Soil-test biological activity with short-term and long-term carbon contributions

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Abstract
Soil-test biological activity (STBA) is a key indicator of soil health because of its relevance to ecosystem processing, broad applicability, and simple and rapid analysis. Short-term and long-term influences of carbon (C) availability on STBA were assessed. A short-term factor was root-derived C inputs from a sudangrass (Sorghum × drumondii) test crop grown under greenhouse conditions. Long-term factors were from soil at two depths in three sections of a field varying in soil texture and exposed to 16 yr of different pasture–crop rotation management. Soil-test biological activity was determined after 0, 14, 21, 31, and 45 d of greenhouse growth. Total variation in STBA was 7% from the short-term factor and 81% from the long-term factors. Soil depth was the primary long-term factor influencing STBA (57%) and soil texture was intermediate (24%). Results indicate that short-term changes in STBA are important but modest compared with variations due to soil depth and texture.

1 INTRODUCTION

Soil is a fundamental natural resource that sustains agriculture and the growing number of people inhabiting the planet (Janzen et al., 2011). Humanity has a responsibility to maintain and improve soil conditions so that future generations will be able to share in this life-supporting natural resource. With appropriate care, soil resources are renewable. However, with reckless management, soil resources can dwindle into barren deserts, rocky fields, or highly contaminated wastelands (Montgomery, 2007). Soil can be managed to meet our needs for today and the future (Franzluebbers, 2010) if we follow some guiding principles to minimize disturbance, keep soil covered, provide living roots for as much of the year as possible, and enhance the diversity of plants and animals. These are commonly referred to as soil health principles (USDA-NRCS, 2020).

Soil health is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS, 2020). A variety of soil physical, chemical, and biological properties have been recommended to assess soil health (Stott, 2019). Biologically active fractions of soil organic matter can reveal production and environmental limitations, irrespective of geography and soil type (Hurisso et al., 2016). A key indicator of soil health is STBA, as it reflects both habitat and activity of soil microorganisms (Franzluebbers, 2018) and is strongly associated with potential soil N mineralization (Franzluebbers, Pershing, Crozier, Osmond, & Schroeder-Moreno, 2018) and fulfilling the N requirement of crops (Franzluebbers, 2020). Soil-test biological activity measures the flush of CO₂ released upon rewetting of dried soil. The methodological approach of using dried soil and

Abbreviations: STBA, soil-test biological activity.
relatively rapid analysis time were key attributes for its development as a soil testing approach (Franzluebbers, Haney, Honeycutt, Schomberg, & Hons, 2000). Also, it relies on natural decomposition of organic matter to yield a reliable estimate of respiratory activity when using a standardized laboratory approach (Franzluebbers & Veum, 2020).

With potentially greater utilization of STBA for soil health assessments, a question often arises as to the most appropriate time of the year when measurements can or should be made. One of the concerns with sampling time is whether recent C inputs are present in soil, especially with more year-round growing conditions when using cover crops. Our objective was to determine the relative contribution of short-term and long-term C inputs to soil to better ascertain how recent C inputs from roots might influence estimates of STBA. We hypothesized that changes in STBA due to recent C inputs from roots would be gradual, similar to that of plant development, and overall modest in comparison with long-term differences in soil C from management and edaphic factors.

