Short Communication: Quantifying and Correcting for Pre-Assay CO_2 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 CO_2 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 CO_2 loss and its influence on apparent
15 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

20 The pulse of CO_2 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 CO_2 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),
25 which stimulates C mineralization (Kim et al., 2012).

Although the short-term pulse of CO_2 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.
30 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

35   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

40   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,

45   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

50   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_2O cm^{-3} soil) for each treatment following Eq. 1 (Elliott et al., 1999):

$$VWC = WFPS/100 * (1 - SBD/2.65) \tag{1}$$

55   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_2O 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

60   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

65   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-

70   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 exetainer (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

75   (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

80   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.

85   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

$(Y = \alpha^{\beta X} + \theta)$, 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.

90   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 $(\hat{Y}_i)$ 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

95   sampled residuals to predicted values $(Y_i^* = \hat{Y}_i + e_i^*)$ 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 CO_2 loss during dry-down.

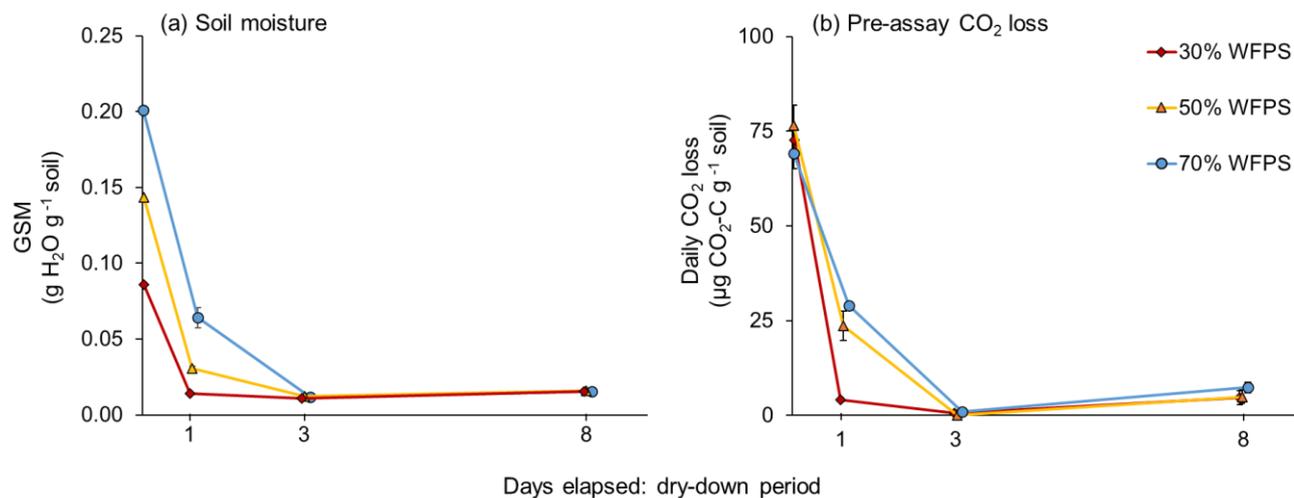
100   Then we divided the total CO_2 loss by three days to obtain the daily rate used to calculate a correction factor following Eq. 2:

$$CF = (daily\ CO_2\ loss\ during\ dry\text{-}down\ /\ 24\text{-}hr\ CO_2\ pulse\ after\ re\text{-}wetting) + 1 \tag{2}$$

The correction factor for each treatment was then multiplied by each replicate's 24-hr CO_2 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

105   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 CO_2 losses

110   **Fig 1. (a) Gravimetric soil moisture (GSM) during air-drying and (b) daily CO_2 losses from each initial water-filled pore space (WFPS) treatment during the dry-down period. Error bars represent standard errors of the mean.**

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 CO_2 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

115   treatment and day ($P = 0.0005$). Soil of even the lowest initial WFPS treatment lost C as CO_2 over three days of drying (26 µg

CO_2-C g^{-1} soil for 30% WFPS), but losses were disproportionately higher from wetter soil (62 and 71 µg CO_2-C g^{-1} soil for 50 and 70% WFPS, respectively).

Initial soil moisture (i.e., WFPS treatment) had a significant effect on 24-hr CO_2 pulses after re-wetting air-dried soil (P =
120   0.007; Fig 2). While final CO_2 pulses were lower for the 50 and 70% WFPS treatments relative to 30% WFPS (Fig. 2), the 50

