

## **Ex situ soil respiration assessment using minimally disturbed microcosms and dried–sieved soils; comparison of methods to assess soil health**

Authors: Comeau, Louis-Pierre, MacKinley, Kyle, Unc, Adrian, and Vallotton, Jeremiah

Source: Canadian Journal of Soil Science, 103(1) : 143-151

Published By: Canadian Science Publishing

URL: <https://doi.org/10.1139/cjss-2021-0143>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.



Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](http://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Ex situ soil respiration assessment using minimally disturbed microcosms and dried–sieved soils; comparison of methods to assess soil health

Louis-Pierre Comeau <sup>a</sup>, Kyle MacKinley<sup>a</sup>, Adrian Unc <sup>b</sup>, and Jeremiah Vallotton<sup>b</sup>

<sup>a</sup>Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, Fredericton, NB, Canada; <sup>b</sup>Environmental Science, Memorial University of Newfoundland, St. John's, NL, Canada

Corresponding author: **Louis-Pierre Comeau** (email: [Louis-Pierre.Comeau@Canada.ca](mailto:Louis-Pierre.Comeau@Canada.ca))

## Abstract

Soil respiration measurements are commonly used as soil health indicators. Several *ex situ* soil respiration methods exist, but comparative performances between them have rarely been analyzed. Specifically, there is a lack of comparisons between intact microcosms and destructive methods. The objective of this study was to analyze and compare three different *ex situ* soil respiration methodologies: minimally disturbed microcosms using fresh soil, dried–sieved 24 h burst test, and dried–sieved 10-day incubation. We hypothesized that (i) the respiration rates for the three methods are correlated to each other; (ii) the respiration rates are strongly correlated with soil physico-chemical parameters; (iii) disturbance caused by drying and sieving reduces regression coefficients compared with microcosms; and (iv) drying and sieving soil produces larger respiration rates. Soil was collected in the Province of New Brunswick, Canada. Total carbon and nitrogen (C:N), pH, aggregate stability, total dissolved C and N, NO<sub>3</sub> and NH<sub>4</sub>, texture, and labile C were determined prior to incubations. Our results showed that the three methods had CO<sub>2</sub> efflux in similar ranges. However, all the methods had low to no significant correlations between soil physico-chemical parameters and respiration. Total dissolved N had the strongest correlation with CO<sub>2</sub> efflux. The results of the microcosm method significantly correlated with the results for 24 h burst test but not with the 10-day incubation method. We conclude that drying and sieving soil prior to performing *ex situ* soil heterotrophic respiration measurements using the 24 h burst tests can produce cautiously reliable results. Despite the disturbance, results from the 24 h burst tests are comparable with the results of the microcosm method.

**Key words:** CO<sub>2</sub> flux, respiration, microcosms, soil disturbances, CO<sub>2</sub> burst, method comparisons

## Résumé

Il est courant de mesurer la respiration du sol pour en déterminer la vitalité. Plusieurs méthodes *ex situ* existent pour cela, mais on s'est rarement attardé à en comparer l'efficacité. Plus précisément, on n'a pas comparé les méthodes qui utilisent le microcosme intact à celles qui le détruisent. Les auteurs voulaient analyser et comparer trois méthodes employées pour mesurer la respiration du sol *ex situ* : l'usage de sol frais au microcosme le plus intact possible, l'essai d'éclatement de 24 heures après tamisage à sec et l'incubation de dix jours, également après tamisage à sec. Ils formulent les hypothèses suivantes : (1) le taux de respiration est corrélé entre les trois méthodes; (2) il présente une corrélation étroite avec les propriétés physicochimiques du sol; (3) comparativement à la méthode du microcosme, les perturbations que suscitent le séchage et le tamisage réduisent les coefficients de régression; (4) le taux de respiration est plus grand quand il y a séchage et tamisage du sol. Pour vérifier leurs hypothèses, les auteurs ont prélevé du sol dans la province du Nouveau-Brunswick, au Canada. Ils en ont mesuré la concentration totale de carbone et d'azote (C:N), le pH, la stabilité des agrégats, le C et le N total dissous, la teneur en NO<sub>3</sub> et NH<sub>4</sub>, la texture, et la concentration de C labile avant incubation. Les résultats indiquent que les dégagements de CO<sub>2</sub> sont du même ordre pour les trois méthodes. Néanmoins, les propriétés physicochimiques du sol ne présentent aucune corrélation importante ou significative avec le taux de respiration, peu importe la méthode. La concentration totale de N dissous est le paramètre qui présente la plus forte corrélation avec les émissions de CO<sub>2</sub>. Les résultats de la méthode du microcosme sont significativement corrélés avec ceux de l'essai d'éclatement de 24 heures, mais pas avec ceux de l'incubation de dix jours. Les auteurs en concluent que sécher et tamiser le sol avant d'en mesurer la respiration hétérotrophe *ex situ* peut donner des

résultats fiables, sous certaines réserves. Malgré les perturbations qu'il entraîne, l'essai d'éclatement de 24 heures donne des résultats comparables à ceux de la méthode du microcosme. [Traduit par la Rédaction]

**Mots-clés** : dégagements de CO<sub>2</sub>, respiration, microcosme, perturbation du sol, éclatement de CO<sub>2</sub>, analyse comparative

## Introduction

Soil heterotrophic respiration (Rh; i.e., CO<sub>2</sub> efflux from soil not including root respiration) releases a significant amount of CO<sub>2</sub> into the atmosphere (Marland 2008). Accordingly, small changes in Rh can have a significant impact on the climate (Schurgers et al. 2018). Therefore, accurate Rh assessments are important for quantifying flux of CO<sub>2</sub> from the soil to the atmosphere and for estimating the activity of soil microbial communities (Kuzyakov 2006). For these reasons, Rh is often used as an indicator of soil health when evaluating different land-uses or management practices (McGowen et al. 2018). For example, the Cornell Soil Health Laboratory soil respiration operating procedures uses standard scores from 0 to 100 to indicate the presence of active soil microbial communities (Moebius-Clune et al. 2017). However, Rh measurements performed in the field or laboratory often produce distinct results (Davidson et al. 1998; Comeau et al. 2018a). Due to soil heterogeneity across the landscape and difficulties in taking representative Rh measurements in different land forms, ex situ (in-lab incubations) Rh assessments are often preferred. In these ex situ incubations, to compare management practices and land-uses or to test specific hypotheses, excavated soil is usually pooled to generate representative samples (Gutinas et al. 2013; Zhou et al. 2014) and Rh measurements are performed under controlled moisture and temperature conditions (Bao et al. 2016; Yan et al. 2017).

