefficient of variation was set at 1.05 times this threshold). Maximum coefficient of variation of 25% was predicted at the minimal soil mass level of 5 g in both evaluations. Minimum and maximum coefficients of variation at 0–3 d of incubation were 7.7 and 15.7%, at 3–10 d of incubation were 9.2 and 29.6%, and at 10–24 d of incubation were 11.8 and 32.2%, respectively. Soil mass for coefficient of variation to be at 1.05 times the minimum was 15 g for soil incubated at 0–3 d, 20 g for soil incubated at 3–10 d, and 50 g for soil incubated at 10–24 d (data not shown). Coefficient of variation when selecting 50 and 100 g of soil at 0–3 d of incubation was 7.0%, at 3–10 d of incubation was 9.3%, and at 10–24 d of incubation was 12.3%.

Preconditioning soil samples with sieving has been shown to impact estimates of C mineralization. Finer sieve size can lead to enhanced C mineralization in some cases (Franzluebbers, 1999; Wade et al., 2018), but not in all types of soil (Franzluebbers & Haney, 2018). This may be a function of antecedent aggregate size and the ability of soils to form aggregates, e.g. sandy soils have limited capacity to aggregate. Chemical soil testing has often used finely ground or pulverized soil to thoroughly homogenize soil and allow for small aliquots (≤ 10 g) to adequately represent the entire soil sample. Although fine grinding can be used to determine soil biological activity, it is not ideal, as soil pores are minimized and readily filled with water creating a more anaerobic environment (Franzluebbers & Haney, 2018). Fine grinding also pulverizes and distributes organic matter that could otherwise be protected from microbial activity (Franzluebbers & Arshad, 1997). On the other hand, coarse sieving may leave a more heterogeneous soil matrix that would require greater mass to obtain representative evaluation of the sample. As conducted in this study, coarsely sieved soil (i.e. < 4.75 mm) retains more of the in situ features of water-stable aggregation and heterogeneous distribution of organic matter and microbial communities.

Relatively low coefficients of variation in this study (i.e. 8% at 0–3 d of incubation and across incubation periods) compare favorably with some other studies using alkali absorption during standardized laboratory incubation. Carbon mineralization estimates from 6 aliquots (i.e. analytical replicates from 50- and 100-g subsamples) of 42 experimental units of a sandy loam soil in North Carolina had coefficient of variation with median value of 4.4% (A.J. Franzluebbers, unpublished data). In contrast, coefficient of variation among triplicate analytical replicates (5 g each) of 16 treatments had median of 17% at 0–1 and 0–3 d periods, 15% at 0–10 d period, and 13% at 0–24 d period (Morrow et al., 2016). Results of the latter study are consistent with those of Wade et al. (2018), who reported 16% coefficient of variation in triplicate samples (10–40 g each) from 20 different soils and conducted at multiple labs. Large analytical variations when small soil mass is incubated was identified in an earlier evaluation, leading to a conclusion that variation in the flush of CO_2 may be too large to be meaningful (Sullivan & Granatstein, 2015).

Mixing assessments between analytical replication (i.e. subsamples from the same bag of soil) and field replication (i.e. different samples from parts of the same field) is not fair. Variations in the field are normal and can lead to greater variation in C mineralization estimates. Coefficient of variation in the flush of CO_2 during 0–1 d of incubation (40 g soil) among field replicates was 24% at a Piedmont location and 23% at a Blue Ridge location in North Carolina (Roper, Osmond, Heitman, Wagger, & Reberg-Horton, 2018). These values were lower than coefficients of variation in soil-test P of 56% at the Piedmont location and 33% at the Blue Ridge location. Coefficient of variation in soil-test biological activity (0-3-d incubation, 100 g soil) among four field replicates from 47 field sites sampled at 0–10, 10–20, and 20–30 cm in North Carolina and Virginia was 14.4% (Franzluebbers et al., 2018b).

