

# Relationship among Different Soil Biochemical Methods to Determine Soil Health

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## Abstract

Numerous soil biochemical methods are used to determine the soil health status, but the relationships among these methods are not well understood. Relationships among soil biochemical tests, 1) chloroform fumigated microbial biomass C (CFMBC), 2) permanganate oxidizable C (POXC), 3) Solvita CO<sub>2</sub>-burst (Solvita), 4) Solvita labile amino nitrogen (SLAN), and short-term soil CO<sub>2</sub> efflux during laboratory incubation using (v) Alkali-base trap (Alkali) and (vi) infrared gas analyzer (IRGA), were evaluated for nine agricultural soils collected across the Red River Valley of North Dakota and Minnesota, USA. Not a single test is comprehensive to relate with all soil biochemical tests. Coefficient of variation percentage for particular method varied with soil type. Among six tests, CFMBC is significantly ( $p < 0.05$ ) related with Alkali ( $r = 0.37$ ), Solvita ( $r = 0.57$ ), SLAN ( $r = 0.52$ ), and POXC ( $r = 0.68$ ). Soil CFMBC correlates with most of soil biochemical tests and can be potential to determine soil biochemical condition.

## Keywords

Chloroform Fumigated Microbial Biomass Carbon, Solvita CO<sub>2</sub>-Burst, Soil Labile Amino Nitrogen (SLAN), Permanganate Oxidizable Carbon, Infrared Gas Analyzer, Soil Organic Carbon

## 1. Introduction

Measuring soil health has been gaining popularity due to the growing consensus about protecting the agricultural sustainability [1] [2]. Different commercial test kits like Solvita CO<sub>2</sub>-burst<sup>®</sup> [3] and standard laboratory methods like permanganate-oxidizable carbon (POXC) [4] and mineralizable C (as determined by short-term aerobic incubation of rewetted soil), are available to assess the soil health condition rapidly. The concept of the labile C pool, behind these me-

thods, is the most biologically active and sensitive to shift in management practices [5]. However, these tests have the potential to predict nutrient availability and supply to crops [6]. The main goal of this study is to determine the associations between rapid soil biochemical tests and soil properties. Understanding their relationships will help them to utilize their potential to the fullest.

The relationships among soil biochemical health tests are not straight-forward [7]. Moreover, their relationship and sensitivity are strongly influenced by landscape characteristics [8], inherent soil properties like texture [9] and crop and soil management practices like tillage and rotation [10] [11] [12]. Some soil biological tests are more sensitive to shift with soil factors than other tests.

For this study, soil samples were collected from nine different agricultural fields of the Red River Valley of North Dakota and Minnesota, USA, to study the relationships among soil properties and selected rapid soil biochemical tests. It was hypothesized that soil health function is significantly correlated with soil properties across soil and crop management practices. Six soil biochemical properties, 1) soil organic matter (SOM), 2) soil pH, 3) soil electrical conductivity, 4) soil nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), 5) Olsen-extracted phosphorus (P), and 6) soil organic carbon (SOC) were selected. Six soil biochemical health tests were determined: a) chloroform fumigated microbial biomass C (CFMBC), b) POXC, c) Solvita labile amino nitrogen (SLAN), and mineralizable C pool using d) NaOH-base trap (Alkali), e) infrared gas analyzer (IRGA), and f) Solvita  $\text{CO}_2$ -burst tests. Main objectives were to understand the i) variability of response in soil properties and soil biochemical health tests and ii) relationships among rapid soil biochemical tests and soil properties for agricultural soils in the Northern Great Plains.

## 2. Materials and Methods

### 2.1. Soil Sampling

During fall 2016, soil samples of 0 - 15 cm depth were collected using a bucket-auger from nine different agricultural fields across the Red River Valley of North Dakota and Minnesota, United States of America. All sites are located under temperate climate. Details about sampling sites are presented in **Table 1**. Soil classification information was collected from the Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>). Soil samples were air-dried, passed through 2 mm sieve.

