EFFECT OF COARSE AND FINE SOIL SIEVING ON SOLVITA CO_2 BURST AND OTHER TRAITS OF NAPT SOIL

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INTRODUCTION

The impact that soil homogenization and soil handling has on tests for CO_2 respiration has become a subject of increased attention. Common lab methods employed to homogenize soils include grinding and sieving and wetting methods include gravimetric, capillary and volume-based. It is thought that any of these may act singly or interactively to influence biological response and Solvita CO_2 burst. This study was conceived in attempt to get to some of the answers. It is a collaboration with Utah State University Plants, Soils and Climate Department (PSC) which processes soils for the NAPT program. For this project, PSC handled and homogenized 4 soils differently to make 2 sieve-size groups which are subsequently tested for CO_2-burst as well as some other parameters.

The potential negative effect of soil processing on Solvita respiration was first observed in 2015 and appeared to be related to over-saturation of soils causing an inhibition of respiration (**Brinton 2015**). Subsequent studies with 8 commercial soil labs indicated that wetting was the principal issue and fineness of texture was indirectly involved with over-saturation linked to sieving and grinding (**Brinton & Burger 2015 a,b**).

There is no surprise that soil handling influences CO_2 respiration results in relation to the structural condition of the soils (**Franzluebbers 1999, Borken & Matzner 2009**). However, the recent observations bring back into focus a deeper, long-standing concern that biological N-mineralization measurements are significantly impacted by grinding and sieving by nature of introducing artifacts, chiefly a result of releasing previously protected organic matter (**Bremner & Waring 1964**). A reappraisal of modern soil handling to arrive at the best practice for biological samples is therefore appropriate. Our hypothesis for this study was that handling soils to produce a finer fraction is a likely factor impacting the magnitude and variability of the CO_2 test.

SOILS & METHODS

Four field soils recently collected across the USA were prepared by PSC. The normal regimen was altered to produce a “coarse” and a “fine” fraction. The preparation entailed 3 different steps to obtain the final samples (**NAPT 2017**).

Soil was initially air-dried followed by coarse-sieving at **2mm**. Soil that did not pass the 2mm sieve was sent to a soil-crusher and then returned to the 2mm sieve, joining the previously sieved sample. Therefore, the 2mm coarse soil included dried, fresh 2mm soil and some exposed to a crusher.

To prepare the fine **0.8mm** sample the process continued with all 2mm blended soil sent to a 0.8mm sieve. Soil not passing the 0.8mm sieve was sent to a flail-mill and then returned to the 0.8mm sieve. This material was blended to become the "fine" fraction.

The two fractions of each of four soil series were subsequently provided to Woods End Labs which ran duplicate tests on different days using two types of wetting: the original bottom-wetting capillary method (**Haney & Haney 2010**) and the newer 50% WFPS method (**Solvita SOP instructions, 2016**).

The Four (4) soils are noted below with Location, Series, Texture and NAPT code

1. NH Fryeburg Very Fine Sandy Loam 2015-120
2. ME Paxton Fine Sandy Loam 2016-112
3. SD Graceville Silty Clay Loam 2016-102
4. ME Fryeburg Silt 2016-101

SIEVE SIZE

1. 0.8mm "fine" ground, sieved
2. 2mm coarse sieved only with some crushing

TESTING

24-hr CO_2-Burst: Solvita Vers 700.6 SOP – water added either statically (fixed volume for capillary action) or based on estimated total pore space (50% WFPS method).

Water Stable Aggregates (WSA) Solvita VAST Method. Woods End Laboratories SOP

Alternate CO_2-Burst: IR pulse ER-10 respirometer, Columbus Instruments

SLAN – Solvita labile-amino-N, 1N NaOH room-temperature treatment, 24 hrs. Woods End Lab SOP

