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Article

Respiration After Rewetting of Air-Dried Soil Has Site-Specific Proportionality to Soil Organic Carbon

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Abstract

Microbe activity in soil can be estimated whereby a soil is air-dried, rewetted to a level favorable to microbes, and determination made of the CO₂ subsequently released. Previous studies indicate this so-called burst respiration has a positive linear response to soil organic carbon (SOC). Investigation was made here of the consistency of this relationship, both regionally for pastures, and among crops fields on a single farm. The three pasture sites were separated from each other by about 100 km across Manitoba, Canada. Sample depths to 100 cm were used to provide a range of values of SOC within a pasture site. Samples were collected from 72 ha at an experimental pasture, as well as from commercial pastures of 3 ha and 16 ha. For all pastures, burst respiration was greater for soils collected at shallow depth. Burst respiration across depths had a linear positive relationship to SOC over the range investigated from 5–80 g C kg⁻¹ at the experimental pasture. Corresponding relationships were found at both commercial pastures, but the regressions were distinct at each pasture. Background variability in SOC at 0–5 cm depth was evaluated among three cropped fields over two years at a single farm near Portage la Prairie, Manitoba. Each field had a different phase of the rotation soybean-corn-canola. Once again, significant but unique positive regressions between burst respiration and SOC were found for each field, despite differences in crop-year combinations among soil samples for a given field, and with SOC across years and fields ranging from 35–60 g C kg⁻¹. Burst respiration was not related ($P=0.65$) to natural variation in clay from 14–38% across the crop fields, indicating that the quality of organic matter from historic management likely dictates the nature of the relationship between burst respiration and SOC at a site locally, and by extension, regionally. The consistency and linearity of the relationship between burst respiration and SOC at a location with consistent management history suggests that SOC can be taken as an index of soil health for a given field. Yet, to compare among sites, or even among fields on the same farm, measurement of burst respiration retains greater utility to estimate soil health.

Keywords: soil carbon; soil health; soil quality; soil depth; loss on ignition; soil respiration

1. Introduction

When a 2-mm sieved soil is air-dried and rewetted, a pulse of CO₂ is released. Stimulation of respiration in this way has been called the Birch effect [1,2] in recognition of the original report of this phenomenon [3]. Following common practice when referring to commercial laboratory analysis of the Birch effect (e.g., <https://agcrops.osu.edu/newsletter/corn-newsletter/2019-08/solvita-%C2%AE-co2-respiration-soil-health-test>), we refer here to the CO₂ released after rewetting of air-dried soil as burst respiration. Compared to full air-drying, partial drying of soil reduces burst respiration [1]. Sequential determinations for a single cycle of drying and wetting reveal this pulse of respiration is most intense over the first 72 hours following rewetting, with 90% of the flush completed within 4–10 days [4]. Measurement of burst respiration to compare soils has monitored CO₂ released only during the first 24 hours after rewetting [5], although a four-day period has also been used [6]. In a

comparison of field-moist soils to paired samples that were air-dried and rewetted, the stimulation of respiration in the latter was sustained through days 6–7 following rewetting [7].

Soil health, or soil quality, is defined in terms of productivity [8]. The search for indicators of soil health has considered various properties, including soil organic carbon (SOC) [9], microbial biomass carbon [10], and burst respiration [11]. These indicators interrelate. Burst respiration is correlated with soil microbial biomass [4]. Microbial biomass carbon has a positive linear relationship to SOC, but site management must also be considered; grasslands give a steeper response to increasing SOC when compared to croplands [12].

In the original study presenting the Birch effect [3], CO₂-C mineralized over four days following rewetting was correlated with SOC across a selection of sites, yet data for other soils fell outside of this relationship. Despite the fundamental importance of SOC for our understanding of the Birch effect, systematic investigation of the relationship between respiration and SOC has been limited. Differences in the response of burst respiration to SOC among regions of North America have been considered in terms of broader climatic effects on the quality of SOC; colder and dryer climates were associated with less burst respiration, likely caused by a greater part of the organic matter being more resistant to breakdown [13]. Differences also arise within a region. For 47 corn fields across Georgia and Virginia, burst respiration was related linearly to SOC for the majority of sites, whereas other sites showed a pronounced difference in this relationship [14]. For 30 Australian soils ranging from 3–76% clay, burst respiration was not related to clay content over 7 days after rewetting of air-dried soil, but a negative impact of clay on respiration was noted for the following 7–17 days of incubation; the study concluded that clay does not restrict respiration of labile substrates, but that it can restrict breakdown of more recalcitrant organic matter [15].