Ex situ assessments of Rh are traditionally performed on dried, sieved, and homogenized (e.g., mixed or pooled) soils (Brinton and Valloton 2019) to create replicates adequate for statistical analysis. However, this physical disturbance affects the original soil structure, which in turn has consequences on microbial activity and composition (Baveye et al. 2018), and as a result, it may not always give an accurate picture of in situ dynamics. Specifically, concerns of microbial life cycle changes brought on by drying, sieving, and rewetting are noteworthy. To address disturbance while at the same time maintaining the advantages of soil mixing and pooling, Comeau et al. (2018b) developed a new method using minimally disturbed soil microcosms. This method involves extracting intact soil cores, gently breaking the cores, pooling the soil (e.g., per treatment or land-use or management), removing the visible live roots, and repacking the moist non-sieved soil into microcosm cores at their original field bulk densities. This microcosm method was demonstrated to be effective in dividing the influence of soil temperature and moisture on Rh and in simulating seasonal climatic variations (Comeau et al. 2018a). Previous studies have analysed the effects of drying and sieving on soil respiration in comparison with intact cores (e.g., Stenger et al. 2002; Herbst et al. 2016) but comparative analysis between the microcosm method and traditional methods using dry-sieved soil has not been performed.

The objective of this study was to assess the effect on Rh of drying and sieving soils and the correlation between soil

physico-chemical properties and CO<sub>2</sub> efflux. Four hypotheses were tested: (i) the three methods will correlate to each other; (ii) the three methods will strongly correlate with soil physico-chemical parameters; (iii) disturbance caused by drying and sieving will reduce the regression coefficients compared to the microcosms; and (iv) dried and sieved soil would have a larger Rh compared to the microcosms because disturbing soil by dry-sieving exposes occluded organic matter to microbial degradation and breaks fungal hyphae (Datta et al. 2014).

## Materials and methods

### Soil sampling

Soil was collected from 34 agricultural sites/farms in New Brunswick, Canada (Supplementary Table 1), encompassing a wide range of arable lands as part of a larger Agriculture and Agri-Food Canada cluster project (PSS2224). Composite soil samples were collected from each site with a Dutch auger (0–15 cm depth) using zigzag random sampling (Zebarth et al. 2021b). In the field, the soil samples were transferred into plastic bags kept in a cooler with icepacks, transported immediately to the laboratory and stored at 4 °C to keep them fresh prior to analyses. Soil samples were either dried (35 °C for 24 h) and passed through a 2 mm sieve or kept fresh for the microcosm method.

### Soil chemical and physical parameters

The soil chemical analyses included pH (1:1 H<sub>2</sub>O), soil texture, aggregate stability, total C, total N, C:N ratio, dissolved C and N, NH<sub>4</sub>-N and NO<sub>3</sub>-N, and labile C, and were analysed at Agriculture and Agri-Food Canada laboratory in Fredericton, New Brunswick. The labile C was assessed with the permanganate oxidizable carbon (POX-C) method in duplicate samples (Culman et al. 2012). Briefly, air-dried soil (2.5 g) was mixed with 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub>. The mixture was shaken for 2 min at 240 rpm on a lateral shaker and allowed to settle for 10 min. A 0.5 mL aliquot of supernatant was diluted in 49.5 mL of deionized water and the absorbance was determined at 550 nm on a Biochrom Libra S60 Spectrophotometer (Biochrom Ltd., UK). The absorbance of four standard solutions was also determined (0.00005, 0.0001, 0.00015, and 0.0002 mol L<sup>-1</sup> KMnO<sub>4</sub>). POX-C was calculated as described by Culman et al. (2012). Wet aggregate stability was determined by slaking using an Eijkelkamp Wet Sieving Apparatus, according to a method modified from Angers and Mehuys (1993). Briefly, 4.0 g of air-dried soil aggregates of 1–2 mm size were placed into a 250 mm sieve, gently moistened, and repeatedly immersed for 3 min in water. The particles and aggregate fragments that passed through the sieve were filtered, dried, and weighed. The particles remaining on the sieve were repeatedly immersed in a 2 g L<sup>-1</sup> NaOH dispersing solution for intervals of 5 min until there were only sand particles remaining in the sieve. Soil aggregate stability was

calculated according to “Manual for Wet Sieving Apparatus” (Eijkelkamp 2008).

Total dissolved organic carbon and nitrogen were determined on fresh soil extracted with 50 mL 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> solution and shaken at 200 rpm for 2 h. Extracts were filtered (40# Whatman), and concentrations of organic C and N in the extracts were determined using a Shimadzu TOC analyzer (TOC-L, Shimadzu Scientific Instruments, Inc.). The concentrations of NH<sub>4</sub>-N and NO<sub>3</sub>-N were analyzed colorimetrically on a QuickChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, CO). Total soil organic carbon and total nitrogen concentrations were determined by dry combustion (Elementar varioMACRO; Skjemstad and Baldock 2007). Soil textural class (sand, silt, and clay) was assessed through the pipette sedimentation method following organic matter removal (Kroetsch and Wang 2007).

### Soil heterotrophic respiration assays

Three assays were employed to carry out the ex situ Rh measurements. First was the 24 h burst test, equivalent to the 24 h burst test of Haney (Haney et al. 2008), which measures CO<sub>2</sub>-C emissions over a 24 h period following rewetting of dry soil. The second assay was the soil respiration rate measured 10 days after soil rewetting, to avoid any burst of CO<sub>2</sub>-C emissions associated with soil rewetting. The third assay used minimally disturbed microcosms that measure CO<sub>2</sub>-C from fresh soil that has been carefully repacked to field bulk density (Comeau et al. 2018b).

