

Soil Organic C:N vs. Water-Extractable Organic C:N

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ABSTRACT

Traditionally, soil-testing laboratories have used a variety of methods to determine soil organic matter, yet they lack a practical method to predict potential N mineralization/immobilization from soil organic matter. Soils with high microbial activity may experience N immobilization (or reduced net N mineralization), and this issue remains unresolved in how to predict these conditions of net mineralization or net immobilization. Prediction may become possible with the use of a more sensitive method to determine soil C:N ratios stemming from the water-extractable C and N pools that can be readily adapted by both commercial and university soil testing labs. Soil microbial activity is highly related to soil organic C and N, as well as to water-extractable organic C (WEOC) and water-extractable organic N (WEON). The relationship between soil respiration and WEOC and WEON is stronger than between respiration and soil organic C (SOC) and total organic N (TON). We explored the relationship between soil organic C:N and water-extractable organic C:N, as well as their relationship to soil microbial activity as measured by the flush of CO₂ following rewetting of dried soil. In 50 different soils, the relationship between soil microbial activity and water-extractable organic C:N was much stronger than for soil organic C:N. We concluded that the water-extractable organic C:N was a more sensitive measurement of the soil substrate which drives soil microbial activity. We also suggest that a water-extractable organic C:N level >20 be used as a practical threshold to separate those soils that may have immobilized N with high microbial activity.

Keywords: Soil Microbial Activity; C:N Ratios; Soil Organic C; N Mineralization; N Immobilization; Soil Testing

1. Introduction

Soil microorganisms are the centerpiece of biogeochemical cycling of nutrients in soil. Soil fertility is directly related to, and defined by, the heterotrophic activity of soil microbes as a whole [1]. While shifts in soil microbial community composition and structure are indicators of altered environmental conditions or management [2-4], the link between microbial composition and soil function is highly variable and thus limited as a general parameter for predicting soil activity rates. Thus, the integrated response of the soil microbial community is needed to elucidate and predict soil nutrient availability for the management of one of our most important resources, soil.

The metabolically-active component of soil can be measured in its simplest form as emission of CO₂, which corresponds to nutrient availability, moisture, and temperature, and can be rapidly quantified [5]. Emission of CO₂ can reveal the broader impacts of management, crop

biodiversity, and climatic changes, but also has predictive capabilities for specifically assessing soil-nutrient release. Measurement of soil microbial activity, in conjunction with other soil physical and chemical properties and processes, can be a valuable tool for developing a complete profile for soil fertility and may be used to increase the efficiency of fertilizer recommendations.

Microbial mineralization/immobilization of soil N can be broadly estimated using soil organic C:N [6]. Based on years of research on conversion rates of decomposable organic matter by soil fungi and bacteria, soil organic C:N of 20 is generally considered to be a threshold point where either net N mineralization or net N immobilization occurs [7,8]. In reality, both N mineralization and immobilization occur simultaneously in soil, making it difficult to predict the amount of available soil N from net N mineralization alone. Correlation between the soil C:N ratio and N immobilization and mineralization is unclear, partly due to early work that was limited to measurements of net N rates. Variations of the C:N ra-

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tios of different pools of organic matter and variations of the C and N assimilation efficiency of the microbial biomass may also confound the usefulness of the soil C:N ratio to predict gross N transformation rates [8].

Current literature suggests that soil respiration rates can be used to predict soil N mineralization/immobilization rates and provide an estimate of soil N mineralization potential [9,10]. While soil CO_2 -C respiration rates have been shown to be correlated to N mineralization [8,11], respiration alone is not an indicator of N immobilization and may not accurately predict net N mineralization/immobilization. As a result, a modified approach couples soil organic C:N with the soil respiration rate for modeling net N mineralization rates in soils [12,13]. High soil microbial activity does not always lead to high N mineralization due to immobilization that can occur; however, determining the C:N ratio from a much smaller more active pool of C and N to soil microbial activity could increase the accuracy of predicting the net mineralization/immobilization. A more sensitive and effective approach may be to assess the much smaller fractions of water-extractable organic C and N, which are highly related to soil microbial activity [14]. Results of a review paper on N cycling focus the importance of both substrate quantity (as C and N concentration) and quality (as C:N ratio) for N cycling rates [9].