2 MATERIALS AND METHODS

Soil was collected in mid-February 2015 from three replicate plots of pasture–crop rotation systems at the long-term Farming Systems Research Unit of the Center for Environmental Farming Systems in Goldsboro, NC (35°22’ N, 78°2’ W). Wickham loamy sand (a fine-loamy, mixed, semiactive, thermic Typic Hapludult) was mapped as the dominant soil type. The actual texture was sandy loam in two blocks and clay loam in one block. Soil organic C was 9.1 ± 3.0 g kg⁻¹, total soil N was .8 ± .3 g kg⁻¹, soil pH was 6.0 ± 0.2, soil-test P was 84 ± 44 g m⁻³, and soil-test K was 147 ± 38 g m⁻³. Pasture–crop rotation systems were established in 1999 and presumed to influence on subsequent STBA. Greenhouse growth was from available nutrients contained in the soil, as no additional nutrients were provided. A total of 24 aliquots of soil from each of the original 18 field samples were scooped into Ray Leach RLC4 Cone-tainers (66-ml volume, 16.1 cm tall, 1.54-cm i.d. at top) (24 × 18 = 432 Cone-tainers). Effective volume was 50 ml and soil mass was 65 ± 6 g (mean ± standard deviation). Pregerminated sudangrass (Sorghum × drummondi) seeds were placed on top of the soil, and racks were placed in a greenhouse (target daytime maximum of 30 °C and nighttime minimum of 20 °C) with supplemental light 2 h in the morning and 2 h in the evening to ensure a full 12 h of total daylight (0700–1900 h). Plants were grown in the same manner as described by Franzluebbers and Pershing (2018). Harvest of plants occurred on four dates, i.e., 14, 21, 31, and 45 d after planting (six replicates per field treatment at each date). The nonlinear time series anticipated a slowing of plant growth due to nutrient limitation. Aboveground dry matter and N concentration were determined as in Franzluebbers and Pershing (2018).

At each harvest, a total of 108 Cone-tainers of soil were dried in an oven at 55 °C for 3 d until constant mass
(18 soil conditions × 6 replications). Cone-tainers of soil were later incubated in 1-L canning jars following rewetting to 50% water-filled pore space at 25 °C for 3 d, just like that of the original soil, to determine STBA. Each of the six replicates for each of the 18 soils was incubated separately.

Soil and plant response data were subjected to analysis of variance using SAS v. 9.4 (SAS Institute) according to harvest date (four levels for plant growth but five levels for soil analysis to include initial condition), management (three levels), soil depth (two levels), field block (three levels), and six greenhouse bioassay replications. Using mean values across the six greenhouse bioassay replications, the relative variation in STBA was calculated from the sums of squares attributed to each of the factors divided by the total sums of squares among all data. The short-term factor was harvest date and the long-term factor was a compilation of management, soil depth, and field block. Repeatability of STBA was determined from variation among the six subsampling replicates of soil in Cone-tainers within each of the 72 experimental units (4 harvest dates × 3 management × 2 soil depths × 3 field block combinations). Initial soil condition was not a part of subsampling replicates. Nonlinear regression of STBA response with time was conducted with SigmaPlot v. 14.0 (Systat Software). Significance was declared at \( p \leq 0.05 \).

### RESULTS AND DISCUSSION

Soil-test biological activity was primarily affected by long-term C inputs rather than short-term C inputs (Figure 1). Long-term C input factors were edaphic, like soil depth and soil texture (which varied according to block), as well as management related from pasture–crop rotation systems. The short-term C input factor was from recent root inputs to the soil during the growth of sudangrass in the greenhouse. As a percentage of the total variation in STBA (\( n = 90 \) from 3 management systems × 3 blocks × 2 depths × 5 greenhouse growth periods), long-term factors explained 81% and the short-term factor explained 7%. Of the long-term factors, soil depth was the largest (57% of the total variation) and agricultural management was lowest (<1% of the total variation). Soil texture was intermediate (24% of the total variation).

Temporal changes in STBA caused by root-induced C inputs (albeit presumed, since we did not measure root mass or rhizodeposition) were relatively uniform across soil textures and depths (Figure 1). Incremental change in STBA from one harvest period to the next was 15 ± 20% among soil textures and depths (\( n = 24 \); Figure 1). As a proportion of the original soil without root-induced C inputs, change in STBA was 53 ± 25% among all four of the harvest dates (\( n = 24 \); Figure 1). Although not temporally significant, there was a trend for greater effect with a greater length of time that the soil was exposed to sudangrass growth, i.e., 43, 46, 56, and 65% at 14, 21, 31, and 45 d, respectively. Therefore, these data suggest that short-term, root-induced C inputs can have a modest to relatively large effect on STBA estimation. The magnitude of the short-term, root-induced effect in this study might be considered near the upper end of expected seasonal changes to STBA in the field, as root mass was highly concentrated in a restricted soil volume during this greenhouse growth trial. Peak change in STBA during the middle of a wheat (*Triticum aestivum* L.) growing season in Texas was ~25% greater than at the beginning of the growing season, probably due to the greatest root mass at that time (Franzluebbers, Hons, & Zuberer, 1995). In Maine, the change in soil biological activity from a 1-d analysis technique with exposure to various crops in the field and greenhouse was 15–40% (Laffely, Erich, & Mallory, 2020). In a 60-wk mesocosm study in Georgia, a large root-mass increase from tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] in loamy sand and clay loam soils led to large increases in soil C and N mineralization (Franzluebbers, 2006).

