

# Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations

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*Soil microbial activity is greatly affected by soil water content. Determining the appropriate moisture content to rewet soils that have been dried in preparation for laboratory incubations to determine microbial activity can be laborious and time-consuming. The most common methods used achieve sufficient moisture content for peak microbial respiration are gravimetric water content, soil matric potential, or percentage of water-filled pore space (WFPS). Alternatively, a fast, simple, and accurate way to ensure that a given soil receives the appropriate amount of water for peak soil microbial respiration is to rely on natural capillary action for rewetting the dry soil. The capillary method is related to the gravimetric method for water uptake and has a strong correlation with WFPS. A microbial respiration test was conducted to compare rewetting methods. The 24-h carbon dioxide (CO<sub>2</sub>) / carbon (C) results were very similar and strongly correlated using the gravimetric method and the capillary method for rewetting dried soil.*

**Keywords** Capillary action, microbial activity, water-filled pore space

## Introduction

The movement of water in soil is dependent on the combined effects of porosity, gravity, mass flow, and capillary action. Soil porosity is influenced by texture, structure (e.g., degree of aggregation), and organic-matter content. For example, coarse-textured soils have larger pores than fine-grained soils, which allow for more water flow. Organic matter greatly increases the water-holding capacity of a soil.

Capillary action is the natural movement of water using adhesion (attraction to solids) and cohesion forces (attraction between water molecules) and is counterbalanced by the effects of gravity and air pockets. Most microbial activity studies that involve wetting or rewetting soils that have been dried using gravimetric water content, soil matric potential, or percentage of water-filled pore space (WFPS) to achieve sufficient moisture content for peak microbial respiration (Franzluebbers 1999; Fierer and Schimel 2003; Maysoon, Rice, and Milliken 2005). Current literature indicates that a range of 30 to 70% WFPS (depending on soil type, organic-matter content, etc.) is sufficient for peak microbial activity (Liebig, Doran, and Gardner 1996; Appel 1998; Bending et al. 2004) and represents roughly 50% of field capacity (Haney et al. 2004). In a paper by Franzluebbers (1999),

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WFPS was used as an acceptable method to determine soil matric potential. To determine the WFPS of a soil, the soil water content, bulk density, soil volume, volumetric water content, and soil porosity must be determined after drying the soil at 105 °C for 24 h with a subsample remaining in moist condition. This method is time-consuming and protracted when a large number of soil samples are being analyzed. The objective of this study was to determine if the natural capillary action of the soil can be used to obtain similar water uptake values using the gravimetric method and to compare microbial respiration (activity) values after rewetting dried soil using the capillary and gravimetric methods.

## Materials and Methods

Twenty agricultural soils from Texas, Oklahoma, and Maine with a wide range of clay content (6 to 55%), soil organic carbon (C; 0.6 to 3.0%), and pH values (5.2 to 8.4) were used for the study (Table 1). Soil samples were received in a dry state (3–12% moisture). Prior to any analysis, the samples were dried overnight at 50 °C to ensure a uniform starting point. After drying, the samples were ground to pass a 2-mm sieve.

### *Gravimetric Moisture Content Determination*

Soil subsamples were weighed (40 g) into 50-mL plastic beakers that had small drainage holes in the bottom. The samples were then wetted with 30 mL water by adding water

**Table 1**  
Soil properties and water-holding capacity

Soil sample ID	Soil pH	Percentage of capillary water held per g soil	Percentage of gravimetric water held per g soil	Clay content (%)
Meyer	5.80	18.23	19.78	16.00
Cj14	6.20	20.45	22.93	18.00
Molar	6.20	18.66	20.74	23.00
33 ac	7.50	24.63	26.21	20.00
Jack	5.20	15.22	17.62	16.00
80 ac	7.00	8.34	11.12	6.50
Franz	6.50	18.65	22.13	22.00
Cr100	8.40	19.87	20.73	13.00
Idaho	6.80	20.20	22.81	34.00
Walnut Creek N	7.80	8.98	12.22	6.00
Walnut Creek 95	7.40	16.55	17.06	8.00
Walnut Creek 01	7.30	17.98	18.70	9.00
Weswood Corn.	8.40	26.30	24.00	28.00
Eric	6.50	38.65	36.90	38.00
Bastrop	8.00	18.30	18.76	33.00
N Carly	6.30	16.55	15.35	12.00
Canton Rd.	5.50	8.90	10.83	6.00
Cj12	6.30	24.80	26.63	24.00
Houston	8.20	48.22	45.63	55.00
Austin	7.90	40.50	39.66	45.00

directly to the beaker of soil, which was placed in a 1-quart mason jar that allowed excess water to drain for 24 h. After 24 h, the beaker was weighed again to determine the wet weight of the sample. The samples were then dried at 105 °C for 24 h to determine the oven-dried weight. Gravimetric water content, also called soil water content, was calculated as follows:

$$\text{Percent water / g of soil} = (\text{Wet weight} - \text{Oven-dried weight}) / \text{Oven-dried weight}$$