The aim of the present study was to better understand the relationship between burst respiration and SOC. In turn, the hope was to gain insight into the value of measurement of CO₂ released after rewetting air-dried soil as a tool to evaluate soil microbial activity and as an indicator of soil health. To investigate the consistency among sites for the relationship between burst respiration and SOC within a defined region, studies were undertaken at two scales: regionally, at three well-separated pasture sites; and locally, at three crop fields on a single farm. To obtain a wide range of values for SOC within each pasture, cores were taken across 100 cm depth and divided into depth intervals. Sufficient spatial variation in SOC was used to evaluate the crop fields, restricting the crop field samples to a 0–5 cm depth interval, and noting soil clay content for each sample.

2. Materials and Methods

For the pasture study, soil samples were collected from June to August of 2020 from the Brookdale Research Pasture (BRP) of Manitoba Beef and Forage Initiatives (<https://www.mbf.ca/farm-station>), located 20 km north of Brandon, Manitoba, Canada. BRP has been described previously [16]. In brief, the 72 ha site was maintained as pasture in a grazing trial running from 2016 to 2021, inclusive. Seven replicates designated A to G varied in size from 4.4 ha to 16.6 ha, and each had approximately equal portions managed as season-long grazing or rotational grazing. However, the portion of D with season-long grazing was not accessible in 2020, and so it was omitted from the study. Three 100-cm deep soil cores of 50-mm diameter were collected from each of the available 13 experimental units across the site, giving 39 cores in total. The location for the three cores within each of the 13 experimental units was determined using coordinates generated with random numbers. Each core was collected using a truck-mounted hydraulic coring system. Compaction of cores was monitored by measuring the length of core recovered and the depth of the hole formed. Cores with compaction exceeding 10% were discarded, with a replacement core taken at fresh coordinates. Mean compaction for accepted cores was 4%. Cores were divided in the field into the following depth intervals: 0–15 cm, 15–30 cm, 30–60 cm, and 60–100 cm. The 39 cores thereby generated 156 soil samples, which were returned to the laboratory in separate bags. Soil samples were air-dried and passed through a 2-mm screen. Cores were similar among grazing treatments for SOC and burst respiration, and so evaluation of grazing systems is not considered further in this study.

Six identical 100-cm cores were also taken similarly over the same period in 2020 from each of two commercial pastures, generating an additional 24 soil samples for each. Pasture A was located near Lauder, Manitoba, Canada (https://en.wikipedia.org/wiki/Lauder,_Manitoba). Pasture B was located near Hargrave, Manitoba, Canada (https://en.wikipedia.org/wiki/Hargrave,_Manitoba). Together with BRP, the two commercial pastures formed a triangle of side length approximately 100 km. The field areas sampled were 3 ha at Pasture A and 16 ha at Pasture B, and each had been maintained long-term under rotational grazing.

For the pasture study, a subsample from each 2-mm sieved soil was ground by pestle and mortar to pass a 0.5-mm screen, before determination of SOC by loss on ignition for 16 hours at 400°C [17]. For determination of respiration, a separate subsample of air-dried soil that had passed the 2-mm screen was used. A mass of air-dried soil equivalent to 20 g oven-dry mass was transferred to a 125-ml Erlenmeyer flask and brought to 25 g moist mass, corresponding to 0.25 g H₂O g⁻¹ oven-dry mass. Measurement of CO₂ evolved used the titration method [18], as follows. An incubation period of one week was selected following rewetting, so that both the pulse of respiration could be characterized and an estimate made of the quantity of organic carbon mineralized over a week following rewetting. The rewetted soil was incubated in the dark for seven days in the sealed flask, along with an inserted tube containing 10 ml 0.2M NaOH. Following incubation, remaining NaOH was titrated against 0.2M HCl using phenolphthalein indicator. Blank flasks without soil were subtracted from experimental determinations. Particle-size analysis was undertaken for each pasture site using additional soil samples collected with a 0–15 cm soil probe, followed by determination using the hydrometer method [19].