### 2.2. Soil Analysis

Samples from each site were divided into five subsamples for the laboratory analysis. Basic soil properties were analyzed as outlined in “Recommended Chemical Soil Test Procedures for the North Central Region” [13]. Soil water holding capacity (WHC) was determined using the Pressure-plate method [14]. Soil pH and electrical conductivity (EC) were measured using pH/CON 450 meter (Oakton Instruments, Vernon Hills, IL, USA) with soil water ratio of 1:2.5

[15]. Soil  $\text{NO}_3^-$  concentration was determined by extracting 5 g soil samples with 25 ml of 2M KCl and subsequently analyzed the aliquot with TL-2800 ammonia analyzer (Timberline Instruments, Boulder, CO, USA) using KCl extraction [16]. The concentration of soil available phosphorus (P) or Olsen-P, was measured spectrophotometrically after extraction of soils with sodium bicarbonate [17]. Soil Organic Carbon (SOC) was determined by the dry combustion method [18] at 1000°C using CA-100 Primacs<sup>SC</sup> TOC analyzer (Skalar Analytic, Norcross, GA). The CFMBC of soil samples were analyzed using chloroform fumigation method [19]. Briefly, a duplicate set of 20 g of each air-dry subsamples was incubated for 7 days at 50% of WHC. After 7 days, the first set of soil was fumigated with ethanol-free chloroform in the dark for 72 hours, while other set were treated as control. Both sets of soil were extracted with 50 ml of 0.5 M  $\text{K}_2\text{SO}_4$  after shaken in a reciprocal shaker (200 strokes per minute) for an hour and filtered through Whatman No. 2 filter paper. Extracts were analyzed for dissolved organic carbon (DOC) using the Shimadzu TOC-VCPH/CPN Analyzer (Shimadzu Corp., Kyoto, Japan). The CFMBC ( $\text{mg C kg}^{-1}$ ) was calculated by dividing the difference in DOC values of the fumigated and non-fumigated soil samples with a correction factor (Kc) of 0.45 [19].

Five rapid soil biochemical tests, 1) POXC, 2) Solvita labile amino nitrogen (SLAN) kit and soil  $\text{CO}_2$  efflux from laboratory using 3) alkali base trap (Alkali), 4) infrared gas analyzer (IRGA), and 5) Solvita  $\text{CO}_2$ -burst kit (Solvita), were analyzed for 45 soil samples (9 samples  $\times$  5 pseudo replicates). The POXC was analyzed as described by [20]. Briefly, 5 g of air-dried soil was weighed into 50 ml polypropylene conical centrifuge tube to which 18 ml of deionized water and 2 ml of 2 M  $\text{KMnO}_4$  were added and vigorously shaken for 2 minutes on a reciprocal shaker (240 oscillations per minute) under room temperature. After 2 minutes, tubes were swirled vigorously by hand to ensure no soil clinging to sides or cap of the tube. Tubes were placed in the dark area to allow the soil to settle precisely for 10 minutes. After 10 minutes, 0.5 ml of supernatant from the upper 1 cm of the suspension transferred fast to a second tube containing 49.5 ml of deionized water and was inverted to mix. The diluted solution was measured for its absorbance in a spectrophotometer, V-1200 (VWR International Ltd., Randor, Pennsylvania, USA) set at 550 nm wavelength. The values for  $\text{KMnO}_4$ -C were determined using the following equation [20]:

$$\text{POXC}(\text{mg kg}^{-1}) = \left[ 0.02 \text{ mol L}^{-1} - (a + b \times \text{absorbance}) \right] \\ \times (9000 \text{ mg C mol}^{-1}) \times \left( \frac{0.02 \text{ L solution}}{0.005 \text{ kg soil}} \right)$$

where, 0.02  $\text{mol L}^{-1}$  is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is mg C oxidized by 1 mol of  $\text{MnO}_4^-$  changing from  $\text{Mn}^{7+}$  to  $\text{Mn}^{4+}$ , 0.02 L is the volume of  $\text{KMnO}_4$  solution reacted, and 0.005 is the kg of soil used.