In this study, we wanted to explore the link between the release of CO_2 following rewetting of dried soil and soil organic C:N vs. water-extractable organic C:N. We hypothesized: 1) soil microbial activity may be more strongly correlated with water-extractable organic C and N concentrations than soil organic C and N concentrations and 2) C:N ratio calculated from the water-extractable organic fraction may be an additional tool in conjunction with the flush of CO_2 following rewetting of dried soil to better predict plant available N and N immobilization.

2. Materials and Methods

Soil was collected from agricultural fields in Idaho, Georgia, Maine, Mississippi Oklahoma, Texas, and Wyoming. Crop management varied: (till/no-till, continuously cropped/crop rotation) along with soil type. Soils had clay content from 10 to 55% (data not shown), pH from 5.56 to 8.02 (**Figure 1**), and soil organic C from 3.63 to 41.31 $\text{g}\cdot\text{C}\cdot\text{kg}^{-1}$ soil (**Figure 2**).

All samples were dried overnight at 50°C and ground to pass a 2-mm sieve. Soil organic C (SOC) and total N (TN) were determined on 2 g subsamples using dry combustion (Elementar; Hanau, Germany). Water-extractable organic C (WEOC) and water-extractable N (WEN) were determined from 4 g of dry soil with 40 mL of deionized water and shaking for 10 minutes on a mechanical shaker (Eberbach, Ann Arbor, MI). Samples were then centri-

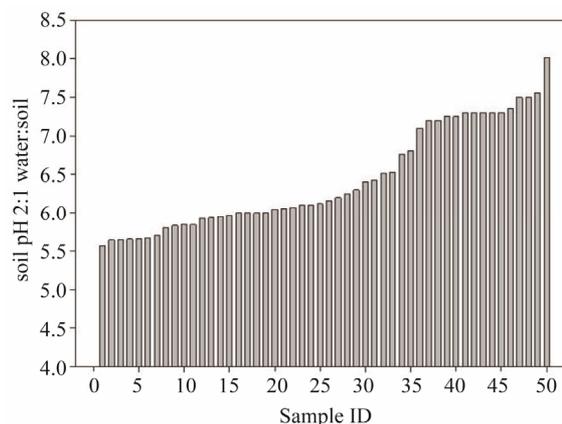


Figure 1. Soil pH range.

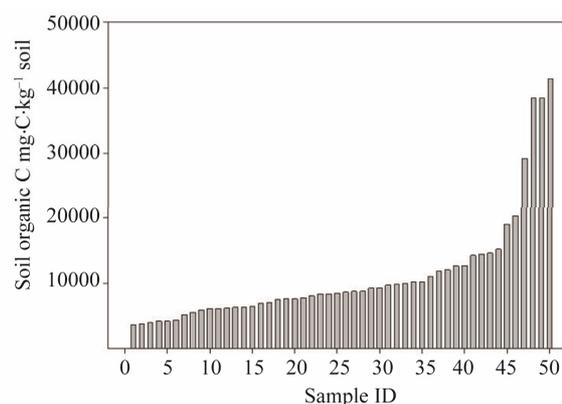


Figure 2. Range in soil organic C.

fused for 5 minutes at 3500 rpm, filtered through Whatman 2 V paper, and analyzed for WEOC and WEN (Apollo 9000, Teledyne-Tekmar; Mason, Ohio). Inorganic NH_4 -N, and NO_3 -N concentrations were also determined (Flow Solution IV, OI Analytical; College Station, TX). Soil organic N (SON) was calculated by subtracting inorganic N content (NH_4 -N and NO_3 -N) from total N. Water-extractable organic N (WEON) was calculated by subtracting inorganic N content (NH_4 -N and NO_3 -N) from WEN.

The release of CO_2 from 24 hour incubation after rewetting dried soil is directly related to the fertility of a given soil; the method is designed to mimic the natural soil drying and rewetting cycle and is designed to be readily adopted by soil testing labs. The flush of CO_2 in 24 hours (1-day) following rewetting of dried soil was determined from 40 g subsamples in 50 ml polypropylene disposable beakers (Fisherbrand Cat. No. 01-291-10) with four to five 6.35-mm holes drilled in the bottom. A Whatman GF/D 4.25-cm glass microfibre filter (Cat No. 1823-042) was placed in the bottom of each beaker to prevent soil loss. The beaker and Solvita[®] gel paddle were placed in a gas-tight 250-mL glass jar filled with 25 mL of water and sealed with a screw-top lid; the jar had a

