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Short communication

Soil CO_2 evolution: Response from arginine additions

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ABSTRACT

Short-term response of soil C mineralization following drying/rewetting has been proposed as an indicator of soil microbial activity. Houston Black clay was amended with four rates of arginine to vary microbial responses and keep other soil properties constant. The evolution of CO_2 during 1 and 3 days following rewetting of dried soil was highly related to CO_2 evolution during 10 days following chloroform fumigation (r^2 = 0.92 and 0.93, respectively) which is a widely used method for soil microbial biomass C, which disrupts cellular membranes. This study suggest that the release of CO_2 following rewetting of dried soil with no amendments other than heat and water can be highly indicative of soil microbial activity and possibly be used as a quantitative measurement of soil biological quality in Houston Black soils.

1. Introduction

Soils dry and are rewetted naturally in the field, the extent of which depends upon climatic and various soil conditions. The mineralization of C and N following drying/rewetting soil may be useful in quantifying the portion of the soil microbial biomass that is most responsive to rainfall events, which can have a strong impact on nutrient availability (Franzluebbers et al., 2000).

During a succession of drying and rewetting events in the lab, a uniform pattern of CO_2 evolution was exhibited, simulation which occurred under field conditions (Birch, 1958). Birch (1959) postulated that the common feature between the evolution of CO_2 and N mineralization after drying/rewetting soil was microbial death and subsequent mineralization. These studies suggest that physical alteration of the soil was not a primary factor for the mineralization of C and N.

The majority of the mineralization of C and N after rewetting dried soil is likely due to the death of heat susceptible microbes, death from water induced osmotic shock, and further renewal of the microbial population which is similar to the chloroform fumigation method for soil microbial biomass C (SMBC) (Jenkinson and Powlson, 1976). The difference being that heat and water are used by drying/rewetting to disrupt cells and chloroform. Microorganisms are tolerant of osmotic shock until they reach a critical level. Halverson et al. (2000) found that *Pseudomonas fluorescens* could survive a 0.5 MPa water potential increase, but greater increases caused a reduction in culturability and an increase in the quantity of solutes. These results supported the notion that increased solutes following drying/rewetting partly originated from the soil microbial community and provided substrates for mineralization. This may explain why the evolution of CO_2 from drying/rewetting soil was correlated so strongly with basal soil respiration (Franzluebbers et al., 2000).

Fierer and Schimel (2003) using ^{14}C, demonstrated that the flush of CO_2 after drying/rewetting soil largely originated from the microbial biomass, although whether this occurred through cell lysis or a cytoplastic release from osmotic shock was not clearly distinguishable. These results provided mechanistic support for the strong correlation observed between short-term C mineralization and microbial biomass C (Franzluebbers et al., 2000, Haney et al., 2001).

Drying and rewetting soils should allow a "snapshot" of the active microbial biomass while at the same time providing a standardized approach to quantify the biological attributes of soil quality.

We hypothesized that variations in substrate addition to a single soil would be an effective means of altering microbial biomass in a controlled manner to eliminate confounding effects of differences in chemical and physical properties on microbial biomass. Our objective was to determine the validity of the relationship between the evolution of CO_2 after drying/rewetting and chloroform fumigation.

2. Materials and methods

Soil (0–5 cm) was collected from under 4 years of an unfertilized corn/wheat rotation in October 2008 at the USDA-ARS research facility near Temple, TX. Soil was a Houston Black clay (fine,

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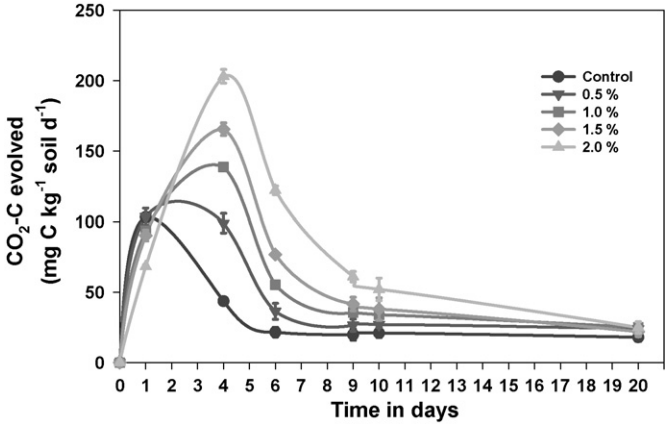