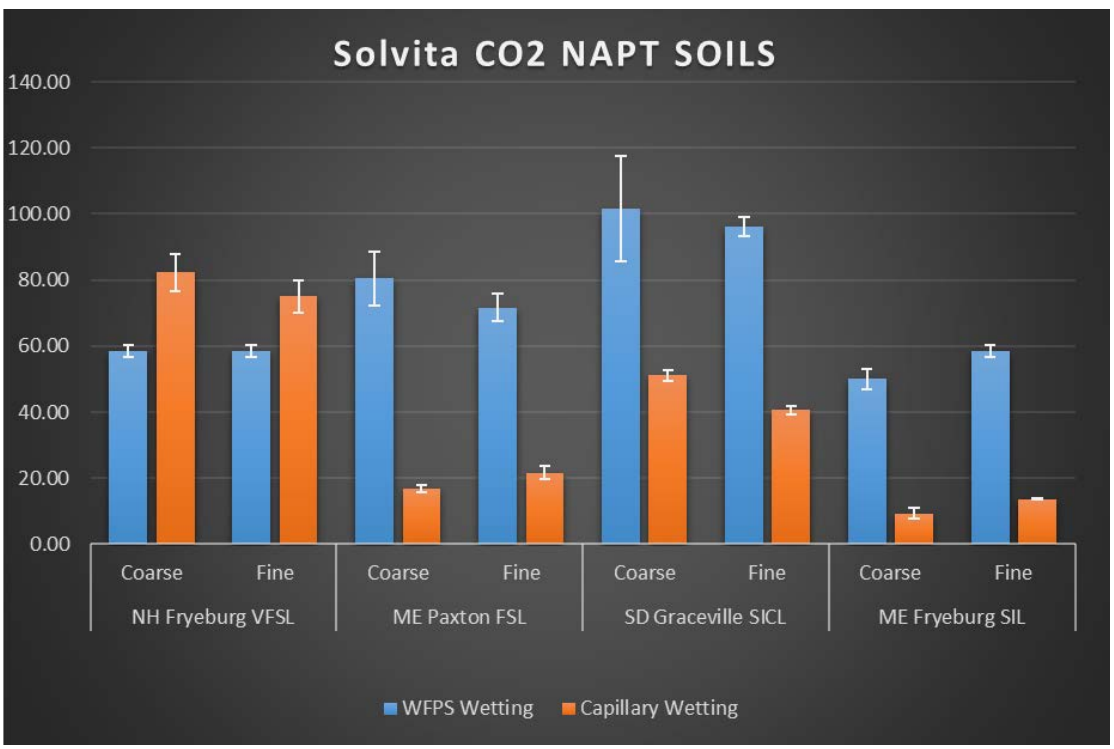
RESULTS

The overall observations indicate that there was little appreciable effect on CO2 respiration attributable to soil sieve size when comparing fine 0.8mm soil to coarse 2mm soil.

In contrast, the wetting methods exerted a significant impact on respiration rate, consistent with earlier observations. For three of the four soils the capillary wetting method caused significant reduction in observed respiration. Only the Fryeburg fine sandy load soil (NH) gave increased respiration with finer sieving; all the others including a Maine Fryeburg series soil gave dramatically reduced respiration attributable to wetting method.

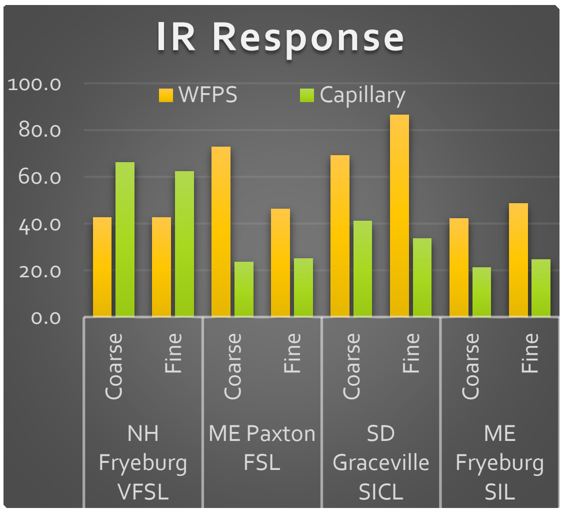
The overall mean CO2 respiration of WFPS vs capillary wetting was **72** vs **39** ppm, respectively or differing by nearly a factor of **2**. In contrast there was no overall effect of sieving with **56.3** vs **54.5** ppm for Coarse vs Fine, respectively. There was a significant interaction effect of soil source x wetting. Excluding the Fryeburg Very Silty soil that behaved as an outlier the other 3 soils averaged a **3.6-fold** impact on respiration attributable to wetting. The ANOVA data is shown in the following sets of tables.

