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Non-instrumental Real-time Soil Respiration Rate and Soil Microbial Biomass Carbon Determinations

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ABSTRACT

Soil microbial property is one of the fundamental indicators of soil health and quality, yet it has not been routinely examined in most soil testing protocols most likely due to the inconvenience of the current procedures. This study was initiated to validate a microrespirometry (MicroRes) method on real-time soil microbial respiration rate determinations with an infrared gas analyzer (IRGA) respirometer. The results showed that the two were agreeable (slope = 0.971, r^2 = 0.977, n = 24). We then used the MicroRes, in place of an IRGA respirometer, to determine the substrate-induced-maximum-initial-respiration (SIR) microbial biomass carbon (SMBC) on the 63 soil samples collected from 28 states of the US and three provinces of Canada. We also determined the10-day incubation mineralized carbon after rewetting the dried soils on the 63 soil samples. The SIR-SMBC determined correlated well with the 10-day incubation mineralized carbon (slope = 0.688, r^2 = 0.816, n = 63). MicroRes is a much simpler and cost-effective alternative to IRGA respirometers in real-time soil respiration rate and SIR-SMBC determinations. Its application makes routine real-time soil microbial property examinations possible under laboratory or filed conditions.

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Real-time respiration rates; substrate induced maximum initial respiration; soil microbial biomass carbon; non-instrumental microrespirometer

Introduction

Soil microbial property is a fundamental indicator of soil quality and soil health because soil microorganisms play a major role in soil carbon and nutrient cycling, organic matter transformations, and other soil processes (Beck et al. 2005; Doran and Parkin, 1994; Gregorich et al. 1994; Nannipieri et al. 2003; Saggar, Yeates, and Shepherd 2001; Turco, Kennedy, and Jawson 1994). Soil microbial properties, however, have not been routinely examined in most soil testing protocols, in part due to the inconvenience of the current analytical procedures.

Soil microbial properties could be indicated by several parameters including soil enzymes, soil microbial biomass, active or labile carbon, microbial respiration, and certain signature molecules such as phospholipid fatty acid (Hill et al. 2000; Killham 2002; Zibilske 1994).

Soil enzyme activities are considered useful indicators of soil quality because of their involvement in decomposition and nutrient cycling and rapid response to changes in soil management (Dick 1994; Dilly, Blume, and Munch 2003). However, relationships between soil enzymes and soil microbial activity or soil qaulity are still not clear and they can only be measured indirectly (Bandick and Dick 1999; Dick 1994). Soil enzyme activity determinations need to be performed in a laboratory under a set of assaying conditions such as a specific substrate, buffer solution, pH, temperature, and reaction time (Hill et al. 2000).

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The culture-independent phospholipid fatty acid (PLFA) method has been used primarily to assess the structure of soil microbial communities and determine gross changes that accompany soil disturbances such as cropping practices (Zelles et al. 1992, 1995). PLFA are potentially useful signature molecules due to their presence in all living cells. Despite their potential, this method has some seriuos limitations because although signature PLFAs can be correlated with the presence of some groups of organisms, they may not be unique under all conditions (Haack et al. 1994). This could give rise to false community signatures and misinterpretations of the status and structure of the microbial community (Hill et al. 2000). In addition, the PLFA analysis has special handling and storage requirements such as freezer storage of fresh soil and lyophilization prior to analysis (Petersen and Klug 1994). Studies have shown that improper storage can lead to shifts in the microbial community composition (Lee et al. 2007) affecting other indicators of microbial function, such as enzyme activity (DeForest 2009) and carbon mineralization (Franzluebbers 1999).

Cultural methods (Alexander 1982; Wollum 1982), microbial respiration rates (Anderson, 1982), and microbial biomass carbon (Parkinson and Paul 1982) have also been used to assess soil microbial property. Cultural methods can only assess a very limited fraction of the total soil microbial population and, hence, have limited representation of a true soil microbial population (Blagodatskaya and Kuzyakov 2013).

