PROFICIENCY RESULTS ON SOLVITA® CO2-BURST and SLAN TESTS FOR SOIL TESTING LABORATORIES PARTICIPATING IN 2016 QC SOIL TRIAL

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SUMMARY

A soil testing survey of Solvita CO2 and Amino-N methods has been conducted with two reference samples supplied to commercial labs able to perform Solvita® CO2-Burst and SLAN tests. Soils were selected based on being either low or moderately-high in biological fertility based on actual test data and review of management history for the prior 20 years. Approximately 26 labs reported data for the CO2-Burst method by employing one of two possible soil wetting methods: a capillary wetting or a 50% water-filled-pore space (WFPS) approach, previously reported to have an influence on the magnitude of CO2 release and between sample variability. ANOVA results for the two soils show that labs distinguished the low from higher fertility soil at a high degree of significance. Difference among wetting methods were significant with stronger separation between the two fertility types when using the WFPS method in contrast to the capillary method. Data results for all labs are ranged from minimum to maximum showing benchmarks for median, median 95% confidence intervals (C.I.) and ± 1 standard deviation (1 SD). Most labs fell within ±1SD of median and a majority fell within the 95% C.I. The magnitude of respiration differences was highly significant based on wetting method, indicating this factor alone will play a large role in apparent variability when making between lab comparisons such as in other national proficiency programs. IN the new WFPS method, the sample preparation to determine volume weight from which estimates of 50% WFPS are made is crucial to reduce between lab variability. Solvita CO2 and SLAN methods are able to accurately delineate depleted from biologically improved soils and it is suggested that a 3-tier system for interpretation low – medium – high values should be used.

INTRODUCTION

Solvita® is soil method for characterizing microbial activity which was first introduced as a basal CO2 respiration technique by Brinton in 1996 (Doran et al. 1997). In 2006 the method was upgraded to a commercial soil lab method by exploiting the phenomena of "CO2-burst" occurring when dried, sieved soils are remoistened, resulting in a large release of CO2 (Birch 1958, Haney & Brinton 2008). The innovation made it possible for commercial labs which routinely process soils to rapidly characterize respiration in 24-hours instead of 72-hours or the more common 7-day method (Haney et al 2008). In addition, the burst of CO2 due to microbial response to sudden re-wetting made it possible to distinguish larger differences between samples of differing origin than occur in the basal mode.

Following a period of several years the Solvita CO2-burst was admitted into two soil proficiency programs (ALP and NAPT) which track lab performance (Miller 2010). The number of entrant labs reporting Solvita data was low and at a minimum to reliably characterize performance (Bob Miller, *personal communication* 2013). However, it was clear that a significant source of variability existed that needed to be further identified. At the time, the only wetting method for the procedure was a rapid approach allowing water to wick-up into the soil by capillary attraction. To obtain this effect an excess of water was supplied to the jars and capillary force drew it into the soil through small Buchner perforations in the bottom of the beaker (Haney & Haney, 2010). Brinton (2015) closely examined water properties for proficiency samples and determined that 95-98% of samples were routinely over-saturated by this form of bottom wetting.

It was already known at the time that over-wetting soil would reduce respiration significantly by providing an obstacle for aeration of microbes during aerobic metabolism (Franzluebbers, 1999b). The trait of over-wetting occurred inconsistently in same samples when repeatedly moistened (Bruce Hoskins, University of Maine Soil lab, *Personal communication*, 2015). This indicated that a physical trait of the samples caused wetting differences of several grams of water per beaker (40g) which appeared to be very significant. The lowest CO2 respiration results were observed in samples with the greatest water infusion.

This report presents the Solvita Soil Reference Program results as conducted July-Sept. 2016 with 24 participating soil laboratories (an additional 7 joined later in the cycle and are not included in the statistical report). The term "reference program" was used as soils were pre-selected based on management histories. Differences in productivity were known and biological fertility differences between the selected soils were validated by other tests, including SOM, total-N and respiration by IR and base-trap.

The primary objective of this program was to determine if a lab using Solvita can accurately distinguish differences in biological soil fertility at practical levels. A secondary objective was to determine the magnitude of the respiration effect caused by how soil is pre-moistened. Previously we reported that unstructured soils may be easily over-saturated by capillary wetting, a cause of reduced respiration (Brinton 2015). It was concluded that this method trait could influence results and variability reported for other proficiency programs.

