

**MO DIRT SOIL HEALTH SURVEY
OF A SORGHUM FIELD AT THE
BRADFORD AGRICULTURAL RESEARCH CENTER**

Report

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INTRODUCTION

The Danforth Center has implemented a citizen science program on soil science called MO DIRT – Missourians Doing Impact Research Together. This program focuses on soil science education, and soil health surveying and monitoring. MO DIRT is funded by the National Science Foundation EPSCoR program through the Missouri Transect. One of the goals of MO DIRT is to collaborate with the EPSCoR scientists by conducting soil health surveys in their research fields. These surveys can provide additional information for interpreting plant responses in agricultural systems.

This report presents the results of a soil health survey conducted at the end of the growing season of 2017 in the sorghum field G17 at the Bradford Agricultural Research Center, a part of the University of Missouri in Columbia. The survey includes the results from measurements of physical, chemical and biological soil health indicators as well as a general interpretation of these measurements and their interrelationships.

This sorghum field has been under study by Dr. Todd Mockler and his team from the Donald Danforth Plant Science Center. They are examining the natural variation in different sorghum genotypes to understand key phenotype-to-genotype associations to optimize breeding strategies for improving the yield and stress tolerance of sorghum.

Erica Agnew provided information on the experimental design and management of the G17 field. The field work was conducted by Terry Woodford-Thomas and Sandra Arango-Caro. The lab work was conducted at the Education Lab at the Donald Danforth Plant Science Center (DDPSC), as well as at Dr. Kristen Veum's Lab (KVL) and the Soil Testing Lab (STL), both at the University of Missouri in Columbia.

Dr. Kristen Veum has been a soil science advisor of MO DIRT and the soil tests conducted in her lab were provided without any cost.

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METHODS

The collection of soil samples and environmental variables was conducted on September 25, 2017 in the field G17 (West and East sides; 38.890147, -92.209486) at the Bradford Agricultural Research Center (4968 Rangeline Rd., Columbia, MO 65201). The field was cultivated with several genotypes of sorghum. The plants were already matured, showing panicles (Figure 1). During the growing season, the field had one application of fertilizer (Urea plus Agrotain, 100 lbs/A) and two applications of herbicides (Application 1: Lexar, RoundUp and AMS, 13 gal/A; Application 2: Atrazine, 15 gal/A).



Figure 1. Sorghum (*Sorghum bicolor*).

Soil sampling design

Twelve composite soil samples were collected at field G17 planted with four sorghum genotypes (P1_229841, P1_297130, P1_297155 and P1_505735). A composite soil sample consisting of three subsamples was collected per row. A row (plot) represented ten individuals of a particular sorghum genotype. Three composite samples were collected per genotype (Figure 2). Two composite samples per genotype were collected in G17 West and one composite sample per genotype was collected in G17 East. Single air temperature and soil temperature measurements were taken at each of the plots (rows). Soil samples were tested in the lab for physical, chemical and biological soil health indicators. The soil samples were collected at least two days after rain in order to give time to the soil to reach field capacity (the amount of water content held in the soil after excess water has been drained away).

Variables

The following environmental variables were measured at the collection site of each composite soil sample:

- Air temperature at 1.5 m above the soil line (one measurement per composite soil sample)
- Soil temperature at 5 cm depth (one measurement per composite soil sample)

The following soil health indicators (variables) were measured for each soil sample at three labs: the Education Lab at the Donald Danforth Plant Science Center (DDPSC), and Dr. Kristen Veum's Lab (KVL) and the Soil Testing Lab (STL), both at the University of Missouri in Columbia.

The soil samples were collected from the first 5 cm of soil depth.

Physical soil health indicators:

- Color (DDPSC)
- Texture (DDPSC)
- Water content (% gravimetric water content) (DDPSC)
- Aggregate stability (KVL)

Chemical soil health indicators:

- pH_s (STL)
- Primary macronutrients: Nitrogen as nitrate (NO₃), phosphorous, and potassium (STL)
- Secondary macronutrients: Magnesium and calcium (STL)
- Cation exchange capacity (STL)

Biological soil health indicators:

- Soil microbial respiration at field temperature (DDPSC)
- Active carbon (DDPSC and KVL)
- % Organic matter (STL)

Protocols

The protocols that describe how the different variables were measured are available at:

Donald Danforth Plant Science Center Lab (DDPSC): The protocols used are from the MO DIRT citizen science program and are included in this report in Appendix 1. Also, they are available electronically at [modirt.missouriepescor.org/sites/default/files/files/Soil%20Health%20Survey%20Manual_Master\(1\).pdf](http://modirt.missouriepescor.org/sites/default/files/files/Soil%20Health%20Survey%20Manual_Master(1).pdf).

Kristen Veum's Lab (KVL): Protocols for active carbon and soil wet aggregate stability are in Appendix 2.

Soil Testing Lab (STL): Information on the protocols for chemical parameters is available at soilplantlab.missouri.edu/soil/soilprocedures.aspx.

RESULTS

Environmental parameters

Air and soil temperature

Soil temperature affects climate, plant growth, soil properties and soil processes such as rate of decomposition of organic matter. It is directly linked to the temperature of the atmosphere. Soil is an insulator for the heat that flows between the terrestrial portion of the earth and the atmosphere. The ideal soil temperature for plant growth ranges between 18°C and 24°C and becomes a limiting factor for plants performance, microbial activity and soil respiration when it rises above 35 to 40 °C.

The sampling day was sunny and bright with low wind. Air temperature in the study field was 26°C, with little variation among sampling locations as expected (Table 1).

Soil temperature at 5 cm depth in the study field ranged between 19°C and 23°C (Table 1) and was consistently lower than the air temperatures as expected. This temperature range indicates that the study sites were at ideal soil temperatures at the time of the sampling.

Very little variation was shown in air and soil temperatures probably due to the buffering effect of the sorghum plants which provided shade at the time of the sampling.

Table 1. Air and soil temperature data (See pages 15-17 for protocols).

Genotype	Replicate No.	Plot No.	Air temperature (°C)	Soil temperature (5 cm depth) (°C)
P1_229841	Rep 1	1072	26	23
P1_229841	Rep 2	2059	26	22
P1_229841	Rep 3	3308	26	21
P1_297130	Rep 1	1137	26	19
P1_297130	Rep 2	2227	26	21
P1_297130	Rep 3	3204	26	22
P1_297155	Rep 1	1083	26	21
P1_297155	Rep 2	2040	26	22
P1_297155	Rep 3	3261	26	22
P1_505735	Rep 1	1102	26	21
P1_505735	Rep 2	2010	26	23
P1_505735	Rep 3	3318	27	21

Physical soil health indicators

Soil texture

Soil texture is determined by the presence and relative proportions of the three types of particles that make up soil: sand, silt, and clay. Depending on their texture, soils will vary in their ability to retain water and nutrients.

In the study field, five soil texture classes were determined (Table 2). This diversity of classes indicates high spatial heterogeneity. Ten of the samples have a clay soil textural classification. Clayey soils have small soil particles that hold large amounts of nutrients, water and gases. These soils are easily compacted, which increases runoff. Under dry conditions, clay soils may be more susceptible to wind erosion since clods (lumps of soil) become pulverized.

Soil color

Soil color is an indicator of various chemical processes acting on soil. These processes include the weathering of geologic material, the oxidation-reduction reactions on soil minerals (mainly iron and manganese) and the decomposition of organic matter. Soil colors were recorded according to the Munsell Color System. The notation of this system has three components: hue (specific color), value (lightness and darkness) and chroma (color intensity) (e.g. hue value/chroma as 10YR 5/3).

A uniform brown color was observed among soil samples. The hue indicated that the colors of the soil samples were in the YR range (yellow-red spectral color range) towards the yellow end, and the value and chroma indicated that the soils were medium-dark with a low color intensity (Table 2). The brown color indicates good air-water relations and may be due to the presence of iron. The level of darkness indicates low amounts of organic matter as confirmed by the lab tests (see table 6).