The incubation-based soil respiration can be used to express the microbial activity in natural soil conditions (Keeney 1982). Those traditional incubation methods, however, are quite lengthy (14 days or more) in order to be reproducible and meaningful. It was found that the CO_2 flush after rewetting dried soils could be an indicator of soil microbial mineralization capability (Marumoto, Anderson, and Domsch 1982; Sorensen 1974; Sparling et al. 1995). The three-day CO_2 flush after rewetting dried soils was also found to be correlated to soil microbial biomass, active soil carbon pool, and net nitrogen mineralization (Franzluebbers and Haney 2018; Franzluebbers et al. 2000; Franzluebbers, Hons, and Zuberer 1996). Soil respiration measurements were usually determined by the exhaustive CO_2 absorption (by alkaline) approach such as alkaline trap-titration (Keeney 1982), Solvita (Haney, Brinton, and Evans 2008) or Draeger tube (Soil Quality Institute 1999) methods. The alkaline trap-titration method is simple and inexpensive but it requires longer incubation time (>24 hrs) and thus can't be real-time measurements. Solvita method uses an alkaline gel/indicator system to determine the amount of CO_2 absorbed during a 24-hr incubation. Solvita is easier to operate than the alkaline-trap-titration method although not as sensitive and accurate (Franzluebbers 2016; Haney, Brinton, and Evans 2008). The Draeger tube method can determine soil respiration rates in less than an hour. It, however, suffers two major drawbacks: First, it is not sensitive. Draeger method measures CO_2 concentration in the percentage (10,000 ppm) range with uncertainty of 500 ppm – 1000 ppm. Second, due to its insensitivity, it can only be applied to a large size soil sample such as in a six-inch diameter soil core in a field. Furthermore, the Draeger tube method is quite tedious and more expensive to operate than the alkaline trap-titration or the solvita methods.

Soil microbial biomass carbon (SMBC) has been one of the more commonly recognized and useful soil microbial property indicators (Carter et al. 1999). SMBC represents the size of the soil microorganism pool, which reflects the quality and quantity of soil organic matter (Carter et al. 1999). SMBC, as a single ecological entity, can provide us a better understanding of some key soil functions such as carbon and nutrient cycling and soil organic matter transformations (Anderson and Domsch 2010).

Various methods for estimating SMBC have been developed over the years. The most commonly referred method for SMBC is the chloroform fumigation incubation (CFI) method (Jenkinson and Powlson 1976). The CFI method uses chloroform to fumigate (kill) soil microorganisms and then re-activates the microbial population by driving off the fumigant, and re-inoculation. The amount of re-mineralized carbon in comparison to the control during a 10-day incubation is used to assess the original SMBC. The CFI method, however, is tedious and lengthy in addition to using a hazardous fumigant. Several extraction alternatives that eliminate the long incubation period were then developed (e.g., chloroform fumigation extraction, Vance, Brookes, and Jenkinson 1987; direct chloroform extraction, Gregorich et al. 1990; hot-water-extraction, Sparling, Vojvodic-Vokovic, and Schipper

1998). The SMBC procedures utilizing CFI and its alternatives, however, are all post-time methods and considered inconvenient for a routine soil analysis.

Anderson and Domsch (1978) proposed a substrate (glucose) induced maximum initial respiration (SIR) approach to assess SMBC in real-time. The SIR-SMBC method measures the metabolically active portion of the microbial biomass based on the initial change in the soil respiration rate as a result of adding a glucose substrate. This initial respiratory response measured in real time before any growth of microorganisms occurred, can be used to assess the SMBC. Anderson and Domsch (1978) showed that the results of the SIR-SMBC method correlated well with those of the CFI method. The SIR-SMBC was later confirmed by corresponding measurements carried out with pure cultures of soil fungi (Martens 1995). The CFI, CFE, and SIR methods for estimating SMBC were given identical ranking for a range of 20 arable and forest soils in an inter-laboratory comparison (Beck et al. 1997; Carter et al. 1999). The SIR-SMBC method, however, requires a sensitive infrared gas analyzer (IRGA) for real-time soil microbial respiration determinations. An IRGA respirometer analysis requires calibration of the instrument, air-tight connection/control of gas flow, estimation of the headspace volume of the setup, correction for moisture and volatile interferences on CO_2 measurements to obtain a good result. Therefore, although the SIR method has the real-time advantage, it is still not routinely used in soil testing because of the technical sophistication of the IRGA respirometer involved.

A non-instrumental microrespirometer (MicroRes) method for real-time microbial respiration rate determinations has been proposed (Hsieh and Hsieh 2000). The MicroRes method does not use the traditional exhaustive CO_2 absorption strategy for determination of the respiration rate that requires a much higher alkaline concentration (and thus low sensitivity) and a longer (>24 hrs.) incubation period for a test. Rather, the MicroRes uses a unique negative-feedback mechanism, in which the CO_2 absorption rate of the reagent adjusts itself to be equal to the CO_2 evolution rate of the sample in a 10–40 minutes "conditioning period". After the steady state of the CO_2 absorption and evolution between the reagent and the sample has been established, the CO_2 evolution rate of the sample can be determined by the CO_2 absorption rate of the reagent.