For the study in crop fields, soil samples were taken from 0–5 cm as part of a previously described experiment on tillage [20]. Briefly, four tillage systems were imposed in a randomized complete block with four replicates, the experiment repeated in each of three fields at the same farm in Portage la Prairie, Manitoba. Fields were coded by number in relation to respective rotations, for 2021 and 2022, as follows: Field 2, corn-canola; Field 5, canola-soybean; and Field 7, soybean-corn. Three sample dates were spring 2021, fall 2021, and spring 2022. Thereby, for the data presented here, n = 48 for each field. Tillage effects were previously evaluated for a number of response variables, with focus on soil nitrate [20], and they are not further discussed here. The present analysis of the crop fields at Portage la Prairie considers responses of burst respiration to both SOC and soil clay on a field-by-field basis across replicates, tillage treatments, and times. Analytical methods for the crop field were similar-or-different to those of the pastures described above, as follows: soil clay used the identical hydrometer method [19], SOC was determined by an equivalent protocol for loss on ignition [21], but burst respiration in this case used the Solvita method [5], with CO₂ release over the initial incubation period of 24 hours, as provided commercially by Agivse Laboratories, North Dakota, USA. The use of NaOH titration to determine CO₂ release for the pasture sites and the use of Solvita gel to determine CO₂ release for the crop fields resulted from the combining of two originally separate studies to generate a larger body of data and allow comparisons among varied field systems.

The responses of burst respiration to both SOC and clay content was determined in all cases by linear regression. Slopes and intercepts were compared by analysis of covariance [22] for the three pastures and separately for the three crop fields.

3. Results

Burst respiration at each pasture site was greatest for soil in the top 15 cm, with values decreasing in order for each incremental interval of depth (Figure 1). Visual inspection of core samples prior to sieving indicated that root occurrence was sparse below 30 cm depth. However, soils collected from 30–60 cm and 60–100 cm released an average of 7–21 mg CO₂-C kg⁻¹ d⁻¹ (Figure 1). As expected, shallow depths had more SOC: 25–80 g kg⁻¹ was recorded in the top 15 cm, whereas 5–17 g kg⁻¹ was recovered from 60–100 cm in the profile (Figure 2). Taking a weighted average for the presented data (Figure 1) over pastures, burst respiration was 45 mg CO₂-C kg⁻¹ day⁻¹ for 0–30 cm but only 19 mg CO₂-C kg⁻¹ day⁻¹ for 30–100 cm, so that approximately twice the respiration per unit soil was found

in the upper layer. The relationship between burst respiration and SOC across depths at BRP was linear (Figure 2) and significant at the level of $P < 0.001$ (Table 1a), with respiration ranging from 2–40 $\text{mg CO}_2\text{-C kg}^{-1} \text{d}^{-1}$ (Figure 2). Using the slope of $0.495 \text{ mg C g}^{-1} \text{C day}^{-1}$ from the regression for BRP (Figure 2), the proportion of SOC respired of 7 days was $(0.495 \times 7) / 1000 = 0.0035$, or 0.4%. Thereby, 0.4% of SOC was released by burst respiration over a week at BRP. Corresponding regressions were also significant at Pasture A (Table 1b) and Pasture B (Table 1c), but the linear equations were distinct by site (Figure 3); to compare slopes, $F_{2,198} = 5.53$ with $P = 0.0046$; to compare elevations, $F_{2,200} = 145.65$ with $P < 0.001$. Pasture A was decidedly sandier than the other two pastures (Table 2), with both BRP and Pasture B falling under the designation sandy loam (Table 2).

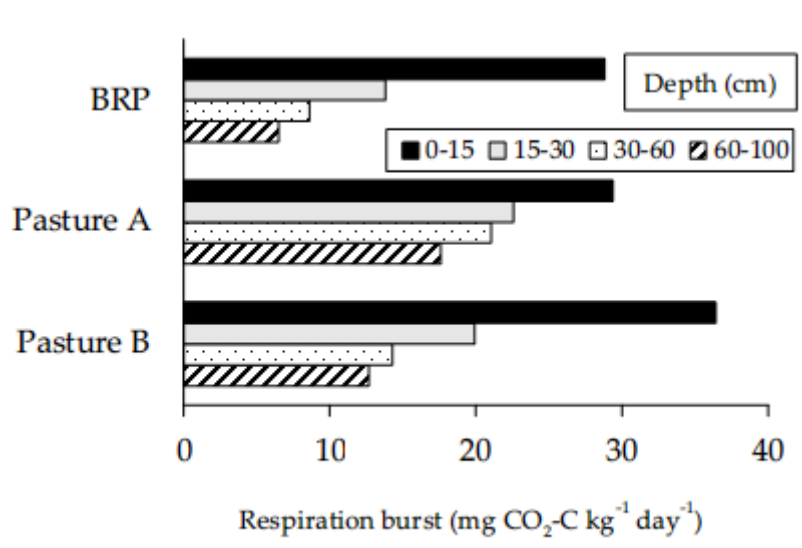


Figure 1. Mean respiration after rewetting of air-dried soil, in relation to depth interval and site. For BRP, $n = 39$; for Pasture A and Pasture B, $n = 6$.