For the 24 h burst test and the 10-day incubation, 40 g of soil was placed in a 50 mL perforated beaker developed for the Solvita test (Solvita and Woods End Laboratories). The perforated beaker was then placed into a 500 mL Mason jar containing 20 mL of water, which allowed the water to wet the soil. The Mason jar was immediately covered with a lid containing a septum and 20 mL of CO<sub>2</sub>-free compressed air was added. Samples were incubated at 25 °C for 24 h and a 20 mL gas sample was collected and injected into a 12 mL pre-evacuated exetainer. The burst test value was calculated as the mass of C in the headspace at the end of the 24 h incubation per unit weight of oven-dried soil. Following the collection of 24 h burst test gas sample, the air in the chamber was flushed, the lid was replaced with parafilm, and samples were incubated another 9 days at 25 °C. After this, the parafilm was removed and the headspace was flushed with CO<sub>2</sub>-free compressed air for 20 s. A lid containing a septum was placed on the jars and 80 mL of compressed air was added. A 20 mL gas sample was collected at 0, 30, 60, and 90 min using a syringe and placed into a pre-evacuated exetainer. Taking into account the volume of air in the headspace at each time point and the removal of CO<sub>2</sub> by gas sampling, the mass of C was determined at each time point. The respiration rate was then calculated as the slope of the regression of the mass of C per kg of oven-dried soil against time (Haney et al. 2008).

For the microcosms, the fresh soil was repacked to bulk density of 1.1 g cm<sup>-3</sup> in the 56.7 cm<sup>3</sup> microcosm cores (inner diameter 3.8 cm and height 5 cm) following Comeau et al. (2018b). Each soil core was placed individually into a hermetically sealed 2.9 L plastic container and left to stabilize in the

dark for 2 weeks at 25 °C. After the preincubation was completed, for all microcosms, 20 mL gas samples were collected with an airtight syringe at 0, 24, 48, and 72 h after container closure. All incubations were performed at 40% gravimetric water content. In the microcosms, the water content was adjusted following Comeau et al. (2018b). For all methods, gas samples were analyzed with a gas chromatograph (Varian, Mississauga, ON) at the Dalhousie University in Truro Nova Scotia as described by Burton et al. (2008). Briefly, an electron capture detector (ECD) was connected with a CombiPAL autosampler. The autosampler removes a 2.5 mL volume from the sample tube and injects into a sample valve that delivers 0.5 mL to the ECD. The ECD was operated at 300 °C, with an argon/methane carrier gas (90% Ar and 10% CH<sub>4</sub>) delivered at 10 mL/min, through a Haysep N 80/100 precolumn (0.32 cm diameter × 50 cm length) and Haysep D 80/100 mesh analytical columns (0.32 cm diameter × 200 cm length) both in a column oven operated at 70 °C. The precolumn was used in combination with a valve to remove water from the sample. Operational conditions and data handling were performed with the Varian Star software. In each analytical run, three concentrations of standard gas mixtures were included for quality assurance/quality control purposes. The Ideal Gas Law was used to determine the amount of CO<sub>2</sub>-C and fluxes (Lang et al. 2011).

### Statistical analyses

Calculations and descriptive statistics were performed with Microsoft Excel XP<sup>®</sup>. For the 3 assays, an analysis of variance test to compare the Rh averages between the 3 methods and verification of the homogeneity of variance were performed with the statistical program R version 2.8.1 (R Development Core Team 2008) with the function linear model (anova.lm). Spearman correlations analyses were performed for the soil physico-chemical parameters using SigmaPlot 14.5 (Systat Software, Inc.). Statistical differences were deemed significant at  $\alpha = 0.05$ .

## Results and discussion

Ex situ assessment of Rh is regularly used to infer soil health and microbial activity. Different methods to determine Rh are commonly performed; however, until now, there have been few studies that perform comparative analyses between procedures. This study verified the correlation of Rh with 11 physico-chemical parameters of 34 different soils collected in New Brunswick, Canada to judge the strength of the different methodological approaches. The premise was that superior methods will show more and stronger correlations between Rh and soil physico-chemical properties at a fixed standard temperature and moisture level.

The 24 h burst tests, 10-day incubations, and microcosms had ex situ Rh of 1.42 ± 0.47 (average ± standard deviation), 1.22 ± 0.58, and 1.60 ± 0.45 mg CO<sub>2</sub>-C/kg dry soil/h, respectively (Table 1). Equivalent values of 0.4–3 mg CO<sub>2</sub>-C/kg dry soil/h have been previously reported for the Atlantic Region of Canada (Miller et al. 2009; Cooper et al. 2011; Zebarth et al. 2022). Drying and sieving of the soil did not increase Rh compared with the microcosms as originally expected.

**Table 1.** Average (SD) of soil respiration and chemical and physical properties.

Variable	Value
24 h burst test (mg CO <sub>2</sub> -C/kg dry soil/h)	1.42 (0.47)ab
10-Day soil incubation (mg CO <sub>2</sub> -C/kg dry soil/h)	1.22 (0.58)b
Microcosms (mg CO <sub>2</sub> -C/kg dry soil/h)	1.60 (0.45)a
pH H <sub>2</sub> O	5.97 (0.44)
Total C (%)	2.07 (0.32)
Total N (%)	0.21 (0.02)
C:N ratio	9.93 (0.87)
Aggregate stability (%)	66.27 (8.95)
Total dissolved organic C (ppm)	11.14 (3.65)
Total dissolved N (ppm)	37.17 (14.37)
N-NH <sub>4</sub> (ppm)	0.21 (0.03)
N-NO <sub>3</sub> (ppm)	38.21 (14.74)
Clay (%)	16.61 (3.81)
Silt (%)	42.17 (5.82)
Sand (%)	41.21 (7.78)
POX-C (mg kg <sup>-1</sup> )	509.38 (16.61)

Note: SD, standard errors; number of experimental units  $n = 34$ . Letters beside the SD indicate statistical differences at  $\alpha = 0.05$ .