Fig. 1. Carbon dioxide–C evolved (0–20 d) after addition of arginine (prior to drying/rewetting–fumigation). Graph is presented in CO_2–C released per day. Error bars represent one standard deviation.

montmorillonitic, thermic Udic Pellustert) with pH 8.3 (1:2 soil/water), organic C concentration of 17.6 g kg^{-1} soil, 6.0% sand, 42% silt, and 52% clay. Soils were ground and passed through a 2-mm sieve with obvious roots and plant residues discarded. The design was a completely randomized design with a factorial combination of four treatments replicated three times. We hypothesized that the response from CO_2 release from drying/rewetting would be similar to that of chloroform fumigation–incubation, since both methods induce cellular substrate release and subsequent mineralization (Fierer and Schimel, 2003; Haney et al., 2001).

Soil was initially dried for 24 h at 50 °C. A total of 30 individual soil samples (40 g each) were prepared (5 arginine additions × 2 disturbances × 3 replications). Arginine was added to a solution in increasing amounts to achieve 0, 5, 10, 15, and 20 mg arginine g^{-1} soil and 50% water-filled pore space. Samples were incubated for 20 d at 25 °C in sealed 1-L glass containers with a vial of 10 mL of water to maintain humidity and a vial of 10 mL 1 M KOH to trap CO_2. Vials of KOH were replaced at 1, 4, 6, 9, 10, and 20 d after arginine addition and titrated with 1 M HCl to determine CO_2–C

