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Estimating Soil Carbon, Nitrogen, and Phosphorus Mineralization from Short-Term Carbon Dioxide Respiration

R. L. Haney,[1] W. H. Brinton,[2] and Eric Evans[2]

[1]United States Department of Agriculture, Agricultural Research Service, Temple, Texas, USA
[2]Woods End Laboratories, Inc., Mt. Vernon, Maine, USA

Abstract: The measurement of soil carbon dioxide (CO_2) respiration is a means to gauge biological soil fertility. Test methods for respiration employed in the laboratory vary somewhat, and to date the equipment and labor required have limited more widespread adoption of such methodologies. A new method to measure soil respiration was tested along with the traditional alkali trap and titration method. The new method involves the Solvita gel system, which was originally designed for CO_2 respiration from compost but has been applied in this research to soils with treatments of increasing dairy manure compost. The objectives of this research are to (1) examine the relationship between the CO_2 release after 1 day of incubation from soils amended with dairy manure compost that have been dried and rewetted as determined using the titration method and the Solvita gel system, and (2) compare water-soluble organic nitrogen (N), as well as carbon (C), N, and phosphorus (P) mineralization after 28 days of incubation with 1-day CO_2 release from the titration method and Solvita gel system. One-day CO_2 from both titration and the Solvita gel system were highly correlated with cumulative 28-day CO_2 as well as the basal rate from 7–28 days of incubation. Both methods were also highly correlated with 28-day N and P mineralization as well as the initial water-extractable organic N and C concentration.

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Address correspondence to R. L. Haney, United States Department of Agriculture, Agricultural Research Service, 808 E Blackland Rd, Temple, TX 76502, USA. E-mail: rhaney@spa.ars.usda.gov

The data suggest that the Solvita gel system for soil CO_2 analysis could be a simple and easily used method to quantify soil microbial activity and possibly provide an estimate of potential mineralizable N and P. Once standardized soil sampling and laboratory analysis protocols are established, the Solvita method could be easily adapted to commercial soil testing laboratories as an index of soil microbial activity.

Keywords: CO_2 evolution, soil microbial activity, soil testing, water extractable C

INTRODUCTION

Soil respiration is an important aspect of soil quality and an indicator of soil fertility (Staben et al. 1997). As early as 1931, Smith and Humfeld noted that during decomposition of green manures, the numbers of bacteria followed the carbon dioxide (CO_2) evolution, which rose rapidly during the first 4 days and then declined to a fairly constant level. Even earlier, Gainey (1919) noticed a parallel formation of CO_2, ammonium (NH_4)-N, and nitrate (NO_3)-N in soil. In 1924, Lebedjantzev stated that drying soil at a low temperature appeared to increase the fertility of the soil, which he noticed also occurred in nature. For roughly 90 years, CO_2 respiration from soil has been used as an indicator of the relative fertility of various soils (Gainey 1919; Lebedjantzev 1924; Birch 1960). The purposes of many of these studies mainly concern the rates of carbon (C), nitrogen (N), or phosphorus (P) mineralization in an effort to gain a clearer understanding of these natural processes.

Soil CO_2 respiration has been widely used for many years to quantify the impact of various treatment and management inputs on soil microbial activity. Research has shown that soil respiration from drying and rewetting (D/R) soil is simple to employ and can be used to separate treatment differences in soils following the addition of dairy cattle manure (Haney, Franzluebbers, and Hons 2001).

Franzluebbers (1999) found that the C mineralized in 3 days from soils dried and ground for laboratory analysis that were rewetted was strongly correlated with 24-day C mineralization from undisturbed soil. Furthermore, short-term C mineralization (1–3 days) from soil after drying (40 or 60 °C) followed by rewetting correlates strongly with longer term (100-day) CO_2 evolution and soil microbial biomass C (Franzluebbers et al. 2000; Haney et al. 1999). Research also shows that the C mineralized after drying soils at various temperatures, representing laboratory and field moisture, was highly correlated with 24-day N mineralization (Haney et al. 2004). Anderson and Domsch (1978) suggested that the size of the soil microbial biomass is reflected by the short-term flush of CO_2 after amendment with labile substrates. This is the basis for the substrate-induced respiration

(SIR) method used to quantify soil microbial biomass. Substrate-induced respiration also occurs in soil that has been dried and rewetted, where the natural release of intercellular compounds due to osmotic shock from microbial cells and resulting increased availability of previously unavailable organic C and N (Fierer and Schimel 2002) serve as the substrate. Natural soil D/R occurs many times throughout a growing season with each rainfall event and subsequent air drying. Laboratory D/R tends to produce a uniform release of C and N, which mimics natural processes that occurs under field conditions (Birch 1958, 1959, 1960).