To present results, we have adhered to the custom of median and median 95% C.I. for target values and to infer the range for acceptable values. Statistical analysis was by ANOVA with variables FERTILITY and WETTING. Data were examined for Anderson-Darling normality and plotted for cumulative probability.

SAMPLE SELECTION

Two soils numbered #101 and #210 originated as fresh moist farm soils collected in South Carolina and southern Maine. Soil #101 was of low productivity and considered representative of depletion farming with 30+ years of continuous corn, conventional tillage and no specific attention to soil building except addition of inorganic fertilizers. Soil #201 was high-productivity under intense organic management with cover crops and compost as the primary soil amendment.

SOIL #101 – Character: **Low Biology, Low organic-N (SOM 2.0% TN 0.055% pH 4.6)**

Source: Stanly County, North Carolina
Soil Series: Tarrus-channery silty clay
Management: 30-year continuous corn, inorganic fertilizer, no cover-crops, no manure

SOIL #201 – Character: **Medium-High Biology, Medium organic-N (SOM 4.0%, TN 0.149% pH 6.0)**

Source: Sagadahoc County, Maine
Soil Series: Hadley Silt Loam
Management: 27-year organic farming with no inorganic fertilizers, using compost and cover-crops on rotation with cash crops.

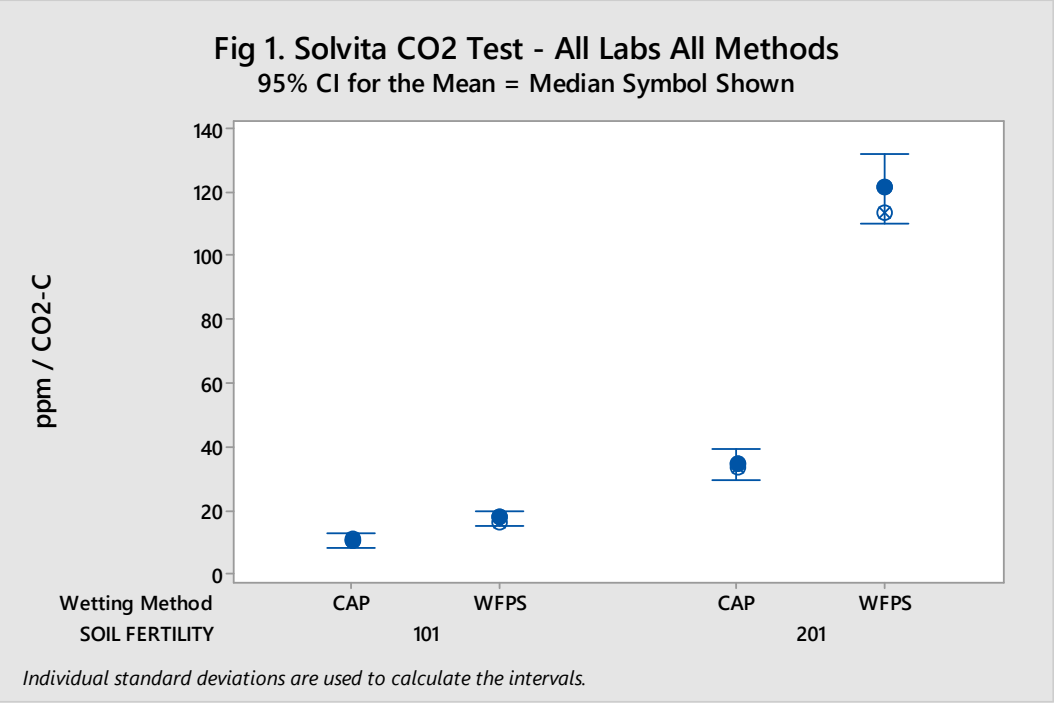
SOIL PREPARATION & EQUIPMENT

To eliminate potential lab-artifacts regarding structure attributable to soil-milling the soils were hand-rolled after air drying to <4% moisture following 10 rpm roller-screening at 1/8" (6 mm). All Labs ran replicate A-B pairs for both soils with their existing Solvita equipment. Five labs were new to all methods and 7 labs had never performed SLAN before. Labs reported temperature ranges from 20 – 26°C. In two

cases of outliers, labs were given opportunity to repeat the tests. In one case, data from an early Digital Color Reader (DCR) Model 400 was arithmetically adjusted to be equivalent to the current 700.6 method.