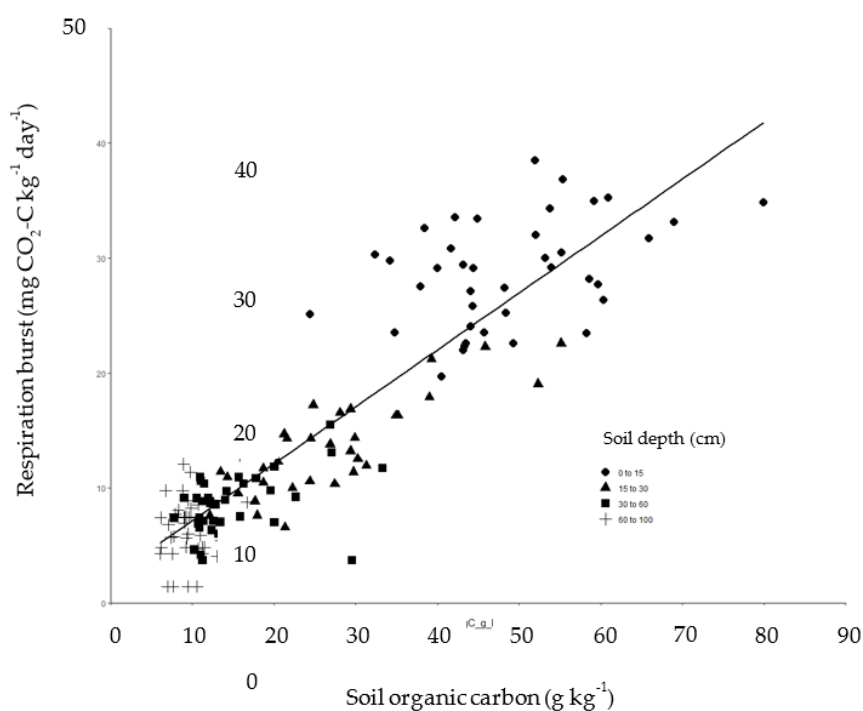


Figure 2. Respiration following rewetting of air-dried soil in relation to SOC for 39 cores taken to 100 cm at BRP and divided into the depth intervals as shown. The regression line is given for $y = 2.2 + 0.495x$, with $r^2 = 0.81$ and $n = 156$.

