Short Communication: Quantifying and Correcting for Pre-Assay CO₂ Loss in Short-Term Carbon Mineralization Assays

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Abstract. The active fraction of soil organic carbon (SOC) is an important component of soil health and often is quickly assessed as the amount of CO₂ released by re-wetting dried soils in short-term (24–72 h) assays. However, soils can lose carbon (C) as they dry and if soil samples vary in moisture content at sampling, differential C loss during the pre-assay dry-down period may complicate interpretations of C availability. We examined pre-assay CO₂ loss and its influence on apparent C availability in the same soil at initial moisture contents of 30, 50, and 70% water-filled pore space (WFPS). We found that 50 and 70% WFPS treatments lost more C during drying than those in the 30% WFPS treatment, which led to a 26–32% underestimate of C availability in wetter soil. We developed a soil-specific correction factor to account for these initial soil moisture effects. Future C mineralization studies may benefit from similar corrections.

1 Introduction

The pulse of CO₂ following the re-wetting of dried soils (Robertson et al., 1999; Franzluebbers et al., 2000) has been widely used to indicate soil C availability because of its association with soil microbial biomass C and the active fraction of SOC. This method is derived from the “Birch Effect,” whereby re-wetted dry soils release a pulse of CO₂ resulting from increased microbial activity (Birch, 1958). Drought stress drives microbial communities to dormancy or death (Borken and Matzner, 2009), and following the reintroduction of moisture, microbes burst or release solutes to avoid bursting (Schimel et al., 2007), which stimulates C mineralization (Kim et al., 2012).

Although the short-term pulse of CO₂ following the re-wetting of dry soils is a widely used method for assessing soil C availability (e.g., Culman et al., 2013; Ladoni et al., 2016; Morrow et al., 2016; Sprunger and Robertson, 2018), we are unaware of efforts to quantify the potential bias introduced by assaying soils of different moisture contents at the time of sampling. Soils that differ in moisture will dry down at different rates, potentially losing different amounts of available C prior to the start of the assay. If sufficiently large, differential pre-assay losses could complicate comparisons of C availability across field treatments or landscape catenas.
Here we investigate the influence of different initial soil moisture levels on pre-assay CO$_2$ release during drying for an Alfisol soil in the upper Midwest, USA. We test the hypothesis that moister soil will have higher pre-assay CO$_2$ loss because a longer dry-down period results in more time for such losses to occur.

2 Materials and methods

2.1 Site description

We collected soil from the Ap horizon (0–20 cm) of an arable grass field at the W.K. Kellogg Biological Station (KBS) in Hickory Corners, MI (42°41'02" N, −85°37'34" W). KBS soils are mixed, mesic Typic Hapludalfs of co-mingled Kalamazoo and Oshtemo series (Crum and Collins, 1995) developed on glacial outwash with intermixed loess (Luehmann et al., 2016). Soil collected in September 2019 for this experiment was from the Kalamazoo series, which describe well-drained fine-loams (43% sand, 38% silt, 19% clay) with ~2% total C (Grandy and Robertson, 2006) and a pH of 7.2 (Robertson et al., 1993). Average annual rainfall at KBS is 1005 mm, average annual snowfall is 1300 mm, and mean annual temperature is 10.1°C (Robertson and Hamilton, 2015). The site was in various corn-soybean-wheat rotations for the past 40 years and before that, corn-soybean-small grain rotations for at least 60 years.

2.2 Experimental design

To examine the influence of initial soil moisture on the pre-assay loss of CO$_2$ during dry-down, we pre-wet recently collected soil to three different initial water-filled pore space (WFPS) levels: 30, 50, and 70%. Then we measured gravimetric soil moisture (GSM) and CO$_2$ loss while soil was air-drying, after which we re-wet them and measured the 24-hr CO$_2$ pulse by standard methods (Robertson et al., 1999; Franzluebbers et al., 2000).

2.3 Laboratory analyses

After collection, soil was sieved through a 4-mm mesh and mixed. We measured gravimetric soil moisture (GSM) and calculated the target volumetric water content (VWC, g H$_2$O cm$^{-3}$ soil) for each treatment following Eq. 1 (Elliott et al., 1999):

$$VWC = \frac{WFPS}{100} \times (1 - \frac{SBD}{2.65}) \tag{1}$$

where soil bulk density (SBD) was 1.5 g soil cm$^{-3}$, a previously assessed value from KBS soils (Robertson, 2016). Then we divided VWC by SBD to obtain a target GSM and thereby determined the amount of water to add to the field-moist soil (11% WFPS; GSM = 0.032 g H$_2$O g$^{-1}$ dry soil). We then weighed 40 g of soil into each of 75 specimen cups. Each cup was randomly assigned to an initial WFPS treatment (30, 50, or 70%), for a total of 25 replicates per treatment. We added sufficient deionized water to each cup to achieve the target initial WFPS and stirred to evenly distribute water. After soil was wet and stirred in the specimen cups, the contents of each cup were transferred to a labeled paper bag. The soil was spread evenly across the bottom of the bag, and the top portion of the bag was cut off to increase air flow. Afterwards, the soil was immediately weighed and set on a laboratory bench to air-dry.
Immediately after wetting, as well as 1, 3, and 8 days later, we assessed GSM and CO$_2$ loss rates for five replicates per initial WFPS treatment. GSM, which was determined after drying the soil at 105°C for 24 hrs, stabilized at 1.5% in the air-dried soil (Fig. 1a), but did not reach zero even when soil was completely air-dry. Because soil in all initial WFPS treatments were air-dry by day 3, with CO$_2$ loss rates close to zero, we terminated GSM and CO$_2$ measurements after day 8.

