Published February 9, 2016

Agricultural & Environmental Letters

Research Letter

Should Soil Testing Services Measure Soil Biological Activity?

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Core Ideas

- Soil biological activity is a key indicator for productivity and environmental quality.
- The flush of CO_2 possesses many qualities of a robust soil test.
- Field calibration is underway to relate the flush of CO_2 to nitrogen availability.

Abstract: Health of agricultural soils depends largely on conservation management to promote soil organic matter accumulation. Total soil organic matter changes slowly, but active fractions are more dynamic. A key indicator of healthy soil is potential biological activity, which could be measured rapidly with soil testing services via the flush of CO_2 during 1 to 3 d following rewetting of dried soil. The flush of CO_2 is related to soil microbial biomass C and has repeatedly been shown strongly related to net N mineralization during standard aerobic incubations. New research is documenting the close association with plant N uptake in semicontrolled greenhouse conditions (r^2 = 0.77, n = 36). Field calibrations are underway to relate the flush of CO_2 to the need for in-season N requirement in a variety of crops. An index of soil biological activity can and should be determined to help predict soil health and soil N availability.

Background

Soil health is a concept borne from its predecessor—soil quality (Karlen et al., 1997). Little difference exists between the two, yet "soil health" has become a more invoking moniker to spearhead a campaign against exploitive soil management that threatens soils around the world (http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/soils/health/), either gradually through high-intensity farming practices that largely ignore soil biological function (relying solely on chemical inputs, leaving soil bare during the nongrowing season, excessively removing plant materials, etc.) or rapidly through frequent and highly disruptive tillage practices on sloping land and in wind-prone flatlands.

Soil health assumes more emphasis on the biological component of soil than the original concept of soil quality because health can only be described for something that is living. Physical and chemical features of soil are still important in the soil health concept, and a variety of indicators can be used (Doran and Parkin, 1994). Indicators ultimately are used to relate to important soil functions, such as decomposing organic matter, cycling water and nutrients, controlling gas emissions, harboring biodiversity, and so on. Characterizing soil function efficiently is desired with a minimum dataset (Andrews et al., 2004). The following are key characteristics of indicators: (i) be easy to measure, (ii) detect changes in soil function, (iii) integrate soil physical, chemical, and biological properties and processes, (iv) be accessible to many users and applicable to field conditions, (v) be sensitive to variations in management and climate, (vi) encompass ecosystem processes and relate to process-oriented modeling, and (vii) where possible, be components of existing soil data bases.

When selecting indicators, researchers should be mindful of the time and expense required for accurate and reliable determination. Franzluebbers and Haney (2006) suggested the following indicators to assess changes in soil health of organic agricultural systems, but they could also be applied to other ecologically based agricultural approaches:

- *Soil organic C and total N*– reflecting the functional capability of soil to supply nutrients to plants, serve as an organic nutrient reserve, mitigate greenhouse gas accumulation, and provide organic resources for stabilizing the soil surface against erosion, for filtering of water, for buffering against nutrient extremes, and for promoting a biologically diverse and healthy microbial population.

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Agric. Environ. Lett. 1:150009 (2016)
doi:10.2134/ael2015.11.0009

Received 9 Nov. 2015.
Accepted 15 Dec. 2015.
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- *Water-stable aggregation and stability*—reflecting the functional capability of soil to provide physical stability and resistance against wind and water erosion.
- *Flush of CO_2 following rewetting of dried soil*—reflecting the functional capability of soil to cycle nutrients, decompose organic amendments, and catalyze and stabilize ecosystem processes through the interactions among a diversity of organisms.
- *Microbial substrate utilization*—reflecting the functional capability of soil to provide biodiversity and habitat, cycle nutrients, decompose organic amendments, filter water passing through soil, and provide biological resilience to soil disturbance.
- *Inorganic N, extractable P, and soil pH*—reflecting the functional capability of soil to supply readily available nutrients, as well as to foster microbial function under optimum pH.