Conversely, the Rh of the 10-day incubation method was significantly lower than the minimally disturbed microcosms. For the 24 h burst test with drying and sieving soil, Rh was slightly and nonsignificantly lower than the minimally disturbed microcosms. Other studies analyzing the effect of soil disturbance on CO<sub>2</sub> efflux from lab incubations have found that disturbance might occasionally enhance microbial activity and respiration. For example, [Herbst et al. \(2016\)](#) and [Datta et al. \(2014\)](#) found that air-drying and sieving can impact the relationship between soil moisture and soil respiration due to alteration of macroaggregates. This was explained by aggregates that protect a fraction of soil organic C from mineralization due to the occlusion of C within the aggregates ([Pulleman and Marinissen 2004](#)). However, other authors found no significant difference in soil respiration between intact and sieved soils ([Stenger et al. 2002](#); [Thomson et al. 2010](#)), while [Adekanmbi et al. \(2019\)](#) found a decrease in soil respiration due to sieving disturbance, same as in our study. The smaller ex situ Rh in sieved soil that we observed might be a consequence of changes in water-holding capacity after sieving. Altering soil structure and aggregate sizes through sieving affects both total porosity and pore size distribution ([Wu et al. 1990](#)). That is, with the same amount of water, the types of pores filled were likely not the same, which in turn could have affected aerobic microbial activity. Specifically, grinding of soil may reduce the water capacity of a soil and therefore increase the likelihood of anaerobic conditions ([Brinton 2020](#)).

As studies show that to avoid destroying the microaggregates, the threshold appears to be around a 2 mm sieve mesh size, this study likely did not cause artifacts that boost microbial activity for the sieved soil. Drying temperature during soil preparation has been linked to changes in total respiration, and it is generally thought that temperatures between 40 and 60 °C are ideal; lower drying temperatures may pre-

**Table 2.** Heterotrophic respiration results of the regression coefficients between the three methods.

	Burst test	10-Day incubation
Burst test	—	—
10-Day incubation	$r^2 = 0.18$ ( $p = 0.01$ )	—
Microcosms	$r^2 = 0.13$ ( $p = 0.03$ )	$r^2 = 0.02$ ( $p = 0.43$ )

Note: Number of experimental units = 34.

vent the burst effect ([Franzluibbers and Veum 2019](#); [Laffely 2019](#)), while higher temperatures (e.g., 100 °C) can cause destruction of microbial communities and greatly reduce 24 h respiration ([Haney et al. 2004](#)). Accordingly, the drying at 35 °C for 24 h used in this study is unlikely to have annihilated major microbial taxa.

The physical and chemical soil properties presented in [Table 1](#) are in the same ranges as for other published studies in the region ([Cambouris et al. 2006](#); [Nyiraneza et al. 2012](#); [Zebarth et al. 2019](#); [Abedin and Unc 2020](#); [Chen et al. 2022](#); [Nyiraneza et al. 2021](#); [Zebarth et al. 2021a](#)). Accordingly, this experiment can be considered representative of the soils of the Atlantic Region that are dominated by shallow podzols and luvisols ([Fhamy et al. 1986](#); [Krzic et al. 2021](#)).

The 24 h burst test significantly correlated with both the 10-day incubation and the microcosm test ( $r^2 = 0.18$  and 0.13, respectively); however, the 10-day incubation method ([Table 2](#)) did not correlate with the microcosm test. [Table 3](#) shows that the 24 h burst test, pH, and total dissolved N positively and significantly correlated with ex situ Rh. As soil extracellular enzymes are pH dependent, previous incubation studies have shown the soil pH to influence Rh ([Yiqi et al. 2006](#); [Wang et al. 2010](#)). Other incubation studies have shown that the amount of total dissolved N significantly enhances Rh due to the influence of fast-growing microbial communities ([Cookson et al. 2007](#); [Soong et al. 2020](#)). Specifically, changes in inorganic N (i.e., NH<sub>4</sub> and NO<sub>3</sub>) often strongly enhance Rh fluxes from incubations due to N limited status of most soil ([Micks et al. 2004](#); [Burton et al. 2012](#); [Zhang et al. 2021](#)). [Table 4](#) shows that in our study, for the microcosm test, only total dissolved N positively and significantly correlated with ex situ Rh. The relationship between total dissolved N and ex situ Rh was similar for the microcosms and 24 h burst tests ([Fig. 1](#)). However, the slope of the regression line was almost three times greater for the 24 h burst test than for the microcosms (i.e., 0.014× vs. 0.005×). For the 10-day incubation, no soil properties significantly correlated with Rh ([Table 5](#)). Accordingly, this method might be suboptimal as an indicator of soil health and soil functional status since reliable ex situ respiration methods ought to have some stable correlations between soil properties and CO<sub>2</sub> efflux.

The soil physical and chemical parameters determined in this study have been shown in previous studies to influence or correlate to Rh from incubations. For example, Rh rates were shown to be proportional to soil organic carbon content without changing microbial communities ([Gan et al. 2020](#); [Nyberg et al. 2020](#)). Due to the fact that microbial respiration per unit microbial biomass depends on organic matter C:N ratio, it has been demonstrated that, in incubations, reduc-

**Table 3.** Summary of analysis of covariance between the burst test Rh and soil physico-chemical parameters.

Soil parameter	Coefficients	Standard error	p value
Intercept	-306.92	213.24	0.16
pH H <sub>2</sub> O	9.18	3.85	0.03*
Aggregate stability (%)	0.07	0.26	0.80
POX-C (mg kg <sup>-1</sup> )	-0.03	0.03	0.29
Total N (%)	1405.68	992.94	0.17
Total C (%)	-87.21	98.22	0.38
C:N ratio	17.61	21.06	0.41
Total dissolved organic C (ppm)	-0.60	0.56	0.30
Total dissolved N (ppm)	0.85	0.41	0.04*
N-NH <sub>4</sub> (ppm)	13.31	67.68	0.85
N-NO <sub>3</sub> (ppm)	-0.63	0.42	0.15
Clay (%)	0.37	0.97	0.71
Sand (%)	0.07	0.39	0.85

**Note:** Overall  $r^2$  of the regression 0.69 with overall standard error of the parameter of 8.02. Number of observations  $n = 34$ .  
\*Significant at the 0.05 probability level.