Table 2. Soil texture and soil color data (See pages 18-22 for protocols).

Genotype	Replicate no.	Plot no.	Soil color	Soil texture
P1_229841	Rep 1	1072	10YR 4/2	Silty clay
P1_229841	Rep 2	2059	10YR 5/2	Clay loam
P1_229841	Rep 3	3308	10YR 4/2	Clay loam
P1_297130	Rep 1	1137	10YR 2/2	Loam
P1_297130	Rep 2	2227	10YR 2/2	Silt loam
P1_297130	Rep 3	3204	10YR 3/3	Clay loam
P1_297155	Rep 1	1083	10YR 4/2	Clay loam
P1_297155	Rep 2	2040	10YR 4/2	Silty clay
P1_297155	Rep 3	3261	10YR 4/2	Clay
P1_505735	Rep 1	1102	10YR 4/3	Clay
P1_505735	Rep 2	2010	10YR 3/3	Silty clay
P1_505735	Rep 3	3318	10YR 3/4	Clay

Soil water content

How much water is stored in the soil determines the soil's ability to moderate the hydrological cycle, influences soil properties (color, pH, horizons) and processes, and limit biological activity.

Soil water content was estimated in terms of grams of water per gram of dry soil (gravimetric water content g/g). In general, soil water content in most soils ranges between 0.05 and 0.50 g/g. The water content in the study field on the sampling day was within this range with a tendency towards low values (Table 3). On average, water content ranged between 0.04 and 0.1 g/g. This indicates that the soil in the study plots had relatively low water content. Soil in the study site was dominated by similar amounts of silt and clay particles that hold water efficiently. Thus, low water levels were likely influenced by temporal environmental conditions.

This preliminary data shows high values of soil water content in soil planted with the three replicates of the P1_229841 genotype.

Soil wet aggregate stability

Soil aggregates are groups of soil particles bound to each other by structures, exudates and “glues” from fungi, earthworms, microbes and organic matter decomposition. Aggregate stability is the ability of soil aggregates to resist disintegration under disruptive forces (e.g. tillage, water, wind). Thus, it is highly dependent on organic matter, biological activity, and the mineral components. It increases as organic matter increases, making the soil less vulnerable to erosion. Wet aggregate stability (WAS) indicates how well a soil can resist the impact of water forces and is rated as: low <40%, medium 40-60%, high 60-80% and very high 80-100%.

The percentage of WAS ranged between 11% and 50% (Table 3). These values are low indicating that the soil at this field is very vulnerable to water impact and could be easily eroded. This is consistent with the low levels of organic matter found in the study fields (Table 6). This preliminary data shows high values of wet aggregate stability in soil planting with the three replicates of the P1_297130 genotype.

Chemical soil health indicators

pH

Soil pH is a parameter used to describe how acidic or basic the soil is. Soil pH is important in determining the availability of nutrients to plants and how easily they can take up nutrients from the soil. In this study, the pH parameter is the active acidity in the soil that indicates the free hydrogen ions in the soil solution. The Soil Testing Lab at the University of Missouri measures the pH from a soil salt solution (pH_s). A pH_s reading is more stable than a pH reading from a soil water solution because even when a soils' acidity is unchanged, the soil pH measurement may vary due to changes in salt levels in the soil (e.g. addition of nitrogen and potassium, decomposition of organic matter and minerals,

leaching, etc.). The rating of soil pH_s for crops is: very low <4.5, low 4.5-5.3, medium 5.3-6.0, high 6.0-7.5 and very high >7.5. Soils with a pH_s between 6.1 to 6.5 have the optimum pH_s for crop growth.

The pH_s of half of the soil samples was in an optimal range (6.1-6.5), and for the other samples was slightly below or above the optimal range (Table 4).

Table 3. Soil water content and wet aggregate stability data (See pages 23-24 and 31-32 for protocols).

Genotype	Rep. no.	Plot no.	Weight of wet soil (g)*	Weight of dry soil (g)	Gravimetric water content (WC) (g/g)	Wet aggregate stability (WAS) (%)
P1_229841	Rep 1	1072	90	83.7	0.08	23.7
P1_229841	Rep 2	2059	90	83.0	0.08	41.6
P1_229841	Rep 3	3308	90	81.5	0.10	41.7
P1_297130	Rep 1	1137	90	85.7	0.05	37.0
P1_297130	Rep 2	2227	90	85.2	0.06	40.2
P1_297130	Rep 3	3204	90	86.8	0.04	38.9
P1_297155	Rep 1	1083	90	82.3	0.09	18.1
P1_297155	Rep 2	2040	90	86.0	0.05	24.9
P1_297155	Rep 3	3261	90	83.3	0.08	33.2
P1_505735	Rep 1	1102	90	83.6	0.08	32.4
P1_505735	Rep 2	2010	90	85.9	0.05	11.5
P1_505735	Rep 3	3318	90	85.6	0.05	50.1

* The wet weight of all soil samples is equal because these samples were previously used for the soil respiration experiments where all the samples had 90 grams of fresh soil.

Soil nutrients

Macronutrients are the nutrients that plants need in large amounts. The fertility of the soil indicates the availability of these nutrients for the plants. The primary macronutrients are nitrogen, phosphorous and potassium, and secondary macronutrients include magnesium and calcium. Potassium, calcium and magnesium form positively-charged ions that are held in the soil by negatively charged soil particles. Nitrogen and phosphorous form negatively-charged ions that are not held well by soil particles. Consequently, these two elements are easily lost from the soil due to leaching (removal from the soil as water passes through it).

Nitrogen was measured as nitrogen in the nitrate form (NO₃-N), a mineralized form that is readily available to plants. Availability of nitrogen in the soil depends on biological activity and therefore fluctuates with changes in temperature and moisture. The levels of nitrogen in nitrate in the soil for crops follow these rating categories: low <20, adequate 20-41, high 41-75, and excessive >75 ppm.

In the study plots, nitrogen ranged between 0.4 and 2.6 ppm, except for plot no. 1083 where the nutrients levels were much higher (10.4) (Table 4). This range indicates nitrogen deficiency in the sampled sites. On average, soil with the genotype P1_297155 showed the highest levels of nitrogen.

Phosphorous was determined as plant-available phosphorous in the soil. In general, the recommended level of phosphorous for sorghum is 45 pounds per acre (lbs/A), and with levels above 200 lbs/A considered excessive.

In the study plots, phosphorous ranged between 42 and 86 lbs/A, indicating good levels (Table 4). On average, soil with the genotype P1_297155 showed the highest level of phosphorous while soil with the genotype P1_505735 showed the lowest levels.

Potassium was determined as exchangeable potassium in the soil. Desired potassium level in sorghum, with average CEC of 10.8 as in the study field is 274 lbs/A.

In the study site, most plots showed potassium levels lower than the ideal, except for two plots (Table 4). Soil with the P1_297155 genotype has the highest values of potassium while P1_505735 genotype showed the lowest values.

Magnesium is determined as exchangeable magnesium, the magnesium available to plants. The soil test categories for this element are: low <120, adequate 120 - 360, and high >360 lbs/A.

In the study plots magnesium levels were adequate to high (257 - 449 lbs/A) (Table 4). On average, soil with the P1_229841 showed the highest levels of magnesium, while soil with the P1_505735 showed the lowest levels.

Calcium was determined as exchangeable calcium, the calcium cations available to plants that are attached to the negatively charged soil particles. Adequate levels for this element in the soil are between 2000 and 4000 lbs/A.

Calcium levels in the study field ranged between 2771 and 4057 lbs/A indicating adequate levels in the soil (Table 4). On average, soil with P1_297130 showed the highest values of calcium and soil with P1_505735 showed the lowest levels.