### *Calculations for Water-Filled Pore Space*

We used the following equations in our calculations:

$$\text{Soil water content (g/g)} = \frac{\text{Weight of moist soil} - \text{Weight of oven-dried soil}}{\text{Weight of oven-dried soil}}$$

$$\text{Soil bulk density (g/cm}^3\text{)} = \text{Oven-dried weight of soil} / \text{Volume of soil}$$

$$\text{Soil porosity (\%)} = \text{Soil bulk density} / 2.65$$

$$\text{Volumetric water content (g/cm}^3\text{)} = \text{Soil water content} \times \text{Bulk density}$$

$$\text{WFPS (\%)} = \text{Volumetric water content} \times 100 / \text{Soil porosity}$$

In summation, WFPS can be determined using the following equation:

$$\text{WFPS} = (\text{Soil water content} \times \text{Bulk density}) / [1 - \text{Bulk density} / \text{Particle density} \times (2.65 \text{ Mg m}^{-3})]$$

### *Capillary Action*

Dry soil subsamples were also wetted through capillary action. Forty g of soil were added to 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 microfiber filter (Cat No. 1823-042; Whatman, Kent, U.K.) was placed in the bottom of the beaker to prevent soil loss. The disposable beaker was placed in a 0.24-liter glass with screw-top lids that has a convex bottom (to allow for drainage), which had been previously filled with 25 mL of water. The wetted soils were weighed 1 h after the water reached the surface (soil appeared moist at the surface). The water-weight results were compared to the WFPS and gravimetric-moisture content results from the same soils.

The gravimetric and capillary-wetting methods were used to rewet subsamples of the same 20 soils prior to incubation in order to compare the soil microbial activity as measured by the CO<sub>2</sub>-C released in 24 h. Forty g of the soils wetted using both the gravimetric and capillary methods were added to 0.2365-L glass jars with plastic screw-top lids. The soils were placed in an incubator at 25 °C. The CO<sub>2</sub> was trapped for 24 h using the Solvita soil test paddle (Solvita, Mt. Vernon, Maine). The amount of CO<sub>2</sub> released was determined using a digital-color reader (DCR). The Solvita system for estimating CO<sub>2</sub> release from soils that have been dried and rewetted for 24 h has been shown to be greatly correlated with the commonly used titration method and CO<sub>2</sub> IRGA method (Haney, Brinton, and Evans 2008).

### Summary of Methods

To use the capillary method, the materials needed are (1) 50-mL plastic beakers with three to five 6.35-mm holes drilled in the bottom, (2) glass microfilters 4.25 cm, (3) 0.2365-L glass jars with plastic screw-top lids, and (4) 25 mL water.

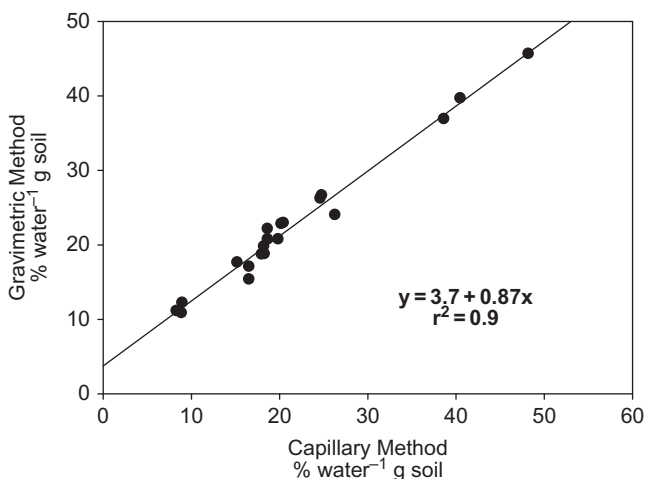
The steps to rewet dry soil for laboratory incubations are as follows: (1) Dry soil samples overnight at 50 °C and grind to pass a 2-mm sieve. (2) Place a filter in the bottom of plastic beaker, and then weigh 40 g soil into the prepared 50-mL plastic beakers. (3) Add 25 mL of water to the 0.2365-L jars. (4) Add the soil in the plastic beakers to the jar and screw on the cap.

The method for 1-day CO<sub>2</sub>-C microbial respiration is as follows: After the beaker of soil is added to the glass jar with 25 mL of water, add the Solvita paddle, screw on the lid, and incubate for 24 h. After the incubation, remove the paddle, place in the DCR, reader and press the button. The reading is in parts per million of CO<sub>2</sub>-C.

