Soil Health Evaluation Manual

Soils Cross Cutting Project
East/Southern Africa Community of Practice
McKnight Foundation
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Manual of Protocols: Soil Health Assessment
McKnight Cross-Cutting Soils Project
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Section 1: Introduction and overview of soil sampling and handling
This manual seeks to provide methods for assessing soil health in smallholder contexts. The methods presented range in complexity, but the idea is that they can all be done either by farmers or by organizations that work with them in research and innovation networks. There are many other works which explain the importance of the measurements we present here, how they relate to soil health, and the overall, integrated concept of soil health; in this manual we will focus narrowly on the technical aspects of the tests and some guidelines for their interpretation. The first two sections of the manual provide guidance on soil sampling strategies as well as the equipment and materials needed to conduct the tests in this manual. Then each method for assessing soil is described in detail in section three.

1.1 **Sampling overview:** The objective of soil sampling is to accurately represent a layer (or layers) of soil at a particular site (e.g. field) and then prepare it adequately for the next steps in analysis. In this guide we will focus on relatively simple soil sampling and analysis methods which do not require special treatment of the soil (soil is only air-dried). It is worth noting that for other types of analysis (for example, analyzing soil microbial communities, types of nematodes, or soluble nitrogen in soil) we would need a more specific and careful handling of soil, which would be defined in other protocols regarding these measurements.

1.2 **Sampling methods:** To generate a sample that represents soil at different depths equally, a soil probe is often used, such as a **sharpened** tube that enters the ground to remove a ground cylinder (Fig. 1). It is also possible to replace this probe with a shovel, plus a knife or machete, by cutting a slice from the edge of the hole in the ground followed by trimming this slice cut with a knife to create a square section (Fig. 1). This shovel sampling method is slower, but not less rigorous than a soil probe (which can cost hundreds of dollars). Another alternative is that if soil macrofaunal assessment will be carried out (see section 3.6 below), three blocks worth of soil will be generated and hand sorted to complete the assessment. These blocks become a strategy for collecting the sample. If fewer blocks are excavated or if greater coverage and representation of soil variability is desired, it may be necessary to collect a few additional samples using the shovel to add to soil from these three blocks (see number of subsamples in 1.4 below). Fig. 3 shows different pathways for sampling and processing samples.
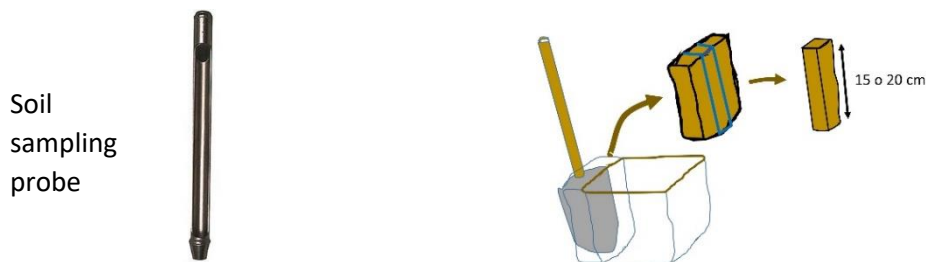

Soil sampling probe

15 o 20 cm

Figure 1. Two methods to simple soil: **Left**: a soil sampling tube or probe; **Right**: using a shovel to cut a slice and then trim it into a square column with an even mix of depths.

1.3 Sampling depth: Generally for agricultural plots a sample is taken to a depth of 15 or 20 cm, since these surface layers are those dominantly explored by roots of herbaceous plants, including crops. This soil depth generally contains most of the plant nutrients and a greater proportion of biological cycling of these nutrients by microbes. Once this depth is chosen it is important not to change it within a single sampling campaign or research project, since changing the depth will change the soil results. If one wants to do modeling of processes in the soil and validate with the field data, it is advisable to find out what is the reference depth for the soil model in question. We note here that the ISRIC international soil database and mapping app (soilgrids.org) uses 15 and 30 cm depth increments, and not 20 cm.

1.4 Combination of subsamples to represent a field: Sampling must adequately represent the variation in soil across an entire field. Five to ten sampling points are generally adequate within each plot, although sometimes only three or four are used, if one is sampling a large number of fields. Usually these sampling points or sub-samples are combined to generate only one sample per plot. This sample averages across the variability in the plot. If there are large differences in a plot that have a strong spatial pattern, more than one combined sample can be generated if this helps to understand the variation in a plot; However, taking different samples within a field will create more analysis work, and if many fields must be sampled this may not be practical.
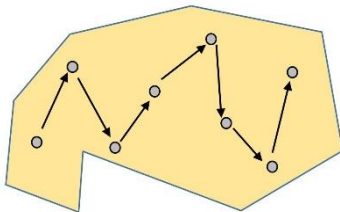

Figure 2.   One typical way of gathering five to ten subsamples across an agricultural field, following a zig-zag pattern that intentionally tries to avoid spatial patterns, such as taking several samples from the same furrow, or from right in the middle of the field.

1.5   Soil Sample Handling: After sampling, soil passes through a few additional steps to get to the point of analysis, which are visualized in Fig. 3. After combining and homogenizing the subsamples (5 to 10 for example) in the field, generally between 1 and 2 kg are bagged to take for analysis, to address losses from drying and sieving and to be sure not to run out of soil for analysis. Space or weight limitations may cause less soil to be taken from the field, but not less than 500 g should be taken. From this point soil can sometimes be directly sieved in the field and analyzed, even in a partly moist state (flow 1 in Fig. 3). More generally this sample is taken and then air-dried in order to sieve it to 2mm size (flow 2). This typical flow can also be combined with the macrofaunal sampling (flow 3). However, if aggregate stability testing is to be carried out (method 3.3 further on in this manual), it is important to sieve at least part of the sample only to a larger size to not destroy large aggregates (8-12 mm, see flow 4 to the right of Fig. 3 and the method 3.3 on aggregates)
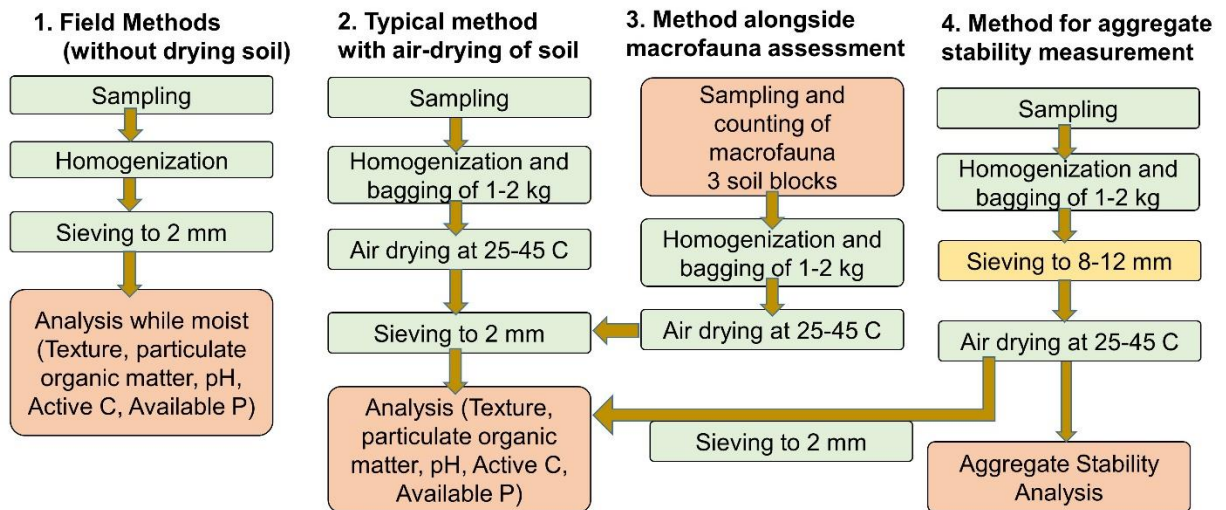
Figure 3. Different paths for soil simple handling depending on the types of analysis to be performed: (1) some analyses can be done in the field (or nearby village site) on the same day of sampling after sieving but without air-drying. (2) more typically, bagged soil samples taken from the field and air-dried to be sieved and analyzed; (3) if macrofaunal assessment is performed in the field, this will generate a sample that can be air-dried, sieved, and analyzed (additional subsamples can be added to assure the inclusion of at least 5 subsamples; (4) for aggregate stability analysis at least a part of the sample should be sieved only to 8-12 mm size rather than 2 mm (see this method below, section 3.3)

After taking the soil sample and while it is still moist, it is best to store it in an open bag in a shaded area at cool to medium temperature (e.g. 5-15°C). This will prevent accumulation of condensation and CO_2 that could alter future results. For almost all analyses here, we emphasize that it is **important to air dry as soon as possible** (for example, in closed paper bags or in open dishes or bowls). Unless we are interested in measuring soil moisture, soil should never be dried in a hot oven (no more than 45 or 50 degrees C, for example). If we want to determine soil moisture, it is best to take only small subsamples of the total homogenized sample that was taken from the field and dry only this part of the soil at 105°C, weighing these small subsamples before and after drying to determine the moisture content (based on the change of weight with drying). You can also see appendix A with a visual guide to soil moisture, for a more approximate measurement of soil water content.

1.6. Sample homogeneity: An important principle of soil sample handling is to mix the sample well before analyzing, so that when a result of the soil is obtained, it represents as well as possible the sample that was collected in the field and in turn the average properties of the agricultural plot (note that this *obscures* micro-level variation across the field, which is not always what we want to do, however mixing everything from one field to an average value is often a practical starting point). Sieving the soil will also remove non-soil components that can have a strong effect on results, such as larger roots or macrofauna, and sieving also creates homogeneity since it fractures and mixes small grains, creating a homogenous mass that guarantees the representativeness of any subsample or analysis.

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Section 2. Equipment, other materials, and reagents

The following section contains description of equipment, materials and reagents that will be used in the methods of soil analysis presented further below. Where possible, we indicate some possible web links for purchasing these items. There are other sellers and other models of the items we note below and you should be able to find acceptable substitutes.

2.1 Equipment

2.1.1 Field pH meter, ExTech stick model

This is a direct reading device, with a flat surface electrode for pH measurements of solutions and soil slurries like those in the pH method.

You can search "Extech pH110" or "Extech pH100" on the internet, or on the site www.testequipmentdepot.com

There are other similar portable pH meters for similar prices between US$70 and $150; we have had relatively consistent and good results with this particular model.

2.1.2 Bluetooth-enabled pH meter

This is a bluetooth wireless pH sensor which can connect to an Iphone or android smart phone, with a plastic body to make it more durable in the field. By using a free app, the phone then becomes a pH meter with more extensive graphical capabilities than a simple handheld pH meter:
http://hannainst.com/hi12302-halo-ph-electrode-with-bluetoothr-smart-technology.html

2.1.3 Portable Colorimeter, Hanna checker high range phosphate model

This is a field model and is the same colorimeter for the test of soil available P (Olsen method) and the test for active soil carbon (carbon oxidizable by KMnO4, or POXC)
The model number from Hanna Instruments is HI-717:

The model number is the Hanna Instruments model number HI-717: *http://hannainst.com/hi717-phosphate-hr.html*

2.2 Materials (some other standard lab or home items may also be needed, see each test)

2.2.1 pH paper: This may also be a viable way to evaluate pH, but the costs of paper and a pH meter may be similar over time. We also have had problems getting pH paper results in line with measurements using a pH meter (see the pH method , 3.2).

2.2.2 Extra vials for use in the colorimeter (11 mL vials with diameter 0.75 inch). It is convenient to have a set of about 10-30 or more vials per kit, to allow multiple tests to be done in series. These are sold by Hanna instruments who also make the colorimeter: https://hannainst.com/hi731315-glass-cuvettes-and-caps-for-checker-hc-colorimeters.html However they can also be ordered much more cheaply with catalog number CT15196525-C-F217-N (and a Teflon seal closure for use with these test solutions) from discount vials in the United States, or other suppliers: https://www.discountvials.com/3-dram-glass-vial-w-cap-pkg-of-25/ A larger package of 144 vials may also be available.

2.2.3 Filter Paper: For many relatively low clay soils, fine grade cone coffee filters may be sufficient to filter soil extracts (Fig. 4). However, these can be clogged by soils high in clay. In this case, laboratory filters may be needed such as Whatman's grade #5 filters with a fine pore size (2.5 microns) and sufficient flow rate so that they do not become clogged during filtering. One provider of these is Cole-Parmer: https://www.coleparmer.com/i/whatman-1005-090-qualitative-filter-papers-9-0-cm-dia-pore-size-2-5-100 -box / 0664822; These cost approximately US $ 20 for a filter box of 100, with a diameter of 9 cm. To be sure of the type note the Whatman catalog number for these: # 1005-090. These 9 cm large circular filters can be cut to smaller circles (for example, 2.7 cm diameter, Fig. 4) in order to filter soil suspensions with a plastic bottle (see section 3.5). In this way four samples per large circle can be filtered so that 400 samples can be filtered with a box of 100 filters.

Fig. 4. Left: cone coffee filters; **Right**: lab-quality filters, Whatman type #5 with 2.5 micron pores. Circles with diameter ~3cm can be cut for filtering soils.

2.2.4 Sieves for general soil sieving, the aggregate stability test and the particulate organic matter (POM) test:

1. **2mm (2000 micron) sieves**: This sieve is fundamental for soil analysis since 2mm is a size threshold for defining soils. Therefore it may be desirable to invest in a high quality metal sieve (brass or stainless steel, 15 or 20 cm in diameter). There are also low cost plastic sieves with stainless mesh that have been found and work well as described below:

    A. **All-stainless sieve, 2mm or #10 mesh:** There are many businesses that sell such a sieve but one economical online source is ZoroTools (United States website), with a diameter of 20 cm (US$55 + delivery). This sieve is product number G3842894 in the zoro tools website www.zoro.com.

    B. **Low-cost 2 mm sieve with stainless mesh** (Fig. 5): these can be found in at Forestry Suppliers for US$ 9. This sieve works well especially if it is mostly used for wet sieving where it will not experience strong scraping or heavy use. This sieve is product number 53935 on the website http://www.forestry-suppliers.com.
       http://www.forestry-suppliers.com/product_pages/ViewItem.php?item=53935

    C. **Home-made 2 mm sieve**: If sieve or perforated plate with 2mm holes can be found, a sieve can be made with pipe or the bottom of a bucket. Sieve material can sometimes be found where materials are sold for mining or from larger supply houses and hardware stores, though it may be not be cost-effective in comparison to buying a sieve.

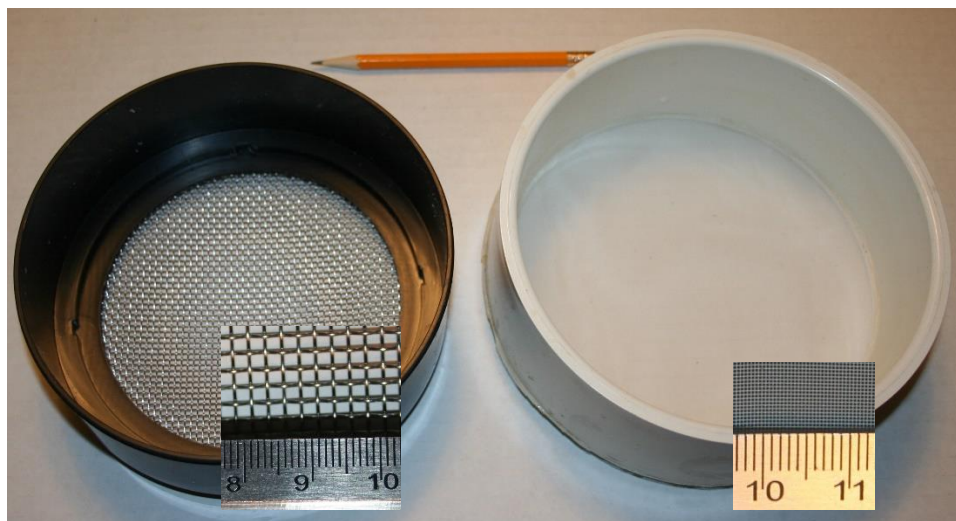

Figure 5. **Left**: low-cost plastic sieve with 2mm hole size; **Right**: Home-made 250 micron sieve using #60 mesh from the silk-screening industry, made with a large-diameter plastic tube. Rulers shown are with cm units and small tick marks are millimeters

2. **250 micron (0.25 mm) sieve**. For this sieve it is possible to purchase a sieve or make a home-made model, as described below:

A. A 0.25 sieve can be purchased on the internet, for example the #60 sieve (250 microns) available from Forestry Suppliers for US$58 + delivery. This is product number 53650 at www.forestry-suppliers.com.

