VARIABLES INFLUENCING SOLVITA CO_2 RESPIRATION RESULTS

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Executive Summary

Respiration testing is a means to determine biological functioning which relates to soil health and soil labs are now able to perform CO2 testing through the use of new available technology (Solvita®). Recently it has been determined that certain factors are accounting for some observed variability in test results. These factors are different from those which soil labs are accustomed to controlling.

Previous studies have shown that biological response of soils can be significantly and predictably altered based on residual moisture in the test sample and by the extent of sieving. Residual water levels that exist after drying have shown a compromised CO_2 burst effect, a trait that varied lab to lab. Additionally the attached paper describes another effect. While increasing soil fineness normally increases CO_2 rate[4,5] we have shown it also increases vulnerability to over-saturation which triggers a CO_2 slump. Therefore it is conceivable that these opposing factors could result in extreme variability in respiration test results from various labs. Differences of only a few grams of water per respiration test have been noted to be sufficient to trigger a CO_2-slump[8]. Other work has found that the best means to obtain reliable estimates of biological activity that compare to field rates for undisturbed soils is by selecting coarsely sieved soil, i.e. 2mm but not finer[4].

In this study we used standardized soils from a proficiency program which processes soil to 0.7mm-minus and smaller to achieve a high degree of uniformity for lab nutrient tests[9]. We identify predictable, abnormal CO_2 behavior and show it relates to over-saturation of the fine, unstructured soil. The CO2-slump caused by excess water is explained by bacteria/fungi under aeration stress. Poor soil structure may be due to actual field forces such as salinity and loose texture, or low organic content. These are not an artifact. If soil processing is triggering variable behavior due to the changed status of the soils, the artifact must be separately addressed.

Short Term Solution: The data indicates that adjusting the watering method may serve to counteract the slump associated with excessive capillary action from soil pulverization. Thus a short term solution for biological testing of disturbed and unstructured soils is to moisten carefully from above to fill a calculated 50% of pore-space. The standard bottom wetting procedure should continue to work for well-structured soils. The challenge is distinguishing an artifact effect (over-grinding) from a real effect ("water-prone" soils) which requires information about lab processing and soil site considerations.

Long Term Recommendation: The longer term solution is to establish soil texture standards appropriate to soil biology. Services validating soil lab biological proficiency could provide minimally processed 2mm soils for biological testing. In the meantime labs offering respiration tests must carefully distinguish over-saturated from normal conditions, and include alternate wetting in their SOP to isolate these factors. We recommend all labs should examine as-yet-difficult to control variables of soil handling, drying, grinding and moistening which clearly influence microbial response, air-delivery and CO_2 diffusion during the test.

INTRODUCTION

Solvita was introduced in 1996 for soil biology and for soil basal CO2 respiration on as-is, unprocessed soil (Brinton, 1996, Doran et al. 1997). More recently it was adapted for use in commercial labs using dried, processed soil (Haney & Brinton 2008). To obtain a Solvita microbial result, soils must be adequately moistened, based on the Birch effect (Birch 1958). A rapid method of wetting was devised using soils inherent capillary force to attract water into the sample by means of perforated beakers (Haney & Haney 2010).

Variance in how soils re-wet has emerged as a potentially major factor influencing respiration results. This is strikingly illustrated in **Figure 1**. Capillary-wetting from below works well for a well-structured soils (*left*) but leads to over-saturation in an unstructured, damaged soil (*right*). The over-saturated condition will yield an anomalously low CO2 response, which is very sensitive to small changes in water content, presumably because the soils are approaching 100% saturation. This paper examines issues surrounding this effect and how to potentially remedy it.

Figure 1*. (Left) A well-structured soil wetted from below and unstructured soil (right) with same treatment. The soil on the left was processed at 2mm sieving and the soil at right is from a soil proficiency program which homogenizes samples to 0.7mm-minus.*

Unstructured soil may result from purely natural causes such as loose, fine-sandy texture, but also from abusive forces such as excessive tillage and salinization. Unfortunately, lab processing of soil may induce this effect, an artifact. Vigorous grinding, pulverization and sieving disrupt natural aggregate-cohesive properties of soils and we have associated this with over-saturation in the Solvita® CO2-Burst test. Other workers have shown that the flush of CO2 is significantly reduced as water contents approach saturation (Franzluebbers 1999), but the interaction with soil lab processing and its sensitivity to small increments of water has hitherto not been thoroughly researched.

Fine milling of soils is considered advantageous for nutrient testing, and has been shown to be conducive to better performance (low variability) by soil proficiency programs (Miller et al 2010). For routine soil nutrient tests as performed in commercial labs it is not relevant that soils' natural structures be maintained prior to testing. This working paper seeks to address the problem by

demonstrating this with proficiency samples, and proposes alternate means to avoid the "CO2-slump" which appears to result from unstructured over-wet soils.