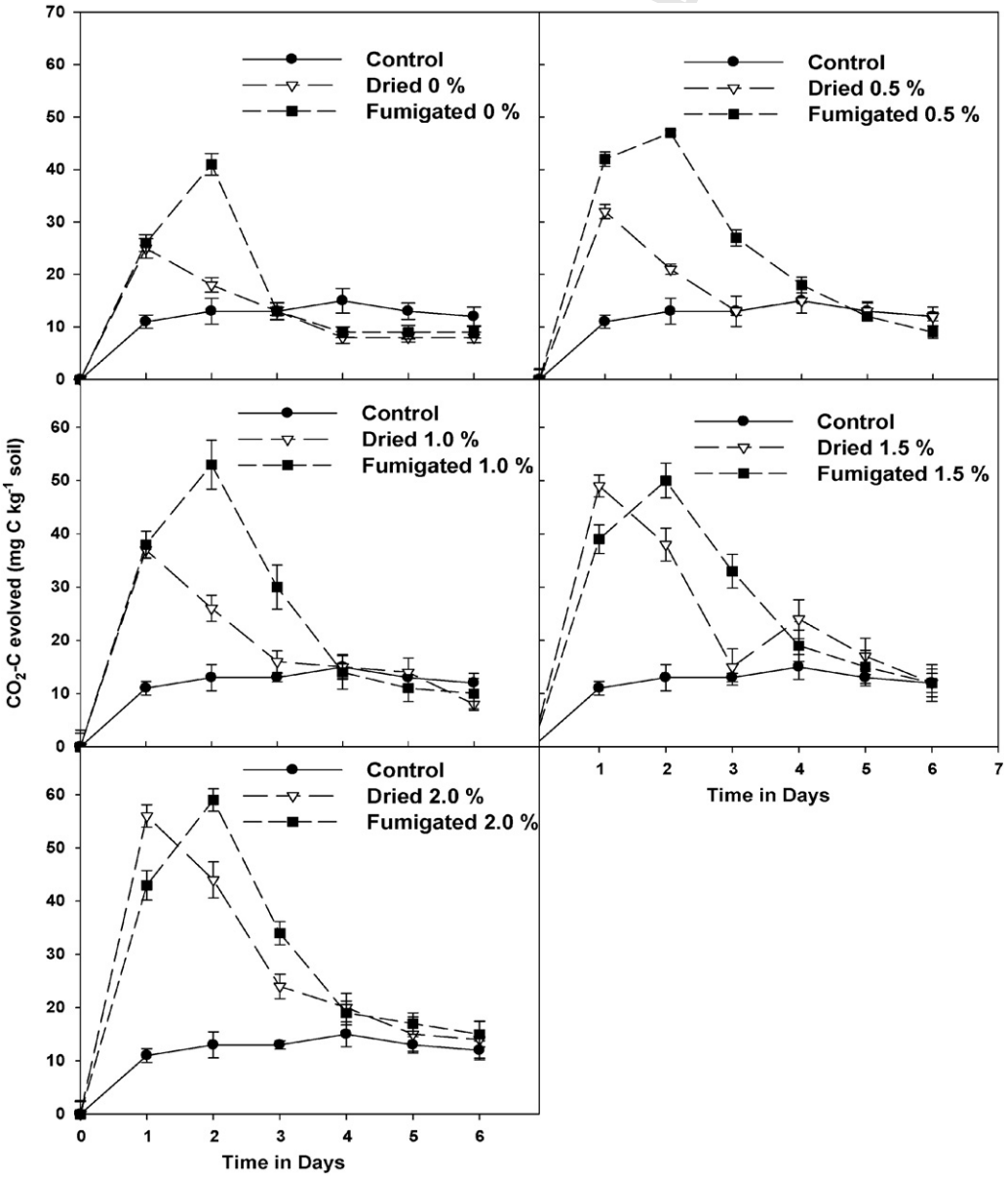

Fig. 2. Rate of CO_2–C released per day after drying/rewetting or fumigation (samples had been amended with four treatments of arginine prior to D/R, fumigation). The control was neither dried nor fumigated and is included as a reference. Error bars represent one standard deviation from the mean. Each point represents three replications.

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evolution (Anderson, 1982). We assumed that the 20-d incubation period after arginine addition would allow microbial biomass to reach a steady-state level. Franzluebbers et al. (1996) showed that dried and rewetted soils exhibited similar microbial biomass and activities as continuously moist samples after an incubation period of 5–10 d.

At the end of the 20-day incubation period, soils were split into two groups, with half dried for 24 h at 50 °C and half fumigated with $CHCl_3$ in vacuum desiccators for 24 h (Jenkinson and Powlson, 1976). The dried soil was rewetted and incubated as before. The fumigated soil was evacuated to remove $CHCl_3$ vapors and incubated as before. Vials of KOH were replaced at 1, 2, 3, 4, 5, 6, 10, and 20 d in both treatments. A continuously moist soil without arginine amendment, drying\rewetting, or fumigation was included as a control.

Statistical evaluation of data was performed using SigmaStat ver 3.5. One-way ANOVA at $p < 0.05$ (Tukey test) separated treatments and linear regression was used to test for strength of relationships.

3. Results and discussion

During the initial incubation phase of 20 d prior to disturbance treatment, increasing the rate of arginine addition led to an increase in CO_2 evolution during the period of 1 to 10 d of incubation. From 10 to 20 d, there was no difference in CO_2 evolution among amendment treatments (Fig. 1). Since arginine contains significant quantities of both C (41%) and N (32%), we expected no inhibitory effect on microbial activity due to the lack of N.

During the second incubation phase following disturbance, evolution of CO_2 peaked the first day after drying/rewetting and the second day of incubation after fumigation (Fig. 2). This temporal difference may have been due to the extent of microbial death and its effects on subsequent population regrowth to mineralize available substrates. During 0–3 d of incubation, C mineralization was lower following drying/rewetting than following fumigation for the 5 and 10 mg g^{-1} arginine amendments, but not statistically different for the other amendment rates. These results indicate that fumigation was more aggressive at releasing cellular components than drying/rewetting.

The release of CO_2 during 1 and 3 d following the rewetting of dried soil was highly related to the flush of CO_2 after fumigation and 10 days of incubation (Fig. 3) and the ratio was approximately equal to that observed before (Franzluebbers et al., 2000). The CO_2 evolution following drying/rewetting was equally sensitive to the change in microbial biomass C as fumigation. We did not measure microbial biomass directly; however, the methods are comparable since the conversion to microbial biomass C from fumigation is a calculation that would not alter the correlations for SMBC from fumigation–incubation. This simple procedure could be useful for quantifying the biological portion of soil quality on a rapid basis, although the separation of treatments was better between treatments during 3 d of incubation than 1 d (Fig. 4).