B. A 250 micron sieve can be also be made from the bottom of a plastic tub or bucket with a diameter of 15 to 20 cm, or a section of a large-diameter plastic tube from hardware stores or salvaged from construction sites (Fig. 5). To this tube or bucket section, #60 mesh (250 microns) can be fastened to the bottom to create the sieve. #60 mesh can sometimes be found in larger cities from silk-screening businesses or supply houses for the silk screen industry. Mesh can also be ordered from Holden Screen Supply Corp., New York City, NY, USA: http://www.standardscreen.com/mesh.aspx , and costs US $14 for 1 meter x 60 inches, from which many sieves can be made. Details on international sales can be found on this website, with a phone number.

2.3. Reagents

It is likely that finding chemical reagents will be a serious challenge to address in many regions. Starting early and identifying potential suppliers in larger cities is fairly important. Here are descriptions of the major chemicals required for the P and active C tests.

2.3.1 **pH buffers for calibrating a pH meter**, with pH values of pH 4 y pH 7 (sometimes pH 4.01 and 7.01 are sold, these are equivalent). pH buffers are usually found in lab supply stores.

2.3.2 **For the test of permanganate-oxidizable carbon or POXC** ("active carbon"):

1. **Potassium Permanganate ($KMnO_4$)**: not much is needed per test: 64 mg per test, or 20 g for 300 tests. This means that if absolutely necessary, a small plastic container of $KMnO_4$ can be flown in with travelers to the region. In the Andes, $KMnO_4$ can be restricted as a drug manufacture precursor, but can be found in small quantities in lab supply stores.

2. **Calcium Chloride ($CaCl_2$; magnesium chloride may be acceptable as well)** – Calcium chloride is needed in the largest amounts for the POXC test: 300 mg (0.3 g) per test, so that finding it locally is preferable. In this test the ion Ca^{++} from $CaCl_2$ acts as a flocculent for clays to help them settle from the test solution. The Mg^{++} ions from $MgCl_2$ will also flocculate clays, though a little less well, so that Magnesium Chloride may also work. About 90 g of $CaCl_2$ is needed for 300 tests.

3. **Citric acid (or just lemon juice):** this is used to clean the containers used with KMnO4 for the test, which will become stained over time from the permanganate. This is not really a reagent so the purity can be quite low, and even lemon juice will work.

2.3.3 **For the available phosphorus (P) test** (Olsen P)

1. **Sodium bicarbonate ($NaHCO_3$)**: Clean baking soda from a supermarket may work well. If this common baking soda is used it needs to be tested to see what level of P it contains as an impurity, by analyzing the Olsen solution without reacting it with soil using the same test outlined in section 3.5. If analytic or reagent grade sodium bicarbonate is available from a laboratory supply house, this is also an

excellent option. 1.05 grams are needed for each soil analysis (i.e. 42 g for each liter of Olsen analysis solution, which can be used to test 40 samples). Each kit for 300 samples thus requires about 350 grams of NaHCO3, rounding up a bit to cover blanks and method testing.

2. **Sodium Hydroxide (NaOH)**. Common lye from a hardware store can usually be used, and impurities are not terribly important since this reagent is only used in small quantities to adjust the pH of the Olsen solution to 8.5. However this reagent is very commonly found in laboratory supply houses and it may be just as easy to find it there. Between 1 and 2 g per liter of solution is used, or about 10 grams for a soil kit to test 300 samples.

3. **Sodium Bisulfate (NaHSO₄).** This is a much safer and easier to dose alternative to sulfuric acid or hydrochloric acid. It is sold very cheaply as a swimming pool chemical in the U.S., and can be found either as a pool chemical, or in laboratory supply houses in capital cities like Nairobi in Africa, for example. Each test uses 450 mg (0.45 g; i.e. 150 g for 300 tests), so it is not out of the question to import it, but could also be found locally. Sulfuric acid purchased as battery acid and then diluted by half for safety is also an alternative and may be easier to find in many locations, see the test instructions below in section 3.5 for this substitution.

4. **Reagent packs** for the analysis of P in water solutions: LOW RANGE reagent pack from Hanna Instruments, for example at the following link:
http://hannainst.com/hi93713-03-phosphate-low-range-reagents-300-tests.html . Note that this is the **low** range reagent pack, but the colorimeter we use is for **high** range (this is on purpose). One reagent pack per test is needed.

2.3.4 Alcohol (Ethanol or Propanol). This may be necessary for the evaluation of soil macrofauna (section 3.7), in those cases where it is desirable to preserve insects or worms from the field for identification in the lab (rather than just rough classification and counting in the field). Alcohol may also be helpful in cleaning the colorimeter vials from the phosphorus and active carbon analysis. Alcohol can usually be purchased in any pharmacy.

2.3.5 Hydrogen Peroxide (H₂O₂): this is the normal type of peroxide that is sold in pharmacies, and is used for the simple qualitative test / demonstration for organic matter in section 3.4.1.

Section 3: Analysis Methods for Soils

3.1. **Soil Texture:** proportions of sand, silt and clay in the soil. Soils high in clay are considered "fine-textured" and can have problems with compaction and drainage. However with good management, clayey soils can store large amounts of organic matter and have good water retention. Sandy soils have good drainage, which implies that they are more vulnerable to drought and that it is difficult to build their organic matter content. In the middle of these extremes are the loamy soils that tend to combine the good properties of clay and sandy soils (see Fig. 7).

3.1.1. **The feel method**: this method was developed by the US department of agriculture soil conservation service. It is quite rapid and practical, and produces results at a precision of +/- 5% to 10% of proportions of sand, silt, and clay. To practice this method, consult Fig. 6 on the following page and the following steps:

1. It is best to used sieved soil (2mm) or remove the stones that are felt in the ball as you begin to knead the wetted soil. Form a ball of wet soil, adding water to form a putty-like ball. To wet easily you can use a small bottle of water or a rinse bottle. It is important to be patient in forming a uniform mass without stones, which is plastic but does not stick too much to your hand. If, when the soil has adequate moisture, a ball can still not be formed, the soil is classified as sand (Fig. 6)

2. After ensuring that the ball has the right level of moisture, try to form a "ribbon" of moist soil with your thumb on the index finger, draped over the index finger (Fig. 6). The length of the ribbon that can be made before it is broken by its own weight can distinguish loam, loamy clays, and clay soils (Fig.6, the three "columns" of alternatives under step 4). Pay attention to the humidity of the soil, since if it is too dry, it will be weak and broken only by lack of water, and if it is too wet it will stick on the hand instead of being moldable into a ribbon. This step should be practiced for understanding and consistency of results.

3. Finally, pinch off a piece of the ball and wet into a paste in order to evaluate the proportions of sand versus silt in the soil (step 5 in Fig. 6). These can be used to add "sandy" or "silty" descriptive words to the main soil types from the previous step (loams, loamy clays, and clays; Fig. 6). This step may take the most amount of practice to estimate correctly, especially regarding the adjective "silty" where it may be difficult to distinguish the feel of clay and silt between the fingers.

4. After this procedure, it should be possible to estimate approximately the percentage of sand, silt and clay by matching the soil texture name in the textural triangle (Fig. 7) to the range of percentages for that type. For example, if the name is determined as clay loam, an approximate level of these fractions would be 35% sand, 30% silt, and 35% clay. For borderline types (based on how the soil feels or the length of the ribbon) you can place the percentages on the border of the two types.

Figure 6 (next page): Flow diagram for determining soil texture classes using the USDA feel method with a ball of moistened soil.

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Start → Place approximately 25 g of soil in palm. Add water bit by bit and knead the soil to break down all aggregates. Soil is at the proper consistency when plastic and moldable, like moist putty, and does not stick excessively to the palm of your hand like mud.

1

Does the soil remain in a ball when squeezed? — **NO** → **Sand**

2

Making the ribbon

Place ball of soil between thumb and forefinger, squeezing it upward into a ribbon. Form of ribbon of uniform thickness and width, about 3 mm thick. Allow the ribbon to emerge and extend over the forefinger, breaking from its own weight.

Loamy sand ← **NO** — Does soil form a ribbon?

Yes

3

How long is the ribbon?

| < 2.5 cm | 2.5 to 5 cm | More than 5 cm |

4 Excessively wet a small pinch of soil in palm and rub with forefinger

Sandy Loam ← Does soil feel very gritty?

Silt Loam ← Does soil feel very smooth?

Loam ← Neither gritty nor smooth

Sandy Clay Loam ← Does soil feel very gritty?

Silty Clay Loam ← Does soil feel very smooth?

Clay Loam ← Neither gritty nor smooth

Sandy Clay ← Does soil feel very gritty?

Silty Clay ← Does soil feel very smooth?
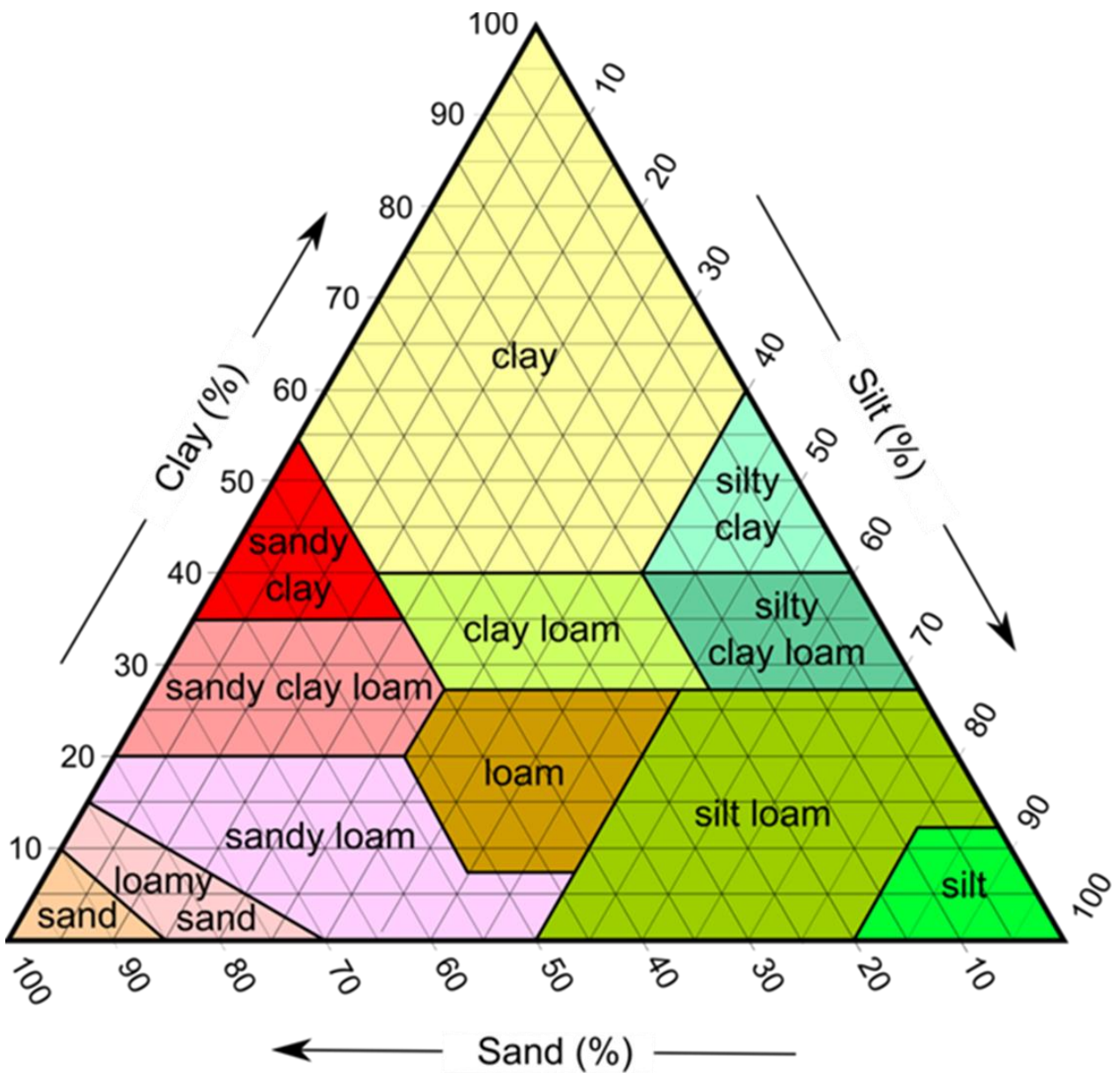
Clay ← Neither gritty nor smooth

Figure 7. Triangular graph of soil texture (the soil *textural triangle*) Different regions of the graph are named as soil textural types according to their percentages of sand, silt, and clay. Pay attention to the labels on each axis and the orientation of these number labels (horizontal and diagonal) which indicate which set of lines within the triangle describes the proportion of each of the three components.

3.1.2. Jar or bottle settling method for understanding size fractions: <mark>(currently in testing)</mark>:
In contrast to the above feel method, this is not really a rigorous test of texture but does allow farmers and others to better visualize the different sizes of sand, silt, and clay particles by observing their settling times in a water column. The sand (size 50 microns - 2 mm) settles first within 40-60 seconds. Silt particles (2 microns - 50 microns) settle for up to about 12 hours, and the clay takes longer to settle. However, these times apply only

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to fully dispersed suspensions of soil, which is difficult to achieve just by shaking a jar and even with a blender. Clays are aggregated into larger size particles in most soils so that these larger particles settle as if they were silt or sand, leading to an underestimate of the clay fraction. For this reason, this method is better to teach about the different particle sizes that exist in the soil, and should be combined with the other tactile methods that can estimate the proportions of sand, silt, and clay more precisely.

3.1.2.1.   Materials
1. Bottle or jar (glass or plastic)
2. Small graduated cylinder or accurate measuring device for liquids to the nearest 10 mL (a balance can also be used to measure 10 mL, i.e. 10 gram, increments).
3. 100 to 200 mL of soil approximately, depending on the total size of the jar or bottle.

3.1.2.2.   Procedure
1. 1. Prepare a jar or bottle with graduations to measure volume, marking the bottle every 10 mL. A thinner bottle or jar will provide more precision to distinguish the percentages of the fractions when they settle in the water column. Sieve the soil to 2mm
2. Add the soil to the jar and then 2-3 times the volume of water. Mix the soil and water well with shaking or a blender, to try to completely destroy the aggregates (in practice it is very difficult to completely destroy microaggregates without chemical dispersant like sodium metaphosphate, which is one reason why this method tends to **_underestimate_** the percentage of clay in the soil and overestimate the percentage of silt.
3. Leave the soil to settle between 4 and 24 hours. You will notice different layers settling, first the sand, then silt, and finally clays, from the cloudy part of the suspension. At the end of this, the supernatant or upper part of the soil suspension will be transparent or translucent.
4. Identify visually the different layers, where possible, between larger, visible sand particles, silt that settled earlier, and a fine clay that settled last. It may be difficult to distinguish between silt and clay in this method, a potential shortcoming.
5. The proportions of sand, silt, and clay are calculated as the proportion of the total volume of soil settled in the jar as indicated by the graduations used to label the jar (or bottle) earlier.
6. A textural name can be approximated for the soil using the textural triangle in Fig. 7. As we note above, this method may not accurately distinguish the proportions especially of clay and silt.

3.1.3. U.N. Food and Agriculture Organization (FAO) method by feel: This method serves as a complement to the USDA feel method in section 3.1.1, which can be used to confirm your findings or as an alternative. Its advantage is that it is quite linear and easy to conceptualize (Fig. 8 below). However, it does not categorize soils into all of the types in the textural triangle of Fig. 7.