MATERIALS & METHODS: Five standard soils (Fig. 2) were selected from the ALP proficiency program. The soils possessed a wide range of organic matter (LOI) from 0.95 to 5.97%. They were run for CO2-burst respiration using the capillary bottom-wetting method (Standard method) and a 50% water-filled pore space method (WFPS Method). The Standard method was performed according to regular protocol. For the WFPS method 40g of soil was weighed into a graduated beaker. The settled volume was noted and free-pore space calculated from particle density and water dribbled on top to attain 50% filled pore space (0.5 m^3 m^{-3}). Both methods were performed in triplicate and were started within 30 min. of each other using routine lab protocols with two lab technicians, one performing weighing and loading, and the other apportioning water and inserting CO_2 probes.

Data for the ALP soils as received are shown in Table 1. Two soils were not fully dried and required additional drying before testing CO2-Burst. It has been previously determined that the initial water content should be less than about 4%, otherwise the CO_2 burst may be suppressed (Franzluebbers, 2014 *personal comm.)*. This is an additional source of potential variation that is not attributable to the CO2 burst protocol but to sampling and lab handling.

Table 1. Properties of ALP Soil Samples utilized in the study

	Fineness			WHC	Water	Capillary Water	% water sat-	
Soil	**>.7mm**	**>0.5mm**	**<.5mm**	**$g \cdot g^{-1}$**	**% as rec'd**	**$g \cdot g^{-1}$**	uration	LOI %
1501 NB	1.2%	8.6%	91.4%	0.460	2.3	0.44	95%	6.50
1502 KS	7.0%	65.0%	35.0%	0.480	**5.0**	0.38	79%	4.25
1503 TX	0.3%	6.0%	94.0%	0.210	0.8	0.21	100%	0.95
1504 ID	0.3%	2.1%	97.9%	0.360	3.4	0.50	140%	1.87
1505 IA	7.7%	21.2%	78.8%	0.900	**7.1**	0.43	47%	5.97

Capillary water is the quantity of water absorbed by the Haney-Haney capillary method

A series of sieves were used to determine particle size separates. All but one sample fell into an extremely fine, unstructured class of 0.5mm-minus. WHC ranged from low to very high and capillary wetting produced a wide result with several over-saturated, corresponding to the quantity of fines. The results of respiration testing for all samples and triplicates are given in Table 2 below showing Solvita® color response which follows Beers Law and the calculation for CO2 based on it.

Table 2. Soil respiration results for 5 ALP soils in triplicate, tested using Standard capillary wetting (Part 1) and WFPS wetting (Part 2).

Part I. Wetting Method: Haney-Haney Bottom Wetting

SAMPLES		Solvita® COLOR			mg kg CO2-C			SUMMARY COLORIMTERY			SUMMARY CO2- RATE		
LAB	ALP ID	Rep A	Rep B	Rep C	Rep A	Rep B	Rep C	COLOR	±	CV%	PPM	±	CV
9318.0	1501 NB	3.44	2.98	3.39	41.4	27.7	39.8	3.27	0.25	7.7%	36.3	7.49	20.6%
9318.1	1502 KS	4.01	3.96	4.17	68.7	65.7	78.4	4.05	0.11	2.7%	70.9	6.64	9.4%
9318.2	1503 TX	1.12	0.82	0.97	5.4	4.15	4.69	0.97	0.15	15.5%	4.7	0.63	13.2%
9318.3	1504 ID	2.16	2.21	2.05	13.5	14	12.2	2.14	0.08	3.8%	13.2	0.93	7.0%
9318.4	1505 IA	3.6	3.5	3.34	47.8	43.5	38.1	3.48	0.13	3.8%	43.1	4.86	11.3%
											33.7	< Means >	12%

Part 2. Alternate Wetting Method: 50% WFPS Approach

SAMPLES		Solvita® COLOR			mg kg CO2-C			SUMMARY COLORIMTERY			SUMMARY CO2- RATE		
LAB	ALP ID	Rep A	Rep B	Rep C	Rep A	Rep B	Rep C	COLOR	±	CV%	PPM	±	CV
9318.0	1501 NB	4.48	4.48	4.53	102.8	102.8	108.2	4.50	0.03	0.6%	104.6	3.12	3.0%
9318.1	1502 KS	4.32	4.32	4.37	90.2	90.2	94.1	4.34	0.03	0.7%	91.5	2.25	2.5%
9318.2	1503 TX	1.33	1.79	1.85	6.5	9.7	10.2	1.66	0.28	17.2%	8.8	2.01	22.8%
9318.3	1504 ID	2.88	3.24	3.19	25.2	34.7	33.1	3.10	0.20	6.3%	31.0	5.09	16.4%
9318.4	1505 IA	3.91	3.96	4.06	62.7	65.7	71.6	3.98	0.08	1.9%	66.7	4.53	6.8%
											60.5	< Means >	10%