The MicroRes has the superior sensitivity of the instrumental IRGA methods in real-time respiration rate determinations (Hsieh and Hsieh 2000; Li and Hsieh 2003; Ren and Hsieh 2005). The results of the MicroRes method have been verified with the IRGA respirometers (r^2 = 0.978, Hsieh and Hsieh 2000; r^2 = 0.914; Sparda et al. 2017) over a wide range of respiration rates. The original MicroRes method requires standardization of the reagent and the exchange of reagent in the device between the "conditioning period" and the "assaying period" (Hsieh and Hsieh 2000). Recently, a "double-indicator" version of the MicroRes method has been introduced that eliminates both the two above mentioned steps of the original method (Microdetect LLC, Tallahassee, FL). This "double-indicator" version of the MicroRes procedure significantly simplifies the original MicroRes procedure but its accuracy needs to be verified.

The objectives of this study were: 1) validate the "double-indicator" version of MicroRes with an IRGA respirometer; 2) use the "double-indicator" MicroRes as an IRGA alternative in the real-time determination of soil respiration rates and SIR-SMBC.

Materials and methods

Soil samples

Forty Standard Reference Soils (SRS) collected from 28 states of the US and three provinces of Canada with a wide range of soil properties (pH 3.70–8.13; organic matter 8.7 mg/g – 104.8 mg/g; texture sand – clay) were obtained from the archives of the Agriculture Laboratory Proficiency (ALP) Program, Collaborative Testing Services, Inc., Sterling,Virginia, USA. Additional 23 samples were obtained from the laboratory of USDA-ARS, Fort Collins, CO, USA. All samples were air-dried, sieved through 2 mm sieve as received. The locations and soil properties of the samples are given in Table 1.

Table 1. Location and properties of soil samples. The first 40 soil samples and their property were provided by the Agriculture Laboratory Proficiency (ALP) Program, Collaborative Testing Services, Inc., Colorado State University, Fort Collins, Colorado, USA. The last 23 soil samples were provided by Lucretia Sherrod of the USDA-ARS, Fort Collins, CO, USA.

Sample ID	Location	Texture	pH	OM(%)	N (%)
SRS-1101	Harington,PE, Canada	SL	4.9	3.73	0.151
SRS-1104	Thor, IA	SCL	7.43	4.11	0.185
SRS-1105	Turlock, CA	SiL	7.78	2.00	0.105
SRS-1107	Tolland, CT	SL	5.42	7.88	0.36
SRS-1108	Raleigh, NC	L	5.64	3.78	0.151
SRS-1111	Auburn, AL	SL	5.59	0.81	0.028
SRS-1113	Kearney, NE	SiL	7.06	2.71	0.13
SRS-1202	Tully, NY	SL	7.4	3.93	0.21
SRS-1207	Deerfield, NH	SL	5.23	5.40	0.257
SRS-1208	Crowley, LA	SiL	8.1	1.27	0.052
SRS-1212	Yahzoo Cty, MS	SiL	7.68	2.12	0.107
SRS-1301	Gainsville, FL	S	6.54	1.67	0.072
SRS-1303	North Scituate, RI	SL	4.37	11.50	0.397
SRS-1308	Madisons, AL	CL	6.85	3.38	0.127
SRS-1311	Flathead, MT	L	7.86	4.67	0.259
SRS-1315	Marianna, AR	SiL	5.21	1.51	0.079
SRS-1403	Bruce, SD	LS	6.3	2.20	0.12
SRS-1409	Emmetsburg, IA	CL	5.93	5.37	0.256
SRS-1411	Bratteboro,VT	SL	5.77	5.69	0.25
SRS-1501	Shubenacadie, NS, Canada	L	5.81	6.19	0.285
SRS-1503	Nacodoches, TX	S	4.97	0.87	0.05
SRS-1504	Jerome, ID	L	5.5	1.72	0.101
SRS-1505	Hubbard, IA	CL	7.57	6.01	0.294
SRS-1507	Pilot Grove, MN	CL	7.64	8.05	0.412
SRS-1508	Maricopa, AZ	SCL	8.1	1.70	0.07
SRS-1509	Summerland, BC Canada	SL	6.88	2.49	0.133
SRS-1510	Clemson, SC	L	5.51	3.07	0.103
SRS-1515	Pryor, MT	CL	5.35	2.72	0.121
SRS-1606	Kanrado., KS	L	7.97	2.48	0.11
SRS-1609	Magnolia, MS	L	5.43	2.41	0.07
SRS-1610	Middleford, DE	SL	6.17	1.62	0.081
SRS-1612	St Francis, AR	SiL	5.11	2.00	0.086
SRS-1615	Viog, IN	SL	5.82	1.78	0.091
SRS-1701	Wislon, AB, Canada	CL	8.13	2.87	0.15
SRS-1702	Deerfield, MA	L	5.25	2.77	0.117
SRS-1704	Alamo, MI	SL	6.13	1.60	0.08
SRS-1706	Vienna, GA	LS	5.34	1.24	0.071
SRS-1708	Arcola, SK, Canada	L	7.65	7.02	0.426
SRS-1709	Eldridge, CA	C	5.68	5.91	0.061
SRS-1710	Ellsworth, MN	SL	6.95	4.35	0.241
ARS-1-1-2	Sterling, CO	SL	5.52	4.68	**NA**
ARS-1-1-7	Sterling, CO	SL	4.75	4.60	**NA**
ARS-1-1-8	Sterling, CO	SL	5.82	4.13	**NA**
ARS-1-1-9	Sterling, CO	SL	5.45	4.52	**NA**
ARS-1-1-12	Sterling, CO	SL	7.06	5.38	**NA**
ARS-1-1-18	Sterling, CO	SL	6.30	4.87	**NA**
ARS-1-1-22	Sterling, CO	SL	5.29	4.57	**NA**
ARS-1-2-2	Sterling, CO	LS/SL	6.91	4.36	**NA**
ARS-1-2-7	Sterling, CO	SL	5.14	6.20	**NA**