3.1.3.1. Procedure:

1. In the same way as the USDA feel method, start by forming a ball of diameter ~ 3cm, like a soil putty with water, without stones that can interfere with the test (using 2mm sifted soil is optimal). The dough has to have just the amount of water to be moldable without sticking too much to the hand, and it is worth kneading with patience until mixing all the dry soil with water. If the ball cannot be formed, it is classified as sand (Fig. 8).
2. If a ball can be formed, next you should try rolling the ball into a sausage, about 6-7 cm long. If the "sausage" falls apart as it is rolled, it is classified as a loamy sand
3. If a 6-7 mm sausage can be formed, try to roll the sausage further into a "pencil" about 15-16 cm long. if the pencil cannot be formed but falls apart, the soil is a **sandy loam.**
4. If the pencil can be formed, try to bend it into a half circle. If the half-circle cannot be formed or falls apart, the soil is a simple **loam**.
5. If the half circle can be formed without breaking, try to continue bending the "pencil" into a complete circular ring with an approximate diameter of 5 cm.
6. If this ring cannot be formed without breaking, the soil can be classified as a **silt loam** or a **silt** soil.
7. If the ring can be formed but some cracks appear as it is bent, the soil may be a number of types that tend to be clayey without having enough clay to be formally called clays, such as a **clay loam**, a **silty clay** or **sandy clay**. These are all the types that border the "clay" type in the textural triangle (Fig. 7), as well as the **sandy clay loam** type. By following the same strategy of feeling a wet pinch of soil in step 5 of the USDA feel method (Fig. 6), these types may also be distinguished.
8. Finally if the ring can be formed with very few cracks, and tends to look more like potter's clay rather than a soil, it is likely the **clay** type of soil.
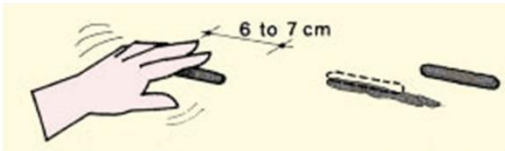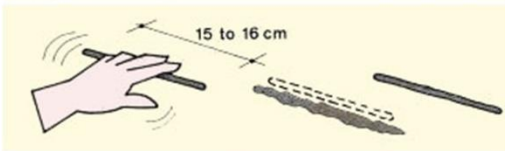
Figure 8. A sequence of tests performed on a kneaded ball of moistened soil, which can be used to classify soil textural type in the FAO "feel method" Image credit and original source: http://www.fao.org/fishery/static/FAO_Training/FAO_Training/General/x6706e/x6706e06.htm

3.2 Soil pH

3.2.1 Materials and reagents

1. **Portable pH meter** for field type, pen-type or similar, with electrode placed in its storage solution if necessary (see the equipment section, 2.1.1).
2. **Buffers** or **calibration solutions** for the pH meter. Calibration should be performed with pH 7 and pH 4 buffers, which gives information on the most important pH range for soils.
3. **Another alternative**: pH paper with a precision of at least one pH units and better if it can indicate pH with gradations of 0.5 pH units. In any case pH paper tends to be less accurate than a calibrated pH meter.
4. **Small plastic cups** or containers for between 50 and 100 mL.
5. **A balance** (1 g or 0.1 g precision) to weigh soils and water.
6. **Distilled water**, or bottled water tested for impact on pH measurement. In order not to interfere with the pH measurement, water with either no or low total mineral content should be used, that is, with a mineral content such as calcium or magnesium below 50 ppm (mg / kg) or even better, less than 10 ppm. The total dissolved solids (TDS) reading can be checked on the label. In some countries reverse osmosis water is sold as bottled water and this water works well. Rainwater can also be collected in a clean container (glass or plastic) to use. If necessary, the readings can be validated in about 4 or 5 soils, using a "candidate" water, in comparison with known distilled water, to verify if the use of bottled water makes a difference for the pH reading. Small differences of ~0.1 pH unit are not a problem.
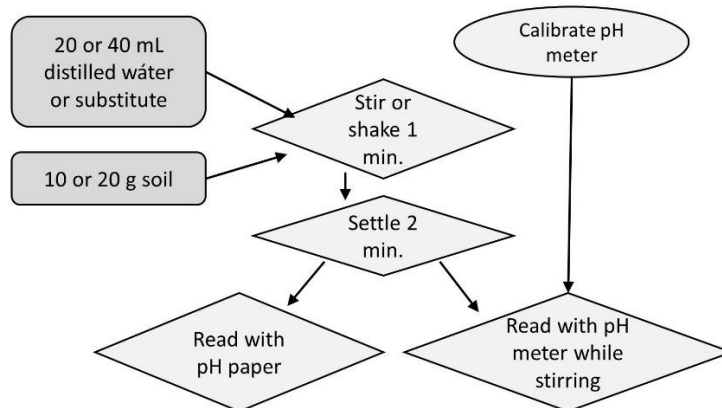
3.2.2. Procedure:

Figure 9. Flow diagram for the measurement of soil pH with pH paper or a portable pH meter

1. **Weigh 20 +/- 0.5 g** of soil in a small glass (or only 10g if you want to economize on the use of the soil sample). If there is no balance, it can also be estimated that a

volume of 14 to 16 mL of soil will weigh approximately 20 g, or another measure of volume can be used based on an approximate density that may be known for soils in the area where the sampling was done. This adjustment to the method works because soil pH measurement is less sensitive to the soil: water ratio than other soil chemical measurements. In any case, when a balance is available it is better to use the indicated weight and not the volume.

2. **Add 40 ml of distilled water**, rainwater, or alternative of low mineral content; see materials list above (or only 20 mL if only 10 g of soil was weighed, in every case, 2x the weight of the soil)

3. **Mix the soil and water** and stir quickly or shake for 1 minute. Shaking with water for one minute in a closed container can be very effective. In this case it is then transferred to the small cup or beaker.

4. **Let stand 2 minutes** or more, mixing from time to time.

5. **Measure with the pH meter**: Place the pH electrode in the cup and stir slowly during the measurement, keeping the pH electrode in the supernatant or top suspension in the cup. Record the pH after the reading stabilizes. The goal is to maintain a stable reading that does not change more than 0.1 pH unit in about 30 seconds. Variation on a finer scale is not important. However, if the meter changes continuously up or down, without stabilizing (e.g.> 0.1 unit in 10 seconds), it is possible that the electrode must be maintained since time electrodes can become dirty and blocked or occluded.

6. **Measuring with pH paper**: after allowing the solution to settle for a few minutes (so as not to stain or color the paper strips excessively with the soil's color, the top liquid or supernatant of the soil suspension can be measured with pH color test strips of the appropriate interval (e.g. pH paper strips with pH range 0 to 14, 4 to 7 or 5 to 8). This paper is then compared with a color chart. We are conducting tests to see how reliable this is, and so far it seems to lead to readings that are 0.5 to 1.5 pH units different (usually lower) than with a pH meter that may be unacceptable. However, if the difference between paper strips and the pH meter is relatively stable for a set of soils from a given zone or region, it may be acceptable to apply this difference or conversion to the paper strip readings and use these as a way to measure pH. Any pH paper alternative should be tested against a calibrated pH meter for the best results.

3.3. Soil aggregate stability
3.3.1. Materials

1. Large size mesh with a hole size of 8 to 12 mm to perform pre-screening of soil aggregates and gently break large soil clods and remove larger stones (Fig. 10).

Fig. 10. Examples of different types of mesh that can be used for pre-screening dry soil, with a hole size between 8 and 12 mm.

2. Soil sieves with mesh size 2 mm (10 mesh) and 0.25 mm (250 microns, 60 mesh), with a sieve diameter of at least 6 "(150 mm) (see section 2.2.3 and Fig. 5). The diameter is important so that the screen does not become clogged with too much material during wet-sieving. As detailed in section 2.2.3, the 250-micron sieves can be made of wide-diameter plastic tubing or the bottom of a plastic bucket, combined with 0.25-millimeter (size #60) plastic mesh that is used in screen printing of fabric (Fig. 5).
3. Water: clean tap drinking water is perfectly sufficient, and any village water supply will work as well.
4. Small basins or tubs that the sieves fit into comfortably for sieving in water (for example 25 cm diameter x 8 cm of height, see photos below).
5. Balance to weigh soils and aggregates (precision 1g or preferably 0.1g).
6. Metronome app on a cell phone, or an audio file that can mark a 50 beats per minute tempo for the timed washing steps.
7. Rinse bottles that allow the rinsing of sieves to move and capture soil and aggregates. A good (perhaps even superior) substitute is a ~500 mL disposable plastic bottle (from water or other) with small holes drilled or poked in the lid to allow a small shower-like stream of water to flow from the bottle when squeezed. The holes in the lid can be made with a pen tip, safety pin, or drill bit (to make approximately 1 mm diameter holes).
8. A medium-sized funnel, 10 to 20 cm in diameter.
9. Squares of cloth (fabric from bedsheets, used T-shirt etc.) or paper filters to capture, visualize, and weigh the stable aggregates from the analysis. It is helpful to weigh these fabrics or filters in advance and write the tare weight on each one with an indelible marker (to 0.1g precision), to facilitate the weighing of the dry aggregates at the end.

3.3.2. Video and other considerations

1. **Video:** a video that shows this method is available at:
   https://www.youtube.com/watch?v=DucBmQBPX6Q

To see a more complete version of this test, and some of the theory behind the test and why aggregates are stable in water, see this video (note: the method performed here is more complex than the adaptation in this manual):

   https://www.youtube.com/watch?v=VOaae2bDDCY

2. **Soil aggregation or soil structure also depends on soil texture** – for example a silty clay will tend always towards higher levels of aggregation than a sandy loam. Therefore, aggregation ideally has to be compared in two soils that are similar in texture if we want to evaluate the management impacts on soil structure. For example, if we compare the aggregation in a sandy loam soil (lower amount of clay) compared to the silty clay (more clay), the differences we observe may be more related to the differences in sand and clay content , and not soil management, so the comparison may be invalid.

3. **Other related tests:** there are other possible structure tests that can be performed, for example the "soil porosity" and "soil structure and consistency" visual evaluations described in the FAO Soil Visual Evaluation Guide by Shepherd et al. (see bibliography). There is also a soil stability analysis developed by the United States Department of Agriculture Natural Resource Conservation Service (NRCS) similar to the procedure below, which produces a stability rating with small "baskets" of window screening mesh. This "tackle box" analysis method appears in the soil quality kit guide of the US soil conservation service: page 20 of the following document:
   https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1044790.pdf
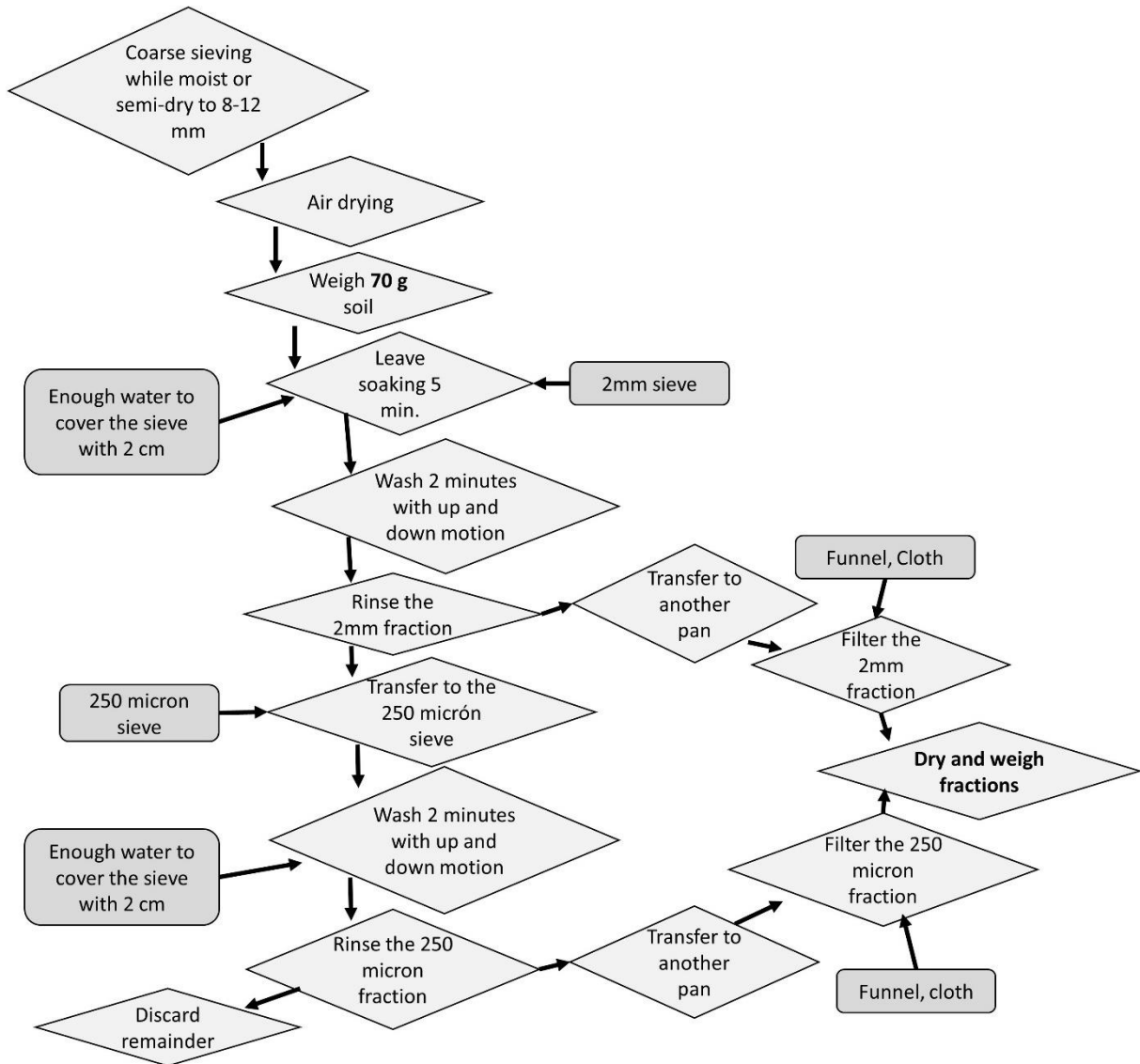
3.3.3. Procedure

Fig. 11. Flow diagram for the aggregate stability test.

1. Prepare 70 g of air-dried soil (this can be dried in oven, but no more than 45° C) sieved to a size between 8 mm and 12 mm. For most soils it is easiest if the soil is coarse-sieved when partially dry and then fully dried afterwards. To perform the sieving a mesh of 8 to 12 mm size can be used. If no mesh is available, aggregates larger than 8-10 mm diameter can be broken and rocks removed by eye. During this process you should in every case remove stones that do not pass the mesh. Also, when soil is broken, natural planes of weakness should be found in the clods and large aggregates, rather than forcing the soil through the mesh. In this way, at least 300g of moist soil should be

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sieved (to maintain a representative subsample) to then take a 70 g as a representative part for analysis. However if there is very little soil in the sample, as little as 40 or 50 g can be used.

2. Immerse the 2mm sieve with a depth of at least 2 cm water above the mesh, and then gently pour the 70 g of soil onto the submerged mesh (Fig. 12).

3. Leave the soil in the water to wet up and slake (break apart) the aggregates naturally for 5 minutes.

Figure 12. Adding coarse sieved soil (sieved to ~10 mm) to the 2mm sieve in a basin of water.

4. Move the sieve in and out of the water slowly, 50 times in 2 minutes (Fig. 13). This can be done using a metronome app on a smart phone, or an audio file (available at www.smallholder-sha.org) to create a 50 beat per minute rhythm, and then raising the sieve out of the water in one beat and lowering it back into the water on the next, repeating this cycle 50 times in the two minutes. Many metronome apps will also measure the time since beginning the rhythm, or you can also use a stopwatch to measure out the two minutes. Make sure you are not doing this action at twice the pace: you should NOT lower and raise the screen on each beat of the rhythm, which would be 100 times in two minutes.

Figure 13. Lowering and raising the sieve 50 times in two minutes.

5. After this washing action with the sieve for 2 minutes, a rinse bottle is used to wash the sides of the sieve and wash any residue of organic matter or small particles of clay through the sieve. The aggregates should not be rinsed so strongly that additional aggregates are destroyed, since these aggregates were already defined as stable during washing; for example do not spray them directly with the stream of the bottle. Then put the bottom pan with water and all the material <2mm on one side for the next wash in step 8. If you want, you can also proceed directly to step 8 and come back to capture and dry the 2mm fraction later, which may be more efficient with time.