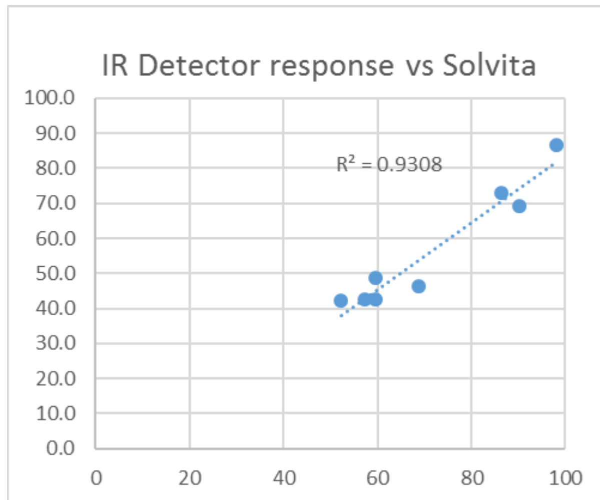
Effects for wetting were significant at $p < 0.005$**, for the interaction effect of source of soils and wetting $p < 0.001$**. As expected, the respiration for actual soil source (location of sampling) were highly significant at $p<0.001$***.



ALTERNATE RESPIROMETRY

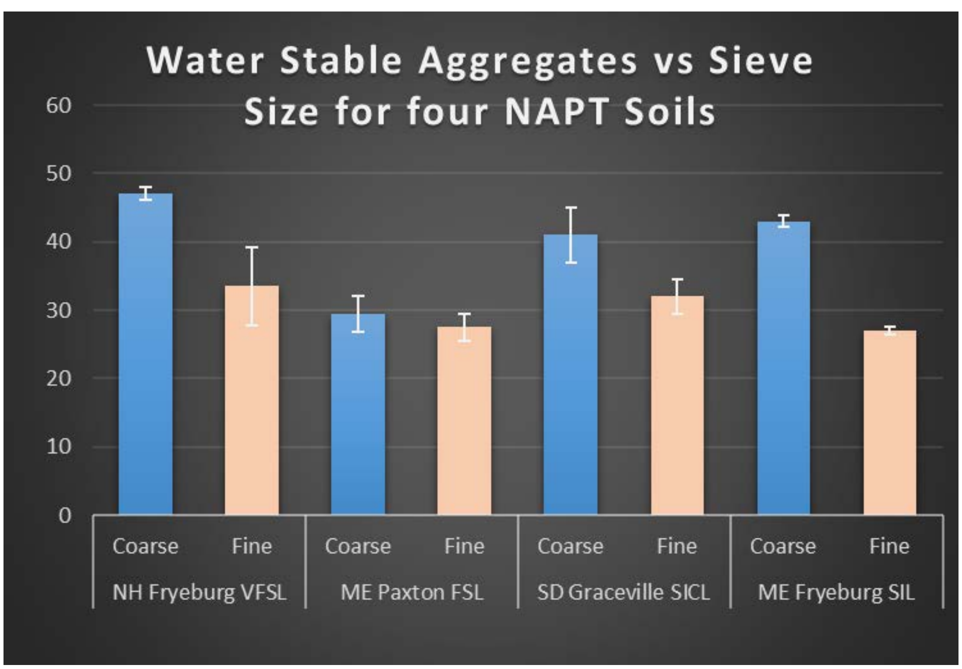
There has been some speculation that Solvita which is a gel-chemistry method differs from infrared (IR) which is an electronic-optical measure of CO_2. We ran the same set of soils by both wetting methods by 24-hr IR respirometry and obtained virtually identical results related to sieve size and wetting methods. The r2 of Solvita and IR response was **0.93 - 0.96** for **WFPS** and **Capillary Wetting**, respectively. These data confirm that observed effects are not particular aspects of Solvita technology.





SOIL DE-STRUCTURING

Based on earlier reported studies, our hypothesis is that the potential negative effects of handling soils should not necessarily be confined to respiration per se, but extend to include other biological traits and



especially physical structuring on which respiration depends in a feedback loop of porosity and dynamic aggregate aeration. In order to evaluate this water stable aggregates (WSA) were measured in duplicate on the same set of soil samples Aggregates are micro-structures of soil in the range of 50 µm - 2mm and are associated with positive biological factors interacting with physical texture.