All samples pre-dried at 45C for 48 hrs

Both sets of tests showed a high-degree of repeatability based on the low variances observed between the individual triplicates. The responses for the two moisture methods were significantly correlated with each other (r^2 = 0.87). However, the Standard Method with bottom-capillary wetting showed an average of 45% lower CO_2-burst compared to WFPS. The ratio of the difference between the two methods varied from 1.3 to 3.0 with the largest difference observed for highest organic matter sample (source: New Brunswick, Canada). This soil may have been a well-structured soil in the field but reacted poorly to pulverization. Had it not been finely ground, it may have behaved normally in the Standard Method. Soils that exhibit a very large difference in respiration between the Standard and WFPS method are likely to be moisture-sensitive soils, although the significance of this is not well understood. Wood End Lab first observed it in irrigated soils with elevated salt content. The CO_2-burst reduction observed herein was also correlated with the saturation levels. Thus high water content is adversely and variably influencing apparent respiration and the magnitude of the effect can be very large. These data suggest that proficiency-program soils that are highly pulverized are prone to over-saturate with the Standard method.

CONCLUSIONS

Soil testing for biological traits such as 24hr CO_2-Burst depends on certain factors being suitably accounted for which are different to those soil labs are accustomed to controlling. Previous studies have shown that increasing soil disturbance due to drying and extent of sieving alters biological responses predictably. While increasing soil fineness normally increases CO_2 rate (Franzluebbers, 1999), we have shown it also increases vulnerability to over-saturation which triggers a CO_2 slump. Therefore it is conceivable that these opposing factors could together result in extreme variability in test results for respiration. Differences of only a few grams water per respiration test have been noted to be sufficient to trigger a CO_2-slump in the same sample (Bruce Hoskins, 2014, *personal communication*). Other work has found that the best means to obtain reliable estimates of biological activity that compare to field rates for undisturbed soils is by selecting *coarsely sieved soil* defined as at least 2mm sizing (Franzluebbers, 1999).

A short term solution may exist for highly-disturbed and unstructured soils by moistening carefully from above to fill a calculated 50% of pore-space. The standard bottom wetting procedure should continue to work for well-structured soils. The challenge is distinguishing an artifact effect (pulverization[9]) from a real effect ("water-prone" soils).

A longer term goal would be that soil services validating lab biological proficiency could provide minimally processed 2mm soils for biological test services. In the meantime labs offering these tests must carefully distinguish over-saturated from normal conditions each time a test is performed. Sources of variation ascribed to the CO2-burst approach and Solvita® technology should be directed towards as-yet-difficult to control variables of soil handling, drying, grinding and moistening which clearly influence microbial response, air-delivery and CO2 diffusion during the test.