CEC (Cation exchange capacity) is a measure of the soil's capacity to retain and release cations (positively charged elements) such as potassium, calcium and magnesium. These cations are held in place in soil by particles that are negatively-charged (anions) found in clay and organic matter. Thus, soils with high clay content and or organic matter content have high CEC, which translates into higher fertility. The CEC rating system is: <5 sand, 5.1-10 sandy loam, 10.1 – 18 silt loam, 18.1 – 24 clay loam, and >24 clay meq/100 g.

The CEC at the study plot was similar among the soil sampling sites (9.8 - 12.3 meq/100g) (Table 4). This indicates that the soil samples have mostly silt loams soil textures that show relatively poor soil fertility levels.

Table 4. Results from the Soil Testing Lab at the University of Missouri.

Genotype	Rep. no.	Plot no.	pH_s	Nitrogen in nitrate (NO₃-N) (ppm)	Phosphorus (P) (lbs/A)	Potassium (K) (lbs/A)	Magnesium (Mg) (lbs/A)	Calcium (Ca) (lbs/A)	Cation exchange capacity (CEC) (meq/100g)
P1_229841	Rep 1	1072	5.7	0.5	52	247	360	3133	11.6
P1_229841	Rep 2	2059	6.1	0.4	52	242	449	3612	12.2
P1_229841	Rep 3	3308	6.5	0.4	84	241	323	3155	10.0
P1_297130	Rep 1	1137	5.8	2.6	45	176	314	3080	11.2
P1_297130	Rep 2	2227	6.7	0.9	66	257	428	4057	12.3
P1_297130	Rep 3	3204	6.4	0.6	76	263	351	3371	10.7
P1_297155	Rep 1	1083	5.9	10.4	72	294	305	2771	10.1
P1_297155	Rep 2	2040	6.0	0.7	52	238	364	3210	11.3
P1_297155	Rep 3	3261	6.6	2.2	86	317	312	3193	10.2
P1_505735	Rep 1	1102	5.9	1.1	42	174	257	2803	9.8
P1_505735	Rep 2	2010	6.0	0.4	50	227	359	3110	11.1
P1_505735	Rep 3	3318	6.5	1.8	74	235	302	3103	9.8

Biological soil health indicators

Soil respiration

Soil respiration (SR) is the gaseous flux of carbon dioxide (CO₂) from soils to the atmosphere. Soil respiration results from ecological processes such as decomposition of soil organic matter and plant litter by soil microorganisms, as well as from respiration from plant roots and soil fauna. It is an important indicator of soil health because it measures microbial activity that is critical for the conversion of nutrients into forms that plants can use. SR is affected by environmental factors. As soil temperature increases, microbial activity also increases up to a point when temperature becomes a limiting factor (around 35 °C). If temperature continues to rise, microbial activity declines. A similar relationship is found between SR and water content. SR is optimal until water displaces the air, restricting oxygen availability.

In this study, soil microbial respiration was expressed as average content of carbon in carbon dioxide at field temperature (CO₂-C pounds/acre/day). Soil respiration among plots ranged between 10 and 110 lbs/A/d (Table 5). All plots had medium-low to very high biological activity. This indicates, in general, that the soil in the study plots offers a suitable environment for the microbial community. On average, soil with P1_229841 showed the highest levels of soil respiration, and soil with P1_297130 the lowest levels.

Table 5. Microbial soil respiration data after 24 hours at field temperature (See pages 25-28 for protocol).

Genotype	Rep. no.	Plot no.	Soil resp.*	Interpretation of biological microbial activity based on Solvita values for soil respiration (CO₂-C lbs/A/day)
P1_229841	Rep 1	1072	110	High biologically with excellent supply of organic matter
P1_229841	Rep 2	2059	110	High biologically with excellent supply of organic matter
P1_229841	Rep 3	3308	42.5	Very active biologically with high organic matter turnover
P1_297130	Rep 1	1137	20	Active microbe population and good organic matter supply
P1_297130	Rep 2	2227	10	Medium activity - may be accumulating organic matter
P1_297130	Rep 3	3204	20	Active microbe population and good organic matter supply
P1_297155	Rep 1	1083	42.5	Very active biologically with high organic matter turnover
P1_297155	Rep 2	2040	20	Active microbe population and good organic matter supply
P1_297155	Rep 3	3261	42.5	Very active biologically with high organic matter turnover
P1_505735	Rep 1	1102	20	Active microbe population and good organic matter supply
P1_505735	Rep 2	2010	20	Active microbe population and good organic matter supply
P1_505735	Rep 3	3318	20	Active microbe population and good organic matter supply

* Soil respiration as average CO₂-C (lbs/A/day) after 24 hours at field temperature.

Soil active carbon

The active carbon test indicates the amount of carbon in the organic matter readily available for microbes as a source of energy and carbon, thus driving biological activity and nutrient cycling.

Based on the MO DIRT protocol, the level of active carbon was poor to fair in the sampling sites (Table 6). This is consistent with low levels of active carbon based on results from Veum’s Lab with values ranging between 126 and 244 lbs/A (Table 6). Since the soil respiration was relatively high, this may have contributed in part to the depletion of active carbon by the end of the growing season. Soil with P1_297155 had much lower levels of active carbon than soil with other genotypes.

Organic matter

Soil organic matter is the organic component of soil and consists of plant residues, living soil organisms, decomposing organic matter and stabilized organic matter (humus). Soil organic matter is the most important soil health indicator. It provides food for microorganisms that facilitate the availability of nutrients for plants, minimizes leaching of nutrients, buffers the effects of high acidity, increases the moisture retention of the soil, the available water capacity and the water filtration, helps to minimize soil compaction, holds soil aggregates together, decomposes toxic substances, and acts as a carbon sink.

The amount of organic matter in the soil ranges between 1 and 20%. In most productive agricultural soils, organic matter ranges between 3-6%. In the study field relatively low levels of organic matter were found (2.0% - 2.4%) (Table 6).

Table 6. Active carbon and percent organic matter data (See pages 29-30 and 33-34 for protocols on active carbon).

Genotype	Rep. no.	Plot no.	MO DIRT (DDPSC Lab)		Veum’s Lab	MU Lab
			Soil quality	Active carbon (lbs/A)	Active carbon (lbs/A)	Organic matter (%)
P1_229841	Rep 1	1072	Poor	> 0 - 232	139.6	2.0
P1_229841	Rep 2	2059	Fair	> 232 - 464	194.5	2.4
P1_229841	Rep 3	3308	Fair	> 232 - 464	227.5	2.1
P1_297130	Rep 1	1137	Poor	> 0 - 232	190.6	2.1
P1_297130	Rep 2	2227	Fair	> 232 - 464	193.2	2.4
P1_297130	Rep 3	3204	Fair	> 232 - 464	179.6	2.3
P1_297155	Rep 1	1083	Fair	> 232 - 464	164.8	2.4
P1_297155	Rep 2	2040	Fair	> 232 - 464	126.6	2.3
P1_297155	Rep 3	3261	Fair	> 232 - 464	203.5	2.0
P1_505735	Rep 1	1102	Fair	> 232 - 464	150.2	2.1
P1_505735	Rep 2	2010	Fair	> 232 - 464	156.4	2.2
P1_505735	Rep 3	3318	Fair	> 232 - 464	244.3	2.3

CONCLUDING REMARKS AND RECOMMENDATIONS

Overall, the soil at the sorghum field G17 at the Bradford Agricultural Research Center was relatively healthy.

- Physical indicators: The study field has silty-clay soils that hold water. However, the gravimetric water content was low, as was soil aggregate stability indicating that the soil is vulnerable to erosion. The inconsistency in the soil texture results between the feel method and the CEC can partially be explained due to the nature of the feel method. This is a qualitative method while the CEC is an indirect but quantitative estimate of soil texture that is more accurate.
- Chemical indicators: pH was ideal. Phosphorous, calcium and magnesium levels were adequate in the study field. On the other hand, potassium and nitrogen, which are two of the three macronutrients critical for plant growth, were at low levels.
- Biological indicators: Soil respiration indicated healthy biological activity. Organic matter was below the range expected for a cropland. Consequently, nitrogen levels as well as soil aggregate stability were low, likely affected by limited organic matter.