Short-term laboratory incubation method was used to determine soil  $\text{CO}_2$  evolution, to estimate the mineralizable carbon. Soil  $\text{CO}_2$  flux from lab incuba-

tion was measured using 1) alkali trap (Alkali), 2) infrared gas analyzer (IRGA) and 3) Solvita gel system (Woods End Laboratories Inc. Mt. Vernon, ME). For all three methods, 50 g of air-dried soils were weighed into a 0.5 L mason jar, and deionized water was added to bring soil to 50% WHC. For alkali trap method, 20 ml of 0.5 M NaOH in the vial was inserted in the jar and incubated for four days at 25°C. The vial containing NaOH was titrated with 0.5 M HCl to determine CO<sub>2</sub> evolved during incubation [21]. A separate set of incubated soils was used to determine soil CO<sub>2</sub> efflux using IRGA, Li-800 (LI-COR Bioscience, Lincoln, Nebraska, USA), after five days of incubation. Headspace air samples were collected inserting 5 ml syringe through rubber septum fitted on the jar lid. Headspace CO<sub>2</sub> concentration (mg kg<sup>-1</sup>) was converted to CO<sub>2</sub>-C µg g<sup>-1</sup> day<sup>-1</sup> using ideal gas equation. For Solvita gel system, (Woods End Laboratories, Mt. Vernon, ME), 40 g of air-dried soils were weighed in 50 ml plastic graduated beaker provided in the kit. Deionized water was dispensed using a hand sprayer to avoid forming of the crater in the soil to bring soil in 50% water holding capacity. Solvita-CO<sub>2</sub> probe was inserted into the glass jar alongside the beaker with the gel facing out for observation and lid was tightly screwed. Jars were kept at a stable temperature of 25°C. After 24 hours, the detector probe was removed, and reading was observed in CO<sub>2</sub> mg kg<sup>-1</sup> using Sovita Digital Color Reader (DCR). Similarly, NH<sub>3</sub> probe was used to determine SLAN (mg NH<sub>4</sub>-N kg<sup>-1</sup>) after adding 2N NaOH.

### 2.3. Statistical Analysis

For statistical analysis, five-pseudo replicates (subsamples) were used to calculate the standard deviation and mean. Relationships among soil parameters were statistically analyzed using Pearson correlation coefficient and regression equation fit using SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC) at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Soil Properties

Collected nine soil samples represent a significant range of agricultural soils comprising seven different soil series with the textural class ranging from fine to coarse-silty, covering mainly corn-soybean or wheat based rotation (Table 1). Soil WHC ranged between 0.19 to 0.38 g g<sup>-1</sup>; soil pH was neutral to moderately alkaline with EC ranged between non-saline (<1 dS m<sup>-1</sup>) soils at Inkster to very high saline soils at Embden (8.69 dSm<sup>-1</sup>). Soil NO<sub>3</sub>-N concentration ranged between low (<6 mg kg<sup>-1</sup>) to very high (>30 mg kg<sup>-1</sup>) availability, but Olsen-P was mostly high (16 - 20 mg kg<sup>-1</sup>) to very high (>20 mg kg<sup>-1</sup>). Soil OM content ranged from 22.8 to 45.7 g kg<sup>-1</sup>. Soil OC ranged from 15.1 g kg<sup>-1</sup>, at Gardner to 39.8 g kg<sup>-1</sup>, at Dilworth. Ranges of CV% for pH, EC, NO<sub>3</sub>-N, Olsen-P and SOM are 0.25 - 1.42, 1.32 - 10.2, 1.68 - 15.5, 4.48 - 17.4, and 1.89 - 7.61, respectively.

These values are a close match with the average ND soils as reported in previous literature [22] reported a soil pH range from 5.3 to a high of 8.5 for 0 - 15 cm

**Table 1.** Geographical location, soil taxonomic classification, cropping system and mean (coefficient of variation %) of basic soil properties of nine agricultural field surface (0 - 15 cm) soils across the Red River Valley of ND and MN.