6. Next, empty the stable 2mm aggregates (particles> 2mm left in the sieve), to a new, dry pan or basin, and then capture them in a cloth or filter. This is easily done by just turning over the 2mm sieve, over a wider pan, and washing all the contents from the back of the sieve out the front into this pan. You can then do a final rinse with a wash bottle to completely empty the sieve, working from the back and the front. As you do this, you can remove any large organic residues such as sticks and long root segments from the sample (these are not aggregates).

7. When all the 2mm stable aggregate material (and likely some stones) have been moved to the second basin, use the wash bottle to move this material to the pre-weighed cloth sitting within a funnel. The funnel is placed in a cup or other container to catch the water passing through, and leaving the stable aggregates in the cloth (see Fig. 15 for an example of this funnel/cloth combination for the smaller aggregate fraction). This cloth is set aside for drying while we turn attention to the smaller 250 micron fraction.

Figure 14. Rinsing all the material <2mm into the 250 micron sieve to perform the next wash of stable aggregates at the 250 micron size.

8. Next we consider the fraction that passed the 2mm sieve, i.e. aggregates <2 mm and any smaller soil components. This material is poured gently into the 250 micron (0.25 mm) sieve sitting in another small pan or basin similar to the others (Fig. 14). Any remaining soil is then washed from the first basin into the sieve. In addition, water can

be added if necessary so that the 250 micron sieve sits with about 2cm water above the mesh, just like in the first washing step with the 2mm sieve.

9. Repeat the washing movement of the 250 micron sieve in the new basin, 50 times in 2 minutes. After this step, what remains in the sieve will be a mixture of sand and aggregates that are between 0.25 and 2 mm in size. As in the first step, this fraction should be gently rinsed with a wash bottle before the next step.

10. Wash the sieve contents (aggregates and sand) with the wash bottle onto a filter or cloth. This can be done first into another basin to make it easier, washing from the back of the sieve, and then into the funnel. The fraction can also be transferred directly to the funnel with the cloth (Fig. 15), though this may result in more water spillage.

Figure 15. Washing a stable aggregate fraction from the sieve to a funnel with a cloth to retain the soil for drying and weighing.

11. Next, the two aggregate fractions (> 2mm plus stones and the fraction between 250 microns and 2mm) are dried in a hot place or drying oven before weighing and calculation of quantitative results (up to 105°C, since we only want the dry weight and are not interested in chemical properties). However for a simpler, qualitative comparison, such as during an educational program in the field, you can visually compare the amount of aggregates among different management practices or experimental treatments, without weighing, or take pictures to compare afterwards.

12. After this, first we consider the >2mm fraction, where you will need to find two weights (precision 0.1g or 1g):
    a. The dry weight of any stones >2mm, which can be hand-picked from the sample or sieved out with a dry sieve (some soils may not contain such stones) and
    b. The dry weight of the cloth plus soil, without stones, after picking or sieving these out. The weight of the cloth taken previously should also be noted.

    The stones are separated to correct the weight of aggregates as well as the total soil weight, since stones >2mm are not considered aggregates or part of the soil that can be aggregated (se 3.3.4. below for calculations).

13. For the small-sized aggregates between 250 microns and 2mm in size, just weigh the cloth with its dry soil; no separation of stones is needed. The tare weight of the cloth taken previously should also be noted.

3.3.4. Calculations to obtain results: As indicated above, for an approximate assessment it is possible to simply compare the qualitatively stable aggregates (both sizes,> 2mm and> 250 microns) between two fields or management practices according to the approximate volume of stable aggregates which are seen in the cloth. In every case keep in mind that it is most valid to compare the stability of aggregates in two soils that have similar textures. For a more rigorous result you can get the percentage of dry stable aggregates that were left in the two cloths or filters:

1. Percentage of soil in large macro-aggregates (> 2mm) is calculated with the following equation, with all the weights in g. (Note that for this calculation the tare weight of the cloth or filter is needed in advance):

% aggregates > 2mm =

$$\frac{\begin{bmatrix}(Weight\ of > 2mm\ fraction\ without\ stones + cloth\ or\ filter)\\ -weight\ of\ filter\ or\ cloth\end{bmatrix}}{70\ g - (weight\ of\ stones > 2mm)}$$

Referring to the weights taken in the procedure step 12 above, this is:

$$\frac{\begin{bmatrix}(weight\ from\ step\ \mathbf{12b})\\ -weight\ of\ filter\ or\ cloth\end{bmatrix}}{70\ g - (weight\ from\ step\ \mathbf{12a})}$$

As noted above, if there are stones> 2mm in this fraction, these should be removed to find only the weight of the soil in this fraction. Optionally, the weight of these stones as a proportion of the 70 g soil can also be used to characterize the small stone content in the soil.

2. Then consider the cloth or filter with aggregates of size between 250 microns and 2 mm. In this case you do not need to remove small stones from the smaller aggregates, but we still take the proportion out of the stone-free mass of soil as above for the >2mm fraction.

% aggregates from 250 microns to 2 mm =

$$\frac{\begin{bmatrix}(Weight\ of\ the\ 250\ \mu m\ to\ 2mm\ plus\ the\ cloth\ or\ filter)\\ -weight\ of\ the\ cloth\ or\ filter\end{bmatrix}}{70\ g - (weight\ of\ stones > 2mm)}$$

Or referring to the procedure steps above in 3.3.3.,

$$\frac{\begin{bmatrix}(weight\ from\ step\ \mathbf{13})\\ -weight\ of\ filter\ or\ cloth\end{bmatrix}}{70\ g - (weight\ from\ step\ \mathbf{12a})}$$

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3. The value of 70 g is placed because we used 70 g of soil initially. If the initial soil weight was changed, different weight should be used in place of 70 g.

4. Interpreting the results: The following table gives rough guidelines for interpreting the results, expressed as the sum of percentages between the two fractions, 250 microns at 2 mm and> 2 mm. The table is separated into three different categories of soil textural types. We emphasize again that the degree to which a soil can develop and maintain structure by the activity of roots, microbes, and macrofauna has a lot to do with its texture and other factors, so it is best to compare between plots that have the same type of soil and different types of management, or try to measure the impact of management on the structure over time. In general, more water stable aggregation is better.

	Qualitative score, based on % 250 µm + % 2mm water stable aggregates			
Soil textural type	Very low	Low	Medium	High
Very coarse soils: Sands and loamy sands	Aggregate stability is of limited usefulness: look for aggregation in a dry soil, but this structure is not expected to be water-stable			
Coarse and Medium-textured soils (sandy loams, loams, silt loams, silt, < 35% clay)	<15%	15% - 30%	30% - 45%	>45%
Fine- textured soils: (clays, sandy clays, silty clay loams, or >35% clay)	<20%	20% - 40%	40% - 55%	>55%

3.4. **Soil Organic Matter** (**SOM**): three tests are presented below that visualize soil organic matter in different ways. The three methods have different levels of rigor and refer to different fractions or processes in the soil. However, they can all be used to foster learning about soil organic matter.

3.4.1. **Test with hydrogen peroxide** (<mark>in testing</mark>): This test is based on the reaction obtained between hydrogen peroxide (H_2O_2) and microbial life and its enzymes in the soil. It generates bubbles which form a foam. In theory, the reaction of the peroxide is proportional to biological phenomena in the soil. Therefore it is not a direct test on the amount of organic residues or humus in soil (forms of **SOM**) but it is likely proportional to organic matter. Sometimes it is used in learning activities to demonstrate that the soil contains "living beings" with the analogy to a wound of a human being that also forms bubbles with peroxide. Soil conditions can affect this test, especially soil moisture and the degree to which the microbes are active and actively producing the enzymes. However, it is a quick way to demonstrate biological aspects of soil and soil health in the field, without any more complicated procedure. We are validating whether a rough relation exists between this peroxide test and the permanganate test below, which is already established as a measurement of available SOM (and thus soil organic carbon).

3.4.1.1. Materials and reagents

1. **Hydrogen peroxide** (type purchased in a pharmacy for wound cleaning; best to have it inside a bottle with a dropper)
2. **A plastic bottle cap** from a drink bottle or bottled water (~2 cm in diameter)
3. **Field-moist soil** is used, as this is a field demonstration

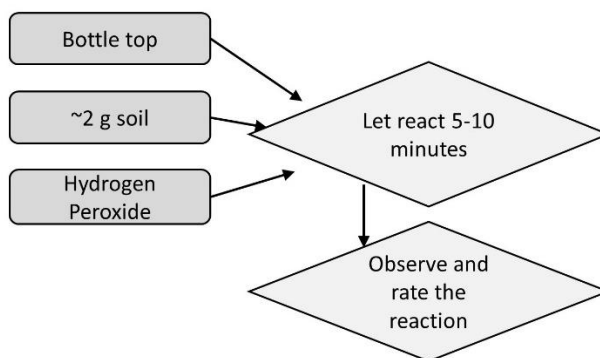**Figure 16.** Flow diagram for the simple demonstration and test of soil biological activity with hydrogen peroxide (H_2O_2).

3.4.1.2.   Procedure:

1. Place a depth of 4 to 5 mm of soil at the bottom of the bottle cap. If the soil is not sieved to homogenize it and remove stones, then stones should be removed by hand since they do not contribute organic matter (SOM) or microbial biomass to

the sample. Before taking this small amount for testing, be sure to mix the soil well to homogenize it and represent the plot adequately.

2. With the dropper, add enough hydrogen peroxide just to soak the soil, or just until a liquid surface shines on the surface of the soil inside the lid.
3. Over time bubbles will appear. Often they will form a foam that rises inside the bottle cap.
4. After 5 minutes, you can rate the amount of bubbles and the speed of the reaction, the speed of reaction being an important parameter in this test. A sample of moist compost or damp manure from a pen can be used for comparison as a "positive control" that represents a very high value, which will also validate that the hydrogen peroxide is still fresh enough to use. Although this is a test or demonstration still in testing, a scale of 5 levels (0 to 4) is proposed, which can be adjusted with experience, as follows:

Scoring table for the hydrogen peroxide soil test

Score	Description of bubbling behavior of hydrogen peroxide
0	Very Little reaction, none or almost no bubbles (like clean sand, for example)
1	Bubbles only on the soil surface or very slow reaction
2	Layer of bubbles with 1-2 mm depth, or slow reaction with a thicker layer of bubbles, but only at the end of 5 minutes
3	Layer of bubbles with 5-10 mm depth, appreciable reaction after only 30 seconds
4	Froth of bubbles with depth > 10 cm, and a quick reaction within 30 seconds, close to the reaction of a wet farmyard manure or moist compost.

3.4.2. Particulate Organic Matter (POM)

This test can be used as a demonstration to show what soil organic matter is, in a very visual way, for farmers or other audiences. POM rinsed out of soil using water can be score qualitatively or weighed as a quantitative measure that in agricultural soils can indicate recent contributions of organic inputs, and may give an idea of what will decompose in the near future and release nutrients to crops.

3.4.2.1. Materials

1. **2 mm and 0.25 mm (250 micron) sieves** of at least 6 "(150 mm) in diameter are used. These relatively sieves are used to avoid clogging with too much material during sieving in water. See section 2.2.3. regarding the purchase and / or manufacture of sieves.
2. **Water**: clean tap drinking water or a village water supply is sufficient.
3. **Basins or pans** that the sieves fit into easily, allowing sieving and washing of the soil on the sieve mesh submerged in water (for example, 25 cm in diameter x 6 cm in height; see Fig. 17 below)
4. **Balance to weigh soil and POM**. To weigh the initial soil amount, only a precision of 1 g to 0.1 g is necessary. To accurately weigh the POM, a more accurate scale (0.001 g or 1 mg) is usually used, and a visual rating may be easier and better depending on context (see the scoring table scale at the end, section 2.5.3) Nevertheless the POM can be saved as small samples in small envelopes or other such for later weighing if desired.
5. **Rinse bottles** that allow rinsing of sieves to transfer and capture soil and aggregates. These wash bottles can be made from a common flexible plastic water or soda bottle (500 mL) by opening gaps in the lid with a thick needle, thumbtack, or a fine drill bit (~1 mm diameter).
6. **A beaker or measuring cup** (~500 mL) that can be used to decant floating organic matter from water (see procedure below)
7. **A plastic funnel**, diameter between 8 and 15 cm approximately.
8. **Pieces of cloth or filters** to capture, visualize, and dry the particles of organic matter at the end of the evaluation, just like those used in the aggregate stability test (section 3.3.1). Cloth can be bedsheet fabric or part of an old T-shirt.

Figure 17. Preparing to wet-sieve the particulate organic matter (POM) out of soil, with the 2 mm sieve submerged in the pan of water.
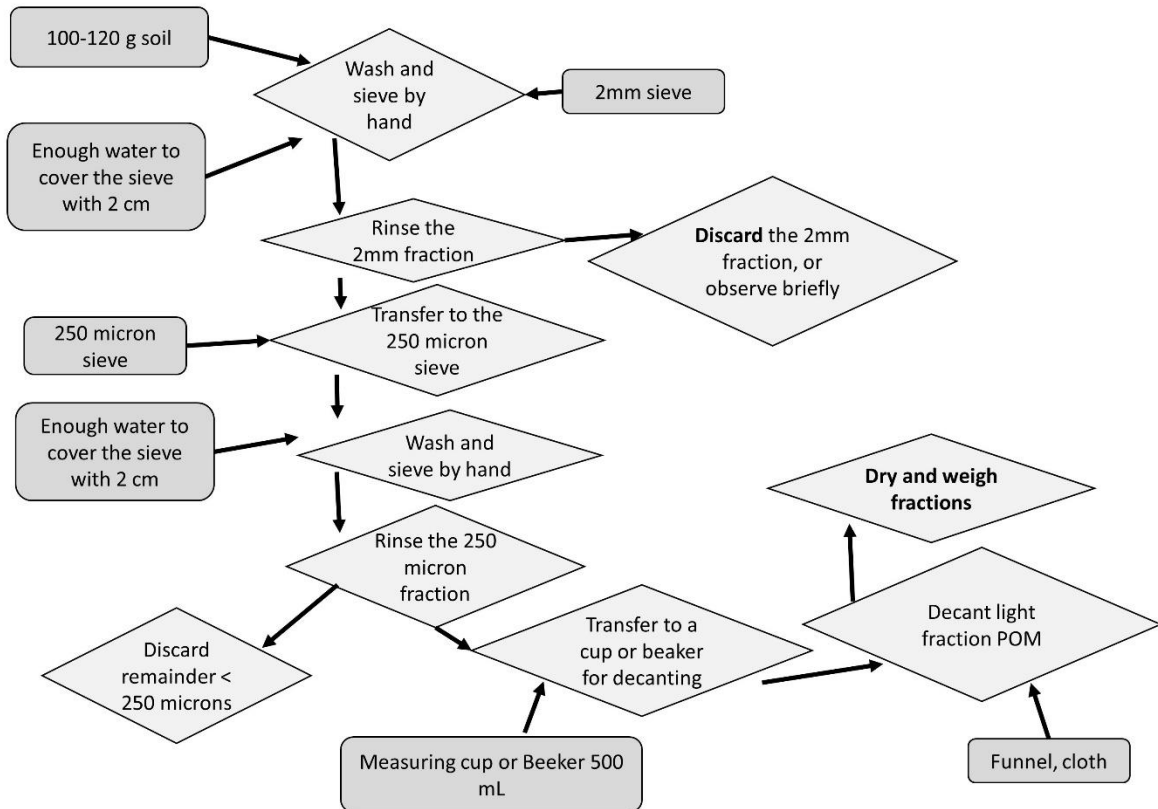
Fig. 18. Flow diagram for measuring particulate organic matter.

3.4.2.2.   Procedure

1. **A video describing this method is available at:**
   https://www.youtube.com/watch?v=zOrG3Ma2ceA

2. Weigh 100 g of air-dried soil or 120 g of moist soil on the balance (Fig. 18). If it is known that the amount of POM in a soil is very low, this amount can be increased even more to 150 or 200 g of soil. In this case it will be necessary to adjust the qualitative scoring at the end of the analysis. If the soil has not been sieved to 2 mm, small rocks should be removed by eye **before** weighing.