Soil health indicators differed among soils planted with the different genotypes:

- Soil planted with the P1_229841 genotype had the highest levels of water content, soil respiration and magnesium. On the other hand this soil had the lowest levels of nitrogen.
- Soil planted with the P1_297130 genotype had the highest levels of water aggregate stability and calcium. On the other hand this soil had the lowest levels of water content and soil respiration.
- Soil planted with the P1_297155 genotype had the highest levels of phosphorous, potassium and nitrogen. On the other hand this soil had the lowest levels of water aggregate stability and active carbon.
- Soil planted with the P1_505735 had the lowest levels of phosphorous, calcium, magnesium and potassium.
- Since only three soil samples were collected per genotype, it is difficult to conclude if the differences in soil health indicators are due to differential adaptations of the genotypes in nutrient and water uptake and other biological activities such as soil respiration.

This soil health survey was based on one-time soil testing at the end of the growing season and generalization cannot be made for the status of soil health at other stages of plant growth. It is recommended that future research on soil health in croplands should include measurements of soil health indicators during early stages of plant development, the peak of vegetative growth, and during the flowering and seeding stages.

This report only provides descriptions of general patterns of soil health indicators. It is recommended to apply statistical analyses to examine if these indicators present significant differences based on sorghum genotypes and other environmental parameters for the growing season.

REFERENCES FOR SOIL TEST INTERPRETATION

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Appendix 1. MO DIRT protocols of soil health indicators.

AIR TEMPERATURE PROTOCOL

Background

Air temperature is a measure of the kinetic energy (energy of motion) of the gases that make up the air. As gas molecules move faster, air temperature increases. In other words, air temperature describes how cold or hot the air is. Air temperature is important to understand how the atmosphere works in order to make weather predictions. For example, air temperature affects the humidity of the atmosphere, influencing the fueling of storms. Also, air temperature influences precipitation since rain, sleet, snow, or freezing rain will fall depending upon the temperature of the air.

Many biological processes are also dependent on air temperature. The metabolism in animals can slow down or increase depending upon the temperature of the environment that surrounds them. Thus, feeding, mating, migrating and other animal behaviors are partially regulated by temperature. In plants, the right temperature is needed to trigger seed germination and to promote plant growth. This indirectly affects the soil environment, for as healthy plants grow under the right environmental conditions, they develop healthy root systems on which a large variety of organisms depend.

Materials

- Clipboard and data sheet
- Measuring tape
- Permanent marker
- Thermometer (the same to measure soil temperature)
- Watch or timer

Measurement procedures

1. Calibrate the thermometer following the instructions that are in the box.
2. At the sampling location determine the height at which you will measure air temperature (1.5 m).
3. Hold the thermometer at this height and wait until the sensor gives a stable reading (~2 minutes).
4. Record the reading on the data sheet.
5. Repeat steps at each of the remaining sampling location.

SOIL TEMPERATURE PROTOCOL

Background

Soil temperature affects climate, plant growth, soil properties and soil processes such as rate of decomposition of organic waste. It is directly linked to the temperature of the atmosphere. Soil is an insulator for the heat that flows between the terrestrial portion of the earth and the atmosphere. During sunny days, the soil absorbs energy from the sun (radiation) and its temperature increases. During the night, the soil releases heat into the air, which affects air temperature.

Soil temperature varies through the seasons. During the summer the temperature of the soil is relatively cool, while during the winter it is relatively warm when compared to air temperature. This can influence the activities of soil organisms, indicate the right time for seed germination or the right time for animals to hibernate or emerge from the ground. For example, soil temperature becomes a limiting factor for plant growth, microbial activity, and soil respiration when it goes beyond 35 to 40 °C. While the ideal soil temperature for plants to grow ranges between 18-24 °C. However, these ranges will depend on species adaptations to local environments.

Soil temperature also influences the state of water (liquid, gas, or frozen), which, combined with the amount of water in the soil, affects soil properties. Furthermore, soil temperature influences decomposition rates that can affect horizon characteristics. In cold environments, the decomposition rate is low because soil microorganisms are less active. This can result in dark-colored soils. In warm tropical climates, weathering is increased, which produces iron oxides and can result in reddish-colored soils.

Lastly, soil temperature influences the evaporation of soil moisture, which affects the humidity of the air, and consequently, the climate. On the other hand, the amount of soil moisture affects the rate at which soil heats and cools. Wet soils heat slower than dry soils because the water in the pore spaces between the soil particles absorbs more heat than air in those spaces.

Materials

- Brush
- Clipboard and data sheet
- Nail
- Permanent marker
- Soil thermometer
- Watch or timer



Soil thermometer at 5 cm depth.

Measurement procedures

1. Calibrate the thermometer following the instructions that are in the box.
2. Find the indent in the thermometer at 5 cm from the tip.
3. At the sampling location remove leaf litter or other debris and insert the thermometer up to 5 cm into the soil and wait until the sensor gives a stable reading (~2 minutes). If you cannot insert the thermometer to the desired depth try again at a different spot a few centimeters away or use a nail to make a hole to place the thermometer. Avoid moving the nail to the sides, as this generates air pockets that alter the temperature reading. Record the reading in the data sheet.
4. Repeat the steps at each sampling location.
5. Clean the thermometer with a cloth or a brush to remove attached soil.

SOIL TEXTURE PROTOCOL

Background

Soil texture is described by the presence and relative proportions of the three types of particles that make up soil: sand, silt, and clay. These particles differ in size as follows (Table 2):

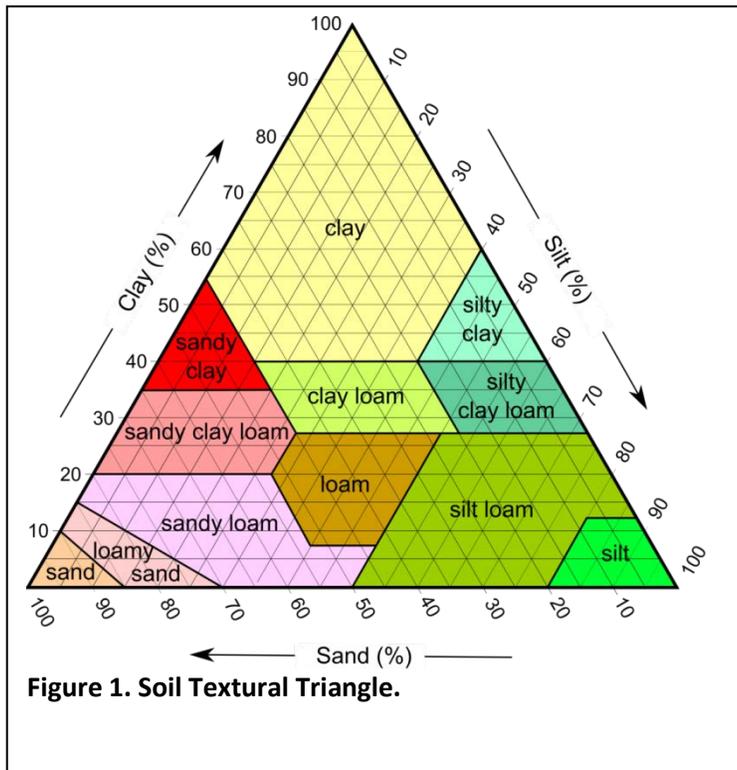
Table 2. Soil particle classification according to the International Society of Soil Science

Diameter of the particles (mm)	Classification
> 2	Stony structure
2 – 0.2	Coarse sand
0.2 – 0.02	Fine sand
0.02 – 0.002	Silt
<0.002	Clay

Particles classification according to the International Society of Soil Science

Depending on their texture, soils will vary in their ability to retain water and nutrients. A simple way to examine soil texture is to physically handle dry and wet soil samples, using your fingers to work with small soil samples. *Sandy soils* feel rough (gritty) because sand particles have hard edges. These soils do not hold many nutrients because they have large pores that allow gases and water to move through them rapidly. The *sand* particles do not adhere to each other and cannot stay together. *Silty soils* are smooth and powdery, and when wet, they make crumbles or ribbons, but are not sticky. Silty soils have smaller pore spaces than sandy soils, therefore, they can hold more water. *Clayey soils* are smooth when dry and sticky when wet, making balls or ribbons that stay together. Because their particles are so small, clayey soils can hold a lot of nutrients, water, and gases.