convex bottom to allow for drainage. Capillary action was used to rewet soil according to its water holding capability [15]. Soils were incubated at 25°C, and respired CO₂ was trapped during 24 h. The quantity of 1-day CO₂-C released was determined using a digital-color reader (DCR) (www.solvita.com). The Solvita system for estimating the flush of 1-day CO₂ following rewetting of dried soil has been shown to be highly correlated with the commonly used titration method and 1-day CO₂-C IRGA method [5]. SigmaStat imbedded in SigmaPlot ver. 12.1 was used for linear regression analysis.

3. Results and Discussion

Soil organic C and SON were highly related ($r^2 = 0.93$), as were WEOC and WEON ($r^2 = 0.84$). Interestingly, ratios of SOC to SON and WEOC to WEON were nearly similar (10.8 and 10.9, respectively), suggesting that WEOC and WEON are a subset of the much larger SOC and SON pools (**Figures 3 and 4**). Some soil test labs currently use SOC data determined from various methods (loss on ignition, titration, and combustion) to estimate potential N release, while few if any use SOC:SON to estimate potential N mineralization. Since both substrate availability and SOC:SON have a strong influence on N mineralization rates [9], it is conceivable that WEOC:WEON could be a better method for determining the state of potential N mineralization/immobilization as an alternative to SOC:SON. However, our data indicate that SOC:SON and WEOC:WEON were poorly related (**Figure 5**). This weak relationship may be attributed to the fact that SOC and SON were roughly 40 times larger than WEOC and WEON fractions (**Figures 3 and 4**). The water-extractable organic fraction, though significantly smaller than total SOC and SON, is a critical component used by soil microorganisms that drive the nutrient cycling system [16-19].

We found that soil microbial activity measured as the flush of 1-day CO₂ following rewetting of dried soil was significantly correlated to SOC, SON, WEOC, and WEON. Water-extractable organic C and WEON, however, had a stronger relationship with the flush of 1-day CO₂ than SOC and SON (**Table 1**).

This finding is consistent with documented findings [16-19] and the authors' theory that the water-extractable C and N pool is the more readily available energy pool for soil microbes as compared to the total SOC and TON pools. In this data set, organic N accounted for 97.4% of the total soil N, while organic N accounts for 53% of the water extractable total N. While SOC:SON may be a useful indicator of soil quality/fertility, it may not be sensitive enough to reliably quantify the quality of organic matter that soil microbes are actively mineralizing due to the sheer size of the C and N pools compared to the water extractable C and N pools. Therefore,

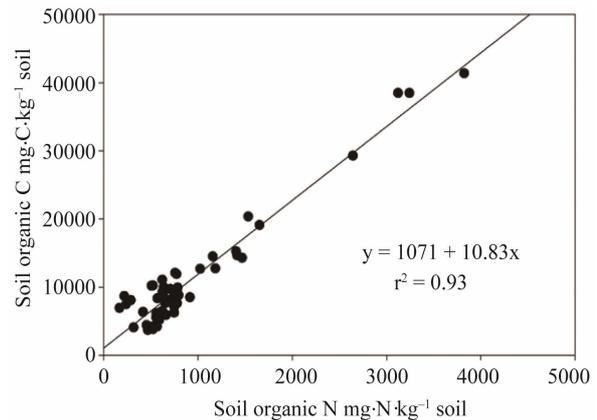


Figure 3. Soil organic N vs. soil organic C.

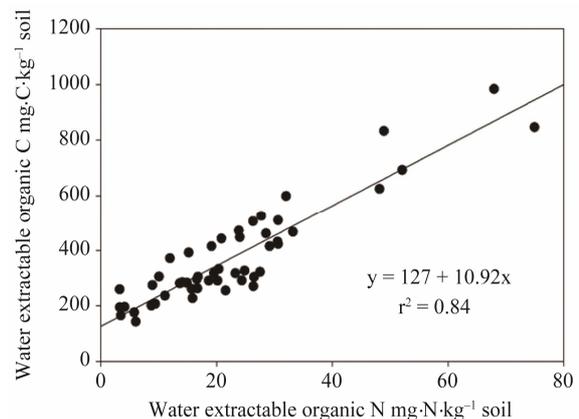


Figure 4. Water extractable organic N vs. water extractable organic C.