**Table 4.** Summary of analysis of covariance between the microcosms Rh and soil physico-chemical parameters.

Soil parameter	Coefficients	Standard error	p value
Intercept	5.72	10.41	0.59
pH H <sub>2</sub> O	0.32	0.19	0.11
Aggregate stability (%)	0.00	0.01	1.00
POX-C (mg kg <sup>-1</sup> )	0.00	0.00	0.80
Total N (%)	-41.17	48.45	0.41
Total C (%)	4.37	4.79	0.37
C:N ratio	-0.92	1.03	0.38
Total dissolved organic C (ppm)	-0.01	0.03	0.65
Total dissolved N (ppm)	0.05	0.02	0.01*
N-NH <sub>4</sub> (ppm)	5.51	3.30	0.11
N-NO <sub>3</sub> (ppm)	-0.04	0.02	0.06
Clay (%)	0.04	0.05	0.39
Sand (%)	0.02	0.02	0.40

**Note:** Overall  $r^2$  of the regression 0.51 with overall standard error of the parameter of 0.39. Number of observations  $n = 34$ .  
\*Significant at the 0.05 probability level.

ing the C:N ratio can increase cumulative respiration (Spohn 2015; Nguyen et al. 2017). In addition, because soil microbes use carbon in labile form, it has been found that Rh is notably higher in fresh soil in comparison to incubation-DOC depleted soil (Birge et al. 2015). Similarly, POX-C, a measurement of labile C availability, has been shown to positively correlate with Rh in incubation where POX-C levels were regulated (Cleveland 2007). Likely, in our studies, none of these soil properties were at levels that would allow them to act as limiting factors for microbial activity, explaining why they did not have the measurable influence on Rh we hypothesized.

Soil physical properties and Rh have been studied in similar incubation experiments. Zezhou et al. (2022) demonstrated that the disruption of the aggregates liberates soil nutrients, which in turn promotes Rh. Similarly, Peng et al. (2017) found that stable microaggregates emit less Rh than dis-

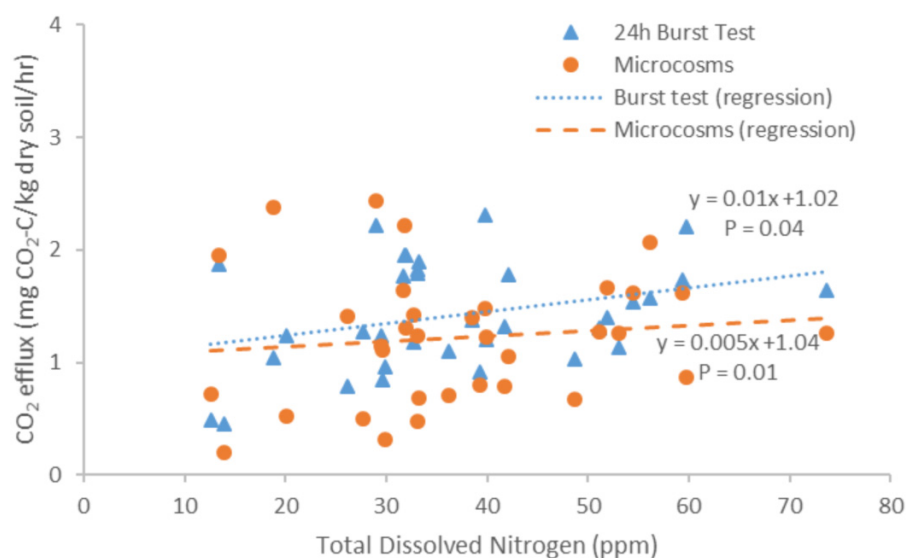
turbed macroaggregates. Regarding soil texture, Cable et al. (2008) found that Rh is more sensitive to disturbance and fluctuation on coarse versus fine-textured soils. Moreover, Chodak et al. (2010) found that soil texture had a greater influence on Rh than microbial diversity. In our study, soil texture and aggregate stability did not have a direct influence on Rh. It is possible that the range of texture and aggregate stability between the samples was too small to be able to detect significant correlations.

Overall, we recommend careful consideration when choosing a method of soil sampling and preparation prior to incubation for ex situ Rh assessment. Both homogenizing soil and the use of minimally disturbed microcosms have advantages and disadvantages. For example, when soils are sieved, it is easier to account for the heterogeneity of field conditions since a larger number of samples from multiple locations can be pooled, homogenized, and assigned to treatments. Thus,

**Table 5.** Summary of analysis of covariance between the 10-day incubations Rh and soil physico-chemical parameters.

Soil parameter	Coefficients	Standard error	<i>p</i> value
Intercept	-25.92	15.96	0.12
pH H <sub>2</sub> O	0.29	0.29	0.33
Aggregate stability (%)	-0.01	0.02	0.55
POX-C (mg kg <sup>-1</sup> )	0.00	0.00	0.59
Total N (%)	134.75	74.31	0.08
Total C (%)	-12.19	7.35	0.11
C:N ratio	2.33	1.58	0.15
Total dissolved organic C (ppm)	0.00	0.04	0.97
Total dissolved N (ppm)	0.02	0.03	0.58
N-NH <sub>4</sub> (ppm)	-4.02	5.06	0.44
N-NO <sub>3</sub> (ppm)	-0.01	0.03	0.72
Clay (%)	-0.02	0.07	0.76
Sand (%)	0.02	0.03	0.57

**Note:** Overall  $r^2$  of the regression 0.31 with overall standard error of the parameter of 0.60. Number of observations  $n = 34$ .

**Fig. 1.** Regression between heterotrophic soil respiration (CO<sub>2</sub> efflux) and total dissolved N.

in this case, the 24 h burst test is a suitable method. On the other hand, in other types of studies, when Rh needs to be analysed together with microbial diversity, the microcosm method should be considered given its minimal disturbance effect.

## Conclusions

To assess the effects of soil management practices on soil health, ex situ assessment of Rh is commonly performed. Different methods to determine ex situ Rh exist, but there have been only a few studies to compare their accuracies. In this study, drying and sieving the soil did not increase the Rh as hypothesized. On the contrary, the 10-day incubation method with dried and sieved soils yielded an average Rh significantly lower than that obtained using the minimally disturbed microcosms. The 24 h burst test using dried and sieved soil was only slightly lower than that obtained using minimally dis-

turbed microcosms. The microcosm method was weakly correlated with the 24h burst test, but did not correlate with the 10-day incubation method. Moreover, all three methods had low coefficients of regression with the 11 soil physico-chemical properties analyzed. Total dissolved N was the factor with the strongest correlation with Rh. We conclude that drying and sieving soil prior to performing ex situ Rh measurements using 24 h burst tests or the minimally disturbed microcosms can produce cautiously reliable and comparable results for soil from Canada's Atlantic region. Further studies should simultaneously compare ex situ and in situ measurements of soil respiration in this region and use a broader diversity of soil types and land managements.