Soil testing services at state-sponsored and private laboratories have historically focused on total soil organic matter, chemical soil indicators of inorganic N, P, and K, soil pH, and various other macro- and micronutrients to assess nutrient availability to crops. Although important, inorganic nutrient availability alone does not offer a complete assessment of soil fertility or of soil biological influences on important soil properties and processes that affect crop yield and environmental quality.

Soil testing could be elevated to a more holistic evaluation of soil fertility and health with the adoption of a test for soil biological activity, such as the flush of CO_2. This specific method fits within the criterion for soil health evaluation to describe soil functioning to cycle nutrients, decompose organic amendments, and catalyze and stabilize a variety of soil microbial processes, as well as the criteria for soil testing of being rapid, inexpensive, reproducible, suitable for a wide range of soils, and correlating to nutrient needs of crops and meeting environmental goals. This paper introduces the rationale, procedure, and potential for this claim, but prior, ongoing, and future research will lay the foundation.

The flush of CO_2 has been reported in a number of studies to characterize the influence of management (e.g., cover cropping, crop sequence, grazing, tillage) (Franzluebbers and Stuedemann, 2003, 2008, 2015). Key features of the procedure that make it suitable for biological soil testing are (i) use of dried soil, (ii) relatively rapid analysis time of 3 d of drying and 3 d of incubation, (iii) strong correlation with a number of other important soil health indicators, and (iv) using inherent biological conditions of the soil.

Procedure for the Flush of Carbon Dioxide

The first step, as for any good soil testing procedure, is to obtain a representative soil sample of a field of interest. Basic approaches to subdivide fields and statistically represent areas need to be used (Petersen and Calvin, 1996). A key consideration is depth of sampling since soil biological activity is closely tied to limited C resources that are highly depth dependent.

Soil processing recommendations based on author experiences over a number of years are described in the following. Soil samples should be oven-dried at 50 to 60°C for at least 3 d. Dried soil is then passed through a screen with 4.75-mm openings after gently crushing with a pestle and large pieces of organic detritus removed. The choice of opening is based on a desire to obtain homogeneity but also to retain aggregates of sufficient size for stability determination and adequate aeration during incubation. Extent of soil disturbance on soil biological activity can be significant (Franzluebbers, 1999b).

Soil is scooped into volume-delimited glass bottles and weighed (actual soil weight can vary by availability and objective, but 100 g is reasonable). Porosity of soil is determined from weight and volume, and then water is added to achieve 50% water-filled pore space. Upon wetting, bottles containing soil are placed into 1-L canning jars along with a screw-cap vial containing 10 mL of 1 M NaOH to trap CO_2 and a vial of water (10 mL) to maintain humidity. Canning jars are sealed and incubated for 3 d at 25°C.

At the end of incubation, the screw-cap vial of NaOH is removed and sealed until titration can begin. In sequence, each vial of NaOH is opened and the following added to solution: (i) sufficient 1.5 M $BaCl_2$ solution (up to 3.5 mL) to precipitate bicarbonate as $BaCO_3$, (ii) a few drops of phenolphthalein color indicator, and (iii) a small magnetic stir bar. The vial is placed on a magnetic stir plate and 1 M HCl is slowly added to solution (initially by stream and near pH endpoint by drop) until the color changes from pink to clear (pH ~9.3). A screw-cap vial of 1 M NaOH incubated without soil is used as a blank (one blank for every 25 soil samples is suggested for sets of ~100 soil samples). Concentration of HCl must be standardized [either certified purchase or through titration against tris(hydroxymethyl)aminomethane]. Quantity of CO_2 evolved from a sample is calculated according to the following:

$$CO_2\text{–C (mg kg}^{-1}\text{ soil)} = (\text{mL}_{[\text{blank}]} - \text{mL}_{[\text{sample}]}) \times N \times M/S$$

where N = normality of acid (mol L^{-1}; e.g., 1), M = mass conversion from $cmol_c$ to g C (6000), and S = soil weight (g; e.g., 100 g).