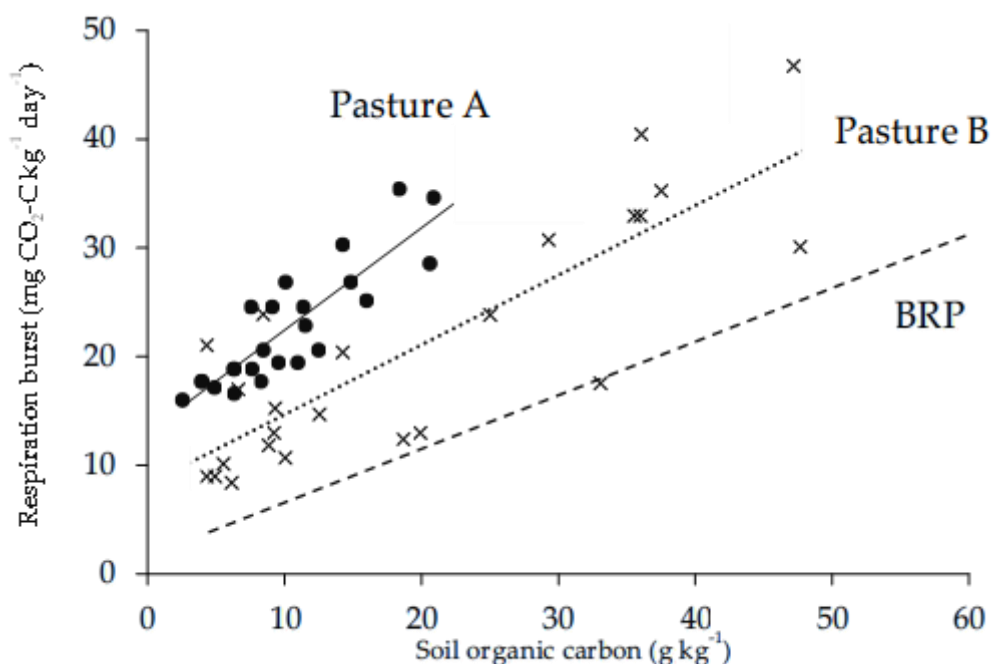


Figure 3. Respiration following rewetting of air-dried soil in relation to SOC for six cores taken to 100 cm at each of Pasture A and Pasture B and divided into the same depth intervals as used for BRP. To simplify, depth intervals are not noted by legend. The regression lines are as follows: for Pasture A with solid line and solid-circle symbols, $y = 13.0 + 0.941x$, with $r^2 = 0.75$ and $n = 24$; for Pasture B with dotted line and X symbols, $y = 8.2 + 0.640x$, with $r^2 = 0.71$ and $n = 24$. For comparison, the regression line for BRP is shown as a dashed line, but with the data points for BRP as previously given in Figure 2 omitted here to provide clarity.

Table 1. Linear regression analyses of variance for the plot of respiration after-rewetting of air-dried soil as a dependent variable against SOC for (a) BRP, (b) Pasture A, and (c) Pasture B.

(a)					
Source	DF	SS	MS	F	P
Regression	1	11116.6	11116.6	642.5	<0.001
Residual	154	2664.7	17.3		
Total	155	13781.3			
(b)					
Source	DF	SS	MS	F	P
Regression	1	535.9	535.9	67.6	<0.001
Residual	22	174.4	7.9		
Total	23	710.3			
(c)					
Source	DF	SS	MS	F	P
Regression	1	1973.6	1973.6	53.3	<0.001
Residual	22	814.9	37.0		
Total	23	2788.5			

Table 2. Particle-size analysis for each pasture.

Site	% sand	% silt	% clay	Texture
BRP	54.7	29.6	15.7	sandy loam
Pasture A	90.0	4.2	5.8	sand
Pasture B	53.3	37.5	9.2	sandy loam

For the crop fields study, burst respiration was proportional to SOC (Figure 4), with correlations significant (Table 3) yet distinct for each field in terms of slope elevation: to compare slopes, $F_{2,138} = 2.94$ with $P = 0.056$; to compare elevations, $F_{2,140} = 12.96$ with $P < 0.001$. Clay percentage varied from 14% to 38%, but burst respiration was not related to clay percentage across the three fields; $F_{1,142} = 0.2$ with $P = 0.65$.

Table 3. Linear regression analyses of variance for the plot of respiration after-rewetting of air-dried soil as a dependent variable against SOC for three crop fields: (a) Field 2, (b) Field 5, and (c) Field 7.

(a)					
Source	DF	SS	MS	F	P
Regression	1	15999.5	15999.5	15.1	<0.001
Residual	46	48630.2	1057.2		
Total	47	64629.7			
(b)					
Source	DF	SS	MS	F	P
Regression	1	25268.2	25268.2	22.4	<0.001
Residual	46	51937.3	1129.1		
Total	47	77205.5			
(c)					
Source	DF	SS	MS	F	P
Regression	1	19234.7	19234.7	17.9	<0.001
Residual	46	49369.8	1073.3		
Total	47	68604.5			

4. Discussion

The range of SOC from 0–80 g C kg⁻¹ across depths in the pasture systems (Figures 2 and 3) was greater than for the crop fields, which had from 34–58 g C kg⁻¹ (Figure 4), doubtless caused by the shallow depth of 0–5 cm for sampling in the crop fields. Nonetheless, this range in SOC for the crop fields was sufficient to explore the relationship between SOC and burst respiration.

Consistency of the linear response of burst respiration to SOC across pasture depths (Figure 2) suggests that the form of SOC is similar from the surface to 100 cm for the prairie-derived pasture systems here studied. Considering the depth interval 0–40 cm for a temperate forest soil, deeper layers within this interval had organic matter that was both of greater density and was associated with mineral surfaces, the shallow layers having organic matter that was less dense and not associated with mineral surfaces [23]. Foliage inputs to the soil surface of temperate forests doubtless contribute to this effect. In contrast, below-ground inputs from roots are of greater importance for prairie soils [24], possibly explaining the consistency in soil organic matter across depths in the present study.