RESULTS

Figure 1 shows the ANOVA results of all labs for CO_2 respiration by variables soil and wetting method. Labs successfully distinguished the low fertility soil #101 from the medium biology soil (#201) with a high degree of statistical precision for both wetting methods ($p<0.001$).



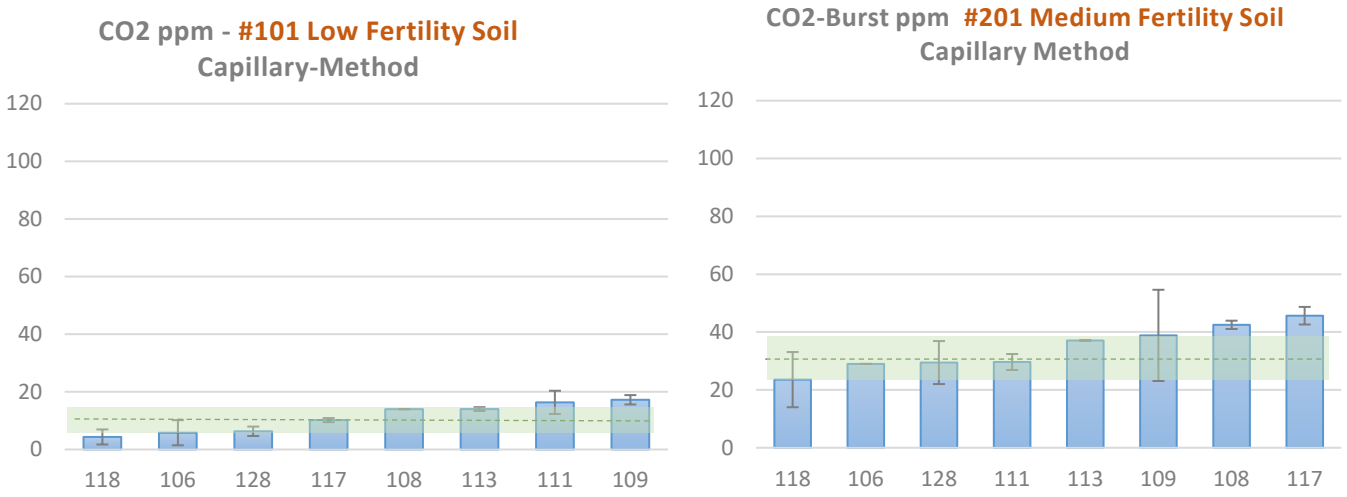
The magnitude of CO_2 difference between the low and medium-high fertility soil was significantly influenced by the wetting method. The Δ of CO_2 (ratio of WFPS to Capillary-method respiration) ranged from 1.8 for #101 soil to 3.4 in #201, therefore as biology increased the difference imposed by wetting method also increased. These results confirm that the CO_2 values labs report will be principally dependent on wetting method, a potential artifact, in addition to the practical differences attributable to management and soil history. Two interactive factors are involved in the behavior: the quantity of water adsorbed and the quality of the soil structure (sometimes improperly referred to as soil texture when discussing respiration effects). Structure can be naturally poor and is related to soil texture and management, but it is unfortunately influenced by lab soil grinding; therefore, a lab artifact component is involved. Based on prior information and this study it is believed that the WFPS method will naturally reduce variability for all soils however handled, but structural abnormality imposed by milling and sieving soil finer than 2mm which is useful for nutrient homogeneity is not helpful for biology.

Figures 2 and **3** provide overall results arranged by lab code in relation to fertility class and wetting method. **Figures 4** and **5** provide descriptive information on the character of data distribution (normality tests). It is thought that these results could guide future method improvements and should be useful in developing interpretation guides congruous with methods applied.