(*Continued*)

Table 1. (Continued).

Sample ID	Location	Texture	pH	OM(%)	N (%)
ARS-1-2-8	Sterling, CO	SL	5.82	4.27	**NA**
ARS-1-2-9	Sterling, CO	SL	5.10	5.02	**NA**
ARS-1-2-12	Sterling, CO	SL	6.08	5.18	**NA**
ARS-1-2-18	Sterling, CO	SL	6.28	4.84	**NA**
ARS-1-2-22	Sterling, CO	SL	6.48	5.16	**NA**
ARS-1-3-2	Sterling, CO	SL	4.98	5.31	**NA**
ARS-1-3-9	Sterling, CO	SL	4.96	7.48	**NA**
ARS-1-3-18	Sterling, CO	SL	4.98	7.34	**NA**
ARS-1-3-22	Sterling, CO	SL	7.13	5.37	**NA**
ALP-DAN 1	KS	SL	3.70	1.85	**NA**
ALP-FRI-15	IA	SL	6.76	10.48	**NA**
ALP-NFL	PA	SL	6.07	4.10	**NA**
ALP-EMB	ND	LS	6.59	2.48	**NA**
ALP-Wayne	NE	SL	5.14	5.64	**NA**

Mineralized carbon in a 10-day incubation after rewetting the dried soils

Twenty-five g of dried soil of each sample were packed in a volumetric cylinder to determine the bulk soil volume. The difference between the bulk soil volume and the calculated soil solid volume (i.e., 25/2.6 = 9.61 mL) was determined as the total soil pore volume of the sample. A 25-g dry soil was transferred to a 300-ml incubation flask and a given amount of water, which could fill 50% of the determined soil pore volume, was added and mixed thoroughly with the soil using a spatula. An alkaline trap cup containing 3 ml of 0.5 M NaOH solution was inserted in the middle of the flask and the flask closed with a stopper. The rewetted soil samples were then incubated for three days in the laboratory at 22 ± 1°C. Three blank flasks were also included in the incubation to serve as the control. After the three-day incubation, the alkaline traps were retrieved and 1.5 mL of 0.5 M $BaCl_2$ added to each to precipitate the carbonate. The remaining alkalinity of the alkaline traps were determined by titrating them with a standardized 0.5 M HCl solution. The total mineralized carbon (μmole CO_2) during the incubation was calculated by the alkalinity consumed (the alkalinity of the blank control minus the alkalinity of the sample in the alkaline traps) divided by 2. Immediately after the three-day incubation, a seven-day incubation was followed to determine the mineralized carbon using the same procedure. The cumulative mineralized carbon in the 10-day (the three-day plus the seven-day) incubations was converted to a weight basis or volume basis by multiplying a factor of 12 (the atomic weight of carbon) or 24.2 (the molar gas volume at 22°C and 1 atm) respectively. Each soil sample was duplicated in the incubations.

The "double-indicator" MicroRes procedure

A MicroRes device consists of a reagent chamber and a sample cylinder (Figure 1). The two are coupled through a hollow rubber chamber seat, which connects the two headspaces and separates the reagent and the sample.