Procedural Developments and Evaluation

Soil microbial activity is an important component of soil health determination, but long-term incubation of field-moist soil has been considered the standard. Determining the flush of CO_2 during 3 d following rewetting of dried soil was an important step toward developing a rapid protocol that could be considered in soil testing laboratories (Franzluebbers et al., 2000). Commercial development of the procedure (https://solvita.com/soillabtest) has led to growing interest in using the flush of CO_2 as a soil health

indicator, although the value of the procedure does not need to be tied to a commercial product. The commercial protocol has some variations of noted interest: (i) 40 g of soil only, (ii) rewetting by capillary action from a pool of water to near saturation, (iii) 1 d of incubation, and (iv) alkali gel impregnated onto a paddle for determination of CO_2 evolved. A strong correlation (r^2 = 0.93, n = 32) between CO_2 evolved during 1 and 3 d was shown for soils in Texas (Franzluebbers et al., 2000). Approximately 2.5 times lower CO_2 can be expected in 1 d than in 3 d. The shorter time and lower weight of soil translate simply into smaller values that may result in lower precision. If the amount of water is considerably greater than 50% water-filled pore space, flush of CO_2 values will be lower (Fig. 1). For example at 80% water-filled pore space, flush of CO_2 was 0.83 ± 0.10 that at optimum water-filled pore space. Haney et al. (2008) compared use of alkali gel with liquid alkali as well as with infrared gas analysis and found strongest relationships between titration with liquid alkali and infrared gas analysis (r^2 = 0.95, n = 36). The digital color reader for the gel technique had weaker correlation with titration (r^2 = 0.82) and infrared gas analysis (r^2 = 0.79).

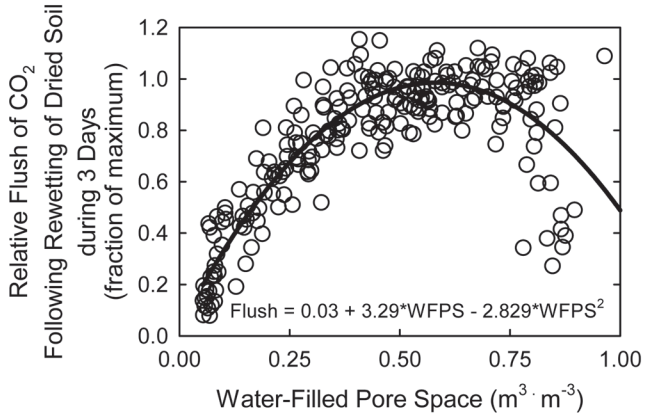

Fig. 1. Relationship between the relative flush of CO_2 following rewetting of dried soil and water-filled pore space from 15 Ultisols in Georgia at two different compaction levels. Data from Franzluebbers (1999a). Maximum flush of CO_2 was 166 ± 25 mg CO_2-C kg^{-1} soil 3 d^{-1}.

Development for Soil Testing

A number of laboratories are now offering the commercialized test of the flush of CO_2 (https://solvita.com/soil/map). This indicates strong interest by customers for this type of information. However, legitimate concerns can be expressed—for example, what do the values indicate, is there a threshold value to be achieved, how should management change based on these values to improve soil health? Certainly, higher values express greater potential soil microbial activity stored in readily available C substrates. Strong correlation of the flush of CO_2 with soil microbial biomass C and potential N mineralization in a number of studies reflects its microbial origins (Franzluebbers and Stuedemann, 2003). The actual soil microbial biomass C level needed to achieve excellent soil health across a diversity of soils has not been determined.