It may be possible to estimate soil C, N, and P mineralization potential by monitoring the fluxes of CO_2 following the rewetting of dried soil (Marumoto, Anderson, and Domsch 1982; Sparling et al. 1995), especially in soils that have had manure or compost amendments (Haney, Franzluebbers, and Hons 2001). Given that soil microbes drive nutrient mineralization, developing a soil-test method based on rapid (1-day) CO_2 evolution after D/R may offer insight into estimating a soil's ability to supply N and P. Castellanos and Pratt (1981) found that soil CO_2 (7-day incubation) respiration was an excellent indicator of available N in soil that had been amended with manure over a 10-month greenhouse experiment. They speculated that soil CO_2 released in as little as 2–3 days might also prove to be equally effective at estimating available N. Research indicates that the quantity and quality of substrates (C and N) available for mineralization may be measured using CO_2 evolution (Sorensen 1974; Sparling and Ross 1988).

Schimel and Bennett (2004) make a strong case for rethinking our approach to estimating N mineralization by also considering the contribution of N from the water-soluble organic N pool. Recent literature has indicated that soil amino acids and amino sugars may be taken up directly by plants after polymerization of organic N compounds (Chapin, Matson, and Mooney 2002; Fierer et al. 2001).

Because organic systems rely almost exclusively on the mineralization–polymerization of N and P from organic sources by microorganisms for acquiring nutrients, the application of this method in soil-testing laboratories is especially important for organic farming. The development of the relationship between potential N and P mineralization and CO_2 evolution from soils after D/R will allow us to account for potential mineralizable N and P and adjust fertilizer recommendations. This knowledge will enable us to make more informed fertilizer management decisions, useful for all growers. In addition, monitoring CO_2 evolution from soils will allow us to track our soil-management performance. After the proper standardization of CO_2 analysis including sampling time and rewetting methodologies, soil-testing laboratories could adopt a method of rapidly quantifying the soil CO_2 release after D/R as an index of soil fertility.

Chemical titration for soil CO_2 respiration is an effective means whereby different soils can be compared for microbial activity. Soils are incubated along with an aqueous solution of potassium hydroxide (KOH) or sodium hydroxide (NaOH) in a small vial. The alkali reacts chemically with CO_2 and barium chloride ($BaCl_2$) and can be back-titrated with hydrochloric acid (HCl) to a phenolphthalein endpoint that is relative to the amount of CO_2 released by soil microorganisms (Anderson 1982). A control vial with no soil is included in the incubation to correct for the CO_2 in the jar at the initiation of the incubation. An equation is then employed to arrive at mg CO_2-C kg^{-1} soil. Although chemical titration has avenues for error associated with the procedure, it is a fairly simple and straightforward method. However, the method requires mixing the alkali and $BaCl_2$, an assumption that the control is accurate, care in titration, and accurately hitting the endpoint, which can induce error. Proper attention to the chemicals involved and especially the disposal of unprecipitated toxic $BaCl_2$ is also a requirement. Soil CO_2 respiration can also be measured with a gas chromatograph or an infrared gas analyzer for CO_2 detector (IRGA); however, these methods require expensive equipment that many soil-testing laboratories do not have readily available.

The Solvita gel system was designed to quantify the relative differences in CO_2 respiration between varying types of compost in an efficient and cost-effective manner. When measuring the CO_2 evolution from compost, a pH-sensitive gel (paddle) is embedded in a one-piece plastic holder that narrows to a point so that it can be pushed into the compost. After a specified time-period, the paddle can be removed from the compost and analyzed visually or optionally with a digital reader. The USDA Soil Quality Institute has listed the Solvita kit as an alternate soil respiration procedure (http://www.woodsend.org/pdf-files/USDA-SOIL-RESPIRATION.pdf) in its national soil-quality test kit program that released a full soil-quality test document (http://www.woodsend.org/pdf-files/kitguide.pdf). The Solvita chemistry gel technology differs from the standard method of measuring CO_2 evolution: it utilizes soil incubation with alkali traps so that it does not absorb all the CO_2 evolved from varying media but absorbs a relative concentration of CO_2 proportionate to the total concentration in the incubation vessel. The absorbed CO_2 produces a colorimetric reaction that is normally interpreted visually by comparison to a color key. In this project, we evaluated a more recent version of the kit in which gel color is read by a digital color reader (DCR) that incorporates diode array detection technology that selects intensity of red, green, and blue (RGB) emission. Using this approach allows very rapid measurement of accumulated CO_2 within the Solvita gel at any time during incubation, improves reliability, and significantly increases accuracy. The reactive gel with DCR appears