Capillary Bottom-Wetting Method

Low Fertility #101 vs Med Fertility #201 Soil - 8 labs Reporting

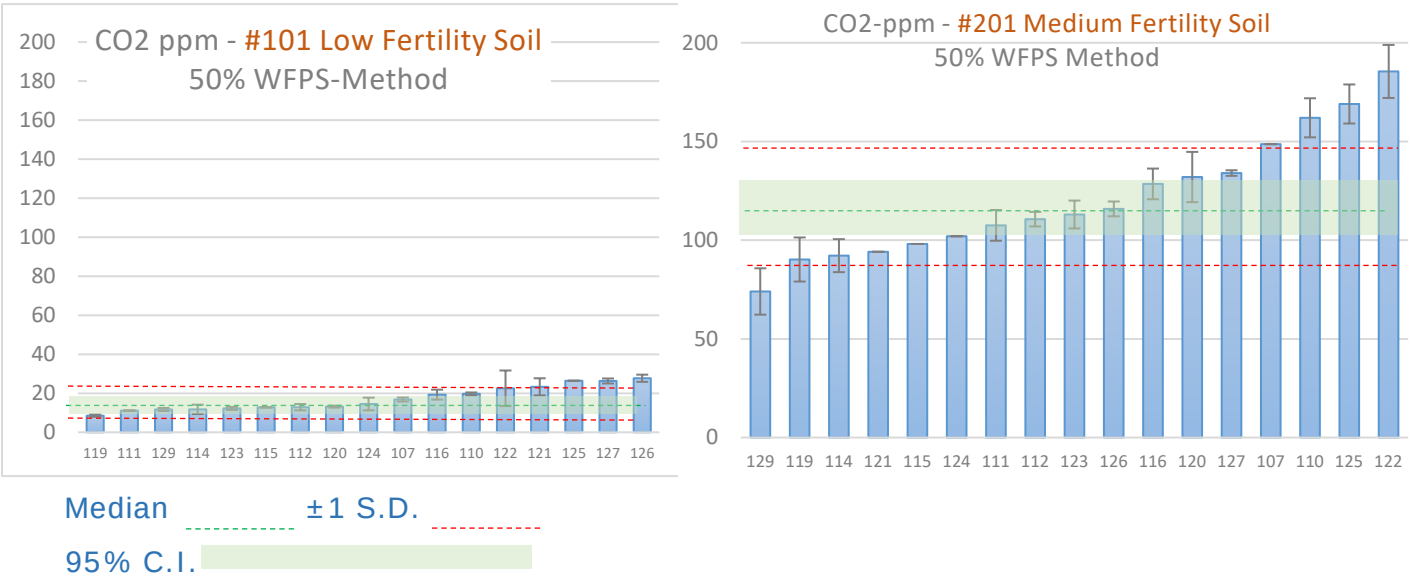
Fig 2 CO_2-Burst, ppm Result



50% WFPS Method

Low Fertility #101 vs Med Fertility #201 Soil - 17 labs Reporting

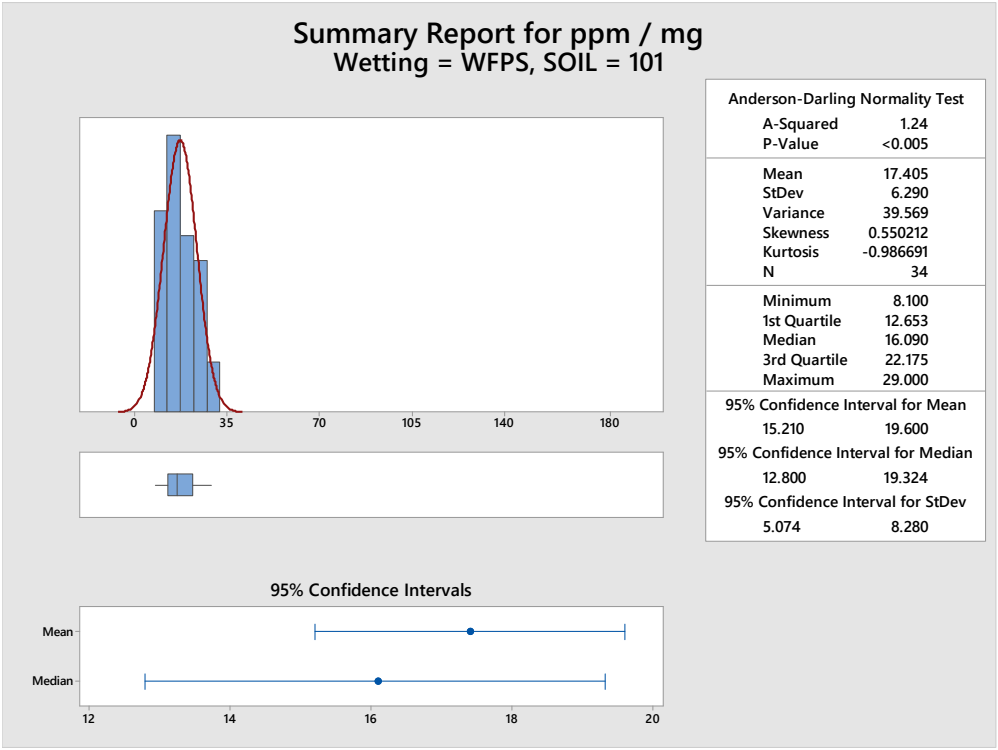
Fig 3. CO_2 ppm Result



Median ±1 S.D.