Three to five g of the incubated soil sample were weighed into a sample cylinder. The sample cylinder was then coupled with a reagent chamber, whose syringe hole was sealed with a septum. An aliquot of either 0.5 mL, 0.8 mL or 1.0 mL of the double-indicator MicroRes® reagent (Microdetect, LLC., Tallahassee, FL, USA) was injected into the reagent chamber, depending on the expected range of the respiration rates. That is, 0.5 mL for rates below 50 μl CO_2/hr, 0.8 mL for rates between 50 μL CO_2/hr and 100 μL CO_2/hr and 1.0 mL for rates above 100 μL CO_2/hr . Using a larger volume of the reagent for a lower respiration rate doesn't affect the result, but requires a longer time to complete

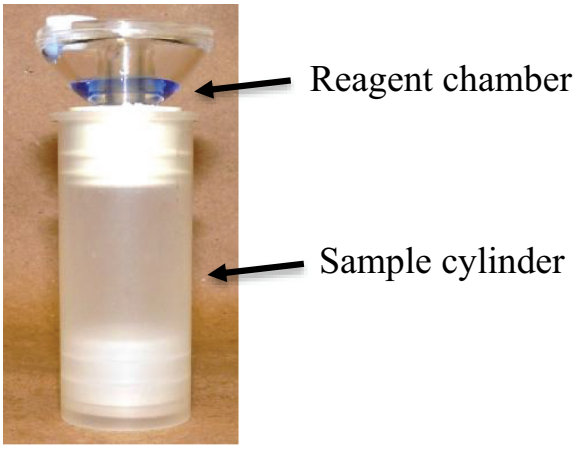

Figure 1. A MicroRes device consists of a reagent chamber and a sample cylinder. The two are coupled through a hollow chamber seat as such the two headspaces are connected but the reagent and the sample are separated.

a measurement. Using a smaller reagent volume for a higher respiration rate, on the other hand, may affect the result, as the assaying period may be too short to be counted accurately. (The assaying period for the MicroRes method should be longer than three or four minutes.)

After the reagent is injected into the reagent chamber, the MicroRes was placed on a Viber (a vibrational shaker, Microdetect LLC, Tallahassee, FL) for a "conditioning" period, in which the color of the reagent was dark blue with a tint of pink. When the blue hue of the reagent transforms into a pink color (according to the pink color reference provided by the Microdetect LLC), the "conditioning" period is ended and the "assaying" period initiated. This initial time of the "assaying" period was recorded as t_o. The "assaying" period ended when the pink reagent turned into colorless and this end time was recorded as t. The duration of the "assaying" period, i.e., $t - t_o$ in minutes, was used to calculate the soil respiration rate (SRR) according to the following formula:

$$SRR(\mu LCO_2/g/hr) = c/(t - t_o)/drywt.ofsample(g) \quad (1)$$

Where c is the constant that converts the "assaying" period, i.e., $(t\text{-}t_o)$ in minutes, to the respiration rate in μL CO_2/hr at 22°C (MicroRes manual, 2018). The value of c is either 205, 328 or 410 depending on whether 0.5 mL, 0.8 mL or 1.0 mL reagent, respectively, was injected into the reagent chamber. A MicroRes Viber can accommodate up to a maximum of 16 MicroRes (16 samples) simultaneously.

The IRGA respirometer

The IRGA respirometer consisted of an IRGA (for quantifying CO_2 concentration, Li-840A, Li-COR Biosciences, Lincoln, NE), an air-tight metal bellow pump (for circulating the headspace air between the IRGA and a sample bottle, Metal Bellows Inc., Sharon, Mass.) and a sample bottle. The IRGA, the metal bellow pump and the sample bottles were all equipped with two gas-tight shut-off connectors (Cole-Parmer, Vernon Hill, IL), one at the input port and the other at the output port. The shut-off connector shuts off the path when it is disconnected and opens up the path when it is connected. The headspace CO_2 concentration of a sample bottle was measured by connecting the bottle to the IRGA through the metal bellow pump to form a close loop of headspaces. The metal bellow pump circulated the headspace air in the loop at a flow rate of 350–400 mL/min. The total dead headspace in the IRGA and the bellow pump was determined to be 38.8 ± 1.2 mL. The headspace of an empty sample bottle was determined to be 302 ± 3 mL. The volume of the sample was estimated and subtracted from the empty bottle volume as the headspace volume of the sample bottle. The respiration rate of the sample in the sample bottle was determined by the difference

between the initial and final CO_2 concentrations of the incubation period, which ranged from 30 minutes to one hour depending on the respiration rate. The actual CO_2 concentration in the sample bottle at the end of the incubation (CO_{2f}) was calculated after correcting the interference of the residual CO_2 in the IRGA dead headspace before the measurement:

$$CorrectedCO_{2f}(ppm) = (CO_{2a} * (38.8 + V_s) - CO_{2b} * 38.8)/V_s \quad (2)$$