A few other studies have assessed mass and volume treatments on soil biological components. Parkin, Starr, and Meisinger (1987) found that surface area-to-volume ratios of 1 to 2 cm^{-1} did not affect soil denitrification rate. However, they did find that increasing soil mass from 2 to 20 kg reduced coefficient of variation in denitrification rate. In estimating microbial diversity, Ranjard et al. (2003) concluded that large soil sample size (4 g) was most suitable for description of the overall soil microbial community, but large numbers of small samples (0.1 g) were more appropriate for a determination of local microbial diversity. Similarly, variation in microbial community structure was greater in small sample sizes of 0.01 and 0.1 g soil compared with large sample sizes of 1 and 10 g soil when using denaturing gradient gel electrophoresis (Ellinsøe & Johnsen, 2002). Penton, Gupta, Yu, and Tiedje (2016) found that 10-g

samples of soil had greater bacterial diversity, less variation among replicates, and more depth of taxa information than smaller sample sizes (0.25, 1, and 5 g), but suggested the value of the resolution gained needs to be considered relative to (1) the variation of the features/attributes of the system under study, (2) the resolution needed to answer the question and (3) extraction costs. Although soil mass values were smaller in these microbial community structure studies compared with the ecosystem process of C mineralization in this study, the same considerations for choice of sample size are relevant here.

3.4 | Looking forward

These experimental results were determined to obtain a quantitative understanding of how variations in laboratory protocols might impact assessments of soil-test biological activity (i.e., the flush of CO_2, short-term C mineralization, soil CO_2 burst, etc.). There is growing interest by farmers, government agencies, non-government organizations, and university investigators and extension agents to determine some measure of soil biological activity so that better land management decisions can be made to increase sustainability of food, fodder, fuel, and/or fiber production. The Soil Ecology and Management Laboratory at North Carolina State University routinely uses two 50-g aliquots of soil for assessment of soil-test biological activity (i.e. 100-g* treatment), and this approach led to estimations that were consistently near the median estimate of C mineralization with low coefficient of variation. From the significant soil mass and surface area-to-volume effects on C mineralization, it would appear that a standard volume approach to C mineralization methodology might also be reasonable to avoid undesired biases in estimations. Mineralization of C from the 74-ml treatment (i.e. surface area-to-volume ratio of 0.14 cm^{-1}) was as accurate and precise as the 100-g* treatment (0.18–0.29 cm^{-1}). This treatment would allow a known volume of soil to be used with variable soil mass (i.e. depending on soil texture, structure, and organic matter). Volume and mass are the two variables needed to calculate soil porosity. With this information, it would then be easy to calculate and precisely apply water needed to achieve ideal water content during incubation (i.e. 50% water-filled pore space).

This research was conducted to support better assessment of soil health. Soil health management systems use the principles of (1) minimizing soil disturbance via tillage (or optimizing sward and soil-surface disturbance in the case of grazing), (2) maximizing soil cover, (3) encouraging year-round root activity, and (4) promoting biological diversity in space, time, and functional attributes. Robust soil-test biological activity estimates can be obtained by optimizing soil mass and volume during incubation.

4 | CONCLUSIONS

Soil mass and volume had the greatest effect on analytical variability of C mineralization per unit of total organic C, as well as a statistically significant effect on absolute estimates of C mineralization per kilogram of soil. Many incubation protocols are possible to compare relative values, but absolute values may not be directly comparable. Relative results need calibration against independent ecosystem processes, or against other laboratory protocols. Increasing soil volume in the incubation vessel appears to limit immediate CO_2 release, so constant surface area-to-volume ratio should be considered. To obtain the most comparable results across a diversity of soil types and experimental conditions, standardized soil mass or volume should be selected. Since large soil mass with small surface area-to-volume ratio limited accuracy of estimation and low soil mass limited precision of estimation, a moderate soil mass of 50–100 g is recommended to optimize estimations. Alternatively, a constant volume of soil lightly packed into a standard-sized vessel (e.g. 74 ml) could be reasonable and allow for an easy way of determining water addition to 50% water-filled pore space. Standardized approaches are needed to compare results of soil-test biological activity across a diversity of laboratories.

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