## Acknowledgements

Funding for this study was provided by the Potato Early Dying (PED) cluster project PSS2224 of Agriculture and Agri-Food

Canada, and the Enabling Agricultural Research and Innovation Program of the New Brunswick Department of Agriculture and Aquaculture. Technical support was provided by Karen Terry, Ginette Decker, and Mohammed Islam. The cooperation of the growers is gratefully acknowledged. Special thanks to Prof. David Burton from Dalhousie University for processing the gas chromatograph samples.

## Article information

### History dates

Received: 3 October 2021

Accepted: 27 June 2022

Accepted manuscript online: 5 July 2022

Version of record online: 23 November 2022

### Notes

This paper is part of a Collection entitled “Soil Health Evaluation: Methods and Directions”.

### Copyright

© 2022 Authors Unc and Vallotton and Her Majesty the Queen in Right of Canada, represented by the Minister of Agriculture and Agri-Food Canada. This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

### Data availability

Data generated or analyzed during this study are provided in full within the published article and its supplementary materials.

## Author information

### Author ORCIDs

Louis-Pierre Comeau <https://orcid.org/0000-0001-5362-3701>

Adrian Unc <https://orcid.org/0000-0002-7265-9758>

### Author contributions

L-PC: Conceptualization, Formal analysis, Project administration, Writing – original draft, Writing – review & editing.

KM: Formal analysis, Methodology, Writing – review & editing.

AU: Writing – review & editing.

JV: Writing – review & editing.

### Competing interests

The authors declare there are no competing interests.

## Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjss-2021-0143>.

## References

- Abedin, J., and Unc, A. 2020. Addition of biochar to acidic boreal podzolic soils enhances micronutrient availability and crop productivity. *Open Agric.* **5**: 188–201. doi:[10.1515/opag2020-0021](https://doi.org/10.1515/opag2020-0021).
- Adekanmbi, A.A., Shaw, L.J., and Sizmur, T. 2019. Effect of Sieving on Ex Situ Soil Respiration of Soils from Three Land Use Types. *J Soil Sci Plant Nutr.* **20**: 912–916. doi:[10.1007/s42729-020-00177-2](https://doi.org/10.1007/s42729-020-00177-2).
- Angers, D.A., and Mehuys, G.R. 1993. Aggregate stability to water. In *Soil sampling and methods of analysis*. Edited by M.R. Carter. Canadian Society of Soil Science, Lewis Publishers, Ann Arbor, MI. pp. 651–657.
- Bao, X., Zhu, X., and Chang, X. 2016. Effects of soil temperature and moisture on soil respiration on the Tibetan plateau. *PLoS One*, **11**: 9–15. doi:[10.1371/journal.pone.0165212](https://doi.org/10.1371/journal.pone.0165212)
- Baveye, P.C, Otten, W., and Kravchenko, A. 2018. Emergent properties of microbial activity in heterogeneous soil microenvironments: different research approaches are slowly converging, yet major challenges remain. *Front. Microbiol.* **9**: 1929–1939. doi:[10.3389/fmicb.2018.01929](https://doi.org/10.3389/fmicb.2018.01929). PMID: [30210462](https://pubmed.ncbi.nlm.nih.gov/30210462/)
- Birge, H.E., Conant, R.T., Follett, R.F., Haddix, M.L., Morris, S.J., Snapp, S.S., et al. 2015. Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations. *Soil Biol. Biochem.* **81**: 304–310. doi:[10.1016/j.soilbio.2014.11.028](https://doi.org/10.1016/j.soilbio.2014.11.028)
- Brinton, W.F, and Vallotton, J. 2019. Basis for comparisons of soil CO<sub>2</sub> respiration test procedures. *Agric. Environ. Lett.* **4**: 1–2. doi:[10.2134/ael2018.10.0053](https://doi.org/10.2134/ael2018.10.0053).
- Brinton, W.F. 2020. Laboratory soil handling affects CO<sub>2</sub> respiration, amino-N and water stable aggregate results. *Agric. Res. Tech: Open Access J.* **24**: 556262. doi:[10.19080/ARTOAJ.2020.24.556262](https://doi.org/10.19080/ARTOAJ.2020.24.556262).
- Burton, D.L., Zebarth, B.L., Gillam, K.M., and MacLeod, J.A. 2008. Effect of split application of fertilizer nitrogen on N<sub>2</sub>O emissions from potatoes. *Canadian Journal of Soil Science*, **88**: 229–239. doi:[10.4141/CJSS06007](https://doi.org/10.4141/CJSS06007).
- Burton, A.J., Jarvey, J.C., Jarvi, M.P., Zak, D.R., and Pregitzer, K.S. 2012. Chronic N deposition alters root respiration–tissue N relationship in northern hardwood forests. *Glob. Chang. Biol.* **18**: 258–266. doi:[10.1111/j.1365-2486.2011.02527.x](https://doi.org/10.1111/j.1365-2486.2011.02527.x).
- Cable, J.M., Ogle, K., Williams, D.G., Weltzin, J.F., and Huxman, T.E. 2008. Soil texture drives responses of soil respiration to precipitation pulses in the Sonoran Desert: implications for climate change. *Ecosystems*, **11**: 961–979. doi:[10.1007/s10021-008-9172-x](https://doi.org/10.1007/s10021-008-9172-x).
- Cambouris, A.N., Nolin, M.C., Zebarth, B.J., and Laverdière, M. 2006. Soil management zones delineated by electrical conductivity to characterize spatial and temporal variations in potato yield and in soil properties. *Am. J. Potato Res.* **83**: 381–395. doi:[10.1007/BF02872015](https://doi.org/10.1007/BF02872015)
- Chen, D., Zebarth, B.J., Goyer, C., Dixon, T., Nahar, K., and Comeau, L-P. 2022. Effect of biofumigation and fumigation on population of root-lesion nematodes and *Verticillium dahliae* and potato yield and soil property in New Brunswick. *Am. J. Potato Res.* **99**, 229–242. doi:[10.1007/s12230-022-09875-2](https://doi.org/10.1007/s12230-022-09875-2).
- Chodak, M., and Niklinska, M. 2010. Effect of texture and tree species on microbial properties of mine soils. *Appl. Soil Ecol.* **46**: 268–275. doi:[10.1016/j.apsoil.2010.08.002](https://doi.org/10.1016/j.apsoil.2010.08.002).
- Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., and Townsend, A.R. 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry*, **82**: 229–240. Available from <http://www.jstor.org/stable/20456454>. doi:[10.1007/s10533-006-9065-z](https://doi.org/10.1007/s10533-006-9065-z)
- Comeau, L.-P., Lai, D.Y.F., Cui, J.J., and Farmer, J. 2018a. Separation of soil respiration: a site-specific comparison of partition methods. *EGU Soil*, **4**: 141–152. doi:[10.5194/soil-4-141-2018](https://doi.org/10.5194/soil-4-141-2018).
- Comeau, L.-P., Lai, D.Y.F., Cui, J.J., and Hartill, J. 2018b. Soil heterotrophic respiration assessment using minimally disturbed soil microcosm cores. *Elsevier MethodsX*, **5**: 834–840. doi:[10.1016/j.mex.2018.07.014](https://doi.org/10.1016/j.mex.2018.07.014).
- Cookson, W.R., Osman, M., and Marschner, P. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. *Soil Biol. Biochem.* **39**: 744–756. doi:[10.1016/j.soilbio.2006.09.022](https://doi.org/10.1016/j.soilbio.2006.09.022)
- Cooper, J.M., Burton, D., Daniell, T.J., Griffiths, B.S., and Zebarth, B.J. 2011. Carbon mineralization kinetics and soil biological characteristics as influenced by manure addition in soil incubated at a range of temperatures. *Eur. J. Soil Biol.* **47**: 392–399. doi:[10.1016/j.ejsobi.2011.07.010](https://doi.org/10.1016/j.ejsobi.2011.07.010)



- Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J. Lal, R., et al. 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. *Soil Sci. Soc. Am. J.* **76**: 494–504. doi:10.2136/sssaj2011.0286.
- Datta, R., Vranová, V., and Pavelka, M. 2014. Effect of soil sieving on respiration induced by low-molecular-weight substrates. *Int. Agrophys.* **28**: 119–124. doi:10.2478/intag-2013-0034.
- Davidson, E.A., Belk, E., and Boone, R.D. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Glob. Change Biol.* **4**: 217–227. doi:10.1046/j.1365-2486.1998.00128.x.
- Eijkkelkamp. 2008. Method 08.13 wet sieving apparatus operating instructions. Eijkkelkamp Soil and Water, the Netherlands [online]. Available from <https://en.eijkkelkamp.com/products/laboratory-equipment/wet-sieving-apparatus.html> [accessed 30 July 2019].
- Fahmy, S.H., Rees, H.W., and MacMillan, J.K. 1986. *Soils of New Brunswick, a first approximation*. New Brunswick Department of Agriculture and Aquaculture, Fredericton, NB, Canada
- Franzluebbers, A.J., and Veum, K.S. 2019. Comparison of two alkali trap methods for measuring the flush of CO<sub>2</sub>. *Agron. J.* **112**: 1279–1286. doi:10.1002/agj2.20141.
- Gan, H.Y., Schöning, I., Schall, P., Ammer, C., and Schrumpf, M. 2020. Soil organic matter mineralization as driven by nutrient stoichiometry in soils under differently managed forest stands. *Front. For. Glob. Chang.*, 1–15. doi:10.3389/ffgc.2020.00099.
- Gutinas, M.E., Gil-Sotres, F., Leiros, M.C., and Trasar-Cepeda, C. 2013. Sensitivity of soil respiration to moisture and temperature. *J. Soil Sci. Plant Nutr.* **13**: 445–461. doi:10.4067/S0718-95162013005000035.
- Haney, R.L., Brinton, W.F., and Evans, E. 2008. Soil CO<sub>2</sub> respiration: comparison of chemical titration, CO<sub>2</sub> IRGA analysis and the Solvita gel system. *Renew. Agric. Food Syst.* **23**: 171–176. doi:10.1017/S174217050800224X.
- Haney, R.L., Franzluebbers, A.J., Porter, E.B., Hons, F.M., and Zuberer, D.A. 2004. Soil carbon and nitrogen mineralization: influence of drying temperature. *Soil Sci. Soc. Am. J.*, **68**: 489–492.
- Herbst, M., Tappe, W., Kummer, S., and Vereecken, H. 2016. The impact of sieving on heterotrophic respiration response to water content in loamy and sandy topsoils. *Geoderma*, **272**: 73–82. doi:10.1016/j.geoderma.2016.03.002.
- Kroetsch, D., and Wang, C. 2007. Particle size distribution. In *Soil sampling and methods of analysis*. 2nd ed. Edited by M.R. Carter and E.G. Gregorich. CRC Press, Boca Raton, FL. pp. 713–725.
- Krzic, M., Walley, F.L., Diochon, A., Paré, M.C., and Farrell, R.E. (Editors). 2021. *Digging into Canadian soils: an introduction to soil science*. Canadian Society of Soil Science, Pinawa, MB. Available from <https://openpress.usask.ca/soilscience/>.
- Kuzuyakov, Y. 2006. Sources of CO<sub>2</sub> efflux from soil and review of partitioning methods. *Soil Biol. Biochem.* **38**: 425–448. doi:10.1016/j.soilbio.2005.08.020.
- Laffely, A.E., 2019. Root biomass and other soil properties affecting the CO<sub>2</sub> flush from laboratory dried and rewetted soils. *Electronic Theses and Dissertations*, University of Maine. Electronic theses and dissertations. p. 2952.
- Lang, M., Cai, Z., and Chang, S.X. 2011. Effects of land use type and incubation temperature on greenhouse gas emissions from Chinese and Canadian soils. *J. Soils Sediments*, **11**: 15–24. doi:10.1007/s11368-010-0260-0.
- Marland, G. 2008. Uncertainties in accounting for CO<sub>2</sub> from fossil fuels. *J. Ind. Ecol.* **12**: 136–139. doi:10.1111/j.1530-9290.2008.00014.x.
- McGowen, E.B., Sharma, S., and Deng, S. 2018. An automated laboratory method for measuring CO<sub>2</sub> emissions from soils. *Agric. Environ. Lett.* **3**, 1–5. doi:10.2134/ael2018.02.0008.
- Micks, P., Aber, J.D., Boone, R.D., and Davidson, E.A. 2004. Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests. *For. Ecol. Manage.* **196**: 57–70. doi:10.1016/j.foreco.2004.03.012
- Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., and Trevors, J.T. 2009. Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. *Soil Sci. Soc. Am. J.* **73**: 1843–1851. doi:10.2136/sssaj2008.0357.
- Moebius-Clune, B.N., Moebius-Clune, D.J., Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., et al. 2017. Comprehensive assessment of soil health—the Cornell framework manual, 3.1. ed. Cornell University, Geneva, NY.
- Nguyen, T.T., and Marschner, P. 2017. Soil respiration, microbial biomass and nutrient availability in soil after addition of residues with adjusted N and P concentrations. *Pedosphere*, **27**: 76–85. doi:10.1016/S1002-0160(17)60297-2