Where CO_{2a} and CO_{2b} were the CO_2 readings (ppm) of the IRGA after and before connecting the sample bottle to the IRGA, 38.8 (mL) was the dead headspace volume of the IRGA and the metal bellow pump and V_s was the headspace volume of the sample bottle in mL. The respiration rate (RR) was calculated by the formula,

$$RR(\mu LCO_2/hr) = (CO_{2f} - CO_{2i}) * V_s/1000/t \quad (3)$$

Where CO_{2f} and CO_{2i} were the final and initial CO_2 concentrations, respectively, of the sample bottle, and t is the duration of the incubation in hours.

Comparison between the "double-indicator" MicroRes and the IRGA respirometer

We compared the soil respiration rates determined by the "double-indicator" MicroRes with those by the IRGA respirometer in selected soil samples with a wide range of respiration rates. Five g of incubated soil samples were used for the respiration rate determinations. Respiration rates of each soil sample, in four replicates, were determined by the two methods, respectively, in paired comparison experiments. The results of the 24 experiments were analyzed by the two-tailed t-tests and one-way analysis of variance (ANOVA).

SIR-SRR and SMBC determinations

SIR-SMBC method determines real-time SMBC in microbial active (fresh or incubated) soils. We determined the SIR-SMBC on the 63 soil samples after they were rewetted and incubated for 10 days. For the SIR-SRR procedure, 25 mg of the 20% glucose-talc mixture was added to five g of the incubated soil. The soil and the glucose-talc mixture were mixed thoroughly with a spatula. The amount of 25 mg of the 20% glucose mixture added was according to the results of a pre-experiment that determined the optimal amount of glucose added, which was sufficient to induce the maximum initial respiration rates for all the soil samples tested. The glucose-soil mixture was transferred to the sample cylinder of a MicroRes. Following a 20-minute wait time, the SIR-SRR was determined by the MicroRes® method described above. The SIR-SRR were calculated according to Eq. 1. All respiration rates of soil samples were measured in four replicates.

The SIR-SRR (mL CO_2/h/100 g dry soil) was then used to calculate the SIR-SMBC (mg C/100 g dry soil) according to the formula of Anderson and Domsch (1978):

$$SIR - SMBC = 40.04 * SIR - SRR + 0.37 \quad (4)$$

Results

Comparison between the "double-indicator" MicroRes and IRGA methods

Paired comparison of the means (n = 4) between the MicroRes and IRGA methods on soil respiration rate determinations showed that 23 out of the 24 tests were not significantly different at the 1% probability level ($\alpha = 0.01$) or 21 out of the 24 tests were not significantly different at the 5 % probability level ($\alpha = 0.05$). The one-way ANOVA of all the tests (n = 24) indicated that the two methods were not significantly different at the 5 % probability level ($\alpha = 0.05$). Linear regression analysis of the results between the "double-indicator" MicroRes method and the IRGA method (Figure

2) indicated that the two methods were well agreeable in a wide range of respiration rates with the slope of 0.971 and the *r*-square of 0.977. The standard error shown in Figure 2 included both the errors from sampling and from the methodology. The coefficient of variation (CV) of the MicroRes method ranged from 1.4 % to 34.2 % (average = 14.5 %). The CV of the IRGA method ranged from 1.6% to 32.6% (average = 9.81%). Although it is difficult to separate the variation of sampling from the error of the methodology, the error of the MicroRes method apparently was greater than that of the IRGA method. The CV of the lower respiration rates (<25 μL CO_2/hr.) were not significantly different at the 5% probability level between the two methods (average CV = 10.62 ± 4.54% for the MicroRes method and 6.31 ± 4.23% for the IRGA method). But those at the higher respiration rates (> 25 μL CO_2/hr.) the CV for the MicroRes method (average CV = 17.1%) were significantly greater than that of the IRGA method (average CV = 8.9%) at the 5% probability level. The higher variation of the MicroRes method in comparison to that of the IRGA method was likely due to the subjectivity of judging the starting time of the "assaying" period when the blue color changed to the pink color. Nonetheless, the "double-indicator" MicroRes procedure was much easier to be operated than the original MicroRes® procedure (Hsieh and Hsieh 2000). The range of time required for the "double indicator" to determine the SIR-SMBC was 19 minutes to 48 minutes in this study, excluding the sample preparation time. Which is shorter than the 60 minutes to 90 minutes required for the original MicroRes procedure and comparable to that of the IRGA respirometer (30 minutes to 60 minutes). The MicroRes method, however, is technically much simpler and less expensive than the instrumental IRGA respirometer.