to closely obey Beer–Lambert's optical law over a wide range of concentrations of CO_2 and suffers only small interference from volatile fatty acids that form a positive response with CO_2 gels, consistent with an unstable compost condition. The Solvita system is almost error free, because it involves placing the paddle in the soil or compost, removing it after the allotted time period, placing it in the reader, and pressing a button. In our research, the Solvita system is used to measure microbial respiration in soils amended with compost.

The objective of this research is to (1) examine the relationship between the CO_2 release after 1 day of incubation from soils amended with dairy manure compost that have been dried and rewetted as determined using the titration method and the Solvita gel system and (2) compare water-soluble organic N, as well as C, N, and P mineralization after 28 days of incubation with 1-day CO_2 release from the titration method and Solvita gel system.

MATERIALS AND METHODS

Sample Characterization

The soil used in this study is classified as Houston Black by the Natural Resource Conservation Service and was obtained from the U.S. Department of Agriculture–Agricultural Research Service farm in Temple, Tex. Initial analysis of the soil shows that it contains 2.0% total organic C and 55% clay and has a pH of 8.1. Yearly rainfall at the research station is approximately 76 cm. For this experiment, the Houston Black soil was amended with composted dairy manure. Initial analysis of the dairy manure compost shows that it is comprises 12% total C, 1% total N, and 0.4% total P. The soil and the compost were dried at 40 °C for 24 h. The soil and the compost were ground to pass a 2-mm sieve. The composted dairy manure was added to the soil at the following rates: 0, 4.46, 8.82, 13.38, 17.84, 22.3, 26.76, 31.22, and 35.68 Mg ha^{-1} (0, 10, 20, 30, 40, 50, 60, 70, and 80 tons/acre). The compost and soil contained less than 3% moisture and was calculated on a dry basis.

The amended soils were extracted with water using a 10:1 extractant-to-soil solution and was shaken for 30 min. The soils were also extracted with H^3A (Haney et al. 2006) and shaken for 5 min. After shaking, the samples were centrifuged for 5 min at 3500 rpm and filtered through Whatman 2V filter paper. The water and H^3A extracts were analyzed for initial inorganic N and P using an OI Analytical Flow 4 autoanalyzer (OI Analytical, College Park, Tex.). Initial water-extractable total N and total organic C (TOC) were determined using an Elementar CN analyzer.

Initial organic N was calculated as the total N in the water extract minus the inorganic N from water extract.

Mineralization Analyses and CO_2 Evolution

Forty g of dried soil from each treatment were weighed into 50-ml plastic beakers in duplicate. Each sample was wetted to 50% water-filled pore space and placed into 1-quart mason jars with alkali traps consisting of 10 ml of 1 *M* KOH. The samples in the mason jars were incubated at 25 °C for 4 weeks. The alkali traps were replaced at 1, 3, 7, 14, 21, and 28 days. Unreacted alkali in the KOH traps was back-titrated with 1 *N* HCl to determine CO_2-C (Anderson 1982). The C mineralized from the amended soils equals the amount of CO_2 evolved in 28 days as measured from titration. The soil basal respiration (SBR) rate is calculated as the cumulative 28-day CO_2 minus the initial 7-day CO_2.

A beaker of soil was removed from each duplicate treatment at day 7, 14, 21, and 28 of the incubation. The soils were dried and extracted with H^3A as described previously for the initial analyses. The H^3A extracts were analyzed for inorganic N and P using an OI Analytical Flow 4 autoanalyzer. Nitrogen and P mineralized were calculated as the 28-day inorganic N and P minus the initial inorganic N and P, respectively.