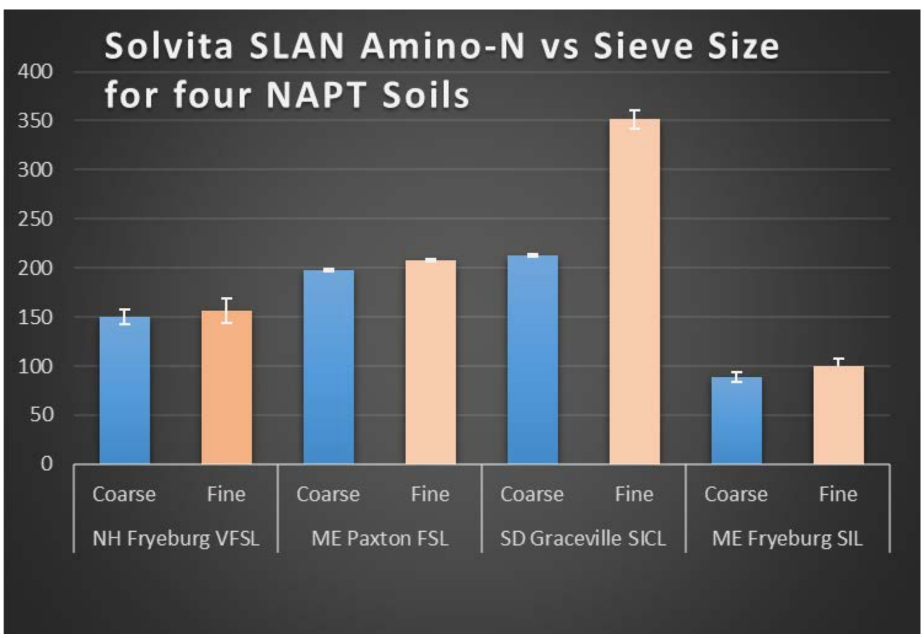
The data show that additional sieving from 2mm down to 0.8mm resulted in a significant loss of aggregate structure for each soil group. The effect of soil sieving on WSA in was very highly significant ($p<0.001$), with adjusted r^2 for the study of 75%. It is noteworthy, however, that the overall average reduction of WSA was only 25% which indicates that a considerable amount of microaggregates survived this sieving. Some soils were only sieved in the study and others required grinding to obtain the smaller sieve sizes.

SLAN

The Solvita SLAN test or labile-amino-N is a trait dependent on organic-matter and employs a regent for extraction, therefore it is not strictly a biological test but an extraction test. The overall impact of sieving was highly significant for all levels of comparison and significant at $p=0.014$ for sieve size alone.

For three of the 4 soils the observed magnitude of SLAN increase was small, and for the heavier clayey soil (SD) which very likely required more crushing and grinding, the impact was relatively large. The interaction effect of series x sieving was very significant ($p<0.001$).

These results reinforce the concept that soil sieving and grinding introduces differential factors which may have an indirect bearing on respiration and conceivably could influence longer term respiration over 1-day results.



GRAND ANOVA OF CO2 BURST EFFECTS DUE TO SIEVING AND WETTING METHOD

SOURCE of Variance: **grind**

Soils	grind	wet	N	MEAN	SD	SE
	Coarse		16	**56.2813**	31.2706	7.8177
	Fine		16	**54.4562**	26.9346	6.7337

SOURCE: wetting

Soils	grind	wet	N	MEAN	SD	SE
		WFPS	16	**71.9000**	19.1404	4.7851
		CAP	16	**38.8375**	27.4908	6.8727

Soils	x	wet	N	MEAN	SD	SE
NH120		WFPS	4	58.4500	1.4434	0.7217
NH120		CAP	4	78.6750	6.0096	3.0048
ME112		WFPS	4	76.0500	7.3777	3.6888
ME112		CAP	4	19.2000	3.0518	1.5259
SD102		WFPS	4	98.8500	9.9695	4.9847
SD102		CAP	4	45.9000	6.1736	3.0868
ME101		WFPS	4	54.2500	5.2855	2.6428
ME101		CAP	4	11.5750	2.7342	1.3671