Most soils contain different combinations of sand, silt, and clay. The Soil Textural Triangle (Figure 1) shows the twelve possible soil classes based on the relative percentages of these combinations of textures. The most appropriate soil class for plant growth is loam, which can absorb water very efficiently. The loam soil is composed of mostly sand and silt, with a smaller amount of clay.



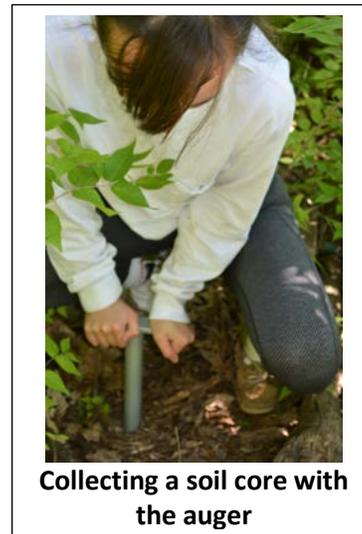
Materials

- Clipboard and data sheet
- Distilled water
- Permanent marker
- Plastic squeeze bulb pipette
- Sealable bag
- Soil texture feel-method diagram
- Stick
- Table knife
- Trowel

Measurement procedures

How to take the soil texture reading:

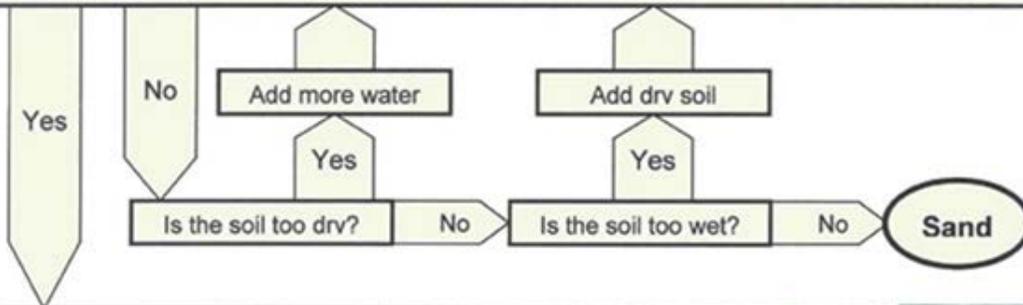
1. Use the feel-test method to determine the texture of each of the soil samples. Use the flow diagram in the next page.
2. Record the information on the data sheet.



Soil Texture by Feel

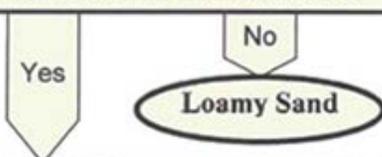
Start: Place soil in palm of hand. Add water drop-wise and knead the soil into a smooth and plastic consistency, like moist putty.

Does the soil remain in a ball when squeezed?

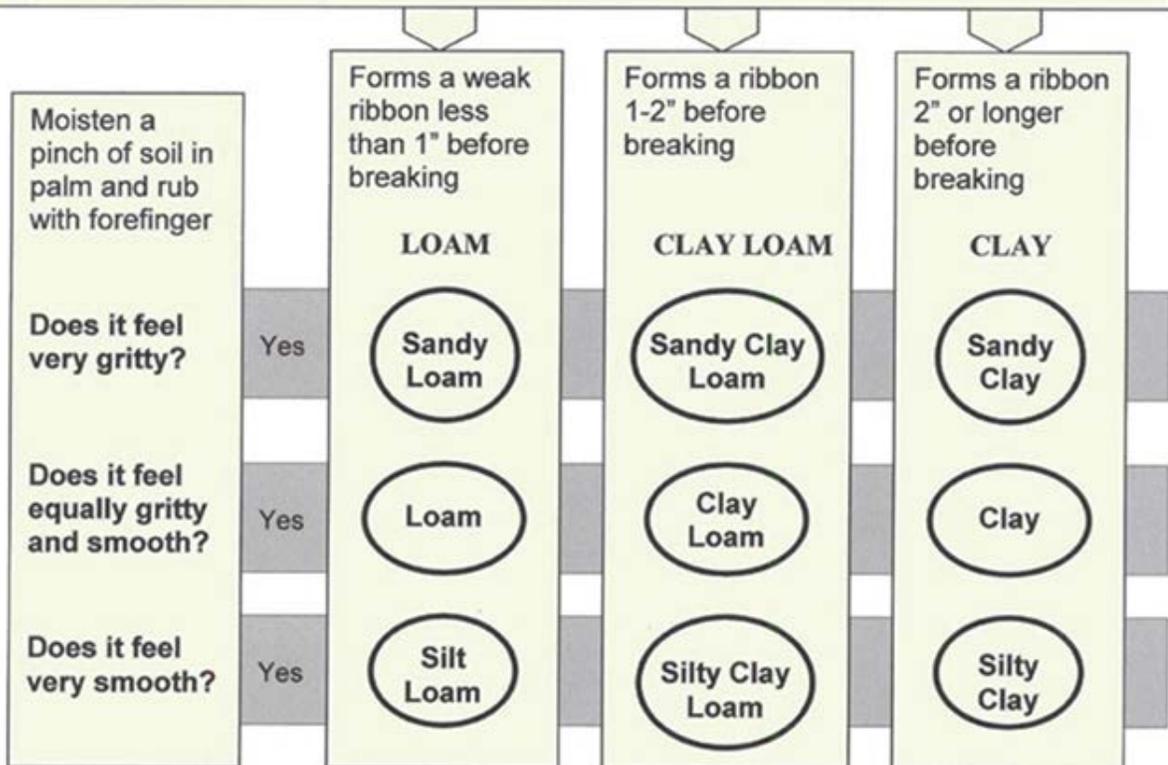


Place ball of soil between thumb and forefinger, gently pushing the soil between with the thumb, squeezing it upward into a ribbon. Form a ribbon of uniform thickness and width. Allow ribbon to emerge and extend over the forefinger, breaking from its own weight.

Does the soil form a ribbon?



What kind of ribbon does it form?



SOIL COLOR PROTOCOL

Background

Soil color is an indicator of various chemical processes acting on soil. These processes include the weathering of geologic material, the oxidation-reduction reactions on soil minerals (mainly iron and manganese), and the decomposition of organic matter. Climate, physical geography, and geology influence these processes.

Soil color can be used to estimate the organic matter content of the soil, to indicate the effects of human disturbance and past vegetation, to identify, classify and evaluate soils, and to locate where the soil water table is, among many other soil activities.

There are two primary coloring agents in soil: organic matter and iron. Dark surface soil usually indicates high content of organic material, while shades of red, yellow, and gray usually relate to the quantity and form of iron present.

Color development and distribution of color within a soil profile are part of weathering. Also, as organic matter decomposes into black humus, it coats surfaces of soil as it permeates through the soil. Humus color decreases with depth, and iron pigments become more apparent. So, as depth below the surface soil increases, colors become lighter, yellower, or redder. See Table 1 below for the interpretation of soil colors.