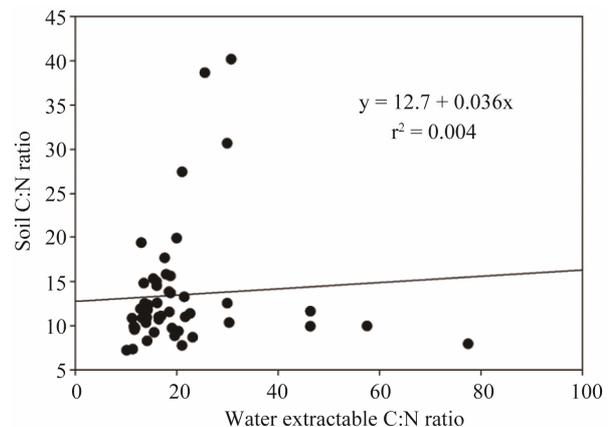


Figure 5. Soil C:N ratio vs. water extractable soil C:N ratio.

WEOC:WEON could be considered a more accurate property to predict variations in mineralization/immobilization potential of a given soil as compared with SOC:SON.

It may be possible to combine the flush of CO₂ following rewetting of dried soil with WEOC:WEON and develop a potential N mineralization/immobilization

Table 1. Correlation table showing correlation coefficients (r) comparing 1-day CO₂-C (mg·C·kg⁻¹ soil) and total and water-extractable organic C and N concentrations (mg·C·kg⁻¹ soil; mg·N·kg⁻¹ soil). All correlations were significant ($P \leq 0.0001$; $n = 50$).

	TOC	TON	WEOC	WEON
1-Day CO ₂ -C	0.704	0.682	0.874	0.869
TOC	.	0.965	0.755	0.737
TON	.	.	0.725	0.736
WEOC	.	.	.	0.919

indicator (**Figure 6**). Soil organic C:N > 20 reflects reduced N availability due to greater immobilization of N [20,21], although the breakpoint can be as high as 30, depending on the [22]. Using a threshold SOC to SON value of 20, above which no net N mineralization would occur, our results indicate N immobilization in 4 of 50 (8%) soil samples, whereas when using WEOC to WEON, 16 of 50 (30%) soil samples immobilized N (**Figure 6**). This information suggests that the WEO C:N ratio may be more indicative of the quality (water extractable organic C) of the organic C as opposed to quantity (soil organic C) of substrate available for soil microbial activity, which is an important point since easily decomposable substrate should translate into N mineralization release. The importance of characterizing the quality vs. the quantity of organic C is a furtherance of the sensitivity that is revealed by a nutrient cycling system that is driven by C. We define the active soil C pool (WEOC) as one that is easily mineralizable and therefore a direct driver of microbial activity (1-day CO₂) responsible for providing N and P mineralization in soil. Utilizing these three pools (WEOC, WEON, 1-day CO₂) in a soil testing environment could increase our awareness of soil microbial activity and possibly help us account for an often overlooked source of N that can be easily subtracted from fertilizer recommendations. This will have a two-fold effect: 1) Save producers input costs in terms of reduced fertilizer input and 2) reduce the N and P loading in soil which is subject to loss from erosion, leaching, denitrification, and leaching that will ultimately affect the quality of our drinking water and water bodies, both freshwater and saltwater.

A more sensitive C:N indicator combined with a rapid method for soil microbial activity would help soil test labs offer reliable estimates of the mineralization/immobilization state of soil. Soil samples are usually taken prior to planting, which is a time when this information would be most needed for fertilizer decisions by producers. Spring sampling would be at a time of the year when the soil microbial community would have stabilized over winter following decomposition of the previous crop.