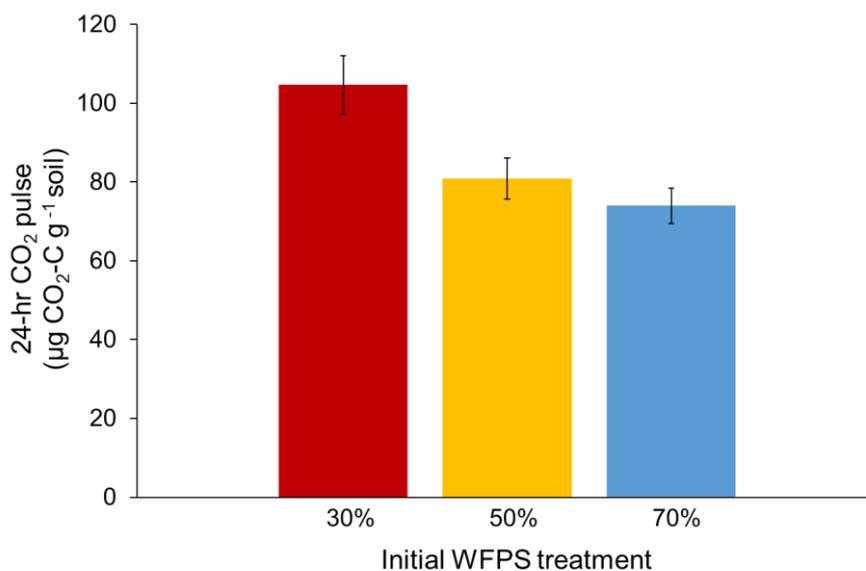



Fig 2. 24-hr CO_2 pulses after the re-wetting of air-dried soil for each initial water-filled pore space (WFPS) treatment. Error bars represent standard error of the mean.

and 70% WFPS treatments also tended to have greater pre-assay CO_2 losses during three days of dry-down, which represented
125   77 and 95% of their 24-hr CO_2 pulses, respectively. After accounting for these losses with correction factors, the 24-hr CO_2 pulses were similar across initial WFPS treatments (P = 0.28; Fig. 3).

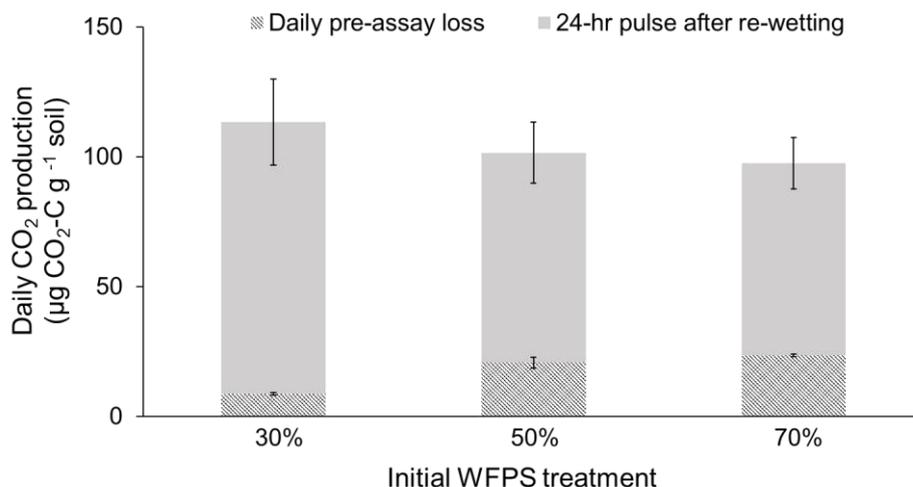



Fig 3. Daily CO$_2$ production rates for each initial water-filled pore space (WFPS) treatment. Lined bars represent the average daily rate of pre-assay CO$_2$ loss during a 3-day dry-down period and solid bars represent the 24-hr CO$_2$ pulses after re-wetting the air-
130 **dried soil. Together both bars represent the 24-hr CO$_2$ pulse corrected for pre-assay losses of CO$_2$ 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 CO$_2$ pulse assay (Robertson et al., 1999; Franzluebbers et al., 2000). Wetter soil lost more C during dry-
135 down, presumably because soil microbes remained active for a longer period of time. These losses decreased the short-term CO$_2$ 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 CO$_2$ losses during the dry-
140 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 CO$_2$ pulse after re-wetting air-dried soil. However, we were able to account for the pre-assay CO$_2$ losses for our soil with a correction factor that made C availability approximately equivalent across all initial WFPS treatments.

145 These trends suggest that efforts to characterize C availability via short-term CO$_2$ 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.

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A correction factor that accounts for pre-assay CO_2 losses may help to normalize such comparisons. In our soil, pre-assay CO_2 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 CO_2 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 CO_2

155   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 CO_2 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).

160   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 CO_2 pulse following the re-wetting of a dried soil to evaluate soil C

165   availability can be misleading for soils with different moisture contents at time of sampling. For such soils a correction factor based on pre-assay CO_2 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-ESVy8wxpnk-kbrZicM9eMuMrX2F5oJpg039DWGQ.

170   **Author contributions**

CV and GPR designed this study, MAB and CV performed the laboratory assays, MAB analyzed the CO_2 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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References

Birch, H. F.: The effect of soil drying on humus decomposition and nitrogen availability, Plant Soil, 10(1), 9–31, doi:10.1007/BF01343734, 1958.