3. Place the soil in the 2 mm sieve inside a pan with water, so that the soil is covered with a depth of 2 cm of water (as in the case of stability of aggregates, section 3.3.3). Note that in this case it is not necessary to wait and soak the soil before performing the sieving, as was done in the case of aggregate analysis.

4. Then, start wet-sieving the soil, stirring it in the sieve and lifting it out and into the water. Aggregates may be gently broken by hand, since they may contain particles of organic matter. Be careful not to be so aggressive with your hand on the sieve as to break pieces of organic matter and force them through the screen.

5. A relatively clean mixture of stones and large POM (> 2mm) will be obtained within a short time after this wet-sieving.

6. Rinse the 2mm sieve (inside and outside) with a rinse bottle, to wash this fraction and pass any particle <2mm to the next screening step.

Figure 19. Adding water to the sieve and soil before wet-sieving (note: the graduated cylinder is just a convenient container, it is not necessary to precisely measure the water)

7. The fraction> 2mm is set aside, still on the sieve. In general this fraction is discarded, but it can be treated as a separate fraction or used to illustrate the breakdown of organic residues into smaller and smaller pieces.

8. Then, pour the water and the soil / OM mixture in the pan under the sieve (the fraction <2mm) onto the 0.25mm (250 micron) sieve in another new pan or bin, taking care to transfer all the material and rinse the first pan through the 250 micron sieve. After this, water can be added if needed to fully submerge the sieve and soil 2 cm deep in the new pan.

Figure 20. Transferring the material which passed the 2 mm sieve to the 250 micron sieve.

9. Repeat the step of wet-sieving but now using the 250 micron sieve in water, gently breaking any aggregates by hand. After a few minutes of sieving, it is preferable to replace the water in the pan with new water and discard all material < 250 microns under the sieve, in order to rinse the >250 micron fraction.

10. Rinse the contents of the 250 micron sieve (outside and inside, and possibly including your hands) and pour the fraction (fine sand plus > 250 micron POM) into a beaker or other container that can be used for decanting (300-500 mL; Fig. 21).

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11. Then decant all the floating material in the vessel (or other container) through one side of the 250 micron sieve, so that this POM will accumulate in the corner of the sieve (Fig. 21, right). The sand will remain at the bottom of the beaker or measuring cup. The beaker is filled and repeatedly stirred with water to perform this decanting. The repeated decanting will also have the effect of washing the suspension of remaining clay content.

12. When you finish decanting each round, you will see some particles that look organic (darker), but that are left behind at the top of the sand layer (that is, their density is between the density of the sand and the rest of the POM). These are usually organic matter complexes with clay and other mixed forms of organic matter, perhaps with a little charcoal. If possible they should also be captured in the 250 micron sieve. However, there will always be some grains of this type and at some point the evaluation should be declared finished.

13. Continue this decanting process until the water above the sand washed in the beaker is clear, and almost 100% of the POM has been captured in the sieve.

Figure 21. Left: Transferring the material from the 250 micrometer sieve (mineral mixed with POM) to a beaker for decanting of organic matter. **Right:** Decanting the 250 micron POM to a 250-micron sieve corner (at one edge of the sieve to facilitate moving the POM to a funnel for drying)

14. Transfer the contents of the 250 micron sieve (Fig. 22, right) to a filter paper or cloth inside a funnel (Fig. 22, right). This process may be easier if the contents of the sieve are first rinsed into an empty pan or bowl (together with water), and then poured from this bowl into the filter or cloth. The cloth or filter should be weighed in advance, to allow this dry weight to be used in the final calculations. A light colored cloth will also allow the visual scoring of organic matter (next section) or demonstration of POM to workshop participants if this is needed, or to take a photo for comparison with other soils.

Figure 22. Left: POM that was decanted into the 250 micron sieve, at one side. **Right:** rinsing the POM onto a cloth or filter in a funnel, to later dry or score the amount captured.

3.4.2.3. Observations and calculations to generate results:

1. To generate a particulate organic matter (POM) data, one strategy is simply to score the amount of POM visually, using a visual guide based on experience and comparison with other soils (e.g. Fig. 23):
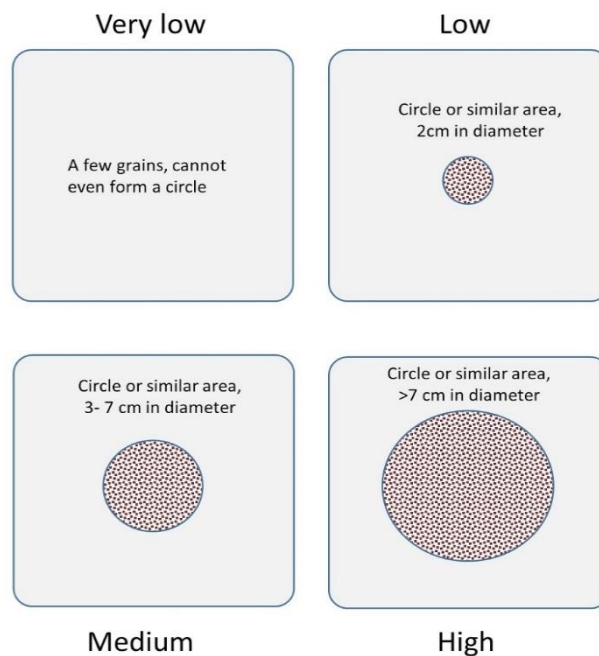

Figure 23. Guide for visual scoring of POM density in soil. Note that if you use more soil initially in the test (greater than the suggested 100g), you may need to adjust the interpretation of the test up or down.

2. In addition to this visual qualification, you can store the small samples of MOP particles in the cloths or filters and then dry them in the air or in an oven (40 ° to 60 ° C), and weigh them on a precision scale (0.001 g or more accurate) which will produce a more precise quantitative result.

3. If this POM weight is obtained, including the weight of the cloth or filter, the fraction of particulate organic matter is calculated as:

$$\frac{[Weight\ of\ the\ decanted\ POM + cloth\ or\ filter] - weight\ of\ cloth\ or\ filter\ (mg)}{initial\ weight\ of\ soil\ (g)(e.g.\ 100\ g)x\ 10}$$

This result will have units of percent. (%), and can be compared between agricultural plots or practices in an experiment.

4. Another alternative is to measure the volume of dry POM, and to develop a conversion factor between the volume of POM and the dry weight over time, based on a set of samples with known weights and volumes. To do this, a consistent method of measuring a volume of POM must be used.

3.4.3. Permanganate-oxidizable ('active') carbon (POXC)3.4.3.1. Materials and Reagents.

1. **Water**: Unlike concerns about phosphorus-free water in the available phosphorus test (section 3.5 below), there are usually no substantial impurities of organic carbon in public water supplies, and tap water can be used. However, the additional cost of using bottled water (for the $KMnO_4$/$CaCl_2$ solution below) that is low in salts and organic matter will not increase costs greatly and is recommended.**Potassium permanganate ($KMnO_4$)** – this is purchased locally or can be shipped in small quantities. It is important to test $KMnO_4$ that is purchased in pharmacies or other less formal settings to make sure it behaves in the manner indicated in this method, since KMnO4 deteriorates (under exposure to light and other environmental factors) to other forms of K-Mn oxides (with a greenish color, which is a way to see if the reagent is expired) and this will not lead to a reliable measurement. One way is to check the source of $KMnO_4$ in practice is to do the analysis on the same sample with a "pure" or reliable lot, compared to a lot of $KMnO_4$ to be tested.**Calcium chloride ($CaCl_2$)** - This is sometimes easier to find than other reagents (for example, in supply houses for the local food industry). This is only used in the solution as a soil flocculent and it is possible that magnesium chloride will also work (it is the divalent Ca ++ ion, or on the contrary Mg ++ ion, which is important)**Citric acid or lemon juice:** citric acid is not necessary for the analysis, but it is useful to clean brown permanganate staining from the containers used in the method, by soaking them for a few hours followed by normal washing.**Centrifuge tubes** (50 mL) or other small containers of 50 to 100 mL in which to shake the digestion solution with the soil and then allow the suspension to settle. A tall, narrower-style container is desirable to allow pipetting from the top of the suspension.

6. **Soil** sieved to 2 mm and air dried (if it is necessary to use moist soil, a correction for the water content must be applied, see end of method)
7. **Digestion solution:** 0.015 M potassium permanganate ($KMnO_4$) + 0.1 M calcium chloride ($CaCl_2$) in the SAME solution. See the recipe below
8. **Transparent glass vials** with volume 11 ml and a diameter of 0.75 inches, to perform the color reading with the Hanna colorimeter (see photos below and equipment section 2.1.3).
9. **Centrifuge tubes** or other small container (volume about 50 ml), to dilute the digested $KMnO_4$ solution before reading.
10. **Graduated dropper** or graduated transfer pipette with volume measurements. These can be purchased with graduations of 0.5 mL. You can also make a dropper graduated to a volume of 0.5 mL with a precision balance to mark the level at which the dropper contains 0.5 mL (equal to a weight of water of 0.5 g).

3.4.3.2.   Recipe for the KMnO4 / CaCl2 digestion solution

This is a solution of 0.015 KMnO4 and 0.1 M CaCl2 in the same solution. The original method published by Weil et al. (available here) uses a 0.02 M solution of $KMnO_4$, but we are trying to save on reagents and, therefore, use a slightly more dilute solution.

For each 100 mL of solution (multiply in case of larger volumes):

1. Measure with a graduated cylinder, or weigh, 100 mL (which is equal to 100 g water) in a transparent bottle or beaker (to be able to see that the reagents are completely dissolved.
2. To each 100 mL of water, add 1.11 g of $CaCl_2$. For volumes greater than 100 mL, multiply the amount of $CaCl_2$ proportionally to the volume, e.g. for 1000 mL or 1 L, multiply by 10 = 11.1 g $CaCl_2$.
3. Mix the solution well until all the calcium chloride is dissolved. If the solution is made inside a bottle, you can cover the bottle and shake or swirl to dissolve it quickly.
4. *To this same solution*, add 0.237 g $KMnO_4$ (potassium permanganate) per 100 mL of solution (or 0.24 g if there is only one precision balance 0.01 g). Mix well again, until completely mixing the $KMnO_4$ particles. This amount (0.24g) is a small amount for many non-precision balances, and it may be better to make a larger volume, for example 500 mL (with 1.185 g $KMnO_4$), to achieve greater precision in weighing the permanganate. Other amounts of solution are also possible.
5. It is best to make just enough of this solution, plus a small margin, to analyze a batch of soils, calculating 20 mL for each analysis. If the solution is to be stored between evaluations (less than a week), the bottle must be covered against light (with tape or aluminum foil for example) so that the $KMnO_4$ does not decompose.
6. If you want to store this solution for a few weeks to a month, you can add a small amount of NaOH to adjust its pH to 7.2 (just after mixing, and assuming distilled or very pure water, it will have a pH of around 5.7). This will help to preserve $KMnO_4$ in solution. The solution can be stored for a few weeks in a refrigerator, in a wrapped bottle to prevent light from entering. However, it is a better idea to mix the solution in small batches and use the entire solution within a week.
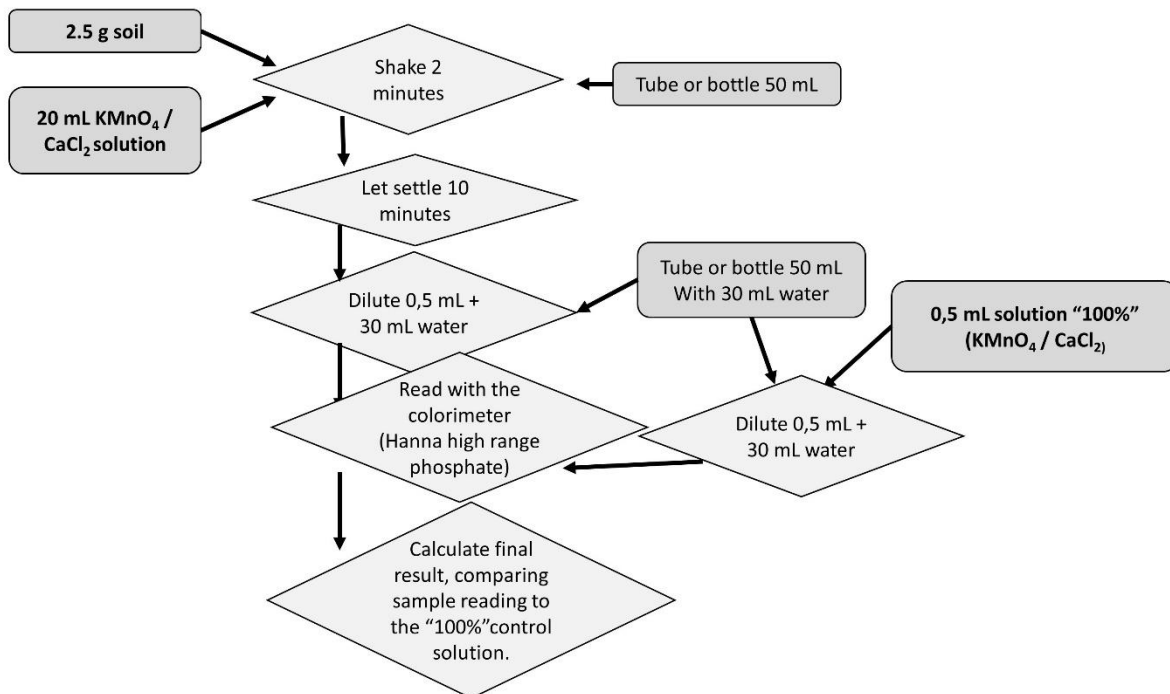
Fig. 24. Flowchart for the test of KMnO$_4$-oxidizable carbon ("active carbon" or POXC).

3.4.3.3.   Procedure (see flowchart in Fig. 24)

1. **A video for this method is available at:**
   https://www.youtube.com/watch?v=89Bn5P4y3n4&t=601s
2. Mix 2.5 g +/- 0.3 g of soil in 20 mL of digestion solution (recipe above). Record the exact weight for the calculations at described below, e.g. "2.61 g". More soil can be weighed out (e.g. 3.5 g) if it is estimated at the outset that the soil contains very low levels of SOM, or less soil for soils very high in SOM.
3. Shake 2 minutes, shaking with your hand or with a shaking machine.
4. Let stand 10 minutes. The CaCl$_2$ will cause the clay to flocculate and settle, to leave a clear solution except for the color of KMnO$_4$. The timing of these two steps is relatively important, e.g. not more than 20 seconds imprecision in shaking and not more than one minute imprecision in settling.
5. During this time, if it has not been prepared in advance, you must fill a second centrifuge tube or bottle with 30 mL or 30 g of water, to prepare a dilution of 0.5 mL KMnO$_4$ + 30 mL water. This dilution will allow reading of the color in the colorimeter.
6. Dilute the settled solution of KMnO$_4$ in the tube with 30 mL water (the KMnO$_4$ solution without dilution is too dark to read). Take 0.5 ml of this settled solution with a graduated dropper or transfer pipette from the top layer of liquid in the vial, being careful to make sure that there is exactly 0.5 mL in the vial. Add this 0.5 mL to the 30 ml of water in the tube and then rinse the pipette or dropper with the 30 mL (sucking and expelling) to transfer all the color to the tube.
7. A "control" tube containing 100% KMnO$_4$ solution, direct from the bottle and without having reacted with soil is also needed for comparison to the soil sample. To make this, dilute 0.5 mL of the solution directly from the reagent bottle where it was prepared and dilute by adding to 30 mL water.
8. The final step of the measurement is to read the color of the diluted solution (step 5) as compared to the color of the 100% diluted solution (step 6). The measurement with the colorimeter is done in the following way (See Figs. 25 and 26):
   a. Insert a vial in the colorimeter with clean water as a blank value.
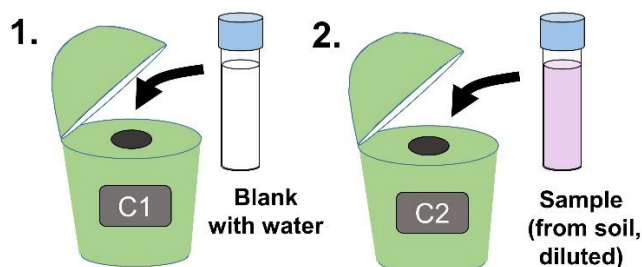   b. Push the button to turn on and wait for "C1" on the screen (Fig. 25)

Figure 25. Measuring the sample value for permanganate solution reacted with soil.

c. Re-push the button to measure the blank vial, and wait for "C2" on the screen
d. Pour approximately 10 mL of the solution diluted with soil and diluted (step 5) into another clean vial to read in the colorimeter. Push the button.
e. Wait for the value of the result on the screen, which will usually read between 0 and 22.
f. To measure the 100% control value, repeat steps (a) through (e), repeating the blank as C1 and inserting a vial with the 100% diluted solution (from step 6) instead of the solution reacted with soil as C2; see Fig. 26.
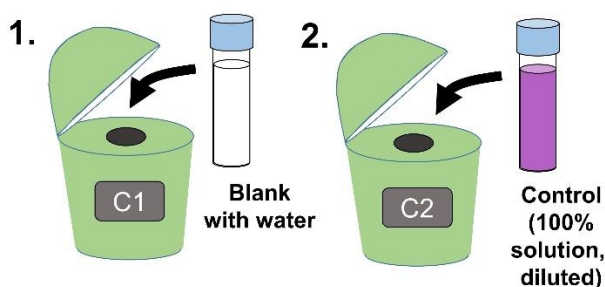

Figure 26. Measuring the 100% control value of the unreacted $KMnO_4$ solution.

g. It is not necessary to repeat the measurement of the 100% solution for each soil sample. The 100% control solution can be re-measured only every 3 or 4 samples. In the calculations below, for the 100% value, the two closest 100% measurements are used.