CO$_2$ loss rates at each sampling interval were measured by placing 10 g of soil into a 235 mL mason jar equipped with a gas-sampling septum. Then we sampled 5 mL of headspace from each jar at 4 intervals (0, 0.5, 1, and 2 hr), injected it into an evacuated 3 mL extainer (Labco Limited, Lampeter, Wales, United Kingdom), and replaced the jar headspace with laboratory air. CO$_2$ samples were analyzed within 24 hrs using a LI-820 CO$_2$ Gas Analyzer (LI-COR Biosciences, Lincoln, NE, USA).

On day 15 we re-wet the remaining five replicates of air-dried soil from each initial WFPS treatment to 50% WFPS (Franzluebbers et al., 2000). We then assessed subsequent 24-hr CO$_2$ pulses by sampling headspaces at 0, 2, 4, 8, and 24 hrs.

**2.4 Statistical analyses and correction factor**

CO$_2$ pulses were calculated as the positive slope of the linear regression of CO$_2$ concentrations through time after accounting for headspace dilution, and then converted to a standardized rate using the ideal gas law. In 17 of 75 cases, we omitted one of the four data points within a jar, which were clear visual outliers. In two cases, we rejected jars with leaks. CO$_2$ loss rates during the dry-down period were analyzed with a two-way analysis of covariance (ANCOVA), where initial WFPS treatment and days elapsed since wetting were factors and GSM at the time of sampling was a covariate. Additionally, a one-way analysis of variance (ANOVA) was used to determine whether initial WFPS treatment had an effect on the 24-hr CO$_2$ pulses upon re-wetting the air-dried soil.

We also calculated a correction factor to account for pre-assay CO$_2$ loss prior to the 24-hr CO$_2$ pulse assay. To calculate the total amount of CO$_2$ loss during dry-down for each initial WFPS treatment, we calculated a best-fit exponential decay curve \( Y = a^{bX} + \theta \), where \( Y \) = daily CO$_2$-C loss and \( X \) = length of dry-down period, until soil was air-dry (i.e., immediately after wetting through day 3). Total C loss was equivalent to the area under the curve.

Because we used sacrificial sampling, we could not calculate standard deviation or standard error in the usual way. Instead, we used a bootstrapping approach in which we computed predicted values for CO$_2$ losses \((\hat{Y})\) and residuals \((e_i = Y_i - \hat{Y}_i)\). All zeroes for CO$_2$ losses were set to 1 for the sake of fitting the regression because an exponential decay curve can approach but never attain 0 and because 1 was lower than any value we observed. Then we created a bootstrap sampling of residuals specific to each dry-down interval (0, 1, or 3 days), sampled randomly from each interval with replacement, and added randomly sampled residuals to predicted values \((\hat{Y}_i^* = \hat{Y}_i + e_i^*)\) for each dry-down interval (after Hesterberg, 2015). Residuals were
bootstrapped 10,000 times to derive multiple estimates of coefficients for the exponential decay curve (α, β, and θ). We also integrated under the curve 10,000 times to get an error estimate (i.e., coefficient of variation) associated with the total amount of pre-assay CO₂ loss during dry-down.

Then we divided the total CO₂ loss by three days to obtain the daily rate used to calculate a correction factor following Eq. 2:

\[ CF = \frac{\text{daily CO}_2 \text{ loss during dry-down}}{24\text{-hr CO}_2 \text{ pulse after re-wetting}} + 1 \]  

The correction factor for each treatment was then multiplied by each replicate’s 24-hr CO₂ pulse following re-wetting. Finally, we verified that the correction factors worked by conducting a one-way ANOVA to determine whether initial WFPS treatment still had an effect on the corrected pulses. For all analyses, we confirmed that assumptions of normality and homogeneity of variance were not violated.

### 3 Results

Soil in the 50 and 70% WFPS treatments took longer to dry than did soil in the 30% WFPS treatment (Fig 1a). A day after wetting, soil from the 30% WFPS treatment was completely air-dry, but soil had lost only 79% and 68% of its initial moisture in the 50 and 70% WFPS treatments, respectively. All soil was air-dry by three days after wetting. Pre-assay CO₂ losses mirrored soil moisture loss, reaching zero for all WFPS treatments by day 3 (Fig 1b). Both GSM at the time of sampling and day had effects on pre-assay CO₂ loss rates \(P < 0.0001\), but initial WFPS treatment did not \(P = 0.28\) probably because GSM captures more variation in soil moisture as the soil dries than WFPS treatment. However, there was an interaction between treatment and day \(P = 0.0005\). Soil of even the lowest initial WFPS treatment lost C as CO₂ over three days of drying (26 μg
CO₂-C g⁻¹ soil for 30% WFPS), but losses were disproportionately higher from wetter soil (62 and 71 µg CO₂-C g⁻¹ soil for 50 and 70% WFPS, respectively).