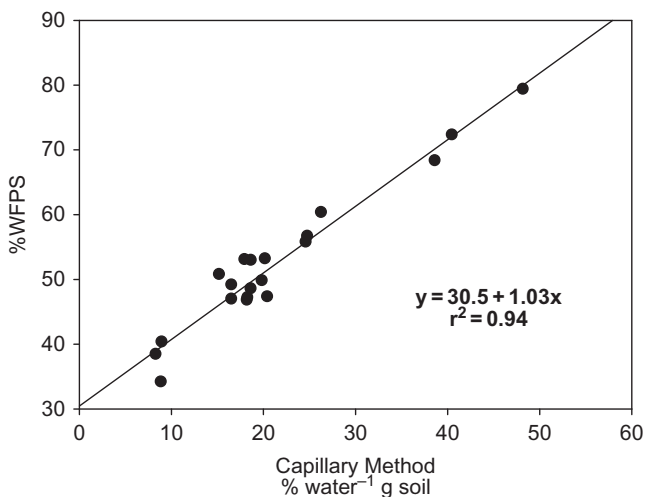
The plastic beakers are reusable. After the incubation is complete, we placed the glass jars with the soil into the oven and dried them overnight. The next day, the beakers could be removed and the soil and filter easily discarded. The beaker can be rinsed out and used indefinitely.

### Results and Discussion

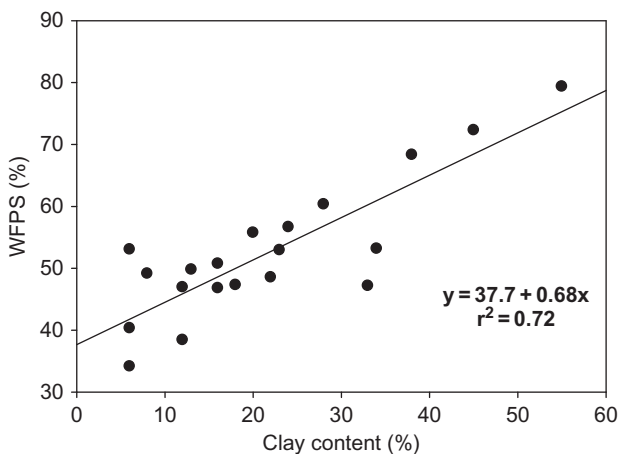
The amount of water taken up by a dry soil via capillary action is highly correlated to the amount of water retained by the same soil when wetted using the gravimetric method in a nearly one-to-one relationship ( $r^2 = 0.98$ , Figure 1). Gravity did not appear to be a limitation on the uptake of water in these samples because water had reached the top of all 20 soils within 5 min after the beakers were placed into the jars containing 25 mL of water. The average amounts of water held in the soils by the gravimetric and capillary methods are 22.49% and 21.45%, respectively. The amount of water taken up through capillary



**Figure 1.** Percentage of water held per gram of soil by various soils when rewetting dry soil from capillary action vs. gravimetric action.



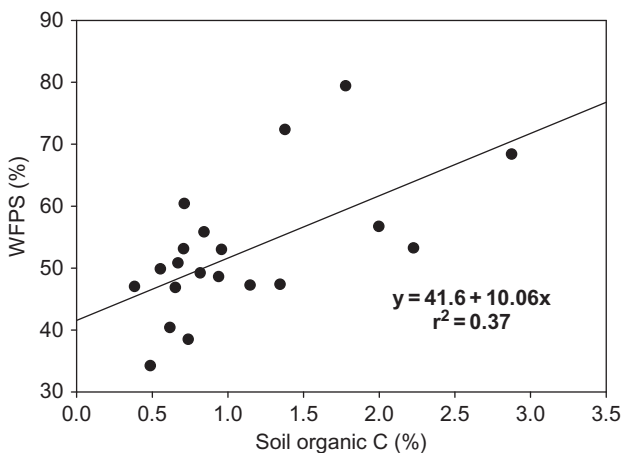
**Figure 2.** Percentage of water held by soil using capillary action compared to water-filled pore space.



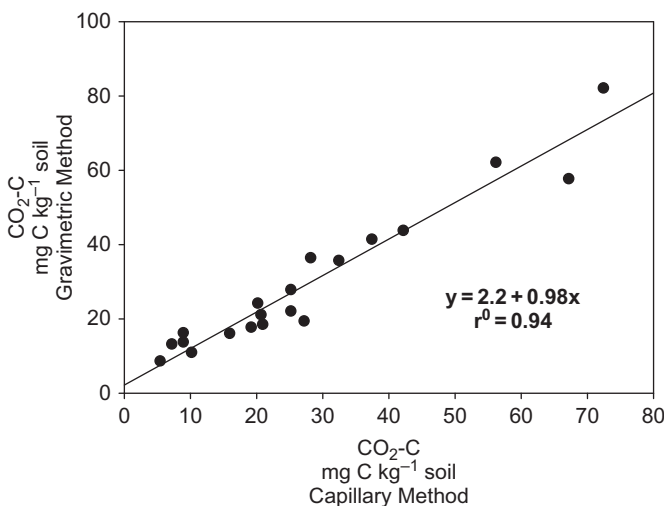
**Figure 3.** Relationship between clay content and water-filled pore space.