3.4.3.4. Calculations to determine permanganate-oxidizable carbon or active carbon:

1. Three pieces of information come from each sample: the weight of the soil introduced to the test (about 2.5 g, for example), the reading of the sample reacted with soil in the colorimeter, and the reading of the 100% $KMnO_4$ solution (without reacting with soil). Note that if the sample solution loses a lot of its color and the sample reading on the colorimeter is low, this means there was a lot of oxidation of carbon in the soil and the value of activated carbon will be high. When there is not much change in color, the level of active carbon is low.

2. Example: Let's say that exactly 2.50 g of soil was weighed to analyze, that the reading of the 100% solution is 17.6, and that the reading of the soil sample is 13.2. The result of active carbon or POXC would be calculated in this way:

   a. First, the change in the concentration of the $KMnO_4$ solution is calculated:

$$\boldsymbol{Change\ in\ concentration\ KMnO_4} = \left(1 - \frac{sample\ reading}{100\%\ solution\ reading}\right) \times 0{,}015\ M$$

   b. For the example we get Change in concentration= **[1-13.7/17.6) x 0.015 = 0.00375 M**, where M stands for moles per litre.

   c. In the next step we use the volume of permanganate solution to figure out the change in the actual amount of permanganate based on this change:

$$\boldsymbol{Change\ in\ quantity\ KMnO_4} = change\ in\ concentration \times 0{,}02\ L$$

Since the 20 mL solution used is equal to 0.02 litres.

   d. For the example we get: Change in amount KMnO₄ = **0.00375 M x 0.02 L = 0.000075 moles KMnO₄**

   e. To convert this amount in moles to mg of activated carbon in the soil oxidized by KMnO4, the authors of this test determined that a conversion factor of 9000 can be used:

$$Soil\ carbon\ oxidized\ (mg) = change\ in\ the\ amount\ of\ KMnO_4 \times 9000$$

   f. In the example, Soil carbon oxidized = **0.000075*9000 = 0.675 mg**

   g. This amount of soil carbon oxidized (in mg) is then divided by the initial amount of soil used (in kg) to yield a result in mg/kg or parts per million (ppm):

$$POXC\ or\ \text{"active" carbon (mg/kg)} = \frac{Soil\ C\ oxidized\ (mg)}{initial\ soil\ mass\ (kg)}$$

   h. In the example, POXC = **0.675 mg / 2.50 g soil = 0.675 mg / 0.0025 kg = 270 mg/kg soil**.

   i. Comparing with the histogram of values in figure 27 and the scoring chart in scoring table below, we can see that this represents a very low to low level of POXC.

In Fig. 27, a range of typical values of POXC or active C of the soil is indicated, in a graph that shows the distribution of values from highland Bolivia as well as Western Kenya.
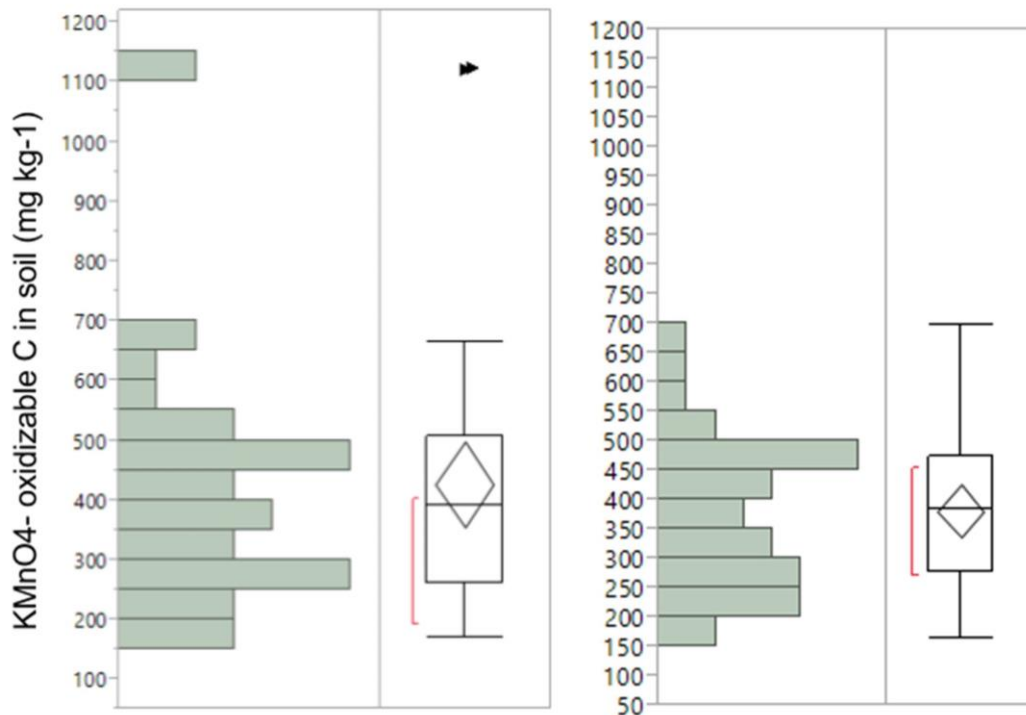
Figure 27. The frequency distribution of values for active carbon measured with this method, from 17 smallholder fields in a mountainous region of Bolivia at left, and 36 fields from

Western Kenya at right (Nandi and Vihiga counties) with a box and whiskers plot at the right of each plot. The range of the data represents the full range of the POXC test outlined here (0-1200 ppm). In the data from Bolivia, the two high outlier values at top are from high-elevation peat soils, however, these values could also occur in soils that are frequently amended with compost and manure. Kenyan data courtesy of the CCRP Multipurpose Legumes project and Blessing Magonziwa.

Scoring table for interpreting the results of the "active carbon" or permanganate-oxidizable carbon test (POXC):

Ranges of POXC (mg/kg)	Score or qualifier	Description
<250	Very Low	Indicates soils that have not received substantial organic inputs for many years, or where subsoil is present due to erosion. These values will also occur more frequently in warm, light textured (sandy) soils where organic residues break down quickly and where there are abundant and dense sand particles that dilute the organic matter reading.
250-400	Low	Indicates a soil that may still need work in building organic matter to better support microbes, water holding, and building of soil structure.
400-600	Medium	Likely available organic matter is supporting good function of microbial nutrient cycling, water-holding capacity, etc. These values may also occur in heavy-textured soils that store more carbon, even when soil organic matter is suboptimal. By contrast, in a sandy soil this range of values may already indicate very good levels of organic carbon.
600-1000	High	Soil either has high levels of remnant organic matter from forest conversion, and/or substantial effort has been made to supply crop residues and manures to these soils.
>1000	Very high	These values are found in intensively manured or composted home gardens, in soils converted recently from forest, or in highland peat soils. They are good for many types of crops, but may rarely be attained in more extensive cereal fields where limited organic inputs are available.

3.4.3.5. Example data table and program to perform evaluations of several samples in sequence:

1. Often several samples need to be analyzed in sequence (10 or more samples). For this it is important to realize the importance of maintaining the exact times of the analysis (shake 2 minutes, let sit 10 minutes in the same way for all samples). The following table shows an example of a way to record the active carbon data and also gives cues for the suggested times for each sample, for example the "start time" when the soil is added to the solution, the "settling time" two minutes later when the tubes are left to stand after shaking, and the "measuring time" when they should be read with the colorimeter.

2. As shown in the table, if two people are working together, 5.5 minutes between the sequential samples allows adequate time for all the different steps needed to shake

and read the samples, and so evaluate the samples in series one after the other. However this sequence time can be adjusted once a team has experience. It is especially important that the samples be read 10 minutes after they have been allowed to settle. It may take practice to accomplish this, but try not to be more than 1 minute late (or early) as this will provide the best data. If it is data that has to be rigorous and comparable as in the case of an experiment, it is preferable to practice with samples in sequence that are not important (e.g. batch of 3-4 "expendible" samples) before starting with samples where the data is important.

Table of example data for the POXC (active carbon) test, including times in which the different steps for multiple samples have to be performed.

Sample ID	Weight soil in (g)	Start time	Settling time	Measuring time	Reading in the colorimeter
101	2.52	0	2	12	13.7
102	2.47	5 .5	7.5	17.5	5.2
100% solution control	--				17.4
etc.	2.54	11	13	21	etc.

3.5. Extractable Soil Phosphorus (Olsen method)

Video on YouTube : https://www.youtube.com/watch?v=R1lFrMjoraE

3.5.1. Materials y Reagents

1. **"Phosphorus-free" water**. In general, tap water (public water supply) will have too much phosphorus (P) to be useful. We have tested different brands of bottled water in several countries and generally there are one or two brands that have a P level below 0.5 ppm (mg / L) that makes it acceptable for this method (there are some brands that have no detectable P, which is even better. Some companies publish their chemical analysis value on the bottle label and if the content of phosphorus (P) is 0.5 ppm or less, the water can be used for this test.

   Phosphorus (P) content test for water:

   In case you want to test the level of phosphorus (P) in the water, it can be done with the following method and the same reagents and colorimeter that is used in the soil test, as follows:

   - Place 10 mL of the water to be tested in a vial for the colorimeter, and 10 mL in another vial for a blank measurement without color.
   - To one of the vials, add a reagent packet as detailed in the method below (step 14); **Note**: it is not necessary to neutralize the water sample with sodium bisulfate as in the soil method, you can directly add the reagent.
   - Cover the vial with reagent, shake well, and wait 10 minutes. In general the water will turn either a very weak color of blue, or no obvious color at all.
   - Read the color of the vial with the colorimeter as described in step 17 below, using the water without reagent added as the C1 blank. The number that appears in the colorimeter must be subtracted from the phosphorus reading in the soil extract before calculating the concentration of P in the soil (see the calculations section in 3.5.5). If the number that is read is zero, no correction is applied.

2. **Sodium bicarbonate** ($NaHCO_3$): This must also be low enough in P to avoid introducing errors in the method, as described above. Tests in Malawi, for example, showed that sodium bicarbonate purchased in supermarkets had low levels of P and could be used, so local options may work if they can be tested, by preparing the Olsen solution and testing it with the procedure below but without reacting it with soil. Reagent-quality bicarbonate will generally be pure enough without testing.

3. **Sodium hydroxide** ($NaOH$), this is not necessary in large quantities in the solution - so it will not generally contribute large amounts of impurities of phosphorus.

4. **Sodium bisulfate** ($NaHSO_4$) to acidify the soil extract in preparation for the color reaction; approximately 0.45 g per sample. It is possible to substitute battery acid for this reagent, see the recipe below for sodium bisulfate solution and the instructions below for acidifying the soil extract (step 8) for more details.

5. **Hanna low-range phosphate reagent** packet (Hanna product number 93713-03, see the materials and reagents at the beginning of this manual); one packet per analysis.

6. **Balance**, best with at least 0.01 g precision

7. **Olsen extraction solution** (See recipe below). This solution does not maintain its properties and it is better to prepare shortly before the analysis (maximum 1 week if stored in fridge).
8. **High-range Hanna field colorimeter** (see the materials section at the beginning): Note that we INTENTIONALLY use the low range reagents with the high range colorimeter.
9. **Graduated cylinder**, 25 mL with at least 1 mL graduations (see photos below in manual)
10. **A bottle or tube** to shake and extract the soil with the Olsen solution, which can be a well-washed 250 ml plastic bottle (<300 mL, for example) or a 50 mL centrifuge tube.
11. **A second filter bottle**, with a wider lid, for example 4 cm in diameter, which can have 300 to 500 mL of volume, e.g. from milk or liquid-style yogurt. This second bottle is modified with holes drilled in the lid with a sewing needle. If the inside of the lid is not completely flat and has a ring raised inside to seal with the mouth of the bottle, it is necessary to remove this ring. See a video that describes the entire soil extract filtering rig at: https://www.youtube.com/watch?v=FEcQOSA_ur4
12. **Important**: Both bottles must be thoroughly washed with water and rinsed with clean bottle water or distilled water, so as not to contaminate the sample with soluble P.
13. **Paper filters:** cone-shaped coffee filters for cutting circles, or laboratory filters (Whatman # 5, see the equipment at the beginning of the manual). For very clayey soils, the coffee filters will be clogged and are too slow, so in this situation the laboratory # 5 filters are required.
14. **Transparent 11 ml, 0.75 inch diameter vials** that are used with the Hanna colorimeter to read the blue color of the phosphate reaction; the same vials as for the POXC test above, section 3.4.3.
15. **Small plastic cups** to capture the filtered extract and to acidify the extract (2 cups per test, about 6 cm wide 8 cm high)
16. **A rinse bottle** with a nozzle will facilitate rinsing and precise addition of water to bring dilutions up to volume.

3.5.2. Preparing Olsen Solution:
1. The standard definition of Olsen extraction solution a 0.5 M (moles per liter) solution of $NaHCO_3$ adjusted to pH 8.5 with the required amount of NaOH. As follows:
2. For each 100 mL of solution, put 100 mL of "P-free" water in a clean bottle (rinsed with P-free water) and add 4.20 +/- 0.01 g of $NaHCO_3$ (0.05 moles) It is better to make just enough solution plus a small margin for each analysis batch, to use within a few days. Each analysis uses 25 ml of solution.
3. Swirl or shake the solution until all $NaHCO_3$ dissolves. This can take 5 to 10 minutes, it is not a very soluble salt.
4. The pH of this solution will be approximately 7.7 or 7.8. After measuring the pH of the solution with a calibrated pH meter or pH paper strips, add small amounts of NaOH (e.g. 0.1 g if it is 100 mL, 0.2 to 0.5 g if there is more solution) and stir with a spatula or small spoon, or by swirling the bottle. Measure the pH and add more NaOH until the solution has a pH of 8.5 +/- 0.05 (that is, any value between 8.45 and 8.55 is acceptable).

3.5.3. Preparing sodium bisulfate neutralizing / acidifying solution

1. Mix 15.0 g (+/- 0.2 g) Sodium bisulfate (NaHSO₄) into 100 mL water, measuring the water either by weighing or with a graduated cylinder
2. Store in a plastic bottle and avoid contact with skin and eyes. This is an acidic solution and should keep quite well, and can be used for several months. You will need 3 mL for each analysis and therefore may want to mix 100 mL at each time for up to 33 samples.
3. **TO USE DILUTED BATTERY ACID AS A SUBSTITUTE:** carefully and with gloves and goggles, and in a well ventilated space or outdoors, accurately dilute battery acid v:v 3+1 with water (Add 3 parts "phosphorus-free" water considered above in the materials to one part battery acid). This concentration of acid (~7.5%) can now be handled in a plastic bottle and with a dropper, without representing an inhalation hazard. Care should still be taken since it will destroy clothing and burn skin if not rinsed quickly with water.