We expected to observe differences in soil organic C and N fractions due to long-term management and soil depth. However, long-term management among these crop–pasture rotation systems had little influence on any of the soil organic C and N properties (data not shown). The density of rooting would be expected to be greater in
the 0–10-cm depth than in the 10–20-cm depth (Ma, Wood, & Bransby, 2000; Roder, Mason, Clegg, Doran, & Kniep, 1988). Blocking of the experimental design was according to sandy loam and clay loam soils (95 ± 10 g clay kg⁻¹ in Block 1, 199 ± 45 g clay kg⁻¹ in Block 2, and 326 ± 36 g clay kg⁻¹ in Block 3).

Aboveground dry matter production from the sudangrass test crop in the greenhouse was 50 ± 9% greater (among harvest dates) from soil collected at a depth of 0–10 cm than at 10–20 cm. This result suggested that nutrients bound in the soil organic matter were probably an important source of fertility. Many soil properties were greater in concentration in the 0-10-cm depth than in the 10-20-cm depth prior to the greenhouse bioassay (data not shown). It could be argued that any one of these soil properties might have been responsible for the difference in greenhouse growth between soil depths. However, we contend that organic N cycling properties were the most important based on (a) the difference in residual inorganic N between depths being only 3.7 mg kg⁻¹ and the difference in plant N uptake being 8.0 ± 0.5 mg kg⁻¹, (b) F values of the soil depth effect from soil organic C and N properties being 56 ± 30, while those from soil chemical properties were 13 ± 12, and (c) across a wider diversity of soil types, STBA, total soil N, and residual inorganic N having the greatest predictive power (76, 3, and 7%, respectively) for plant N uptake in a similar greenhouse bioassay (Franzluebbers & Pershing, 2018).

The coefficient of variation of STBA among the six subsamples of the same treatment was 15 ± 7% (n = 72 sets of data). This level of variation in STBA with root-enhanced soil was similar to the 14% observed among four field replicates of soils collected from 47 farms and three soil depths (Franzluebbers et al., 2018). The coefficient of variation in field and greenhouse studies in Maine was ~8% (Laffely et al., 2020). Therefore, seasonal variations in STBA due to root-induced C inputs at the field level are likely to be significant but may also be near the limit of discernment from random variation. Additional field studies are warranted to determine whether season, plant growth stage, soil sampling depth, and/or temperature and moisture conditions might be important in describing variations in STBA. A test of root-induced C inputs on soils with greater organic STBA levels would also be important, as short-term variation appeared to be high against the relatively low STBA in this study. The magnitude of change in STBA was 52 ± 35 mg kg⁻¹ during 3 d at the 0-to-10-cm depth (41 ± 23%) and 32 ± 17 mg kg⁻¹ during 3 d at the 10-to-20-cm depth (92 ± 51%). Irrespective of this, net N mineralization during 24 d and plant N uptake during 45 d of greenhouse growth were highly associated with the level of STBA (r² = .74 and .82, respectively, n = 18).

4 | CONCLUSIONS

Both short- and long-term C inputs influenced STBA in sandy loam and clay loam soils from North Carolina. Long-term contributions were greatest from sampling depth (57% of total variation) and soil texture (24% of total variation) factors and least from management differences in pasture–crop rotations (<1% of the total variation). Short-term contribution following growth of a sudangrass test crop in the greenhouse for different lengths of time was also an important factor that accounted for 7% of the total variation. Short-term, root-induced changes in C input increased STBA by 53 ± 25% compared with control soil prior to plant growth bioassay. Soil-test biological activity of samples from different textures, depths, and management systems in the field was highly predictive of sudangrass growth and plant N uptake in the greenhouse growth trial. Our results illustrate the potential for root-induced seasonal changes in C inputs from cover crops or cropping systems to alter STBA in the short term. We conclude that STBA is a sensitive indicator of soil health condition.

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