Values for burst respiration averaged over seven days (Figures 2 and 3), as found here in the range 2–45 mg CO₂-C kg⁻¹ d⁻¹, were in keeping with those previously reported: from 4–18 mg CO₂-C kg⁻¹ d⁻¹ over 25 days [25], and from 7–43 mg CO₂-C kg⁻¹ d⁻¹ over six days [7]. Respiration rates are typically higher over the initial 24 hours following rewetting: from 5–80 mg CO₂-C kg⁻¹ d⁻¹ [5], and from 20–100 mg CO₂-C kg⁻¹ d⁻¹ [26]. Rates for the commercial method uses here (Figure 4) in some cases exceeded 200 mg CO₂-C kg⁻¹ d⁻¹, in keeping with the 24-hour monitoring period used.

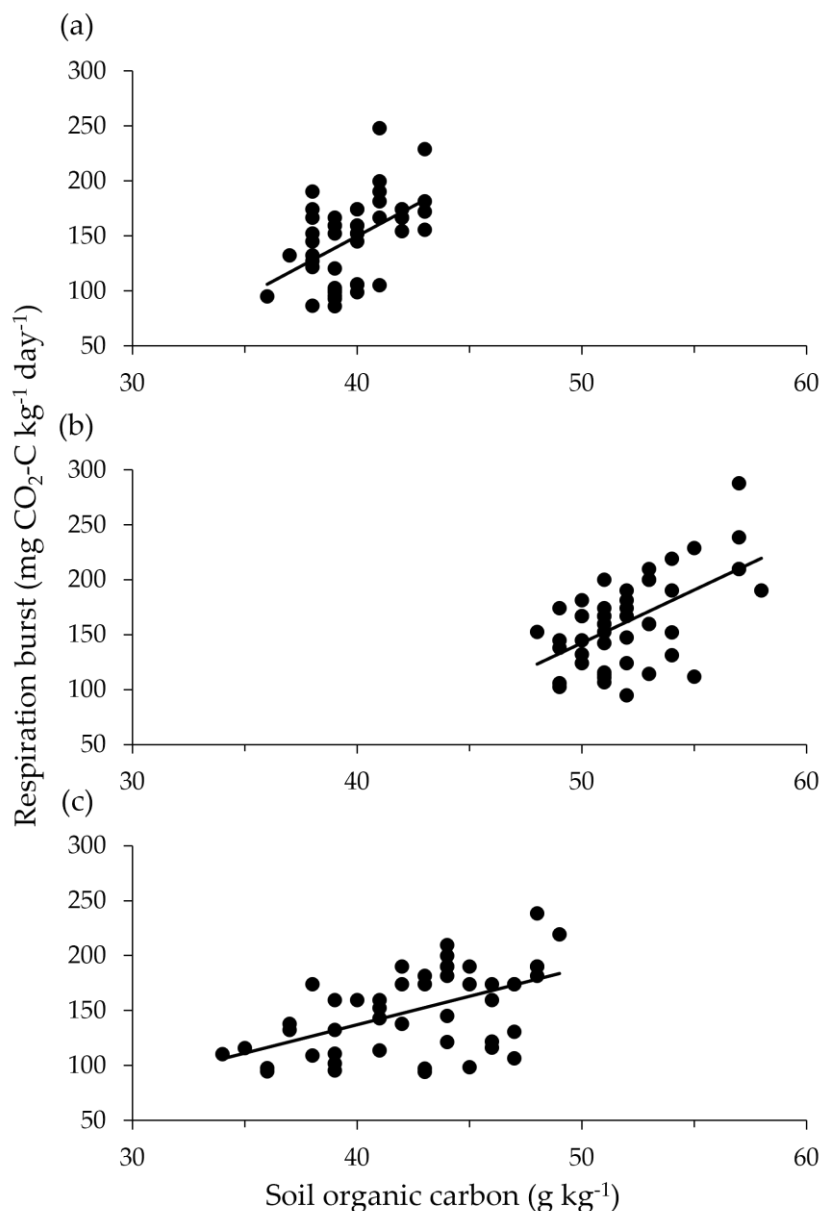


Figure 4. Respiration following rewetting of air-dried soil in relation to SOC for 48 cores taken 0–5 cm at each of three crop fields on a single farm. The r^2 values are (a) 0.25 for Field 2, 0.33 for Field 5, and 0.28 for Field 7.