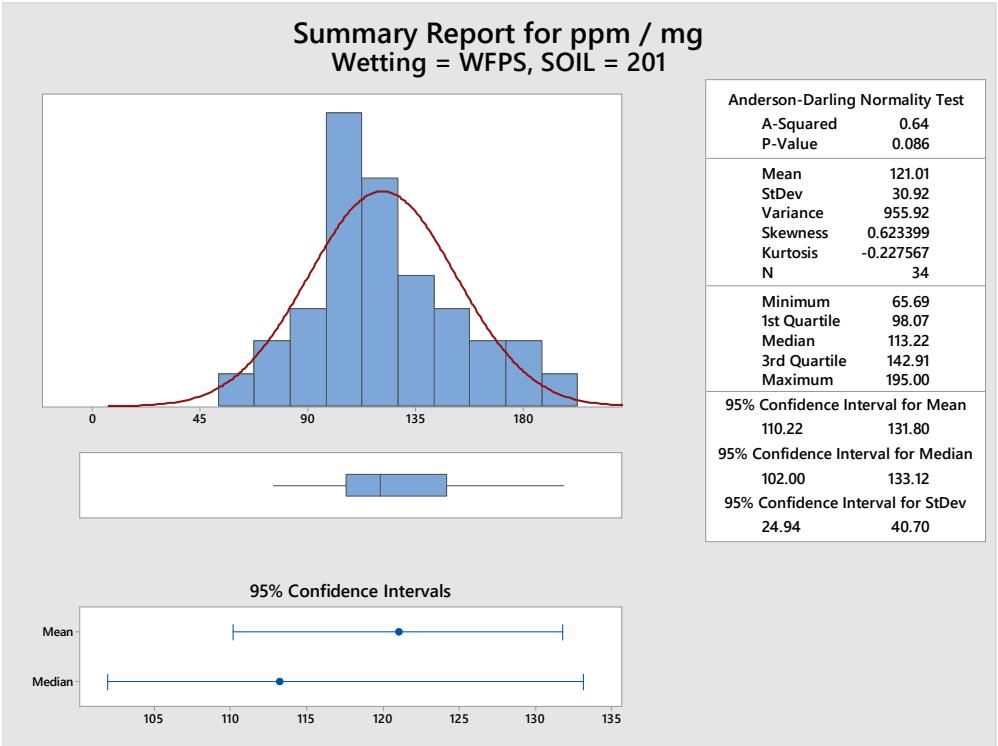
95% C.I.

Fig 4A. DISTRIBUTION HISTOGRAMS FOR LOW RESPIRATION SOIL



Anderson-Darling Normality Test	
A-Squared	1.24
P-Value	<0.005
Mean	17.405
StDev	6.290
Variance	39.569
Skewness	0.550212
Kurtosis	-0.986691
N	34
Minimum	8.100
1st Quartile	12.653
Median	16.090
3rd Quartile	22.175
Maximum	29.000
95% Confidence Interval for Mean	
15.210	19.600
95% Confidence Interval for Median	
12.800	19.324
95% Confidence Interval for StDev	
5.074	8.280

Method: WFPS



Anderson-Darling Normality Test	
A-Squared	0.64
P-Value	0.086
Mean	121.01
StDev	30.92
Variance	955.92
Skewness	0.623399
Kurtosis	-0.227567
N	34
Minimum	65.69
1st Quartile	98.07
Median	113.22
3rd Quartile	142.91
Maximum	195.00
95% Confidence Interval for Mean	
110.22	131.80
95% Confidence Interval for Median	
102.00	133.12
95% Confidence Interval for StDev	
24.94	40.70