Solvita Analysis

Additional samples (40 g) of each treatment were weighed into 50-mL plastic beakers and wetted to 50% water-filled pore space. The wetted samples were placed into 8-oz jars with lids accompanied by a Solvita gel paddle (http://www.solvita.com). The samples with the Solvita gel paddles were placed in the incubator at 25 °C for 1 day. After the 1-day period, the paddles were removed and placed in the Solvita digital reader for analysis of the CO_2 concentration.

Regression Analysis

Regression analysis (SigmaPlot ver. 10) was used to assess the strength or weakness of the relationship between the CO_2 evolved in 1 day as measured by the titration method and the Solvita method. The relationship between 1-day CO_2 evolved from each method was compared to initial inorganic N, 28-day mineralized N and P, CO_2 evolved in 28 days from the titration method, soil basil respiration (SBR), and water-soluble organic C. Water-soluble organic C was also compared

to water-soluble organic N and CO_2 evolved in 3 days as measured using the titration method.

RESULTS AND DISCUSSION

A surge of CO_2 was observed after D/R the soils (titration method), which leveled out by day 3 of the experiment (data not shown). The flush of CO_2 observed after D/R the soil is not thoroughly understood but may result from (1) nonliving soil organic matter becoming more susceptible to microbial attack, which induces the rapid mineralization of C from exposed aggregates (Adu and Oades 1978), and (2) the contribution of cellular lysing from water-induced osmotic shock to an easily mineralizable C pool that is consumed by the surviving soil microbes (Halverson, Thomas, and Firestone 2000). Studies indicate that both of these mechanisms are involved in the increased CO_2 release after D/R (Scheu and Parkinson 1994); however, it has been suggested that the increase in CO_2 production after D/R has more to do with intracellular water potential equilibrium than a physical disturbance of soil aggregates (Fierer and Schimel 2003).

The 1-day CO_2 release from both methods after D/R were compared with the cumulative 28-day CO_2 evolved (C mineralization) and 7- to 28-day C mineralization (Figure 1). The 7- to 28-day C mineralization data excludes the flush of CO_2 after D/R, yet is highly correlated with 1-day CO_2 measured from both the titration and Solvita methods ($r^2 = 0.84$ and $r^2 = 0.80$, respectively). The 7- to 28-day C mineralization is considered the SBR. These data support the hypothesis that the rapid release of CO_2 during the first day after D/R may be a snapshot of the active microbial biomass. If it were not, we could expect that the SBR would not correlate well with the flush of CO_2 occurring after the first day of incubation. These data suggest that the substrate providing the flush of CO_2 is proportional to the active microbial biomass, which is responsible for the mineralization of C and N.

The release of CO_2 (1-day incubation, titration, and Solvita methods) after D/R was highly correlated with initial organic N and C (Figure 2). The high correlation between initial organic N and C (Figure 3) indicates that the organic N measured may represent a portion of the microbial community present in the soil/compost mixture. In addition, the strong relationship between the 1-day CO_2 measured and initial organic N indicates that the organic N pool may serve as part of the food source for the microbial community surviving the osmotic shock after D/R. Water-soluble organic C is likely the main driving force behind the rapid release of CO_2 after D/R because microbial cells need roughly 10 times the amount of C as N to grow (Alexander 1977). The water-soluble organic C may represent C from both living and nonliving biomass: however, the