One important area of prediction that has high monetary value to producers and high ecological significance to society is determining changes in N fertilizer requirements of crops. An indication of this predictive capacity was shown in the strong relationship (r^2 > 0.91 in each year, r^2 = 0.31 across 2 yr) of the flush of CO_2 with crop N uptake in a field study in Texas (Haney et al., 2001). Calibration of the flush of CO_2 to N availability in the field remains a weak link but is absolutely critical in utilizing the test as a predictive measure of biologically available N. Since most soil testing facilities lack a test for biologically derived N, the flush of CO_2 could fill a critical void.

Characterizing N availability from inorganic and organic sources is needed to improve fertilizer N recommendations (Fig. 2). Inorganic N in surface soil, as well as residual inorganic N in the profile before possible leaching below the root zone, is necessary to quantify and understand but difficult to assess due to it spurious nature. Currently, the weakest link in characterizing available N sources is from biologically active organic matter. Research is underway to conduct calibration studies relating the flush of CO_2 to N availability for various crops in a number of soils and management conditions. Greenhouse growth studies, for example, are being conducted to verify the relationship between the flush of CO_2 and plant N uptake (as indicated by plant dry matter accumulation in Fig. 3). The size of the flush of CO_2 relates well to net N mineralization during

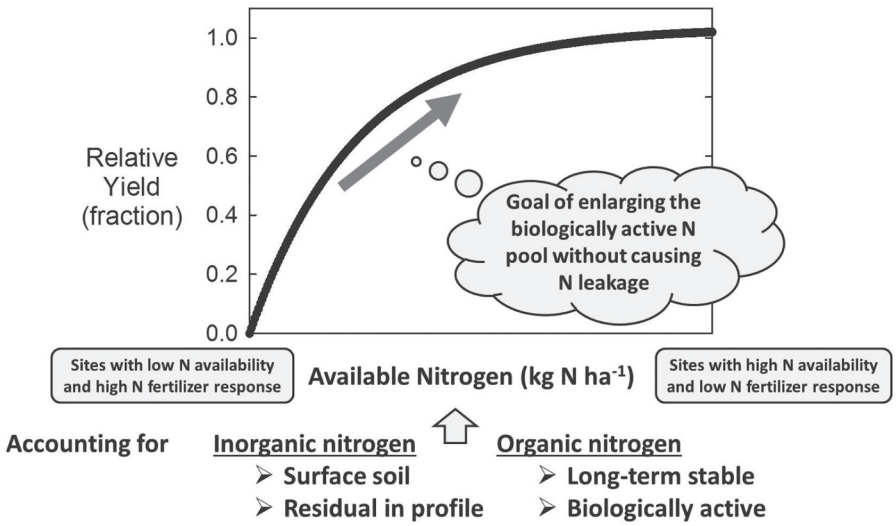

Fig. 2. Idealized N response accounting for inorganic and organic sources of soil N and conceptual scheme for separating N responsive and nonresponsive sites.

longer-term soil incubations and plant growth trials. Field trials are also being conducted to assess pre-plant soil testing of the flush of CO_2 against yield response to in-season N applications (Fig. 4). Strong relationships continue to be found between the flush of CO_2 and N availability—previously mostly in highly controlled laboratory incubations (r^2 = 0.90, n = 5, Franzluebbers [1999b]; r^2 = 0.72, n = 120, Franzluebbers and Brock [2007]; r^2 = 0.82, n = 646, Franzluebbers and Stuedemann [2008]) but now also in semicontrolled greenhouse conditions and noncontrolled field conditions (preliminary data in Fig. 3 and 4; unpublished data). Great potential exists to expand such calibration studies in a wide array of geographical settings.