The Munsell System of Color Notation (www.munsell.com) is a system used to compare soil colors anywhere in the world. This system helps scientists to be consistent in the interpretation of colors. It has three components: hue (specific color), value (lightness and darkness), and chroma (color intensity) that are arranged in books of color chips. Soil samples are held next to the chips to find a visual match and assigned the corresponding Munsell notation. For example, a brown soil may be classified as: hue value/chroma (10YR 5/3).

Materials

- Clipboard and data sheet
- Distilled water
- Permanent marker
- Plastic squeeze bulb pipette
- Sealable bag
- Soil texture feel-method diagram
- Stick
- Table knife
- Trowel



Soil color identification

Table 1. General interpretation of soil colors

SOIL COLOR	DUE TO THE PRESENCE OF:	COMMENTS
Dark or black	Organic matter	Mostly found at soil surfaces. Associated with well-aggregated soils with above-average nutrient levels.
Clear or white	Calcium and magnesium carbonates, soluble salts or high proportion of sand (quartz crystals)	May indicate considerable leaching and low organic matter.
Red and bright yellowish	Iron is oxidized and not hydrated with water	Under dry conditions or well-drained soils. The iron oxides have strong surface charge properties that promote good aggregation of soil particles with sufficient porous that allow air and water for root development.
Yellowish brown/orange	Less oxidation of iron and hydration	Average air and moisture conditions.
Mucky soil mass or clay with spots of red, yellow, and gray colors	Ferrous and ferric compounds	In soils that are waterlogged for at least one part of the year, or due to the activity of plant roots living in ponding.
Grey/green/bluish-grey	Iron and manganese in reduced state	In waterlogged soils with lack of oxygen with colorless forms due to the loss of pigments.

Measurement procedures

How to take the soil color readings:

1. For each soil sample, assign a soil color using the soil color book.
2. Take a ped (soil aggregate) from the sample with your fingers and note whether it is moist, dry, or wet. If it is dry, moisten it slightly with water using a plastic squeeze bulb pipette.
3. Break the ped and hold it next to the color chart. Stand with the sun over your shoulder so that sunlight shines on the color chart and the soil sample you are examining.
4. Find the color in the color book that most closely matches the color of the inside surface of the ped. Be sure that all group participants agree on the choice of color.
5. Record the chosen color in the data sheet.

SOIL WATER CONTENT PROTOCOL

Background

How much water is stored in the soil determines the soil's ability to moderate the hydrological cycle, influences weather and climate, and maintains soil-water balance. Soil moisture also influences other soil properties (color, pH, horizons) and processes. Soil processes such as soil respiration and decomposition of organic matter are influenced by soil moisture's effect on microbial activity. Soils saturated with water can be unhealthy, supporting only anaerobic microbial activity and promoting plant roots decay over time. On the other hand, in dry soils, the relatively few water molecules are strongly attached to soil particles preventing the use by soil organisms.

Soil water content is expressed as the mass (weight) of water in a soil sample (Gravimetric water content) and as the volume of water in a known volume of soil (Volumetric water content).

Materials (some are optional)

- Balance or scale (0.1 grams precision and 400 grams minimum capacity)
- Brush
- Clipboard and data sheet
- Compostable bowls (5)
- Hot pad/oven mitt
- Knives: table knife and flat-bladed knife (pocket knife)
- Oven capable of maintaining a temperature not exceeding 105°C, **or** a 250 Watt infrared heating lamp (1 or 2 bulbs) that reaches temperatures of 65-90°C, **or** a fan
- Newspaper (if you air dry the soil samples)
- Permanent marker
- Sealable bags (5)
- Soil sample
- Tray
- Trowel



Measurement procedures

1. Make a composite soil sample from three locations within your sampling unit (e.g. row, plot, subplot).
2. At each of the three locations collect approximately 1.5 cups of soil from the first 5 cm depth.
3. Clean the sample from plant material (e.g. leaves, bark, roots, etc.) and rocks.
4. Weight the fresh soil sample (wet soil) and record the reading in the data sheet.

5. Air dry the soil sample.
6. Weight the dry soil sample and record the reading in the data sheet.
7. Calculate the percent gravimetric water content of your sample using the formula below and record it in your data sheet.

Calculations of gravimetric water content

% Gravimetric soil water content (I) = [(weight of wet soil (C) – weight of dry soil (F)) / weight of dry soil (F)] x 100. Where ...

- Weight of wet soil (C) = (weight of wet soil + bag (A)) – weight of bag (B)
- Weight of dry soil (F) = (weight of dry soil + container (D)) – weight of container (E)

SOIL RESPIRATION PROTOCOL

Background

Soil respiration is the gaseous flux of carbon dioxide (CO₂) from soils to the atmosphere. It represents one of the largest fluxes in the global carbon cycle. Soil respiration results from ecological processes such as decomposition of soil organic matter and plant litter by soil microorganisms, as well as from respiration of plant roots and soil fauna. It is an important indicator of soil health because it measures microbial activity that is critical for the conversion of nutrients into forms that plants can use.

Soils store a vast amount of organic carbon that can be released quickly or slowly into the atmosphere depending on soil respiration rates. Such rates are greatly influenced by several factors that make soil respiration very variable in space and time. Climate is a main driver of soil respiration because soil respiration increases as temperature rises, peaks under optimal soil moisture conditions, and decreases when soils are too wet or too dry. Vegetation type and phenology (timing of flowering, fruiting, and budding) also influence soil respiration through photosynthesis, because large amounts of carbon compounds from photosynthesis are allocated to plant roots and their associated symbiotic bacteria and fungi. Also, adding nitrogen to the soil reduces soil respiration because it causes a decline in the allocation of carbon to plant roots. Soils high in peat hold a vast amount of carbon. There is also a large amount of inorganic carbon in the form of carbonate that is associated with rock minerals in soil.

Agriculture and other human activities have a great impact on soil respiration by affecting soil factors that increase the release of soil CO₂ into the atmosphere. Consequently, soil respiration contributes to the dramatic increase of greenhouse gases in the atmosphere that are raising global temperatures affecting climate patterns.

In this protocol, you will use the Solvita[®] method to measure microbial soil respiration. Microbial soil respiration is positively correlated with soil fertility and crop responses. The health of the soil microbial communities is directly associated with the amount of humus and mineralized nitrogen (the nitrogen available to plants as by-product of organic matter decomposition completed by soil microbes).

Materials

- Balance or scale (0.1 grams precision and 400 grams minimum capacity)
- Brush
- Data sheet
- Nitrile gloves
- Permanent marker
- Sealable bag
- Soil sample
- Soil thermometer



Solvita soil kit and set up for measuring soil respiration

- Solvita kit (1 jar with lid, foil pouch with paddle, color chart). Keep the Solvita foil pouches in a cool place or in a refrigerator (must not be allowed to freeze) away from sunlight to prevent changes in temperature.
- Tray
- Trowel
- Watch

Procedures

1. Make a composite soil sample from three locations within your sampling unit (e.g. row, plot, subplot).
2. At each of the three locations collect approximately 1.5 cups of soil from the first 5 cm depth.
3. Using gloves, place the soil sample on the tray. Clean the soil sample as much as you can from roots and any other organic material as well as rocks.
4. Place the Solvita jar on the balance and zero-out the weight of the jar.
5. Fill the Solvita® jar with the soil, using the fill line as a guide, until it weighs 90 g.
6. Tap the bottom of the jar on a hard surface occasionally during filling to eliminate voids or air pockets.
7. Open foil pouch and insert gel paddle into the soil with the gel facing the clear side of the jar. Be careful not to jostle or tip jar. The soil should not touch the gel in the paddle.
8. Screw the lid on tightly and let the jar stand undisturbed for 24 hours, and keep it in a room with a controlled temperature of 20°C (70°F), away from sunlight.
9. Record in the data sheet the start time of the experiment and the color in the paddle at 0, 1, 2, 3, 4, 5, 10, and 24 hours into the experiment. Use the color chart to determine the number for the color on the paddle. Note that the color on the paddle may not exactly match any of the colors on the chart. Select the best match.
10. Do not delay the reading of the gel paddle because the color changes over time with CO₂ release from the soil.
11. Dispose of the soil leftovers after setting up the experiment.
12. At the end of the experiment, clean the jars with mild soap and water and dispose of the paddles.