3.5.4. Procedure (Fig. 28):

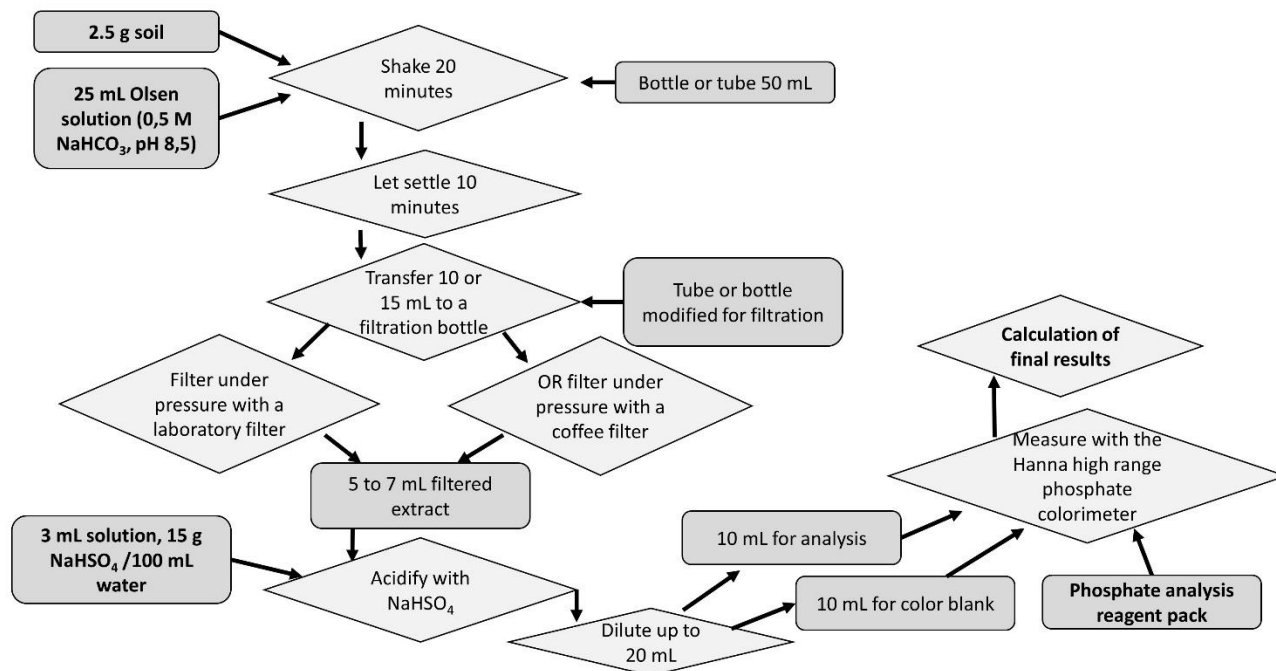**Figure 28.** Flow chart diagram for the analysis of soil extractable phosphorus (P).

1. Weigh 2.5 g of sieved soil (2mm) in the centrifuge tube or other bottle.
2. Using a graduated cylinder add 25 mL of Olsen solution to the sample of soil in the bottle or centrifuge tube (Fig. 29). When many samples are analyzed, Olsen solution can also be pre-measured or weighed in bottles before adding soils, to save time)

Figure 29. Adding 25 mL Olsen solution to the soil in a re-used juice bottle to start the extraction.

3. Cover the bottle with the lid closed and shake 20 minutes. If by hand, it is acceptable to alternate between shaking the bottle and putting down briefly to tend to other tasks.
4. Let the suspension sit 10 minutes. Some of the clays will settle during this time, but a transparent solution is not necessary.
5. There are now two alternatives for filtration: with filter lab paper, Whatman # 5 (with 2.5 micron pores), which is more guaranteed; or with filter paper from cone coffee filters, which can be used for light textured soils. In both cases, a different bottle is used to perform the filtration under pressure, with a filter inserted into a lid with holes in it to allow the filtrate to pass. . See a video that describes the entire soil extract filtering rig at: https://www.youtube.com/watch?v=FEcQOSA_ur4 . It is important to practice this step to gain confidence in producing a filtered extract without turbidity (clay content):

 In the case of coffee filter paper from conical filters:
  1. Transfer the supernatant (clay suspension in the upper part of the bottle) from the extraction bottle to another filtration bottle. This bottle must have a lid with a flat surface inside to place a filter in the shape of a circle, and small holes in the lid (<1mm diameter) to let the filtered liquid flow. See the YouTube video referenced above.
  2. A double layer of coffee filter paper cut into a circle is inserted into the perforated lid of this bottle. Then, by hand or with a wooden press (see Figs. 31 and 32), press the bottle until you sees drops coming out.
  3. Do not initially save these drops as they will come out with some clay and turbidity. Over time the drops should become more transparent, although there will still be a light brown color of the transparent solution, and this is fine.
    After discarding the first drops if these are cloudy, start collecting the clear drops in a new clean glass until you have a volume between 5 and 7 mL. 7 mL is indicated if there are no problems in collecting this solution. This process can take up to 10 minutes so it is recommended to use the wooden press (Fig. 32) to be

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able to free your hands for other work, especially if there are many samples to analyze.

Figure 30. Placing a double layer of coffee filter paper below the lid of the bottle.

Figure 31. Pressing the bottle by hand to create a filtered soil extract.

In the case of laboratory Whatman # 5 paper, with pores of ~ 2.5 microns
1. Leave the extraction bottle without moving it a lot so as not to disturb the settled clays.
2. Using the transfer pipette or a dropper, transfer the upper 10 to 15 mL of the soil suspension in the extraction bottle to the new bottle for filtration. This suspension will still be cloudy, but the idea is to minimize its level of clay so as not to obstruct the filter paper too much. See the filter rig video referenced above for more details on the filtration method.
3. Put a single circle (not double) of filter paper inside the lid with holes and place carefully, but firmly, on the filtration bottle. Overtightening may rip the filter paper, but it does need to be quite tight. Trial and error will indicate the correct tightness.
4. Turn over the bottle and press it by hand or with a filtration press that can be made for the purpose (Fig. 30 below)
5. The drops that come out of the filter should be clear and can be collected in a clean glass directly (unlike the first drops with the coffee filter option above). If they are cloudy, you should check for rips or cracks in the filter.
6. Keep collecting drops until you have between 5 and 7 mL for the next steps (7 mL is best)

Figure 32. A bottle press to help with filtration using bottles and filter paper in the lids, which allows maintaining pressure on the bottles and use a laboratory filter or a coffee filter to extract the clays from the extract. This approach saves time because while pressing the bottles one can proceed with other tasks.

6. **Regardless of the filtering method above (coffee or laboratory filters):** Continue filtering clear drops in the cup until you have 7 mL or a little more. If filtration is very difficult, you can reduce this amount by something between 5 and 7 ml, and you will need to reduce the amount of sodium bisulfate solution proportionally in the acidification step below (see table below)

7. Empty exactly 7 ml of filtrate (or less if the filtrate was difficult) into a clean or recently rinse graduated cylinder (shake out any excess water if rinsed).

mL filtered soil extract	mL of sodium bisulfate solution to add for acidification of solution	Alternative: volume of diluted battery acid (3+1) to add (mL)
5	2.25	1.1
6	2.75	1.3
7	3.10	1.5

8. Pour the 7 mL soil solution into a clean plastic cup, and then using a clean transfer pipet or dropper with graduations, add 3.1 mL of the 15g/100 mL sodium bisulfate solution prepared previously (step 3.5.3 at beginning of this protocol).

9. **TO SUBSTITUTE DILUTED BATTERY ACID:** instead of the 3.1 **,** add 1.5 mL dilute battery acid for each 7 mL of filtered extract. You can add less bisulfate solution or diluted

battery acid if less filtrate was obtained (see the table above for bisulfate and diluted battery acid amounts, assuming battery acid diluted 1+3 acid+water) With soil samples from very calcareous soils, you can add a few extra drops of bisulfate solution or battery acid to neutralize extra carbonates in the solution.

10. The solution will bubble as the bicarbonate ions are neutralized (Fig. 33). The acid bisulfate (or diluted battery acid) lowers the pH of the extract from approximately pH 8.5 to pH 6, so that when the reagents are added then they can lower the pH to approximately pH 1 or 2 where the development of the blue color of the molybdate-ascorbic acid complex can occur. Without this low pH the blue color (proportional to phosphate concentration) cannot be developed.

Figure 33. Mixing 3 mL solution of sodium bisulfate (15g $NaHSO_4$ per 100 mL) to acidify 7 mL of filtered extract. Note the bubbling of the soil extract as bicarbonate reacts with the acidic bisulfate.

11. While allowing the solution to bubble off CO_2 from the bicarbonate, in order to save time, a reagent pack can already be added to a clean, dry colorimeter vial for step 14 below (Fig. 35).

12. After bubbling subsides, pour the acidified extract from the cup back to the 25 mL graduated cylinder. Rinse the cup with a little water (<4 mL for each rinse) with the wash bottle and add this rinsing water to the cylinder as well. Then fill the cylinder up to a final volume of 20 mL +/- 0.2 mL (Fig. 34). This will give you 10 ml diluted extract for the vial with the reagent, and 10 ml as a control without added reagent to be placed into the colorimeter. (to explain: the extract without reagent will be yellow to brown colored, so we want to correct for this color by using it as a blank in the colorimeter).

Figure 34. The extracted, filtered solution has been acidified and brought to a standard volume of 20 mL. It is ready for reacting with the color reagent to develop the blue color and then read with the colorimeter.

13. **Important:** Mix the sample well before placing it in the vial to perform the colorimetric reaction, by pouring it back and forth between the graduated cylinder and the cup where you acidified it.

14. Add the contents of the phosphate reagent packet to a clean, dry vial (Fig. 35). You can cut the package straight on the top, then open the top of the package in a square or diamond shape, and pour a few times, to ensure that all the reagent enters the vial.
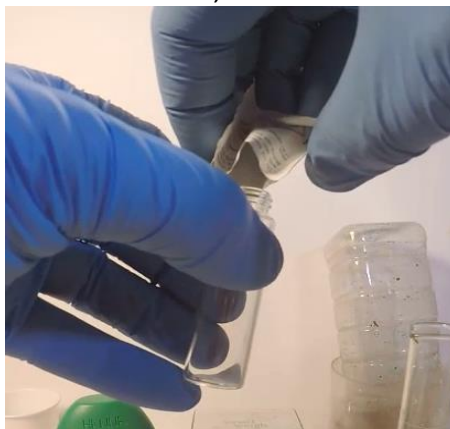

Figure 35. Adding the phosphate reagent packet to a clean, dry vial.

15. Add 10 ml of the 20 mL extract solution in the graduated cylinder to the vial with the reagents. Mark a 10 ml line in advance or pour until the space between the meniscus and the lid is the same as the thickness of the lid, which is equivalent to the 10 mL level.

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16. Add the remaining solution in the test tube to a 'control' vial or blank without added reagent. For samples of the same approximate type of soil, the same control solution can be used for several samples because the yellow or brown color of the extract will be quite similar. When large differences in organic matter occur, the control vial will be darker for soils with more organic matter and a different control vial should be used for these different soils.

17. Cap and shake the bottle with the reagent. You may have to unscrew the vial once or twice to release bubbles. If, when adding the reagent, it releases many bubbles as in the acidification step above, it is a sign that not enough bisulfate was used to acidify, perhaps because it is a very calcareous soil, and it can take another few drops of bisulfate solution (up to 0,2 mL) before reading the result. However, note that it is normal for the solution to release some bubbles with the reagent.

18. A blue color should develop in the reagent vial (Fig. 36). Read the blue color after about 10 to 15 minutes in the high-range Hanna phosphate colorimeter:

● Turn on the colorimeter

● When C1 appears, put the control vial (clear or light brown color, no reagent) and press the button.

● When C2 appears, change the control bottle for the vial with added reagent (blue color, if there was phosphate present) and press the button.

● Record the reading in ppm of phosphate. Remember that this reading is **not** the final result because you have to perform the calibration steps and the final calculations

Figure 36. A blue color will develop in the extract with the reagent packet added. The other yellow colored vial serves as the color blank to be measured in the colorimeter.

19. It is possible that, when the test is performed the first few times, you want to repeat the readings after 20, 25 and 30 minutes, to test if the color development

continues. The blue color should reach a maximum after 15 to 20 minutes, but does not change much after 15 minutes.

20. In many soils, after approximately 30 minutes the blue color is combined with organic matter (MOS) dissolved by Olsen extraction and produces blue particles and a precipitate. It should not be read after this point because the blue color will begin to diminish. In very high soils in organic matter, this process of precipitation can complicate the reading already from the 20 minutes, and it is necessary to make a conjecture as far as the best moment of the reading, and to register this in the observations.

21. In case the level exceeds the calibration limit below, which would be greater than 20 ppm reading in the colorimeter, it is advisable to use less soil in the analysis (eg 1.5 g instead of 2.5 g) to reduce the final level of phosphate that needs to be read by the colorimeter.

22. **Handling reaction wastes**: Placing diluted reagents in an infertile soil or compost will likely not cause any adverse effects or toxicity, and molybdenum may even act as a nutrient for plants. It can also be disposed of in a public sanitation system. The extracts read in the colorimeter (which are acidic) can be neutralized with a little bit of kitchen or fire-pit ash.

3.5.5. Calculation of Olsen available phosphorus in the soil:

1. First, the raw concentration of phosphorus in the final solution that was placed in the colorimeter is calculated by means of a previously developed calibration curve:

$$Raw\ Conc.\ of\ P = P_{raw} = 0.0559 \times colorimeter\ reading - 0.0052$$

2. In the event that a blank value was recorded when the reagents were checked while preparing to do this test, reflecting slight contamination of the water or sodium bicarbonate used (see section 3.5.1, section on "**Phosphorus (P) content test for water**"), this equation is modified slightly to reflect the blank value (C_{blank}):

$$Raw\ Conc.\ of\ P = P_{raw} = 0.0559 \times (colorimeter\ reading - C_{blank}) - 0.0052$$

3. Where C_{blank} is the value of the blank measured with the colorimeter for a sample of clean water. In the event that bicarbonate purchased from a supermarket was used, it is preferable to assume that there is a certain level of contamination and use a value of $C_{blank} = 0.2$)

4. Then the concentration of P in the original extract is calculated after shaking the Olsen solution with the soil:

$$Conc.\ P\ in\ extract = [P_{extract}]$$

$$= P_{raw} \times \frac{20\ mL}{mL\ solution\ used\ for\ neutralizing} (mg/L)$$

5. Where the "mL solution used for neutralizing" generally refers to 7 mL measured to neutralize with sodium bisulfate, and may vary between 5 and 7 (see step 6 above)

6. Next, [**P**_{extract}] is the concentration (mg/L) of phosphorus in the extract and from this we can calculate the *amount* (mg) of phosphorus in the extract, in this way:

$$\textit{Amount P in extract } (\textit{mg}) = \textbf{\textit{P}}_{\textit{extract}}(mg) = [\textbf{\textit{P}}_{\textit{extract}}] \times 0.025 \, L$$

Remembering that there we created a total of 25 mL of extract from the soil, equivalent to 0.025 L.

7. Finally, to calculate the amount of phosphorus available in the soil, we divide this amount in mg by the initial weight of the soil in kg:

$$\textbf{\textit{P}}_{\textit{available}} \textit{ in soil } (\textit{mg/kg}) = \frac{\textbf{\textit{P}}_{\textit{extract}}}{\textit{initial weight of soil } (\textit{kg})}$$

8. Take into account that the weight in grams of the soil must be divided by 1000 to find the weight in kg: for example 0.0025 kg for 2.50 g or 0.00243 kg, for example if we use 2.43 grams of dry soil.

9. In case we use moist soil we have to adjust the results for soil moisture, which would be to divide the result up by [1 - water content in the sample]. Moisture must be measured separately, or can be approximately rated with a visual rating such as that in appendix A.