Initial soil moisture (i.e., WFPS treatment) had a significant effect on 24-hr CO₂ pulses after re-wetting air-dried soil ($P = 0.007$; Fig. 2). While final CO₂ pulses were lower for the 50 and 70% WFPS treatments relative to 30% WFPS (Fig. 2), the 50

![Fig 2. 24-hr CO₂ pulses after the re-wetting of air-dried soil for each initial water-filled pore space (WFPS) treatment. Error bars represent standard error of the mean.](https://doi.org/10.5194/soil-2020-55)

and 70% WFPS treatments also tended to have greater pre-assay CO₂ losses during three days of dry-down, which represented 77 and 95% of their 24-hr CO₂ pulses, respectively. After accounting for these losses with correction factors, the 24-hr CO₂ pulses were similar across initial WFPS treatments ($P = 0.28$; Fig. 3).
Fig 3. Daily CO₂ production rates for each initial water-filled pore space (WFPS) treatment. Lined bars represent the average daily rate of pre-assay CO₂ loss during a 3-day dry-down period and solid bars represent the 24-hr CO₂ pulses after re-wetting the air-dried soil. Together both bars represent the 24-hr CO₂ pulse corrected for pre-assay losses of CO₂ during dry-down. Error bars represent standard deviation as described in the “Statistical analyses” section.

4 Discussion

Initial soil moisture levels played a significant role in our ability to accurately characterize soil C availability (Fig 2) via the conventional 24-hr CO₂ pulse assay (Robertson et al., 1999; Franzluebbers et al., 2000). Wetter soil lost more C during dry-down, presumably because soil microbes remained active for a longer period of time. These losses decreased the short-term CO₂ pulses and therefore the final estimates of soil C availability.

Without knowledge of these losses, one might erroneously conclude that soil from the 30% WFPS treatment had about 35% higher soil C availability than the others (Fig 2), but this trend is instead due to higher pre-assay CO₂ losses during the dry-down period for wetter soil (Fig 3). It is striking that even short drying intervals (i.e., 1 versus 3 days) can affect soil C availability as deduced from the 24-hr CO₂ pulse after re-wetting air-dried soil. However, we were able to account for the pre-assay CO₂ losses for our soil with a correction factor that made C availability approximately equivalent across all initial WFPS treatments.

These trends suggest that efforts to characterize C availability via short-term CO₂ pulses following the re-wetting of dry soil should exercise caution if comparisons involve soils with a range of initial soil moistures. This includes soils compared across seasons; across drought, precipitation, or irrigation gradients; across landscape catenas; across crop, grazing, or forest
management practices; and as well in cross-site comparisons and meta-analyses that include soils collected at different initial soil moistures.

150 A correction factor that accounts for pre-assay CO₂ losses may help to normalize such comparisons. In our soil, pre-assay CO₂ losses led to a C mineralization bias as high as 32%, for which we could confidently correct by applying a correction factor based on measured rates of pre-assay CO₂ loss (Eq. 2). Other soils with moisture contents sufficient to oxidize available C during dry-down will require different correction factors. A soil-specific correction factor can be calculated by measuring CO₂ loss during dry-down on a subset of samples, as we described above (Eq. 2).

An alternate solution is to minimize the dry-down period such that little available C is lost prior to the assay. Strategies to minimize pre-assay CO₂ loss might include exposing soils to temperatures high enough to speed evaporation, but low enough to avoid sterilization (Jager, 1968) or otherwise artificially disrupt the microbial community (Evans and Wallenstein, 2012). This could be performed in a closed vented chamber such as a soil incubator. Alternatively, faster and more even drying might be achieved with a steady flow of air (i.e., a fan or vented system) over exposed soil samples. Even with faster drying, however, a correction factor may be needed.

Overall, our results demonstrate that using the 24-hr CO₂ pulse following the re-wetting of a dried soil to evaluate soil C availability can be misleading for soils with different moisture contents at time of sampling. For such soils a correction factor based on pre-assay CO₂ losses can be applied with confidence.

Data availability
Data will be made publicly available upon publication at Dryad; a pre-publication version is available at https://datadryad.org/stash/share/o4a-ESV8w3pxn-kbrZicM9eMuMrX2F5oJpg039DWGQ.

Author contributions
CV and GPR designed this study, MAB and CV performed the laboratory assays, MAB analyzed the CO₂ samples, CV conducted the statistical analyses, MAB and CV wrote the paper with contributions from SSR and GPR.

Competing interests
The authors declare that they have no conflict of interest.
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