Fig 4B. DISTRIBUTION HISTOGRAMS FOR MEDIUM RESPIRATION SOIL

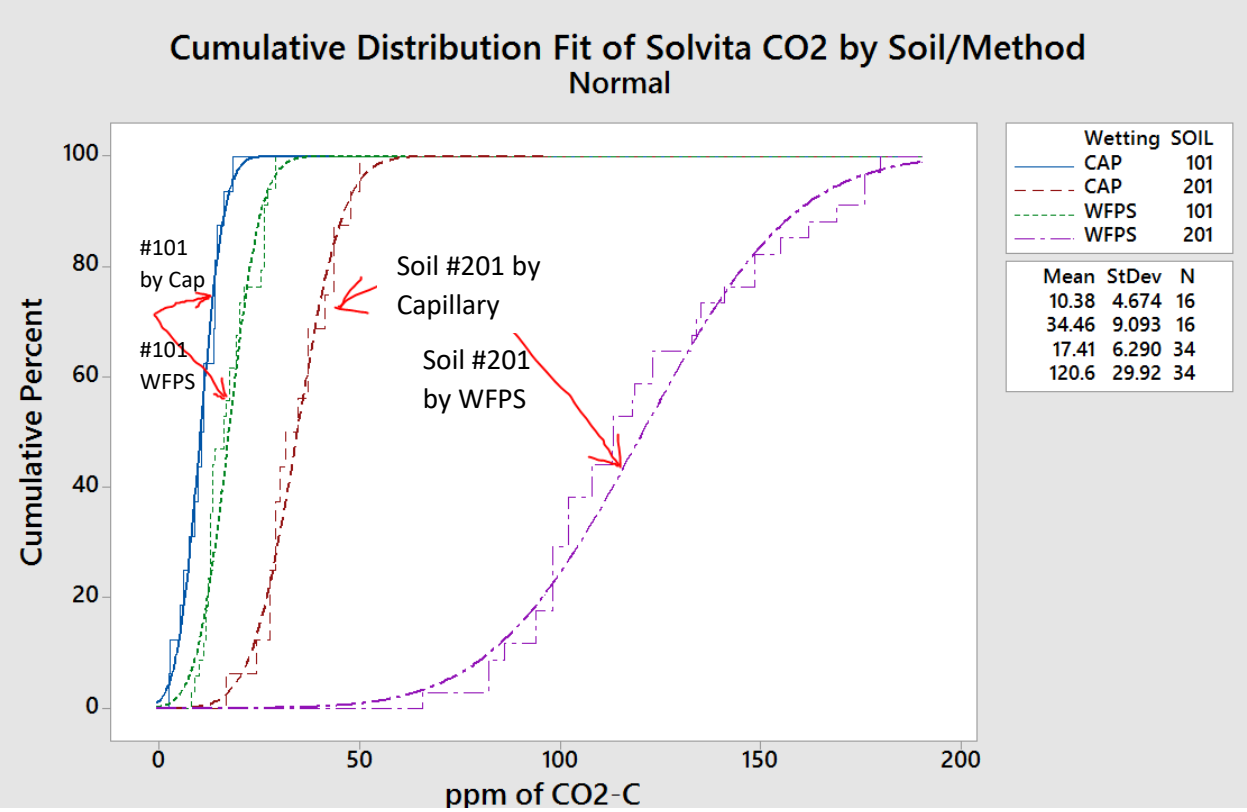
Method: WFPS

The distribution characteristics (**Figures 4A, 4B**) for the #101 and the #201 soil arranged by WFPS show some skew indicative of data slightly weighted to lower values and the relationship to normal distribution. The #101 low fertility group exhibits a compressed curve typical of low to zero test values near a lower limit of detection. It is unlikely that a poorer soil could be found. The #201 med-fertility soil displays a broad-based distribution reflecting a greater range of reported values towards the higher end of Solvita detection, but is undoubtedly not the highest biological fertility soil one can find.

Figure 5 examines the soil CO2 respiration cumulative distribution arranged by wetting and soil. This chart illustrates the potential difficulty encountered in distinguishing practical fertility classes when using the capillary-method. Both wetting methods accurately distinguished the low from the higher fertility sample, but the WFPS method enabled cleaner and more normal separation and distribution of the two selected soils from each other. Healthy soil biology may be partly described as a function of air transport through structured material; both require the other. The need is to reduce methodological interferences or artifacts while optimizing the potential to detect practical differences related to faming systems under examination. The cumulative plot indicates that this potential is being harnessed by WFPS. It is possible therfore that at least 3 biological classes may be able to be clearly distinguished.