action is also strongly correlated to the percentage of WFPS calculated from duplicate soil samples ( $r^2 = 0.94$ , Figure 2). As soil-water retention increases, the amount of WFPS also increases. This result is primarily due to increased clay content in soils, whereas the sandier soils tended to have less WFPS (Figure 3). Clay content has a greater affect on WFPS than does percentage of soil organic C (Figures 3 and 4).

The 24 h CO<sub>2</sub>-C results are very similar and strongly correlated ( $r^2 = 0.94$ , Figure 5) between soils rewetted prior to incubation using the gravimetric and capillary methods. The slope of the regression equation is 0.98, and the y intercept is 2.2 mg C kg<sup>-1</sup> soil, which indicates very similar results when using either method to rewet the soil. The average concentrations of 24 h CO<sub>2</sub>-C for the 20 soils are 27.63 mg C kg<sup>-1</sup> using the



**Figure 4.** Percentage of soil organic C vs. percentage of water-filled pore space.

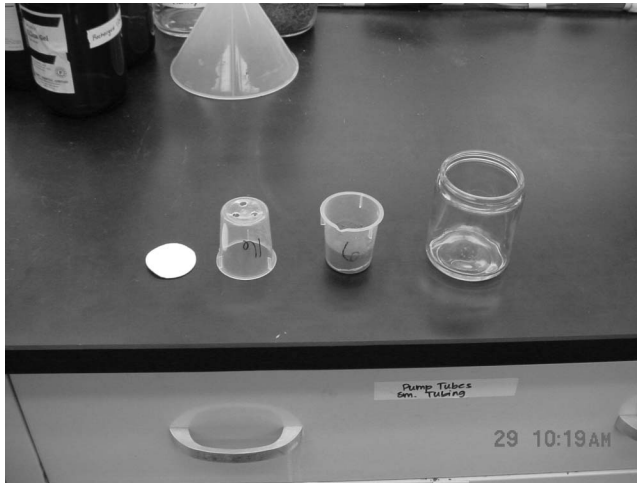


**Figure 5.** CO<sub>2</sub>-C using the capillary method vs. CO<sub>2</sub>-C from the gravimetric method.

capillary method for the 20 soils and 29.33 mg C kg<sup>-1</sup> soil C using the gravimetric method.

To begin soil-test analyses, it is necessary to dry soils at a constant temperature to provide a consistent starting point; otherwise, soils with differing water contents will increase the variability of the results from laboratory incubations. An advantage of utilizing the capillary versus the gravimetric method or WFPS is that the soils do not have to be dried overnight at 105 °C after the initial soil preparation to calculate moisture content for the determination of the correct amount of water to add to the soil prior to the incubation. When using the capillary method, the laboratory can simply weigh the soil into a filter beaker and use the natural capillary action of soil to rewet the sample. In addition, whether the rewetted soil is used for short-term or long-term incubations, the beakers can be rewetted in mason

jars or any container suitable to the needs of the experiment. Because of the shortened length of time needed to prepare for the incubation using the capillary rewetting method, it is more feasible for soil-testing labs to offer a rapid method for soil-microbial activity as an index of soil biological quality using the Solvita gel paddles, DCR reader, and 24 h CO<sub>2</sub>-C method (Figures 6–10).



**Figure 6.** Materials for rewetting dry soil with capillary technique.



**Figure 7.** Solvita paddle in soil with glass jar.



**Figure 8.** Soil and Solvita paddle enclosed in glass jar ready for incubation.



**Figure 9.** Incubated soil samples using the Solvita gel paddles for 24 h CO<sub>2</sub>-C analysis.

## Conclusion

The capillary method is related to the gravimetric method for water uptake for laboratory incubations and has a strong correlation with WFPS. In addition, the 24 h CO<sub>2</sub>-C results are very similar and strongly correlated ( $r^2 = 0.94$ , Figure 5) between soils rewetted prior to incubation using the gravimetric and capillary methods. The results indicate that the capillary method for rewetting dry soil is a fast and simple method that can be used by soil-testing and agricultural research laboratories that perform soil microbial assays. In our experience, we found that the cost for the equipment used is minimal and that the plastic beakers can be reused or discarded. We recommend this method for rewetting dry soil for laboratory incubations as a quick, simple, and reliable precursor to assessing soil microbial activity.



**Figure 10.** DCR digital reader for CO<sub>2</sub>-C analysis.

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