This letter emphasizes a clear linkage of "soil health" sampling via the flush of CO_2 with a worldwide dilemma for better N management, to meet crop N demand with a more accurate exogenous supply of N for optimizing profitability and environmental quality. Sufficient N should be supplied to crops that need it, while excess N must be avoided when crops are satisfied with N supply to avoid environmental deterioration. Fine-tuning N fertilizer recommendations to within 20 to 40 kg N ha^{-1} of actual need should be possible with preplant sampling and analysis of the flush of CO_2 and other soil characteristics—a strategy that could help local producers gain more profit, help the global farming community produce more food, and help protect the environment regionally and globally. The question of whether soil testing services should measure soil biological activity may have been recently spurred by a groundswell of interest in such a test, but the answer must be underpinned by scientific rigor at multiple points of evaluation, that is, correlation, calibration, and recommendation. Correlations have been conducted (Schomberg et al., 2009), and calibration studies are being conducted. The need for further field testing remains to make robust recommendations, but the foundation for this testing is clear.

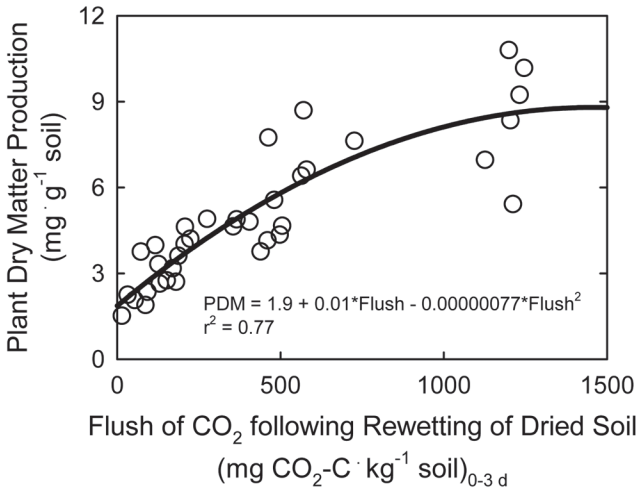

Fig. 3. Preliminary results relating the flush of CO_2 to plant dry matter production under greenhouse conditions (M.R. Pershing, unpublished data). Soils (Typic Kanhapludults) were samples from a long-term pasture–crop rotation study in Watkinsville, GA (Franzluebbers and Stuedemann, 2015).

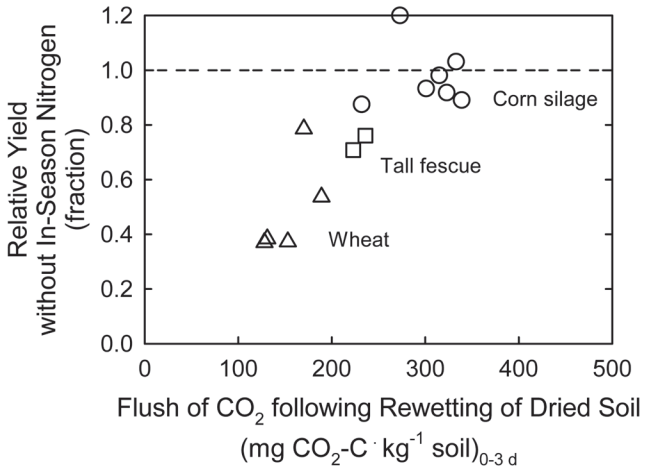

Fig. 4. Preliminary results relating the flush of CO_2 to relative crop yield response to N fertilizer (yield without in-season N compared with yield with highest N rate) (A.J. Franzluebbers, unpublished data; collaboration with S. Gibson on wheat [*Triticum aestivum* L.], C.D. Teutsch on tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.], and J. Cline, A. Lipscomb, T. Mize, and R.S. Shoemaker on corn [*Zea mays* L.]silage).

Acknowledgments

Funding is from USDA-Agricultural Research Service, USDA-NIFA-AFRI Competitive Grant No. 2013-67019-21369, USDA-NRCS-CIG Agreement No. 69-3A75-14-251 (Matt Poore as Principal Investigator), and North Carolina Small Grain Growers Association Agreement No. 58-6070-2-113. I thank Rick Haney for continuous inspiration over the years, as well as many current collaborators for their encouragement. Sound technical support was provided by Steven Knapp, Erin Silva, and Ellen Leonard.

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