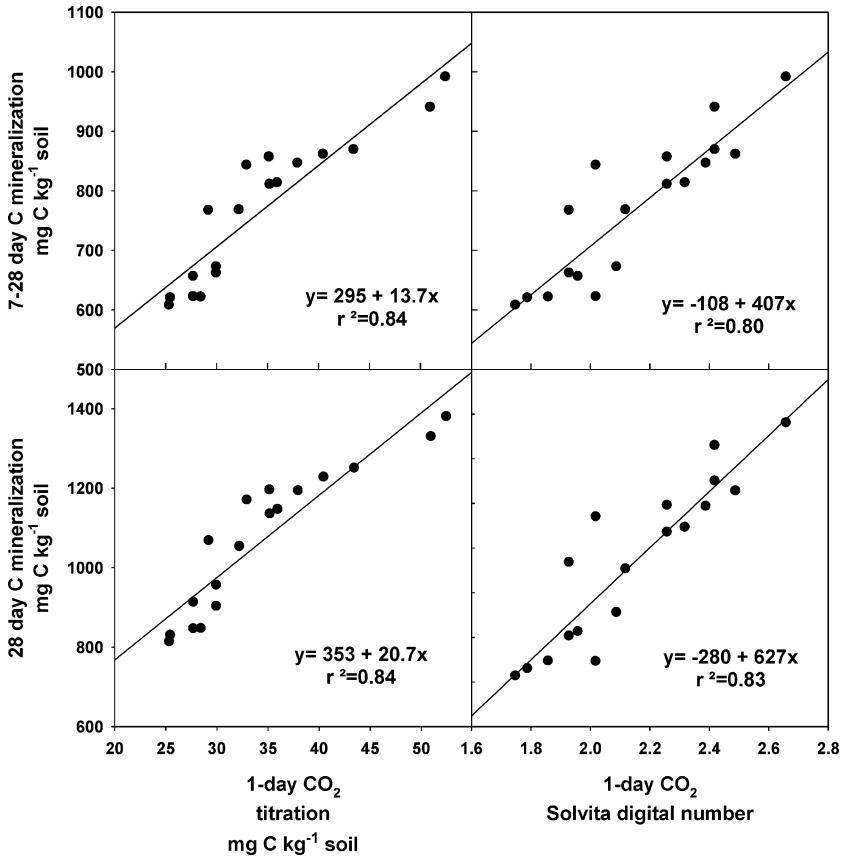

Figure 1. One-day CO_2 from titration and Solvita vs. 28-day C mineralization and 7–28 day C mineralization (basal respiration).

strong relationship between initial organic C and 1-day CO_2 evolution indicate that the contribution is mainly from the living biomass, which would be more easily and rapidly mineralizable than nonliving biomass (Fierer and Schimel 2002). The 1-day CO_2 from titration accounts for roughly 20% of the C rendered soluble by extraction, assuming the soluble C pool is the source of the CO_2 evolved. The remaining 80% of the C initially extracted appears to take another 2 days to deplete when viewing the slope of the regression line, which shows that more C has evolved as CO_2 than was initially water extracted (Figure 4). We have incubated many hundreds of soil samples over the years and usually always find that the increase in CO_2 greater than the basal respiration rate that is due to D/R is completed in 3 days. Based on these results and a review of the literature cited in this article, we believe that the release of CO_2 after D/R reflects the active microbial biomass prior to drying.

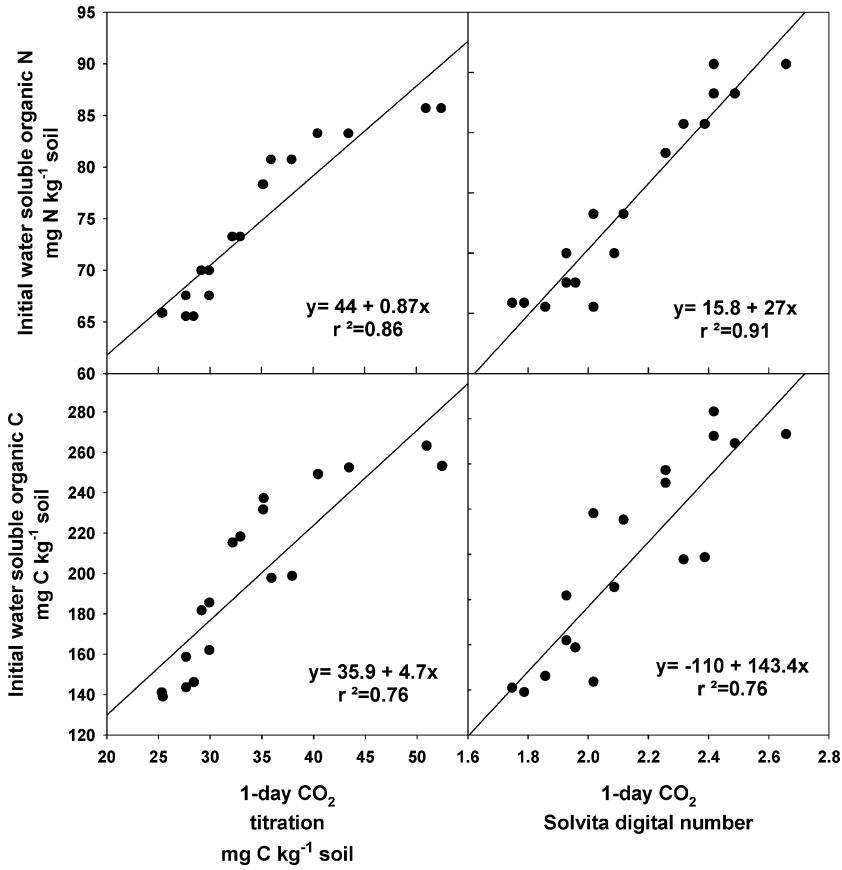

Figure 2. One-day CO_2 titration and Solvita vs. initial organic N and C.