Location	Latitude, and Longitude	Soil series	Taxonomic classification	Rotation	WHC g <sup>-1</sup>	pH	EC (dSm <sup>-1</sup> )	NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	Olsen-P (mg kg <sup>-1</sup> )	SOC (g kg <sup>-1</sup> )
Ada (MN)	47°31'49.7"N 96°40'88.5"W	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	Wheat-Corn	0.19	8.53 (0.82)	1.99 (4.52)	6.04 (3.48)	23.0 (8.61)	29.1 (3.02)
Downer (MN)	46°48'06.3"N 96°32'52.0"W	Elmville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	Soybean-Corn	0.20	8.60 (0.35)	2.27 (1.32)	17.3 (3.05)	21.9 (17.4)	31.3 (3.07)
Embdon (ND)	46°51'12.4"N 97°25'48.8"W	Ryan	Fine, smectitic, frigid Typic Natraquerts	Wheat-Alfalfa	0.27	7.73 (1.16)	8.69 (1.73)	10.9 (15.5)	68.6 (9.65)	22.6 (0.88)
Gardner (ND)	47°16'53.8"N 97°05'41.6"W	Fargo	Fine, smectitic, frigid Typic Epiquerts	Soybean-Corn	0.20	5.98 (0.84)	1.08 (10.2)	14.4 (4.72)	33.7 (8.72)	15.1 (4.70)
Glyndon (MN)	46°54'45.0"N 96°36'35.0"W	Bearden	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	Corn-Soybean	0.31	8.07 (0.25)	4.09 (1.71)	30.5 (2.52)	49.5 (5.29)	44.3 (1.35)
Inkster (ND)	48°09'57.3"N 97°43'12.9"W	Inkster	Coarse-loamy, mixed, superactive, frigid Pachic Hapludolls	Soybean-Potato	0.16	5.64 (1.42)	0.99 (8.08)	9.68 (45.3)	71.5 (4.48)	16.3 (3.62)
St. Thomas (ND)	48°34'05.7"N 97°27'01.9"W	Glyndon	Coarse-silty, mixed, superactive, frigid Aeric Calciaquolls	Wheat-Sugar beet-beans	0.25	7.06 (0.57)	2.83 (2.12)	33.5 (18.8)	50.4 (10.7)	26.5 (0.72)
Walcott (ND)	46°31'45.3"N 96°54'14.3"W	Fargo	Fine, smectitic, frigid Typic Epiquerts	Soybean-Corn	0.38	7.77 (0.51)	2.99 (2.34)	25.7 (1.68)	66.5 (8.18)	27.8 (0.90)
Dilworth (MN)	46°55'10.6"N 96°39'05.6"W	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	Soybean-Wheat	0.34	8.21 (0.37)	2.67 (3.75)	20.7 (6.15)	16.3 (8.60)	39.8 (1.48)

WHC: Water holding capacity; EC: Electrical Conductivity; SOM: Soil organic matter; SOC: Soil organic Carbon.

depth soil samples collected from 53 counties in North Dakota. Critical Bray and Kurtz-P level of ND soil is 20 ppm [23]. [12] also reported similar SOM and SOC values of 6.84% - 9.07% and 30.0 - 37.3 g kg<sup>-1</sup>, respectively for agricultural soils under different crop rotations.

### 3.2. Soil Biochemical Tests

Soil biochemical test results of nine agricultural soils were reported in **Table 2**. Values for Soil CFMBC, POXC, SLAN, IRGA, Alkali and Solvita range between 867.6 - 2609 mg C kg<sup>-1</sup>, 226 - 785 mg C kg<sup>-1</sup>, 52 - 164 mg NH<sub>3</sub>-N kg<sup>-1</sup>, 18.4 - 180 mg CO<sub>2</sub>-C kg<sup>-1</sup>day<sup>-1</sup>, 40.5 - 108 mg CO<sub>2</sub>-C kg<sup>-1</sup>day<sup>-1</sup>, and 61.8 - 135 mg CO<sub>2</sub>-C kg<sup>-1</sup>day<sup>-1</sup>, respectively. Highest and lowest values of CFMBC, POXC, SLAN, IRGA, Alkali and Solvita are observed at Inkster and Walcott, Inkster and Glyndon, Inkster and Glyndon, St. Thomas and Downer, Inkster and Ada, St. Thomas and Downer, respectively. Lowest and highest values of coefficient of variation percentage (CV) are 6.72 - 13.5, 4.27 - 36.6, 7.29 - 22.4, 2.52 - 26.4, 2.67 - 19.1, and 5.48 - 17.4 for CFMBC, POXC, SLAN, IRGA, Alkali, and Solvita, respectively.

It is interesting to notice that the lowest values are consistently observed at either Inkster or St. Thomas; whereas the highest values are found within Walcott, Glyndon, Downer, and Ada. Values of CV indicate that reproducibility varies with site and test method. Reproducibility of determination methods, as indicated by the average CV of soil biochemical tests, follows the order of Alkali (8.16) < POXC (9.04) < CFMBC (9.26) < Solvita (10.28) < IRGA (11.6) < SLAN (12.8).