CITATIONS

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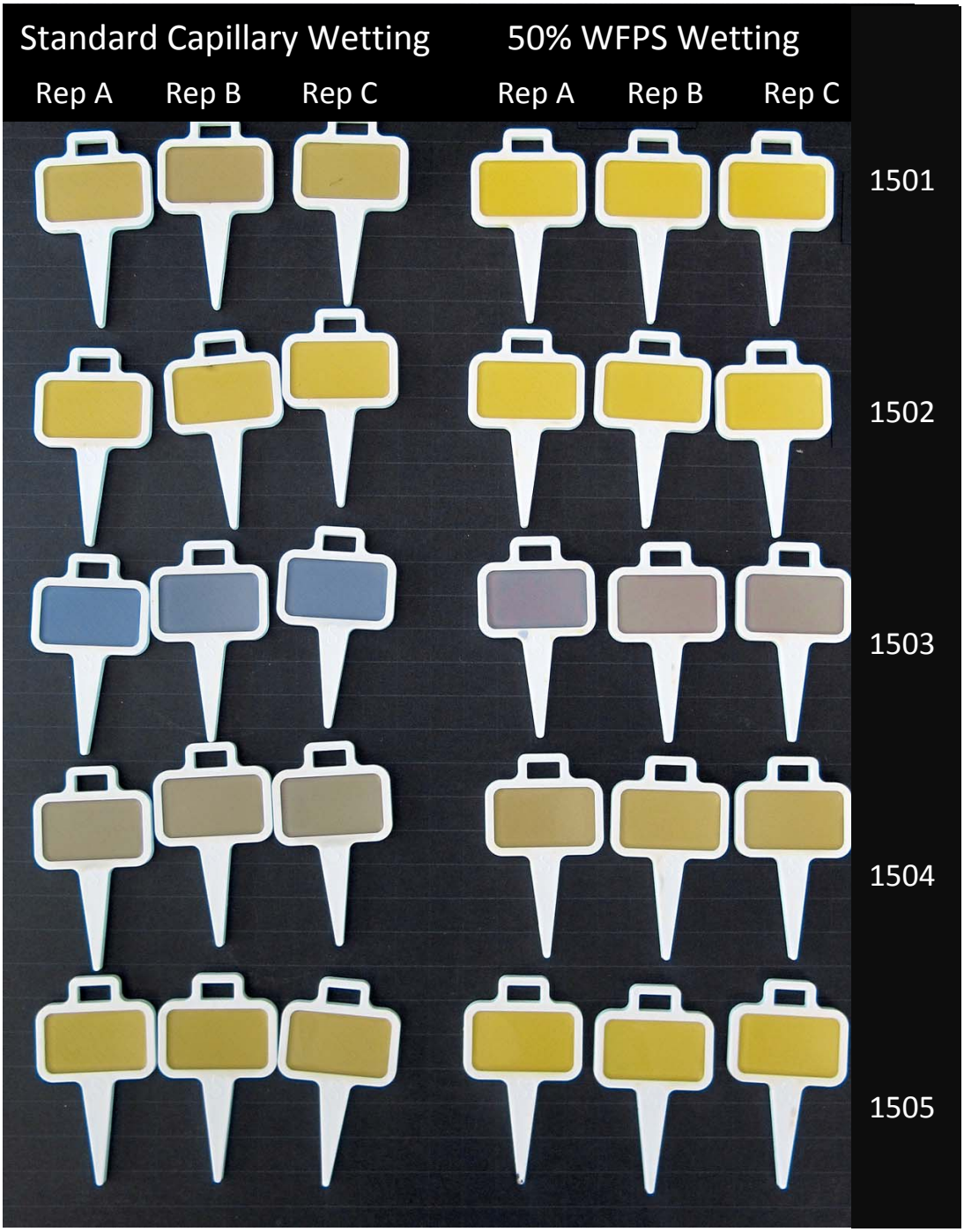
Images:

Figure 2. The five ALP samples used in the test runs, after wetting. Top Row after Standard method capillary wetting and lower row after 50% WFPS wetting. A spatula is required to open the surface to detect excess water in the over wet samples.

Soil	1501	1502	1503	1504	1505
Loc:	NB (Canada)	KS	TX	ID	IA

Figure 3. Solvita results arranged by replicates for each of two wetting methods.



Method Notes:

Identifying over-saturated soils:

1) Allow soil to wick up water completely as per Standard method. Weigh wetted soil in Solvita capillary beaker. Subtract beaker tare weight and calculate water as grams water per gram soil ($g \cdot g^{-1}$). If the soils are not clayey or high in OM, anything > 0.3-.4 $g \cdot g^{-1}$ may be too much water.
2) Over saturation should be confirmed visually after rewetting is completed by using a soil spatula to gently open the soil surface. A glistening semi-liquid appearance most likely indicates over saturation (see Figure 1, right image).
3) Optionally and separately determine water holding capacity by a suitable water absorption method, such as Parnes-Brinton method. Contrast this result to the amount of water from #1 above. Results > 70% of WHC are too high for proper respiration.

Adjusting soil using WFPS if Standard method (bottom-wetting) over-saturates soil:

1) Place 40g soil in Solvita graduated beaker and gently tap. Observe the volume from the graduated lines. Round up to the nearest 5cc, i.e. 26cc = 30cc, 43 = 45cc.
2) Use the Solvita moisture adjustment chart to choose the correct amount of water to add to the soil sample. Dribble it on the top of the sample. An adjustable burette is ideal for this as the amount of water to be added will normally range from 5-12cc.
3) Start the test and record results as "WFPS" method. This approach does not require any more time than the previous method.

Table 3. Determining water addition for 50% WFPS wetting method. After determining volume of 40g soil from (1) above, use Col (A) below find amount of water Col (B) to add to bring sample to 50% water filled pore space. (C)(D) and (E) show related calculations. ⌂

	(A)	(B)	(C)	(D)	(E)
	40g Soil Settled Vol	Water, ml for 50% WFPS	Bulk Density g/cc	solids PD x 40g	Avail. Pore Space (A - D)
	20	**2**	2.0	15.2	4.8
	25	**5**	1.6	15.2	9.8
*	**30**	**7**	1.3	15.2	14.8
*	**35**	**10**	1.1	15.2	19.8
*	**40**	**12**	1.0	15.2	24.8
	45	**15**	0.9	15.2	29.8
	50	**17**	0.8	15.2	34.8

* most common range for ag soils; PD - particle density