Data interpretation

Use Table 1 to translate the colors and numbers in the paddle and color chart to biological soil conditions, and emissions of carbon in the carbon dioxide (CO₂-C) or emissions of carbon dioxide (CO₂) to the atmosphere in cultivated soils. If your survey is in a natural habitat (e.g., forest, woodland, prairie), you may have to do an additional step as explained next.

Cultivated soils

Data interpretation in Table 1 is based on soil samples from moist cultivated soils tested at room temperature of 20 C (70°F) after 24 hours into the test.

Soils from natural habitats

Soils from some natural habitats might be “fast risers”, which means they are associated with high-functioning systems with high levels of organic matter and microbial rates. This might be reflected in a fast change in paddle colors within the first hours into the experiment. If by 5 hours into the experiment the paddle has already changed to the color 3 or higher, the soil is considered a fast riser. In this case, take the color reading at 5 hours and find the respective amount of CO₂-C lbs/acre/day in Table 1. However, continue recording the changes in color until the color 6 is reached. The table will give you a range of numbers for a particular color. Multiply both numbers in the range by 2.5, the conversion factor, to estimate the equivalent numbers after 24 hours. Write your result in the data sheet.

Temperature conversion to field conditions

Use Table 2 to determine the CO₂-C emissions at field temperature based on your results at room temperature at 24 hours. Follow these steps:

- In Table 2, find the average field soil temperature and the respective conversion factor.
- Divide the CO₂-C value at room temperature after 24 hours, found in Table 1, by the conversion factor found in Table 7. Do this calculation for both numbers of the range.
- Find this new CO₂-C value in Table 1. This value indicates the CO₂-C emissions at field temperature at 24 hours. Record this value in your data sheet.
- If you obtain a value that is greater than 160, this indicates that your soil sample has extremely high biological activity. This could be the result of a soil very rich in organic matter and or high soil temperatures.

Table 1. Solvita Field Test – Performed in test jar at 20-22°C (68-77°F) after 24 hours

Color reading of gels in paddles					
Blue-Gray Color 0 - 1.0	Gray-Green Color >1.0 - 2.5	Green Color >2.5 - 3.5	Green-Yellow Color >3.5 – 4.0	Yellow Color >4.0 – 5.0	Bright Yellow Color >5.0 – 6.0
Biological soil condition of cultivated soils					
Extreme Low Activity	Low Activity	Medium – Low Activity	Ideal Activity	Medium – High Activity	Very High Activity
Associated with extremely depleted soils	Marginal biological activity with low organic matter	Medium activity - may be accumulating organic matter	Active microbe population and good organic matter supply	Very active biologically with very high organic matter turnover	High biological activity with excellent supply of organic matter
Emissions (Flux) of CO ₂ -C as lbs / acre / day *					
0.5 - 1	>1 - 5	>5 - 15	>15 - 25	>25 - 60	>60 - 160
International emissions (flux) of CO ₂ as g / m ² / day **					
0.2 – 0.4	>0.4 - 2	>2 - 6	>6 - 10	>10 - 25	>25 - 65

* Units are CO₂-C (amount of carbon in the CO₂ gas). Results are likely to depend on a variety of factors such as depth of sampling, soil temperature, and field moisture.

** International Metric Units based on CO₂. To convert CO₂ values to CO₂-C, multiply CO₂ values by 0.273. To convert CO₂-C values to CO₂, multiply CO₂-C values by 3.7.

Table 2. Conversion of CO₂-C emissions at room temperature (20°C /70°F) to emissions at field temperature

Field soil temperature*	5°C	10°C	15°C	20°C	30°C	40°C
Conversion factor	4	2	1.5	1	0.5	1

*If your average temperature in the field is an intermediate number between the values in this table, use intermediate values of the conversion factor. For example, if the average temperature is 25°C, divide your CO₂-C value by the conversion factor 0.75.

ACTIVE CARBON PROTOCOL

Background

Soil organic matter (SOM) is a widely acknowledged indicator of soil health. However, it does not have a definite chemical composition. The dominant element in SOM is soil organic carbon (SOC). Soil organic carbon contains high levels of recalcitrant forms (slowly altered by microbial activity) and small portions of labile fractions (decomposing readily). The labile fraction or active carbon is the type of carbon in the SOM that is readily available to the soil microbial community as a source of energy and carbon, driving much of the biological activity in the soil and the cycling of nutrients. Active carbon has fractions of microbial biomass carbon, particulate organic matter (particles less than 2 mm and greater than 0.053 mm in size), and soil carbohydrates.

Active carbon as a soil health indicator is positively correlated with percent organic matter, aggregate stability and with soil respiration rate, a measure of biological activity in the soil. Active carbon is very sensitive to land management practices and soil productivity, responding much sooner to changes in land management practices than total organic matter.

Materials

- Balance or scale (0.1 grams precision and 400 grams minimum capacity)
- Brush
- Clipboard and data sheet
- Color chart
- Composite soil sample (air dry)
- Distilled water
- Free-standing tube (30 ml)
- Nitrile gloves
- Plastic squeeze bulb pipette
- Potassium permanganate solution (0.2 M KMnO_4 in 1 M CaCl_2 , 7.2 pH) (5 ml), (store in a cool place).
- Set of measuring spoons
- Tray
- Watch or timer



Preparation of soil solution, mixing, and soil color comparisons.

Measurement procedures

1. From the leftovers of a composite soil sample, take a soil subsample (1/4 cup).
2. Using gloves, crumble the soil gently to give an even, aggregate consistency and spread thinly on the metal tray.
3. Remove organic matter from the soil sample (e.g. roots, leaves, bark, animals, etc.) as well as rocks and any other big debris.

4. If the soil is moist, air-dry it for a couple of days. Mix the soil 2-3 times while drying. Do not use extreme heat.
5. Place 2 ml of the 0.2 M KMnO₄ solution in the free-standing tube and add distilled water to the 20 ml mark. Cap the tube and mix the solution.
6. Add 5 g of soil to the solution.
7. Cap the tube tightly and shake vigorously for **exactly** 2 min (~100 strokes/min) to oxidize the active carbon in the sample. Stand the tube on the tray for **exactly** 10 minutes, avoiding any kind of disturbance. Protect the sample from direct sunlight while the soil particles settle.
8. After the 10 minutes, use the color chart to determine the level of active carbon in the sample and record the results in the data sheet. The purple color becomes lighter as a result of the oxidation of the carbon.
9. At the end of the experiment, dispose of the solution in a sink, flush with water, and clean the materials with mild soap and water.

Safety instructions

- Potassium permanganate is a very powerful oxidizer and should not be stored near acids or fuel sources to prevent fires, explosions, and or toxic gas buildup.
- The storage of this chemical (powder) should be in a clean and dry sealed container. It can be stored for over a year.
- When the powder is mixed with water, it becomes a powerful dye and stains fabrics permanently, stains skin temporarily, and causes corrosion on any metal or masonry.

Data interpretation

Potassium permanganate (KMnO₄) is an oxidizing agent that reacts with active carbon to partially bleach the deep purple permanganate color to light pink or clear. The safety data sheet for potassium permanganate is in Appendix 3). The lighter the color of the KMnO₄ solution after reacting with the soil, the greater the amount of active carbon and the better the quality of the soil.

The table below indicates soil quality based on the potassium permanganate method to estimate relative amounts of active carbon (Table 10).

Table 10. Field color chart to estimate the amount of active carbon in a soil sample using the potassium permanganate test

	Poor	Fair	Good	Excellent
Soil quality				
Active carbon in pounds per acre (lbs/A)	> 0 – 232	> 232 – 464	> 464 – 928	> 928

Appendix 2. Kristen's Veum Lab protocols for wet aggregate stability and active carbon.