- Nyberg, M., and Hovenden, M.J. 2020. Warming increases soil respiration in a carbon-rich soil without changing microbial respiratory potential. *Biogeosciences*, **17**: 4405–4420. doi:10.5194/bg-17-4405-2020.
- Nyiraneza, J., Cambouris, A.N., Ziadi, N., Tremblay, N., and Nolin, M.C. 2012. Wheat yield and quality in relationships with soil texture and nitrogen fertilization. *Agron. J.* **104**: 589–599. doi:10.2134/agronj2011.0342
- Nyiraneza, J., Chen, D., Fraser, T., and Comeau, L-P. 2021. Improving soil quality and potato productivity with manure and high residue cover crops in eastern Canada. *Plants J.* **10**: 1436–1437. doi:10.3390/plants10071436.
- Peng, X., Zhu, Q., Zhang, Z., and Hallett, P.D. 2017. Combined turnover of carbon and soil aggregates using rare earth oxides and isotopically labelled carbon as tracers. *Soil Biol. Biochem.* **109**: 81–94. doi:10.1016/j.soilbio.2017.02.002
- Pulleman, M.M., and Marinissen, J.C.Y. 2004. Physical protection of mineralizable C in aggregates from long-term pasture and arable soil. *Geoderma*, **120**: 273–282. doi:10.1016/j.geoderma.2003.09.009.
- R Development Core Team. 2008. R: a language and environment for statistical computing. Version 2.8.1. R Foundation for Statistical Computing. Available from <http://www.R-project.org>.
- Schurgers, G., Ahlström, A., and Arneeth, A. 2018. Climate sensitivity controls uncertainty in future terrestrial carbon sink. *Geophys. Res. Lett.* **45**: 4329–4336. doi:10.1029/2018GL077528.
- Skjemstad, J.O., Baldock, J.A. M.R., Carter, E.G., and Gregorich. 2007. Total and organic carbon. In *Soil sampling and methods of analysis*. 2nd ed. Edited by. CRC Press, Boca Raton, FL. pp. 225–237.
- Soong, J.L., Fuchslueger, L., Maraňon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., and Richter, A. 2020. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Glob. Change Biol.* **26**: 1953–1961. doi:10.1111/gcb.14962
- Spohn, M. 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio, *Biogeosciences*, **12**: 817–823. doi:10.5194/bg-12-817-2015.
- Stenger, R., Barkle, G.F., and Burgess, C.P. 2002. Mineralization of organic matter in intact versus 201 sieved/refilled soil cores. *Aust. J. Soil Resour.* **40**: 149–160.
- Thomson, B.C., Ostle, N.J., and McNamara, N.P. 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. *J. Microbiol. Methods*, **83**: 69–73. doi:10.1016/j.mimet.2010.07.021. PMID: 20691223.
- Wang, L., Han, Z., and Zhang, X. 2010. Effect of soil pH on CO<sub>2</sub> emission from long-term fertilized black soils in Northeastern China. *Sci. Res.* **1**: 58–61.
- Wu, L., Vomocil, J.A., and Childs, S.W. 1990. Pore size, particle size, aggregate size, and water retention. *Soil Sci. Soc. Am. J.* **54**: 952–956. doi:10.2136/sssaj1990.03615995005400040002x.
- Yan, D., Li, J., and Pei, J. 2017. The temperature sensitivity of soil organic carbon decomposition is greater in subsoil than in topsoil during laboratory incubation. *Sci. Rep.* **7**: 20–79. doi:10.1038/s41598-017-05293-1. PMID: 28154414.
- Yiqi, L., and Xuhui, Z. 2006. Soil respiration and the environment; processes of CO<sub>2</sub> production in soil. Academic Press. Vol. 1. pp. 35–59.
- Zebarth, B., Fillmore, S., Watts, S., Barrett, R., and Comeau, L-P., 2021a. Factors related to within-field yield variation in commercial potato fields in Prince Edward Island Canada. *Am. J. Potato Res.*, **98**: 139–148. doi:10.1007/s12230-021-09825-4.
- Zebarth, B., Moreau, G., Dixon, T., Fillmore, S., Smith, A., Hann, S., and Comeau, L-P. 2021b. Soil properties and topographic features influence within-field variation in potato tuber yield in New Brunswick, Canada. *Soil Sci. Soc. Am. J.* doi:10.1002/saj2.20342.
- Zebarth, B., Islam, M., Comeau, L-P., Cambouris, A., and Burton, D. 2022. Spatial variation of soil health indices in a commercial potato field in Eastern Canada. *Soil Sci. Soc. Am. J.*, **86**, 134–145. doi:10.2136/sssaj2019.03.0087.

Ze Zhou, Z., Dongmei, W., and Mengxun, L. 2022. Soil respiration, aggregate stability and nutrient availability affected by drying duration and drying–rewetting frequency. *Geoderma*, **115743**, 1–10. ISSN 0016-7061. doi:[10.1016/j.geoderma.2022.115743](https://doi.org/10.1016/j.geoderma.2022.115743).

Zhang, J., Li, Y., and Wang, J. 2021. Different responses of soil respiration and its components to nitrogen and phosphorus addition in

a subtropical secondary forest. *For. Ecosyst.* **8**: 37–38. doi:[10.1186/s40663-021-00313-z](https://doi.org/10.1186/s40663-021-00313-z).

Zhou, W., Hui, D., and Shen, W. 2014. Effects of soil moisture on the temperature sensitivity of soil heterotrophic respiration: a laboratory incubation study. *PLoS One*, **9**, e92531. doi:[10.1371/journal.pone.0092531](https://doi.org/10.1371/journal.pone.0092531).