SIR-SMBC and the 10-day incubation mineralized carbon

The SIR-SMBC determined by the MicroRes procedure covered a wide range from 1.90 mg C/100 g to 55.90 mg C/100 g. The 10-day incubation mineralized C also resulted in a wide range from 1.72 mg C/100 g soil to 75.98 mg C/100 g soil. The SIR-SMBC was well correlated with the 10-day incubation mineralized C (Figure 3, slope = 0.688; r^2 = 0.816, n = 63).

The average CV of SIR-SMBC for the quadruplicated samples was 12.6% and that of the 10-day incubation mineralized C for the duplicated samples was 8.75%. The 10-day incubation mineralized C, SMBC, active organic carbon pools and net nitrogen mineralization potential were all well correlated

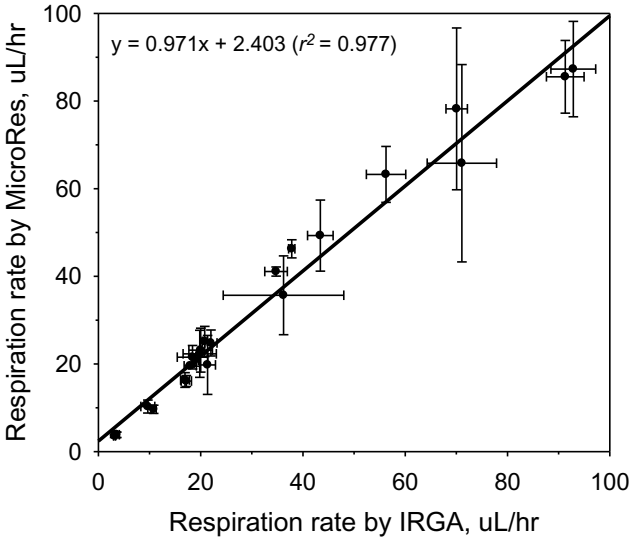

Figure 2. The relationship between the "double-indicator" MicroRes method and the IRGA method on soil respiration rate determinations. Each sample had four replicates determined simultaneously. The standard error bars (n = 4) indicates the magnitude of the combined sampling and methodology errors.

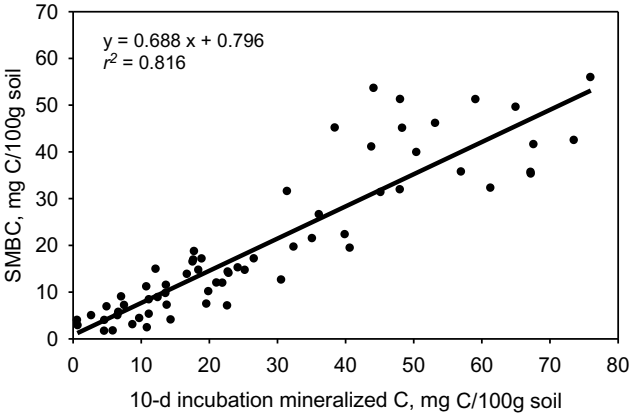

Figure 3. The relationship between SIR-SMBC and the 10-day incubation mineralized carbon (n = 63).

soil parameters that indicate soil microbial property. They are all valuable indicators to soil quality and health. (Franzluebbers et al. 2000; Franzluebbers, Hons, and Zuberer 1996) The SIR-SMBC procedure presented in this study, however, is the only one that can be conveniently determined in real time and under field conditions.

The correlations between the SIR-SMBC and the soil organic matter (Figure 4, r^2 = 0.548) and between SIR-SMBC and the total soil nitrogen (Figure 5, r^2 = 0.387) are also significant but to a lesser extent as compared with the correlation between the SIR-SMBC and the 10-day incubation mineralized C. These lesser correlations are expected because SMBC is not only a function of the quantity but also the quality of the organic matter and nitrogen. The soil organic matter and total nitrogen determined represent only the total but not necessarily the bioavailable quantity.