Borken, W. and Matzner, E.: Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils, Global Change Biology, 15(4), 808–824, doi:10.1111/j.1365-2486.2008.01681.x, 2009.

Crum, J. R. and Collins, H. P.: Soils of the Kellogg Biological Station (KBS), Kellogg Biological Station Long-Term Research Publication, Zenodo, https://doi.org/10.5281/zenodo.2581504, 1995.

Culman, S. W., Snapp, S. S., Green, J. M. and Gentry, L. E.: Short- and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance, Agronomy Journal, 105(2), 493–502, doi:10.2134/agronj2012.0382, 2013.

Elliott, E. T., Heil, J. W., Kelly, E. F. and Monger, H. C.: Soil structure and other physical properties, in: Standard Soil Methods for Long-Term Ecological Research, edited by: Robertson, G. P., Coleman, D., Bledsoe, C., and Sollins, P., Oxford University Press, Inc., New York, NY, USA, 77, 1999.

Evans, S. E. and Wallenstein, M. D.: Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter?, Biogeochemistry, 109(1), 101–116, doi:10.1007/s10533-011-9638-3, 2012.

Franzluebbers, A. J., Haney, R. L., Honeycutt, C. W., Schomberg, H. H. and Hons, F. M.: Flush of carbon dioxide following rewetting of dried soil relates to active organic pools, Soil Science Society of America Journal, 64(2), 613–623, doi:10.2136/sssaj2000.642613x, 2000.

Grandy, A. S. and Robertson, G. P.: Aggregation and organic matter protection following tillage of a previously uncultivated soil, Soil Science Society of America Journal, 70(4), 1398–1406, doi:10.2136/sssaj2005.0313, 2006.

Hesterberg, T. C.: What teachers should know about the bootstrap: resampling in the undergraduate statistics curriculum, The American Statistician, 69(4), 371–386, doi:10.1080/00031305.2015.1089789, 2015.

Jager, G.: The influence of drying and freezing of soil on its organic matter decomposition, Stikstof, 12, 75–88, 1968.

Kim, D. G., Vargas, R., Bond-Lamberty, B. and Turetsky, M. R.: Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research., Biogeosciences, 9(7), 2459–2483, doi: 10.5194/bg-9-2459-2012, 2012.

Ladoni, M., Basir, A., Robertson, P. G. and Kravchenko, A. N.: Scaling-up: cover crops differentially influence soil carbon in agricultural fields with diverse topography, Agriculture, Ecosystems & Environment, 225, 93–103, doi:10.1016/j.agee.2016.03.021, 2016.

Luehmann, M. D., Peter, B. G., Connallon, C. B., Schaetzl, R. J., Smidt, S. J., Liu, W., Kincare, K. A., Walkowiak, T. A., Thorlund, E. and Holler, M. S.: Loamy, two-storied soils on the outwash plains of southwestern lower Michigan: pedoturbation of loess with the underlying sand, Annals of the American Association of Geographers, 106(3), 551–572, doi:10.1080/00045608.2015.1115388, 2016.

Morrow, J. G., Huggins, D. R., Carpenter-Boggs, L. A. and Reganold, J. P.: Evaluating measures to assess soil health in long-term agroecosystem trials, Soil Science Society of America Journal, 80(2), 450–462, doi:10.2136/sssaj2015.08.0308, 2016.

Robertson, G. P.: Soil carbon and nitrogen deep core surveys at the Kellogg Biological Station, Hickory Corners, MI (2001 to 2013) ver. 23, Environmental Data Initiative, https://doi.org/10.6073/pasta/8c9f759d32699902d88c7b5d607fb2f7, 2016.

Robertson, G. P. and Hamilton, S. K.: The ecology of agriculture landscapes: long-term research on the path to sustainability, in: Long-Term Ecological Research at the Kellogg Biological Station LTER Site, edited by: Robertson, G. P. and Hamilton, S. K., Oxford University Press, Inc., New York, NY, USA, 1–32, 2015.

Robertson, G. P., Crum, J. R. and Ellis, B. G.: The spatial variability of soil resources following long-term disturbance, Oecologia, 96(4), 451–456, doi:10.1007/BF00320501, 1993.

Robertson, G. P., Wedin, D. A., Groffman, P. M., Blair, J. M. and Holland, E. A.: Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration, in: Standard Soil Methods for Long-Term Ecological Research, edited by: Robertson, G. P., Coleman, D., Bledsoe, C., and Sollins, P., Oxford University Press, Inc., New York, NY, USA, 258–271, 1999.

Schimel, J., Balser, T. C. and Wallenstein, M.: Microbial stress-response physiology and its implications for ecosystem function, Ecology, 88(6), 1386–1394, doi:10.1890/06-0219, 2007.

Sprunger, C. D. and Robertson, G. P.: Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems, Geoderma, 318, 42–51, doi:10.1016/j.geoderma.2017.11.040, 2018.