Soil CO_2 respiration measured after 1 day using the titration and Solvita methods decreased with decreasing compost additions (Figure 5). These data suggest increased microbial activity following increasing compost addition and support idea that 1-day CO_2 analysis may be used to monitor soil-management strategies. For example, with each 4.46 Mg ha^{-1} (10-ton) rate of our compost, the 1-day CO_2 rate increased by 12.5%; this could be used to standardize the impact that certain composts have on soil microbial activity. The amount of CO_2 absorbed by the Solvita gel after the first day of incubation was highly related to 28-day N and P mineralization (Figure 6). Seventy-six percent of the variability in N mineralization can be predicted from the variance in 1-day CO_2 evolution when measured using the titration method, whereas 82% of the variability in N mineralization can be predicted using the Solvita method. A portion of the variability (64%) in P mineralization can be predicted by the variability in 1-day CO_2 as measured with the

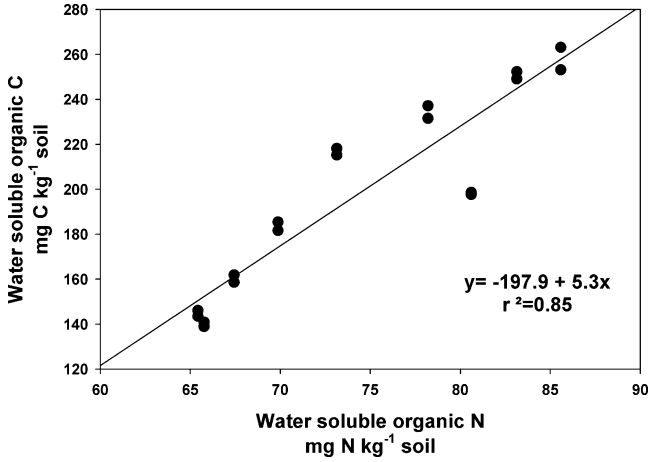

Figure 3. Water-soluble organic N vs. water-soluble organic C. Soils were extracted prior to wetting and incubation.

titration method, whereas 83% of the variability may be explained by the variability as measured with the Solvita method. These data indicate that there is indeed a connection between potential soil fertility and soil microbial respiration in the Houston Black soil amended with compost.

The titration method was highly related to the Solvita gel system for soil CO_2 respiration after a 24-h incubation ($r^2 = 0.84$; Figure 7). The relationship between 1-day CO_2 Solvita and the initial water-soluble organic N pool is stronger than the titration method and initial water-soluble organic N ($r^2 = 0.91$ vs. $r^2 = 0.86$; Figure 2). The titration method and the Solvita gel system were both highly correlated with 28-day N

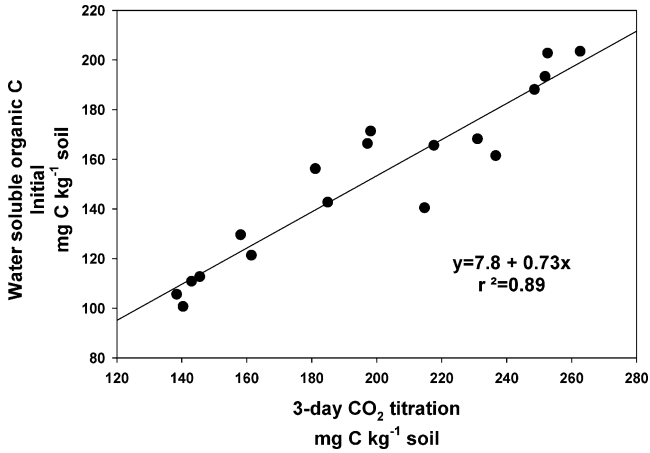

Figure 4. Three-day CO_2 titration vs. the initial water-soluble organic C pool.