### 3.3. Relationship between Soil Biochemical Health Tests and Soil Properties

Pearson correlation coefficient and significance among soil biochemical tests

**Table 2.** Mean (CV%) values of soil biological health parameters of different soil samples collected from agricultural field across the RRV of ND and MN.

Location	CFMBC		POXC		SLAN		IRGA		Alkali		Solvita	
	mg C kg <sup>-1</sup>		mg C kg <sup>-1</sup>		mg NH <sub>3</sub> -N kg <sup>-1</sup>		mg CO <sub>2</sub> -C kg <sup>-1</sup> day <sup>-1</sup>		mg CO <sub>2</sub> -C kg <sup>-1</sup> day <sup>-1</sup>		mg CO <sub>2</sub> -C kg <sup>-1</sup> day <sup>-1</sup>	
Ada	1015	(13.5)	488	(4.27)	98.6	(7.29)	45.4	(18.3)	108	(5.80)	70.0	(12.3)
Downer	1557	(10.3)	503	(5.40)	97.0	(14.5)	180	(26.4)	83.3	(2.67)	135	(5.48)
Embdon	1496	(6.73)	761	(5.48)	109	(16.2)	28.9	(9.48)	44.6	(19.1)	78.2	(11.0)
Gardner	1239	(12.2)	553	(6.27)	101	(16.2)	97.8	(23.5)	63.8	(4.12)	107	(7.23)
Glyndon	1873	(7.91)	785	(4.98)	164	(8.32)	77.6	(11.9)	91.1	(10.6)	92.6	(17.4)
Inkster	867.6	(7.56)	226	(36.6)	52.0	(22.4)	32.6	(2.52)	40.5	(3.23)	63.5	(12.5)
St. Thomas	1272	(6.72)	672	(8.11)	144	(10.4)	18.4	(3.32)	64.7	(13.4)	61.8	(15.4)
Walcott	2609	(7.99)	719	(5.77)	140	(9.76)	75.3	(5.43)	84.9	(8.78)	117	(4.05)
Dilworth	1749	(10.5)	609	(4.50)	147	(11.4)	47.7	(3.96)	99.3	(5.86)	85.1	(7.13)

IRGA: Infrared Gas analysis soil respiration; SLAN: Solvita Labile Amino-Nitrogen; POXC: Permanganate-oxidizable Carbon; SOC: Soil organic Carbon; CFMBC: Chloroform fumigation extraction-microbial biomass carbon; Alkali: Base-trap method; Solvita: CO<sub>2</sub> burst test.

and properties are presented in **Table 3**. Soil CFMBC had a significant relationship with soil properties like SOC, pH, NO<sub>3</sub>-N, and SOM and with soil biochemical tests, Alkali, Solvita, SLAN, and POXC. Regression equation fit of SLAN and POXC with CFMBC showed a significant quadratic fit (**Figure 1(a)** and **Figure 1(b)**). Soil mineralizable C measured by IRGA had a significant negative relationship with Olsen-P and Solvita; and IRGA also had a significant quadratic relationship with Solvita (**Figure 1(c)**). Similarly, mineralizable soil C measured by Alkali also had a negative relationship with Olsen-P and positive relationship with SLAN, SOC, and pH. Solvita had a significant relationship with CFMBC and IRGA.

SLAN had a significant positive relationship with CFMBC (0.52), Alkali (0.37), POXC (0.70), SOC (0.68), pH (0.44) and SOM (0.69). Quadratic fit between POXC and SLAN is presented in **Figure 1(d)** ( $r^2 = 0.59$ ,  $p < 0.001$ ).

Our results suggest that CFMBC had a close relationship with the most soil biochemical health tests. Several authors [20] [5] reported a close relationship between CFMBC and POXC. Besides, SLAN and POXC also showed a close relationship with SOM, pH, SOC and NO<sub>3</sub>-N. [24] found a correlation of 0.94 between organic C and POXC for agricultural soils. [25] also found a positive correlation among CFMBC, acid hydrolyzable C, the amount of C respired after 12-d incubation and light fraction C. [26] also reported a strong relationship between POXC and net N mineralization.