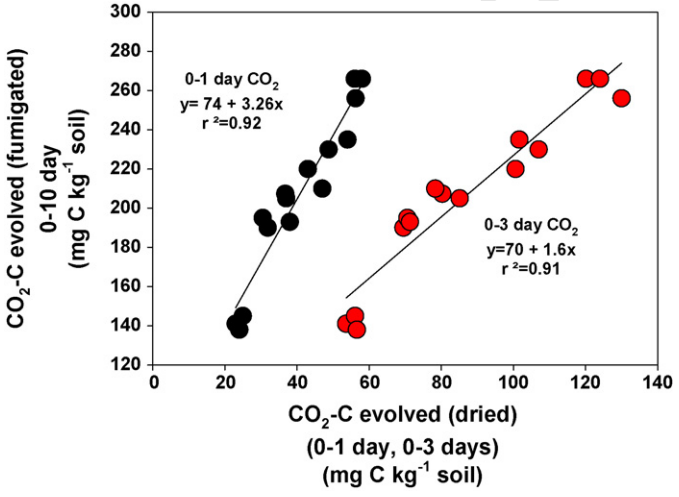

Fig. 3. Relationship between 1-day and 3-day CO_2–C from drying and rewetting and chloroform fumigation (0–10 d). All replicates are included.

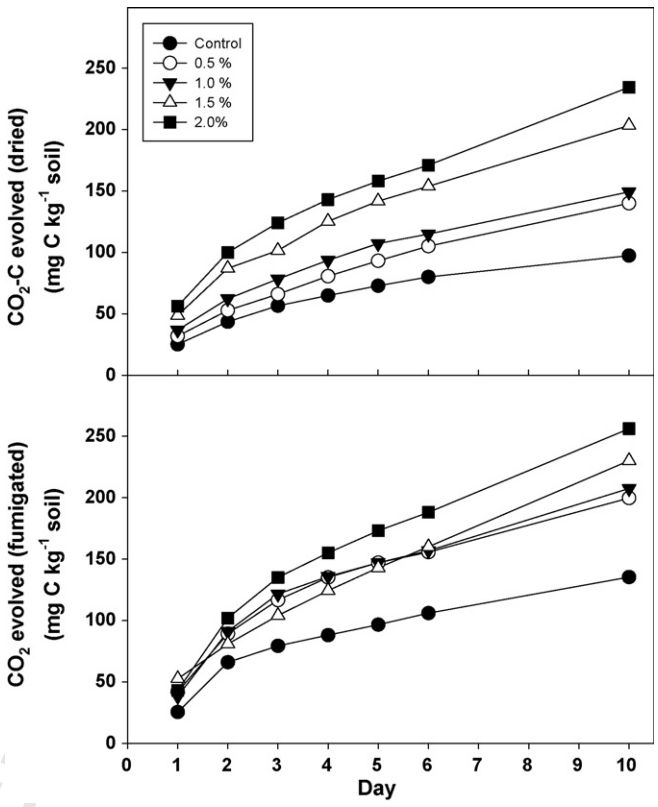

Fig. 4. Comparison between the cumulative release of CO_2–C from drying and rewetting and chloroform fumigation for each arginine treatment. Points are the average of three replicates. Control was neither dried nor fumigated, it was included as a reference.

Our results suggest that C mineralized from drying/rewetting and fumigation likely originated from similar C pools. Drying/rewetting of a soil resulted in the rapid mineralization of some microbial cellular components and a partial turnover of the microbial biomass, similar to the processes involved following fumigation (Fierer et al., 2003).

It has been suggested that a rapid analysis based on drying/rewetting soil and observing CO_2 evolution for as little as 1 or 3 days might be useful for comparing soil from different treatments, tillage regimes, and for quantifying inherent microbial activity (Franzluebbers et al., 2000). The results of this study support the utilization of this procedure to rapidly quantify microbial activity in a Houston black soil. Quantifying microbial activity based on the flush of CO_2 following drying/rewetting would be advantageous for several reasons. Soil could be stored in a dry state, thus freeing refrigerator space. Analysis is easy (oven-drying followed by incubation), using simple equipment (buret, stirring mechanism) and, most importantly, the procedure is based on a natural phenomenon (soil drying/rewetting).

4. Conclusions

As rates of arginine addition increased, CO_2 evolution increased from both fumigation and drying/rewetting treatments, indicating

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an increase in soil microbial biomass. The CO_2 evolved from drying/rewetting soil was highly related to that occurring from chloroform fumigation. Similar to the rupture of microbial cells following $CHCl_3$ fumigation, these data support the hypothesis that CO_2 released following drying/rewetting is also from the mineralization of microbial biomass killed by heat and water induced osmotic shock. The release of CO_2 following drying/rewetting could be an excellent indicator of soil microbial activity and will be valuable as a rapid biological soil quality indicator.

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