Discussion

This study showed that the results of the "double-indicator" MicroRes and the IRGA respirometer are agreeable in soil respiration determinations. The MicroRes is a much less expensive and technically simpler alternative to IRGA respirometry in real-time respiration rate determinations. The application

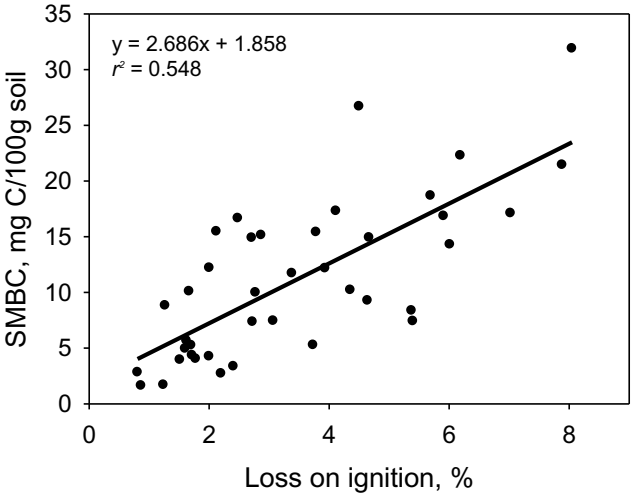

Figure 4. The relationship between the SIR-SMBC and the soil organic matter (loss on ignition, n = 40).

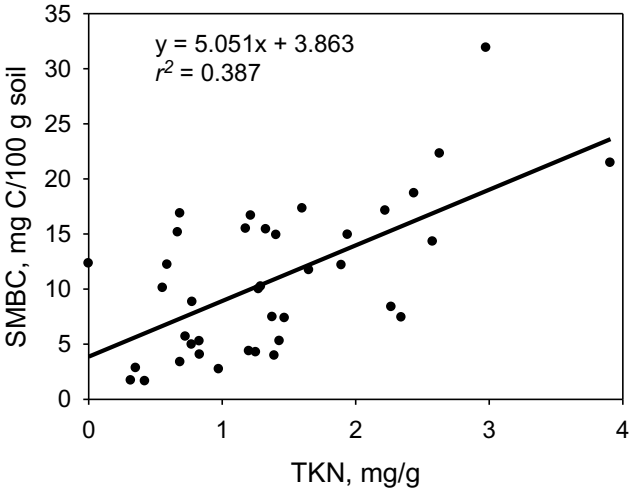

Figure 5. The relationship between the SIR-SMBC and the total soil nitrogen (n = 40).

of the MicroRes to the SIR-SMBC procedure makes real-time routine SMBC determinations possible under laboratory or field conditions. The upper limit for soil respiration rate determination by MicroRes is around 100 μL CO_2/hr. We used five g wet soil sample for this study. For soils that have higher SIR-SRR (> 100 μL CO_2/hr for five g wet soil), we recommend reducing the soil sample size to four or three g.

The original SIR-SMBC formula (Anderson and Domsch 1978) is valid for the temperature of 22°C only. A temperature correction factor of SIR-SMBC needs to be identified and applied to the situation where SIR-SMBC is determined significantly deviate from the 22 ± 1°C.

The SIR-SMBC method was developed for testing microbial active soils when the soil moisture was not a limiting factor for the maximum respiration rate after the glucose was added (Anderson and Domsch 1978). We tested the SIR-SMBC procedure on a field-moist soil (12.2 % moisture content or 23% soil pore space filled with water) and the treatments of adjusting the soil moisture to 22% (or 43% soil pore space filled with water) and 32% (65% soil pore space filled with water). The SIR-SMBC thus determined were 62.67 ± 9.44 mg C/100 g, 104.59 ± 3.07 mg C/100 g and 110.61 ± 8.33 mg C/100 g for the 12.2%, 22% and 32% soil moisture, respectively. The 12.2% moisture was obviously limiting the maximum microbial respiration in the test. The 22% moisture and the 32% moisture had no significant difference in the SIR-SMBC results. This indicates that the SIR-SMBC test should be carried out with a high soil moisture condition such that moisture is not a limiting factor for microbial respiration after the glucose-talc mixture is added. Because the MicroRes SIR-SMBC procedure can be done in less than one hour, a moisture level as high as 65% soil pore space filled with water should not limit the oxygen supply to the aerobic respiration. Moisture level significantly lower than 40% soil pore space filled with water, on the other hand, may underestimate the SIR-SMBC. Therefore, additional moisture needs to be added to a soil sample in the SIR-SMBC procedure as such the soil moist is >20% but less than saturation. This wide range of higher soil moisture level for the SIR-SMBC procedure shouldn't be difficult to be figured out by feeling the soil by hand.

Routine examinations of SIR-SMBC complement the soil testing procedure, which usually includes only the soil chemical and physical properties. Routine testing of soil microbial properties could significantly improves our understanding of soil health and quality in croplands.

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