Based on these data, future developments in protocol should involve examining soil quantity to optimize the distribution histogram for higher fertility soils (Fig 4B) since the Solvita optical densities (DCR value) indicate CO2 in the steep part of concentration curve. This shift is noticeable by labs which have switched wetting methods and now must be concerned with new accuracy and interpretation.

Fig 5. Distribution of Solvita Results by Method and soil



Alkaline Extract SLAN for Labile Amino-N

14 labs participated with SLAN, 7 performing the test for the very first time. SLAN is a chemical extract and does not have wetting issues comparable to CO2-burst. The labs distinguished #101 (Low fertility) from #201 (Medium Fertility) with a very high degree of statistical probability ($p < 0.001$)(Fig 6). Median values fell to the bottom of the CI range close to reference values. The results fit a normal distribution with the skew to the lower values. 40% of labs were within the 95% CI and 70% ±1SD.

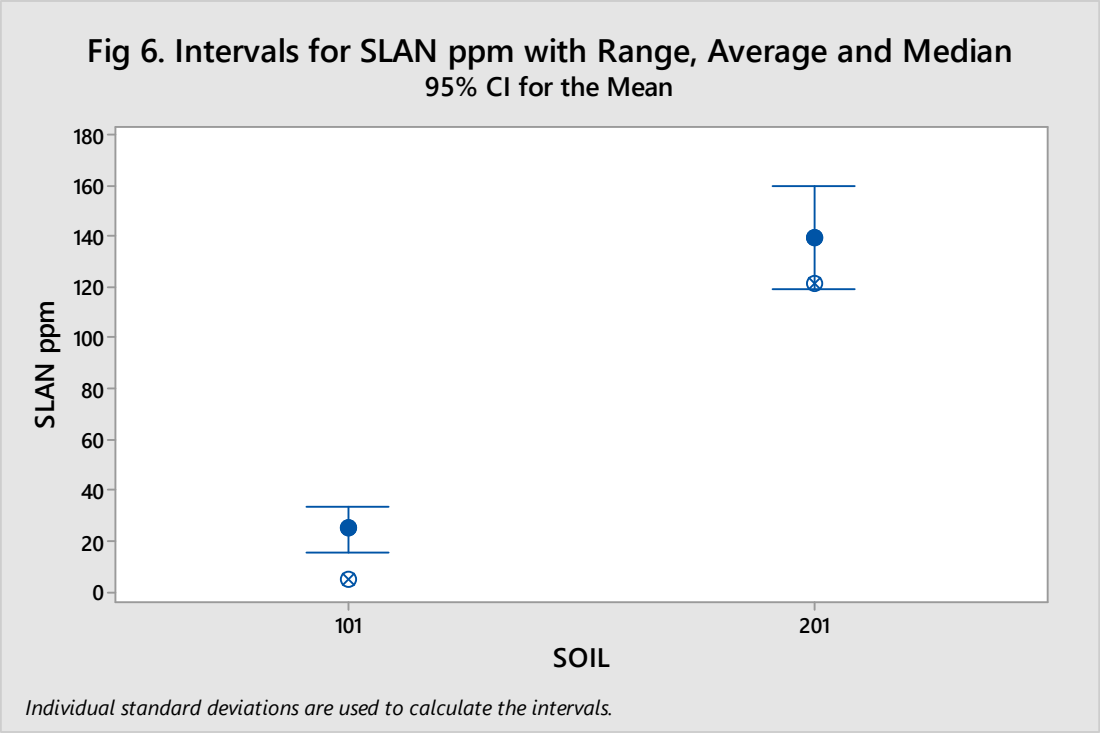
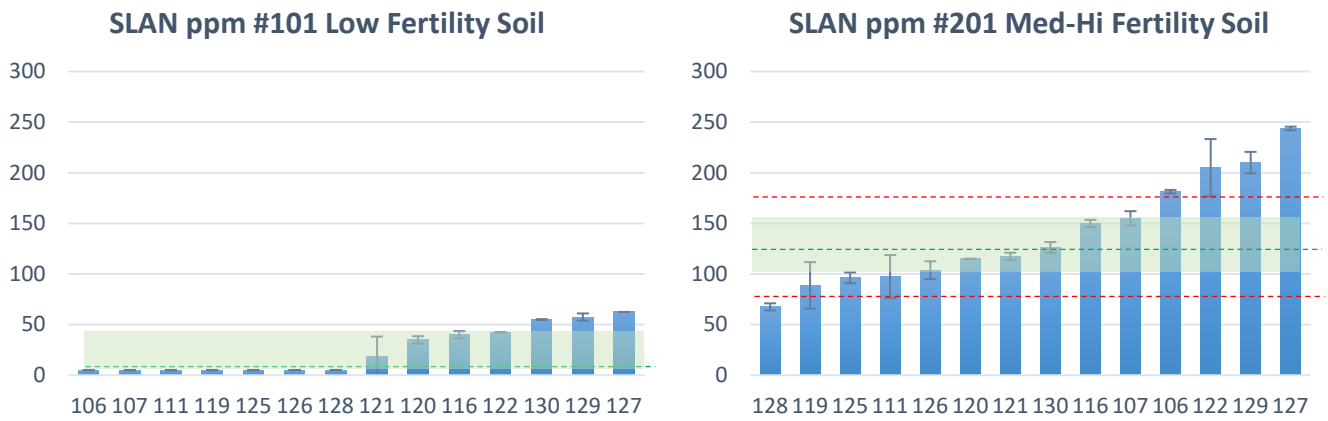