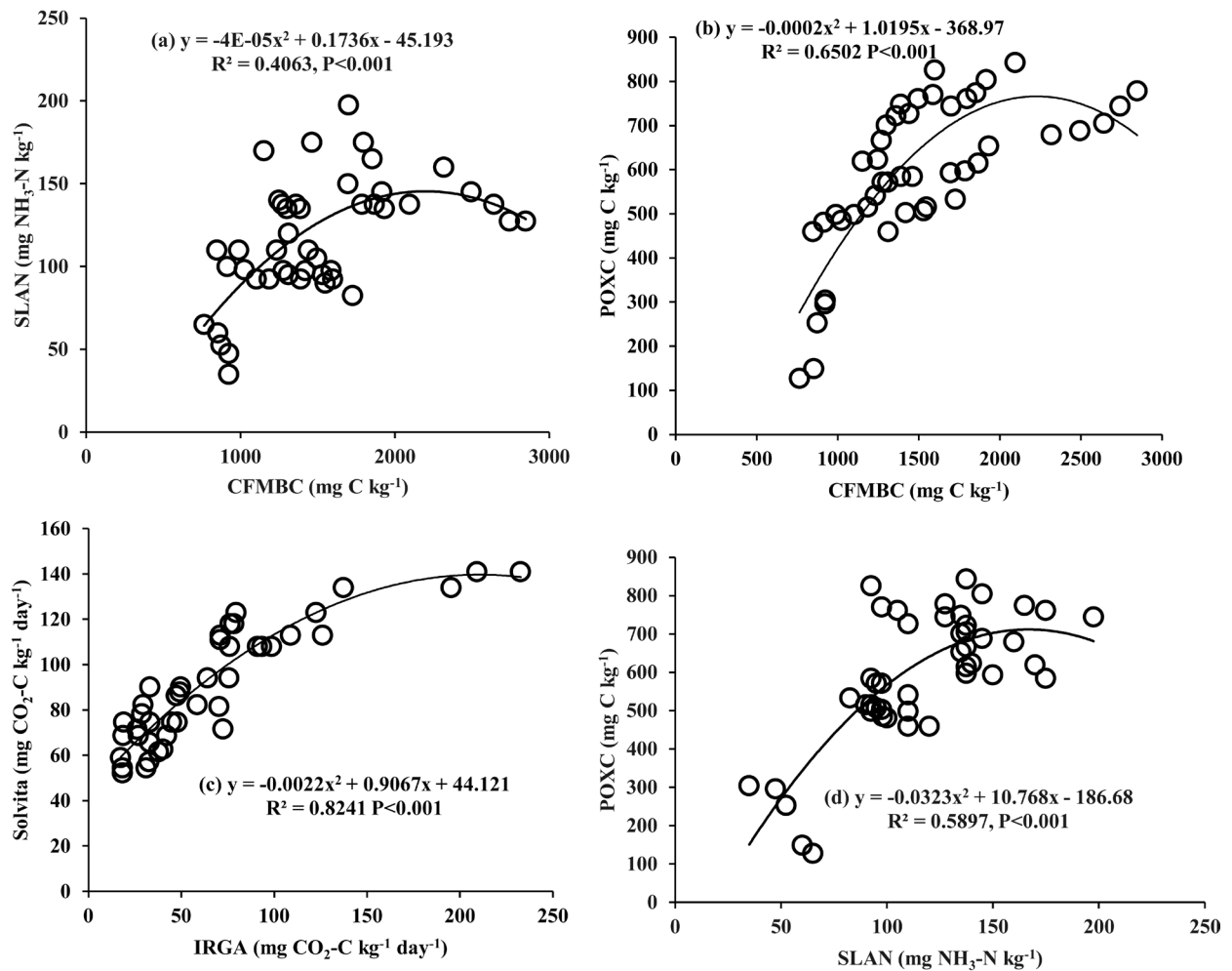
Measurements of soil CO<sub>2</sub> by IRGA and Solvita showed a strong correlation of  $r = 0.86$ , but both did not show any relationship with Alkali. This finding was in contrast with the previous findings of strong correlations of these three methods

**Table 3.** Pearson correlation coefficient ( $r$ ) representing relationship among soil properties and soil biological health indicators of soils collected from nine agricultural fields across the RRV ( $n = 45$ ) (NS indicates not significant at  $p < 0.05$  and \* indicates the  $p$  value).