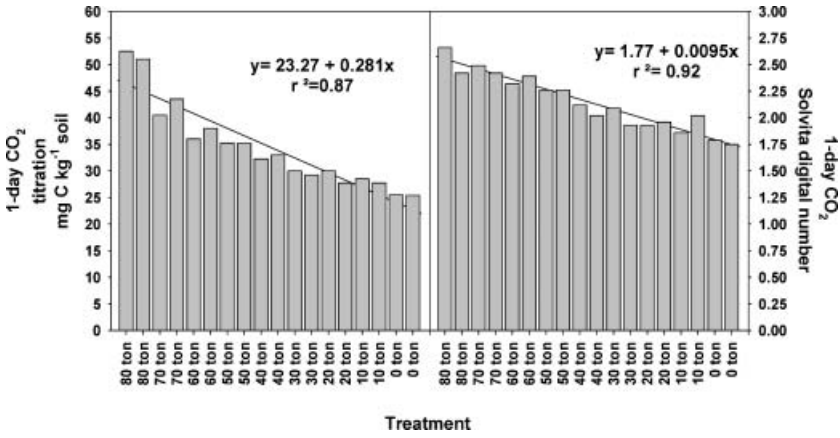

Figure 5. One-day CO_2 titration and Solvita with decreasing compost additions.

mineralization and water-soluble organic N, indicating the flexibility of this rapid (1-day) C mineralization test. These data indicate that the Solvita gel system would be an acceptable alternative to alkali traps for estimating soil C and N mineralization and initial water-soluble organic C and N. The Solvita gel systems as well as the titration method for 1-day CO_2 were strongly correlated with 28-day P mineralization (Figure 6). Both methods appear to accurately estimate the potentially mineralizable P pool in the soil tested, although the Solvita method shows a better correlation ($r^2 = 0.83$ vs. $r^2 = 0.64$).

CONCLUSIONS

Our results from these studies provide a number of indications for interpreting soil respiration data in view of estimating N mineralization and response of soil to amendments of compost. In untreated soils, short-term 1-day release of CO_2 following D/R correlated highly with long-term 28-day respiration rates. We further determined that use of alkali-trap CO_2 absorption techniques or Solvita test kit procedures with digital readout provided essentially the same information, and both CO_2 measures also correlated highly with the mineralization rate for N and P determined for 28 days.

In adding organic amendments to the soil over a range of 0 to 35.68 Mg ha^{-1} in the form of composted manure, a linear and highly significant correlation was observed in terms of short-term CO_2 rate following D/R, also again essentially the same whether estimated with alkali-trap methods or Solvita test kit. The rate of increase of CO_2, therefore, most likely corresponded to the net contribution of highly