Fig 7. Solvita SLAN Amino-N ppm Results, 14 Labs



Summary and Recommendations:

Solvita CO2-Burst were examined between 24 labs and found to be dependent on rewetting which consistently influences microbial activity. Solvita SLAN fits a category of a chemical extract which is independent of this wetting effect. This report provides evidence that both Solvita CO2-Burst and SLAN can very accurately distinguish soils of differing practical biology and management. Additionally, the magnitude of separation of the #101 and #201 soil by CO_2 and SLAN were quite similar, consistent with a view that the biological parameters being measured share common features. Solvita method updates and continued communication s between labs on SOP may undoubtedly further improve the data distribution.

These data support a conclusion that labs employing Solvita should refer to the wetting method used. It also suggests labs should avoid capillary wetting, particularly with machine-ground samples. Until such time as ALP and NAPT alter grinding methods to be more suitable, soils distributed by these programs are not suitable as validation of biological test procedures.

Interpretation of test results should be adjusted based on the method. This is given Table 1 using a heuristic algorithm to derive three general categories which are very likely to be distinguishable by Solvita.

Table 1. 2016 Interpretation Guide for Solvita Results by Test and Method

Solvita Test	Method	Suggested Interpretation		
		LOW	MEDIUM	HIGH
		results as ppm (mg/kg)		
CO2-Burst	50% WFPS	0 - 40	40 - 140	140 - 300
	Capillary Wetting	0 - 20	20 - 60	60 - 120
SLAN	4g/10cc2N NaOH	0 - 40	40- 150	150 -350

Table 2 shows procedural factors controllable in the lab and which influence repeatability and variability.

Table 2. 2016 Procedural Areas for Improved Repeatability.

Solvita Test	***Reliability Factors for Lab Use***	
	Primary	Secondary
CO2-Burst	Is Soil Dry? Time and Temperature of test period	Bulk Density Det'm for WFPS calculation
SLAN	Surface Area for NH3 release: Size of Beakers	Location of Probe (inside/outside beaker)

Samples used in this project are available as check standards for any lab wishing to test the methods. Contact solvita@woodsend.com for more information.

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References and Citations

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

Brinton, W (1996) A new soil test for general biological activity. IFOAM Research Conference, Oxford University, Sussex UK

Brinton W. (2015) Variables affecting Solvita CO2 Respiration Results. Jrnl Woods End Labs April

Doran, J., T Kettler, M Tsivou (1997) Field and Laboratory Solvita Soil Test Evaluation. University of Nebraska USDA-ARS.

Franzluebbers A. (1999a) Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biology and Biochemistry 31 1083-1090

Franzluebbers A. (1999b) Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. Applied Soil Ecology 11 (1999) 91-101

Franzluebbers, A.J., R.L. Haney, C.W. Honeycutt, H.H. Schomberg, and F.M. Hons. (2000) Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613–623.

Haney R. L.; E. B. Haney (2010) Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations. Communications in Soil Science and Plant Analysis

Haney, R.L., W.F. Brinton, and E. Evans. (2008) Soil CO2 respiration: Comparison of chemical titration, CO2 IRGA analysis and the Solvita gel system. Renew. Agric. Food Syst. 23:171–176.

Miller, R (2010) The Impact of Soil Pulverizing on Lab Analysis. Conference presentation, Starkville MS