	CFMBC	IRGA	Alkali	Solvita	SLAN	POXC	SOC	pH	EC	NO <sub>3</sub> -N	Olsen-P
<b>IRGA</b>	NS										
<b>Alkali</b>	0.37 (0.01*)	NS									
<b>Solvita</b>	0.57 (<0.01)	0.86 (<0.001)	NS								
<b>SLAN</b>	0.52 (0.001)	NS	0.38 (0.01)	NS							
<b>POXC</b>	0.68 (<0.001)	NS	NS	NS	0.70 (<0.001)						
<b>SOC</b>	0.47 (0.001)	NS	0.71 (<0.001)	NS	0.68 (<0.001)	0.49 (0.001)					
<b>pH</b>	0.41 (0.005)	NS	0.73 (<0.001)	NS	0.44 (0.002)	0.46 (0.002)	0.76 (<0.001)				
<b>EC</b>	NS	NS	NS	NS	NS	0.65 (<0.001)	NS	0.33 (0.02)			
<b>NO<sub>3</sub>-N</b>	0.52 (0.001)	NS	NS	NS	0.72 (<0.001)	0.56 (<0.001)	0.49 (0.001)	NS	NS		
<b>Olsen-P</b>	NS	-0.41 (0.005)	-0.67 (<0.001)	NS	NS	NS	-0.39 (0.008)	-0.47 (0.001)	0.38 (0.01)	NS	
<b>SOM</b>	0.74 (<0.001)	NS	NS	NS	0.59 (<0.001)	0.59 (<0.001)	0.57 (<0.001)	NS	NS	0.62 (<0.001)	0.33 (0.02)

IRGA: Infrared Gas analysis soil respiration; SLAN: Solvita Labile Amino-Nitrogen; POXC: Permanganate-oxidizable Carbon; SOC: Soil organic Carbon; CFMBC: Chloroform fumigation extraction-microbial biomass carbon; Alkali: Base-trap method. Solvita: CO<sub>2</sub> burst test.





**Figure 1.** Quadratic fit between different soil biochemical health tests of soils collected across nine agricultural fields of the Red River Valley of North Dakota and Minnesota, (a) Solvita Labile Amino-nitrogen (mg NH<sub>3</sub>-N kg<sup>-1</sup>) and CFMBC (mg C kg<sup>-1</sup>), (ii) POXC (mg C kg<sup>-1</sup>) and CFMBC (mg C kg<sup>-1</sup>), (iii) Solvita-CO<sub>2</sub> burst test (mg CO<sub>2</sub>-C kg<sup>-1</sup> day<sup>-1</sup>) and CO<sub>2</sub> flux measured using IRGA (mg CO<sub>2</sub>-C kg<sup>-1</sup> day<sup>-1</sup>), and (iv) POXC (mg C kg<sup>-1</sup>) and SLAN (mg NH<sub>3</sub>-N kg<sup>-1</sup>), n = 45.

[3] [27]. We hypothesized that the variations among these three methods were strongly dependent on the incubation period, the volume of the vessel and wetting methods (gravimetric vs. capillary) [26] [28]. [29] reported that the limit of quantification (lowest level that an analytical result becomes meaningful) of IRGA was significantly lower than Alkali method after day 10 of incubation. They also mentioned that CV of Alkali method was nearly 50% as compared to IRGA on day 1.

Analysis of soil chemical properties pH, and EC were related to rapid soil biochemical tests, SLAN and POXC. [30] concluded that microbial biomass and microbial activity tended to stabilize at pH values between about 5 and 7 because the differences in organic acid, total N, and aluminum concentrations within this pH range are small. Finally, soil biochemical tests did not show any relationship with Olsen-P; rather extractable aluminum and iron are commonly best predictors [31].



## 4. Conclusion

Soil biochemical tests showed different levels of relationship with each other; no single method was found to comprehensively represent the complete soil biochemical health. Our findings emphasized that regular soil tests including, soil pH, EC, NO<sub>3</sub>-N, and Olsen-P are also important, as they related significantly to some tests.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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