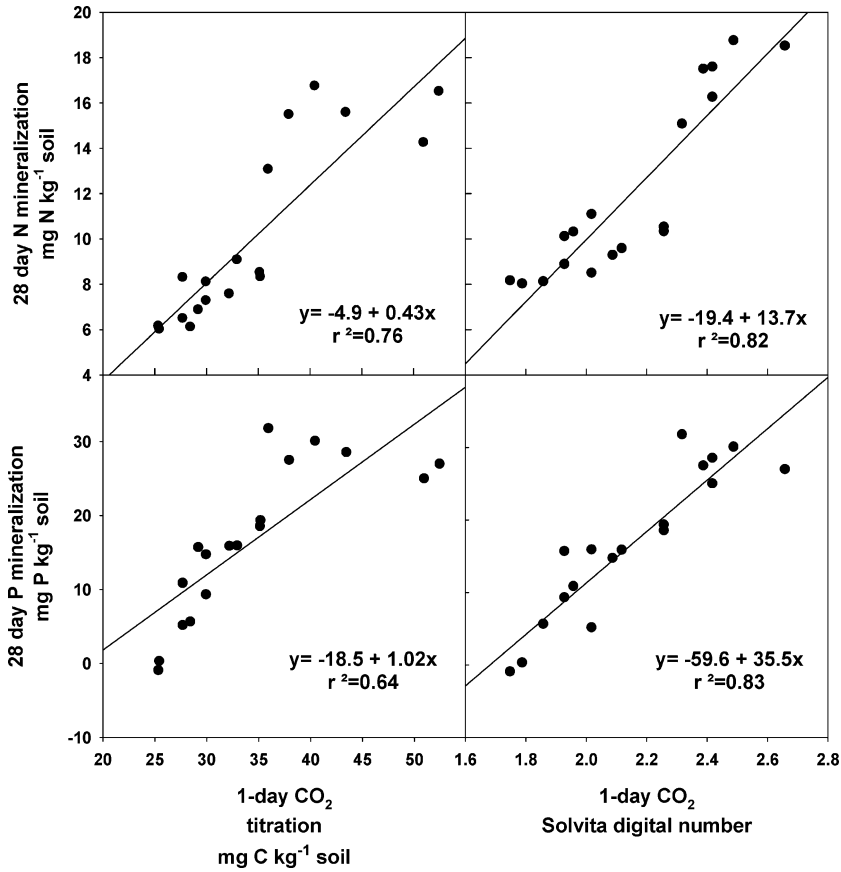

Figure 6. One-day CO_2 titration and Solvita vs. 28-day N and P mineralization.

available C from the composts. This short-term burst of CO_2 in compost-amended soil also correlated highly to the 28-day mineralization response.

It is normally considered that organic amendments added to soil that have a C:N ratio of less than 20:1 are likely to result in net mineralization of N (Alexander 1977). Our composted manure had a C:N of 12 and, therefore, was close to the expected final endpoint for composts. Had the compost not exhibited the normal low C:N expected for matured composts, it is possible that the CO_2 response as observed in our studies might not have indicated the same proportionality to N mineralization. Therefore, it may be important to also evaluate CO_2 response following addition of composts of elevated C:N, such as might be found in the case of yard waste composts, which are increasingly available from municipal green-waste collection programs and exhibit C:N ratios greater than 20. Assuming composts are in the normal range, the level of correlation we have observed both in CO_2 response following application and D/R, plus

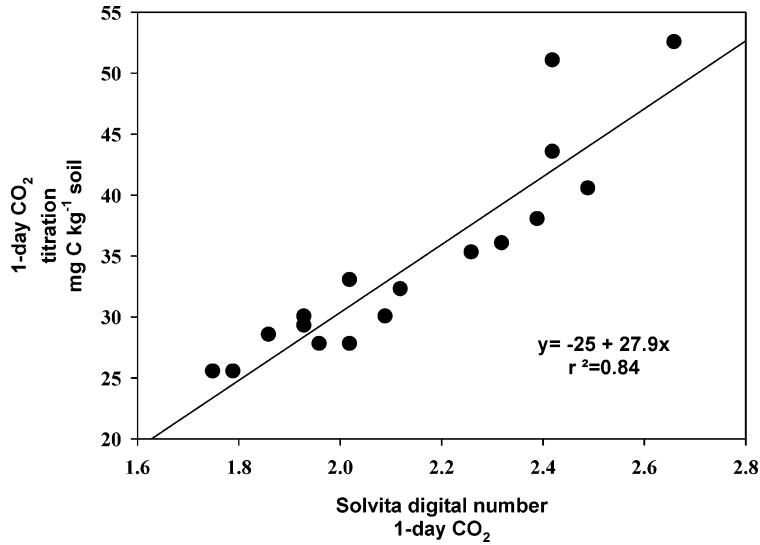

Figure 7. Relationship between 1-day CO_2 from the Solvita digital reader vs. the titration method.

the high degree of correlation to mineralization indices over 28 days, suggests that this approach might lead to a practical means to estimate N response from composts or manures in general.

Our test results comparing traditional alkali-trap methods to that of the new Solvita detection system indicated highly satisfactory correlations. The importance of this observation is that the Solvita approach presents a rapid and relatively low-cost approach to performing respiration analyses, so that laboratories may readily equip and conduct such tests. For example, we compared the time to read CO_2 results with the alkali trap vs. Solvita methods in addition to the preparation of necessary reagents and setup of titration apparatus. It required 1.5 h to titrate 40 soil samples, but only approximately 10 min to perform the same quantity of readings by Solvita DCR. Because our data indicate that the accuracy between the two approaches is comparable, if not slightly better for Solvita, this approach combining accurate, rapid, and fairly easy measurement of D/R treated soils for CO_2 respiration with and without organic amendment may pave the way for a standardized approach of routine evaluation of N-mineralization potentials in soils.

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