Currently there are no standardized universal soil test

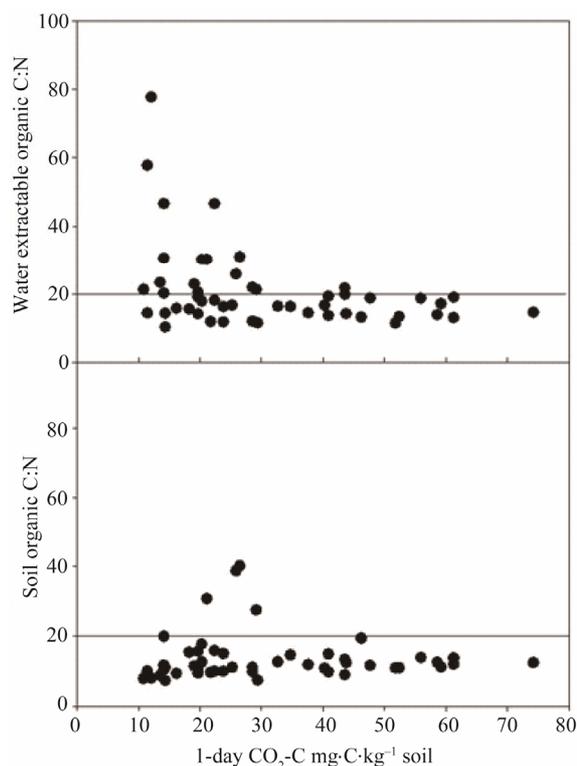


Figure 6. 1-day CO₂-C vs. soil organic C:N and water extractable organic C:N.

methods for quantifying the active portion of soil. Consequently the majority of soil test labs ignore the active soil C pools. In terms of N, soil labs often test for total and inorganic N, while only a few test for ammonium. On the other hand, some labs do not soil test for N at all. Because the cost for fertilizer usually accounts for roughly 30% - 40% of the total budget of a modern farming system, it is a shame that all available N resources are not measured and accounted for. Developing soil test methods to account for soil C and N pools that contribute to plant-available nutrients is a necessary step towards improving resource efficiency and reducing input cost. In addition to using WEOC: WEON instead of SOC:SON, in future research we intend to investigate the possibility of using a sliding scale for WEOC: WEON that can be developed and compared to yield in unfertilized and unamended plots. The sliding scale would begin with a linear response and represent increased N mineralization potential as WEOC:WEON declines. This sliding scale could be quickly evaluated by geographic area using WEOC:N and CO₂-C respiration data and test plots for a variety of crops and forages. For example, higher C:N ratios would predict less N mineralization as compared to low C:N ratios. Setting a break-point of 20:1 above which no potential N mineralization occurs could reduce the possibility of under-fertilizing based on recommendations from soil microbial activity alone. This

would represent a safety factor to guard against high soil microbial activity (CO₂ respiration) with little N released when N is immobilized due to high WEOC:WEON.

In preliminary investigations, we found that soils with low WEOC:N ratios released more N than soils with high WEOC:N ratios. We experienced this in fields here in Texas that had legume cover crops vs. no cover crops for two years. The C:N ratio from a water extraction averaged 10:1 in cover crop soils vs. 16:1 in non-cover crop soils. Both of these fields were in a replicated corn-wheat rotation. Wheat yields increased 10 bu and corn yields increased 20 bu in the cover crop vs. no cover-crop fields, which corresponds to the WEO C:N ratios from the two treatments (data not shown). The sliding scale WEOC:N ratio could be developed by geographic areas based on local climatic conditions which would require crop or forage yield, a total C:N analyzer for water extraction, Solvita DCR and paddle kit, and inorganic N analyzer (both NH₄-N and NO₃-N). Overall this future research could provide insight into the application of the previously described methods and their application to both conventional and organic farming systems.

4. Conclusions

Our data suggests the C:N ratios determined from soil water extractions are likely to be more sensitive than total soil C:N to microbial activity and therefore can be a better measurement of the impact of management inputs. Determining the quality as opposed to the quantity of organic C could enhance our ability to rapidly measure impacts on soil microbial activity which is an active reflection of soil health. This information can be applied to track the relative impact of management decisions on soil fertility ultimately improving the efficiency of management decisions. Combining WEO C:N ratios with microbial activity could be used as a rapid soil biology indicator with healthy soil having a WEO C:N ratio below 20:1 and microbial activity between 30 - 50 ppm C. Tracking soil biology improvement or degradation is currently difficult due to the lack of a proper tool and rapid, accurate analytical methods. Introducing management schemes to improve the C:N ratio and increase microbial activity should result in increased soil fertility/soil biology and highly productive and sustainable systems. Using soil testing labs to measure soil biology and tracking management inputs based on soil biology/soil fertility improvements would be a step towards true sustainability that can be easily acquired through adoption of both the water extraction method for determining organic C and N and the Solvita soil respiration method.

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