ANOVA TABLE

FACTOR :	rep	Soils	grind	wet	ppm
LEVELS :	2	4	2	2	32
TYPE :	RANDOM	WITHIN	WITHIN	WITHIN	DATA

VAR	SS	df	MS	F	p	
Soils	8220.2912	3	2740.0971	171.842	0.001	***
grind	26.6450	1	26.6450	2.313	0.370	
wet	8745.0309	1	8745.0309	19379	0.005	**
s x w	7786.6311	3	2595.5437	125.76	0.001	**
g x w	0.9800	1	0.9800	0.143	0.770	
s x g x w	139.2126	3	46.4042	0.628	0.644	

S x w = soil-source and wetting interaction
G x w is grinding and wetting interaction
S x g x w is all level interaction

CONCLUSIONS

This study segregated four NAPT soils into two size groups, one with minimal processing at 2mm, and one with continued processing until all the sample passed an 0.8mm sieve. The hypothesis that additional sieving disrupts and reduces CO_2 respiration was not confirmed.

Sieving to a finer size did cause a significant change of water stable aggregates (WSA) which declined significantly, and SLAN amino-N, which increased significantly.

Much literature suggests that the disturbance of aggregate structure caused by lab sieving is expected to artificially *increase* the availability of nonmicrobial substrates and therefore microbial activity. However, may if not all early studies do not examine moisture or wetting as a separate variable. Therefore, in cannot be ruled out that there are competing influences whereby grinding and sieving potentially *increases* respiration but may also *compromise* wetting leading to *reduced* respiration.

The sieving effect on increased SLAN observed in this study was of relatively small magnitude except in the case of a clayey SD soil. This is most likely the result of the additional grinding required for that soil to obtain the 0.8mm fraction.

The study confirms earlier Solvita reports that the major influence on respiration appears to be via the wetting method, which in 3 out of 4 soils tested here resulted a significant improvement of CO_2 rate when using the 50% pore-space wetting method instead of the capillary method. The study also employed an infrared respiration method. This produced similar results showing that there is nothing about Solvita itself involved in the observed effects.

It is reassuring that this study disproves that sieving at 0.8mm has a significant influence on CO_2 respiration, but due to the way PSC custom-prepares soils, differing soils may be subjected to different levels of crushing and grinding. This is a good approach which tends to minimize unnecessary homogenization. It is most likely not what commercial labs are routinely doing. It cannot be ruled-out that inter-lab variations in sieving and grinding will continue to exert variables influences on respiration which are not addressed in a study of this nature.

Commercial laboratories may obtain reliable and repeatable results with biological tests by minimally processing soils and moistening to obtain optimal water content. It is readily possible for any lab to evaluate the impact of its grinding and sieving regimen by testing the soils under differing scenarios.

Researchers intending to compare lab respiration tests with other N-mineralization protocols should explicitly document the extent of soil handling and homogenization which is expected to exert direct and indirect influences on results. ⌂

CITATIONS

Borken, W and E Matzner (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology (2009) 15, 808–824, doi: 10.1111/j.1365-2486.2008.01681.x

Bremner, J.M., S.A.Waring (1964) Effect of Soil Mesh Size on the estimation of Mineralizable Nitrogen in Soils. Nature Vol 4937: p 1141

Brinton, W. (2015) Variables Influencing Solvita CO2 Respiration Results. Woods End Laboratory Research Journal Vol 1 April 2015

Brinton W. B. Burger (2015a) Solvita Proficiency Lab Test Results, report to ALP/NAPT labs using Solvita.

Brinton W. B. Burger (2015b) Solvita for Soil Respiration: Influence of Various Soil Factors. Synergy in Science. ASA-CSA_SSSA Meeting, Nov 15-18 Minneapolis

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

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.

NAPT (2017) Soil Handling Protocol: 1 Procedures for drying NAPT soil samples. 2 Procedures for crushing and sieving NAPT soil samples 3) Procedures for Blending NAPT soil samples. SOP Document, North America Proficiency Testing and Soil Sci Soc of America

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