WET AGGREGATE STABILITY PROTOCOL

Based on Kellogg Soil Survey Laboratory Methods Manual (2014).

Procedure

Day 1

1. Assemble a 2 mm sieve on top of a 1 mm sieve, with a bottom pan. Sieve an entire sample of air-dried soil with a Gilson sieve shaker for 4 minutes.
2. Place the material that is retained on 1 mm sieve in a Falcon 50 ml centrifuge tube, discard the remaining material from the analysis.
 - If desired amount is not achieved (many replicates) it is acceptable to slightly crush the sample by hand or with mortar and pestle with minimum reduction in size.
3. Weigh 3.00 grams of the 2-to1 mm particle size in aluminum foil dishes.
4. Fill plastic bowls with 2 liters of DI water.
5. Place 0.5 mm sieves in plastic bowls. There must not be any air bubbles under the sieve screen. Tap the bottom of the sieve, or remove the sieve entirely to start over.
 - To better see the air pockets/bubbles turning off the lab lights helps
6. Evenly distribute the 3.00 grams of soil on the 0.5 mm sieve. Aggregates should not touch the sides.
7. Allow samples to sit overnight.

Day 2

1. Agitate the sample by raising and lowering the sieve in the bowl 20 times in 40 seconds.
 - On the upward strokes drain the sieve, but do not raise so high as to break water tension and air enters beneath the sieve.
2. Remove sieves from water bowls, place on plate, and dry in oven for 2 hours at 110°C.
3. Remove samples from oven, and weigh sieve+plate+dry sample. Record weight (Wt_1).
 - Let sieves cool a bit, extreme temperatures make the balances fluctuate.
 - The soil must now be removed to leave only sand on the sieve.
4. Fill the Sodium Hexametaphosphate labeled bowl with 2 liters of DI water and 25 ml of sodium hexametaphosphate solution.
5. Place the 0.5 mm sieve with sample into the sodium hexametaphosphate bowl, and use your fingers to rub the soil particles through the screen leaving only $>.05$ mm particles.
 - Allowing the sieve to soak in the solution suspended for 20-30 seconds speeds up the soil dispersion. Do not allow sand to stick to fingers for end weight.
6. Remove sieve from solution, and thoroughly rinse sieve with DI water to remove any sodium hexametaphosphate residue. Only sand is left on the sieve.
 - The NaHex solution in the bowl needs to be remade ~every 8 sieves
7. Place sieve on rinsed plate, and dry for ~2 hours at 110°C
8. Remove samples from oven, let cool and weigh sieve+plate+sand. Record weight (Wt_2).

9. Discard any sand or organic matter in the trash by brushing the sieves and plates.
10. Record weights on sieve+plate (W_{t3}).
 - This could possibly be done on Day 1, as there isn't as much time restraint.

Calculations

Aggregates Retained: $W_r = W_{t1} - W_{t3}$

Sand Weight: $S_w = W_{t2} - W_{t3}$

Aggregate % = $\left(\frac{W_r - S_w}{(I_w / (AD/OD)) - S_w} \right) \times 100$

Where

I_w = Initial sample weight (3.00 g.)

W_r = Total weight of aggregates retained on 0.5 mm sieve

S_w = Weight of 2 – to 0.5 mm sand

AD/OD = Air-dry/oven-dry weight (if not available, use 1.00)

Reagents

Sodium Hexametaphosphate solution:

- Sodium hexametaphosphate ($Na_4P_2O_7$): **35.7 g**
- Sodium carbonate (Na_2CO_3): **7.94 g**
 - ✓ Dissolve 35.7 g of Sodium hexametaphosphate & 7.94 g of Sodium carbonate in 900 ml of DI water.
 - ✓ Use a large 2,000 ml beaker and a large stir bar. This solution takes a while to dissolve completely, and needs aggressive stirring.
 - ✓ Transfer to a 1,000 ml volumetric flask and bring to volume with DI water.
 - ✓ Store at room temperature in a glass liter bottle.

ACTIVE SOIL CARBON PROTOCOL - KMnO₄ LABILE

Based on Weil et al. (2003) using 0.2M KMnO₄ / 1M CaCl₂ stock:

Level 1 Stock Standards: *See insert in binder for easy prep.

Final [KMnO ₄] (M)	ml 0.2 M KMnO ₄ stock	ml DI water
0.0025	0.625	49.375
0.005	1.25	48.75
0.01	2.5	47.5
0.015	3.75	46.25
0.02	5.0	45.0

Level 2 Calibration Standards = 100x dilution of stock standards.

Final [KMnO ₄] (mM)	ml stock STD	ml DI water	Example Absorbance
0.0	0	50.0	0.001
0.025	0.5 (of 0.0025M stock)	49.5	0.058
0.05	0.5 (of 0.005 M stock)	49.5	0.115
0.1	0.5 (of 0.01M stock)	49.5	0.227
0.15	0.5 (of 0.015M stock)	49.5	0.290
0.2	0.5 (of 0.02M stock)	49.5	0.433

Sample Handling:

- Run samples in triplicate and include a reference and blank sample in each batch. Run in small batches of 12 to avoid biases due to timing delays.
- Weigh 2.5 g (0.0025 kg) of air-dried and ground soil into a 50 ml plastic Falcon tube. For highly degraded soils, use 5.0 g of air-dried soil instead. If you have highly degraded soils, you will notice that there is little to no color change (still very dark purple) and the final sample absorbance values will be high.
- Add 18 ml DI and 2 ml of 0.2M KMnO₄ stock– (this is a 10x dilution resulting in a 0.02M KMnO₄ solution).
- Cap tightly and place on side-to-side shaker at 120 oscillations/min for 2 min.
- Invert tube manually once to capture the soil that has settled in the top portion of the tube.
- Uncap and allow samples to settle upright for 5 minutes, preferably in the dark.
- Pipette 0.5 ml of sample supernatant into a new Falcon tube with 49.5 ml DI and vortex (100x dilution).
- Zero spec with ultrapure DI water and read absorbance at 550 nm. Record on sample data sheet.

Reagents:

0.2 M KMnO₄/ 1M CaCl₂ Stock Solution_In a 1L volumetric flask:

- Fill halfway with ultrapure DI water
- Add **147g** CaCl₂ dihydrate **OR 110.98 g** CaCl₂ anhydrous
- Dissolve and QS to 1L with DI water
- In a 1L beaker fill 900ml with 1M CaCl₂
- Add 31.607g KMnO₄
- Dissolve completely, may take up to ½ hour on a stir plate with low gentle heat.
- Remove from heat and check final pH and adjust to 7.2 as needed with 0.5M NaOH.
- Transfer to a volumetric flask and QS to 1L with remaining CaCl₂ solution

The pH-adjusted 0.2 M KMnO₄ stock solution should be kept in a dark bottle. Shelf life 3-6 months. KMnO₄ stains! Acid bath takes out any discoloration from plastic or glassware, but be careful with fabric or surfaces.

Notes:

This method was adapted from Weil. et al. (2003), a method that was designed for use in the field. It has been adapted to facilitate batch analysis.

The KMnO₄ concentration (0.02M in contact with soil) is essential to the integrity of the method. It is better to modify the soil mass (2.5g or 5.0g) rather than the KMnO₄ concentration if there are issues with under- or over-oxidation.

Although the serial dilutions may seem unnecessary and it may be tempting to eliminate those steps, they are essential to achieving accurate concentrations. Do not shortcut these steps!

Remember to modify the calculations if you change the soil mass.

1 mol KMnO₄ reduced (Mn⁺⁷ to Mn⁺⁴) = 0.75 mol C oxidized (9000 mg)

Active C (mg kg⁻¹) = [0.02 mol/l – (a + b X absorbance)] X (9000 mg C/mol) X (0.021 solution/0.0025 kg soil)