The amounts of CO₂ released in the present study by soil samples from depth intervals below 30 cm stand in contrast to greatly reduced respiration from samples below similar depths reported in North Carolina [27]. Averaged over pastures (data from Figure 1), respiration in the present study from 30–100 cm was close to 50% of that seen for 0–30 cm. However, respiration for shallow depth in the North Carolina study was several-fold greater than the CO₂ released from below 30 cm [28]. The prairie origin of the Manitoba pastures studied here, with concomitant depth enrichment of soil organic matter by root inputs [28], likely explains this difference.

Using the slope of the regression obtained for the BRP site (Figure 2), calculation indicated 0.4% of SOC was respired over seven days following rewetting. Similar values were reported for soils of Alberta and British Columbia, with mean annual temperature 2°C, where 0.5% and 0.8% of SOC was respired over 72 hours after rewetting of air-dried soil [28]. In contrast, soils from the considerably warmer climates of Texas and Georgia, with mean annual temperature of 17–18°C, had corresponding values of 1.0% and 1.5%, respectively [28]. Estimates of microbial biomass carbon range from 0.5% to 2.7% of soil organic carbon [12,29]. Expiration of soil microbes caused by soil

drying [2], along with concomitant release of water-soluble carbon upon rewetting [1], likely contribute to the respiration pulse. The decrease in respiration seen when repeated cycles are applied for air-drying and rewetting [25] can thus be attributed to substrate exhaustion. The 0.4% of SOC found here to be released as burst respiration aligns with a part of soil microbial biomass, recorded as 2.5–2.8% for BRP [16]. Our data are consistent with the broader interpretation of accumulation of amino acids and monosaccharides, both extracellularly in soil [30] and within microbe cells [31], which fuel respiration on rewetting. Indeed, the appearance in burst CO₂ of label from ¹³C-glucose added at rewetting was delayed compared to native C [32], showing that the substrate for the initial burst response is labile C present before the water is added.

The linear relationship between burst respiration and SOC varied considerably among pasture sites within the same climate region here studied. A negative effect of increasing soil clay content on soil respiration following rewetting of air-dried soil was found for five Georgia soils varying from 30–350 mg clay g⁻¹ in the A_p horizon [33]. However, for the present study, soil texture was not related consistently to the slope of the response of burst respiration to SOC among pasture sites. Both BRP and Pasture B had similar textural classification as sandy loam, yet the slope of the response of burst respiration to SOC was greater for Pasture B than for BRP. Also, burst respiration was not related to soil texture for the crops field studied here. Thereby, it seems that the quality of the soil organic matter, and not soil texture, is the most likely cause of the site-specific relationship of burst respiration and SOC as found here among regions and among fields on a single farm. Such factors are likely to include the form of organic inputs to soil, as affected by management history for crops, forages, and cattle. To illustrate, organic input from wheat management gave a stronger burst respiration than organic input from pasture [34]. The large number of potential variables precludes any attempt to discern among features of management for the present study.

Consideration of management practices to promote soil health is normally focused on yield, but outcomes of management can vary regionally [35]. Measurement of burst respiration to quantify soil health is well established [36], but SOC might instead be used as an indicator at particular sites. The linear relationship between SOC and burst respiration might be taken as an index of soil health for a given location. SOC is related to productivity both globally [37] and regionally [38], although considerable variation exists from the impact of many factors on yield. For example, crop rotation may not change SOC in the short term. Yet, that rotation can act to sustain yields under pressure from pests, pathogens, and weeds [39]. Where SOC is taken as an indicator for soil health at a site, the present data suggest that soil depths to 100 cm might be considered for a prairie location, although sampling within the depth of the plow layer would be not just acceptable but also more practical and of wider applicability to many systems. However, to compare among sites regionally, and even among fields on the same farm, the measurement of burst respiration appears to be a more sensitive measure of microbial activity than SOC. Thereby, CO₂ evolved after rewetting of air-dried soil seems to provide broader utility to estimate soil health, rather than just measuring SOC.

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Data Availability Statement: Data supporting reported results presented herein are held by TPM and SJC.

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Conflicts of Interest: The authors declare no conflict of interest.

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