Example of the results calculation:

10. 2.63 g of soil is initially weighed. Then 7 mL of extract was filtered for neutralization, and when analyzing the color with the colorimeter an intensity of 14.5 units is read. Distilled water and a bicarbonate reagent have been used that have no detectable phosphorus content. Then the level of available P is calculated as follows:

    a. P_{raw} = 0.0559 x 14.5 - 0.0052 (no correction for a blank value)
       = **0.8503 mg / L**
    b. Then, [$P_{extract}$] = 0.8503 x (20/7) = **2.429 mg / L**
    c. And the quantity extracted will be: 2.249 mg / L x 0.025 L = **0.06074 mg**
    d. Then the level of $P_{available}$ in the soil would be: $P_{available}$ = 0.06074 mg / 0.00263 kg = **23.1 mg / kg**
    e. This corresponds to a high level of P available according to the table below for the interpretation of results. Keep in mind that in reality this result is "extractable P" according to a certain method (the Olsen method). It is an estimation of available P that allows us to compare different soils and rate their P fertility, rather than an absolute definition of plant-available P, which depends on many other factors.

Scoring table of qualitative ranges for extractable P with the Olsen method:

Value of Olsen available P (mg/kg)	Score	Description
0 to 5	Very Low	Biomass, vigor, and maturity of most crops will be severely limited and deficiency symptoms may occur, especially when P is the only limited nutrient; if crops are multiply limited, growth may just be limited in an overall way (low biomass)
5 to 10	Low	Crops may exhibit maximal P response since some P, but not entirely enough, is present to fuel growth and vigor.
10-20	Medium	Many crops will still respond to additional inputs of manure, compost, or P fertilizer, especially legumes and many flowering/fruiting vegetables. Some P-efficient cereals may already attain sufficiency.
20 and above	High	Most crops will not be limited by P fertility. Some vegetables and weeds may however continue to respond better at ever higher levels e.g. 30 to 50. Values greater than 50 indicate inefficient, over-allocation of phosphorus to these fields, and nutrient pollution of soils and potentially, watersheds.

3.6. Soil Macrofauna Evaluation:

Evaluation of soil invertebrate communities offers a simple and low-tech option for studying soil biology, and this method offers a number of key advantages. Soil macrofauna are sensitive to changes in their environment and shifts in their community structure offer an integrative assessment (i.e., combining changes in multiple soil properties into a single measure) of ecosystem impacts over time. Also, soil macrofauna, particularly ecosystem engineers (e.g. ants, earthworms), can have significant influences on soil and ecosystem functioning and thus their populations reflect key ecological processes within soils and ecosystems. Finally, large soil invertebrates are relatively simple to measure, ubiquitous, and familiar to land managers, as they are frequently encountered during soil management activities.

This procedure takes more time than some other evaluations. It is good to perform the evaluation as a team, to share the work with a group and also foster group learning and observations about life in the soil. The evaluation requires the soil to be in a productive state, generally during the rainy season and with growing plants and crops present. Although it appears at the end of this manual, it can be an excellent starting point for observing and learning about a soil because soil life can be observed and because the soil is extensively handled, leading to a general sense of its tangible qualities before pursuing the other chemical and physical tests. In addition, as described in the introduction of the manual (see Fig. 3) this procedure will generate a sample of "clean" soil to be used in the chemical tests such as pH, activated carbon, and available P, and can be thought of as a sampling method. As noted earlier however, this procedure should not be used to create a sample for aggregate stability (section 3.3), because in the macrofauna evaluation we deliberately destroy natural aggregates looking for macrofauna, invalidating the aggregate stability method.

3.6.1. Materials
1. **Shovel** with square tip for digging a regular, square hole.
2. **Knife or machete** for trimming the edges of a hole
3. **Ruler**, at least 20 cm long, to measure the dimensions of the hole. Sometimes two 20-cm rulers can be taped to form a right angle, which makes it easy to measure the hole precisely.
4. Feed sacks or other similar strong sack to collect soil before looking for macrofauna
5. Trays to use for distributing and searching soil for macrofauna
6. Vials, tubes, or bottles (50 to 100 mL size) to store macrofauna samples (if you want to perform a detailed classification
7. Tweezers or forceps to transfer macrofauna to vials or bottles.
8. Alcohol to conserve the macrofauna, and possibly formaldehyde for long-term storage, but the latter only as part of the lab work to avoid carrying this toxic substance in the field
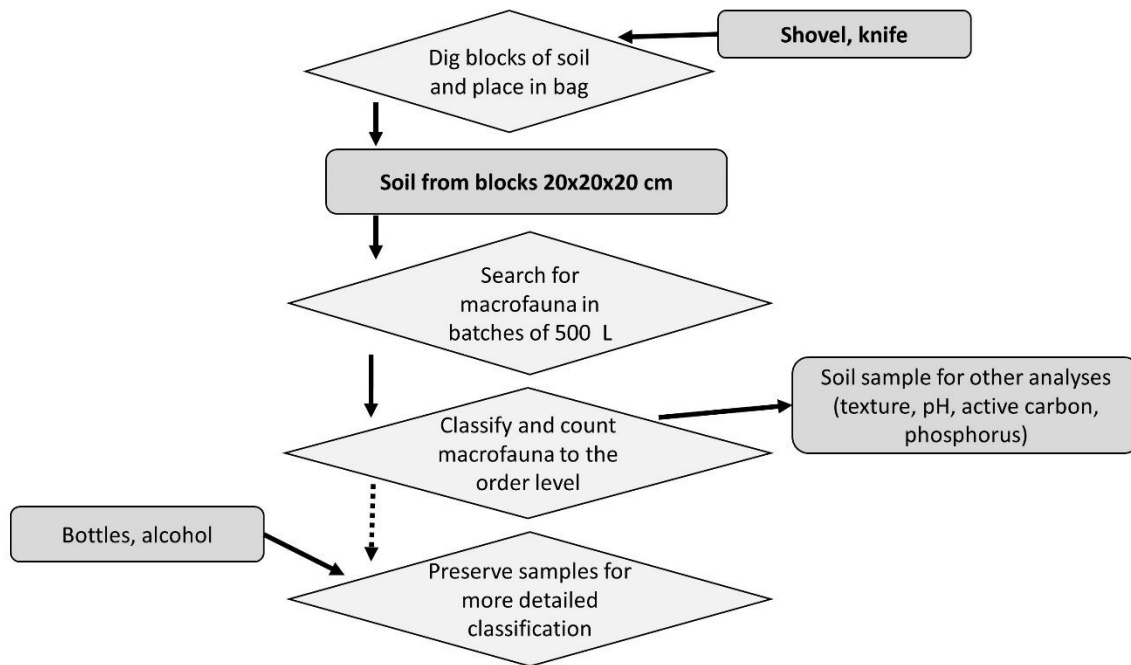
3.6.2. Procedure:

Fig. 37. Flowchart diagram for the soil macrofaunal evaluation.

1. Using a ruler or small quadrat, mark out an area of 25 x 25 cm (square) using flags or similar marker. Try to avoid heavy footsteps, as some macrofauna (especially large anecic earthworms, with deep vertical burrows) can escape fairly quickly. In general macrofauna are heterogeneous in their spatial distribution, so it is good to collect at least 3 samples per treatment plot being evaluated.

2. Using a flat edge spade or shovel, excavate the soil quickly into a large woven plastic sack (Fig. 38 below). Try to ensure from the start that the walls of the pit are vertical and excavate the pit in as few shovel loads as possible to avoid damaging macrofauna. It is possible to reduce the volume of the sample slightly for demonstration purposes or when labor or time is in short supply (e.g., 20 x 20 cm), but it is important to recognize that this introduces greater error and can result in damaging a greater proportion of macrofauna (this can make tallying more difficult in later steps).

| **Figura 38a.** Excavating a square hole (20x20x20cm deep) to assess the macrofauna in this volume. | **Figura 38b.** Soil is rapidly transferred from the hole to the bag to keep macrofauna from escaping. |

3. **Hand-sorting of macrofauna from soil:** Find a comfortable place to work with adequate light, and with shelter from the sun, rain and wind. Scoop handfuls of about 500 mL, onto trays to sort through the soil, use tweezers to gently pick out any 'critters' that are > 2mm in size (in practice, most anything that is easily visible will be included, such as earthworms, ants, termites, beetles, spiders, and insect larvae). Fill two specimen cups (~120ml) about ¼ full with 70% ethanol and label them according to the sample being collected. Place soft-bodied organisms (these are organisms without an exoskeleton; generally limited to earthworms, slugs, and earthworm cocoons) in one specimen cup. Everything else goes into another specimen cup (these are all arthropods, and have legs). If there are lots of small ants or termites (more common for warm tropical soils), it can be helpful to use a small paintbrush dipped in ethanol to collect the rapidly moving macrofauna (as they generally stick to the brush when wet). A team of at least 3-4 people handling each sample bag should be able to finish sorting a sample in 20 to 40 minutes, depending on the number of organisms encountered. One challenge is to not undercount (i.e., become rushed or sloppy) as one grows tired of sorting.

4. Back in the lab, macrofauna can be sorted and counted. Typically, sorting to the level or order (i.e., beetles, ants, spiders, etc.) is sufficient for understanding the functional composition of soil macrofauna communities, but further identification is useful for better understanding impacts on diversity depending on interest and expertise. Sorting to the level or order can often be done with the naked eye, but it is useful to have a dissecting microscope or good hand lens for some specimens. Also, for longer-term storage (>2 weeks) it is important to dump off the old ethanol and replace it with clean 70% ethanol for the arthropods. Soft-bodied organisms can be stored in formalin if long-term preservation is desired. Earthworms can become difficult to identify if stored in ethanol for more than a few weeks; replacing the dirty ethanol with clean ethanol and

storing samples in the refrigerator can extend this time substantially, but one must use air-tight vials to avoid possible fire risk.

Figure 39: Hand-sorting of macrofauna from a tray. Note collection vials for macrofauna at right, if this is desired for precise identification and archiving of samples.

Figure 40. Arthropods collected in 70% ethanol

5. It is important to note that macrofauna data can often be quite "noisy" and conclusions are not always absolute or clear-cut. For this reason, analyses are often best conducted at the level of orders and/or with the most abundant taxonomic groups (often earthworms, ants, and beetles). Results are typically reported on a basis of individuals per square meter (so multiplying abundance numbers by 16 is necessary if using a 25 x 25 cm pit).

6. An excellent field key which is simplified to capture most orders of soil macrofauna is available from resources prepared by IRD / FAO. This guide is reproduced as appendix B of this manual and is also available at (http://www.fao.org/3/a-i0211e.pdf ; or also http://ftp.fao.org/docrep/fao/011/i0211e/i0211e.pdf).

3.6.3. Results calculations:

After sorting into either orders or genera, which can include morphotypes common at a site with a photo to help log them, each count of an organism or group can be expressed as a number per square meter. Note that if a 20x20x20 cm block were excavated, then this means multiplying all the raw counts in the data by 25 = 5 x 5 since each side of the block has a length of one-fifth of a meter (20 cm) so that 25 such blocks would fit in a square meter. If a 25x25cm block is excavated then the numbers should be multiplied by 16, not 25.

3.7. Water Infiltration (<mark>under testing,</mark> <mark>check for updates before using</mark>)

This method is the simplest of the methods used by the conservation service of the USDA of the United States. Its advantage is that it is very simple and can give a relative comparison between fields (of similar soil texture), and indicate the presence of compaction or crusting in the soil. It is necessary to understand the results to have an idea or an exact measurement of soil moisture, because the "initial" or instantaneous infiltration measured in this test depends on humidity and not only on factors such as aggregation or compaction.

This measurement provides an indication of how the soil will perform in infiltrating water during the initial part of a rain event, and ignoring the destructive effects of large raindrops on soil structure and crusting (the aggregate stability test earlier in this manual is better for understanding that aspect). Because it is an initial rate it is not equivalent to such "classic" measures of infiltration as the saturated conductivity or K_{sat}. We are currently working to improve this test so that it can measure both initial and more saturated or longer-term behavior of infiltration during a longer period of rain, say.

Other related tests: See the evaluations "waterlogging in the soil", "identification of a plow pan", and "surface crusting of the soil" in the FAO visual soil evaluation guide by Shepherd et al. (see bibliography)

3.7.1. Materials
1. Metal ring of 15 cm diameter and 10 to 20 cm in height (a large can, or other such fabricated in a sheet metal workshop)
2. Ruler with cm and mm markings.
3. Chronometer or stopwatch app on a smart phone
4. 500ml container with a mark at the level of 450 mL (the volume required)
5. Flexible plastic sheet at least 40 x 40 cm
6. Scissors to trim vegetation at the soil surface
7. Larger hammer (small sledge) or a medium-sized stone
8. Board to distribute the force of the hammer when pushing the ring into the ground
9. Knife to cut roots (sometimes necessary)

3.7.2. Procedure
1. Cut plants to leave a bare soil surface in an area 20 x 20 cm for the ring, or in some cases just enough to fit the ring. Try not to alter the soil surface at all when cutting.
2. Insert the metal ring about 3 cm into the ground, using the board on top of the ring and hammering the ring. If there are roots that obstruct the ring from entering the ground, it can be adjusted with a sharp knife (be careful not to break the structure too much); or a new site can be selected.
3. Use your fingers to gently press and fill the outside edges of the ring to prevent leakage under the ring. Trial and error will show when this needs to be done (in what type of site or soil).

4. Put the loose plastic inside the ring on the soil surface and then pour 450 ml (2.5 cm deep, check with ruler) of water over the plastic, so that it does not yet start to infiltrate.
5. Gently pull the plastic from the ring as you start the stopwatch. Record the time necessary for the water to infiltrate into the soil, that is, until the surface is only glistening, without standing water. Very roughly, this will be between 30 seconds and 10 minutes.
6. To understand the data it is important to take into account the soil moisture at the time of the test. However, if two fields have approximately the same moisture and texture, the data can be compared.
7. Because infiltration is highly variable in space, it is important to replicate the measurement in 2 or 3 parts of the plot and take an average of the results.

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APPENDICES

Appendix A: Visual and feel guide for soil moisture:

Here estimates are presented for moisture based on how typical soils look and feel. These estimates can be used to adjust measurements of nutrient or carbon content to what the measurement would have been in fully air dried soil, which is the best, standardized way to express these measurements. The adjustment to air dried soil can be calculated by multiplying by the factor [1/(1-%moisture)]. For example if a moisture content of 9% is estimated, chemical results using this soil moisture would be multiplied by [1/(1-0.09)] = 1/0.91 = 1.10 which uses the factors in the table below. If many samples are to be evaluated in a single region it is probably best to create a local guide by associating how local soils look and feel with their real moisture content.

Table 1. Moisture contents of different textured soils at different stages (% moisture given)

	I. Very moist	II. Moist	III. most crumbs Moist	IV. most crumbs dry	V. air dry	VI. Air dry[1], many days or heated (~45 C)
loamy sands and sands	9%	7%	5%	3%	2%	1%
Sandy loams	13%	10%	8%	5%	3%	2%
sandy clay loams, loams, silt loams, silts	18%	14%	10%	6%	3%	2%
Sandy clays, clay loams, silty clay loams, silty clays, clays	23%	18%	14%	7%	4%	2%
High organic matter soils (e.g. > 5% SOM; see note below)	+3%	+2%	+2%	+2%	+1%	+1%

Moisture levels (see corresponding images below):

I. **Very moist**. the soil is wet enough that when handled, clumps form that are larger than the normal crumb size aggregates from sieving (5-15 mm), and is very difficult to sieve at 2 mm without clogging the screen. With just a little more water we would start to see glistening soil (free water not held in the aggregates); when pressed with the fingers and thumb, the soil almost forms a smeared together clod, but still shows some grain structure of the individual crumbs.

II. **Moist**. Larger clumps (5-15 mm) are no longer forming from soil being handled, but all crumbs are still visibly moist. Sieving is still difficult from moisture, but doable. When pressed with the fingers and thumb, the soil holds together loosely but does not smear together, and the clump formed is relatively easily broken. In the sandiest soil the clump with fingers and thumb does already not quite hold together.

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[1] For air dried soils, in most cases the moisture content is assumed to be zero and no adjustment is made to any chemical analysis results.
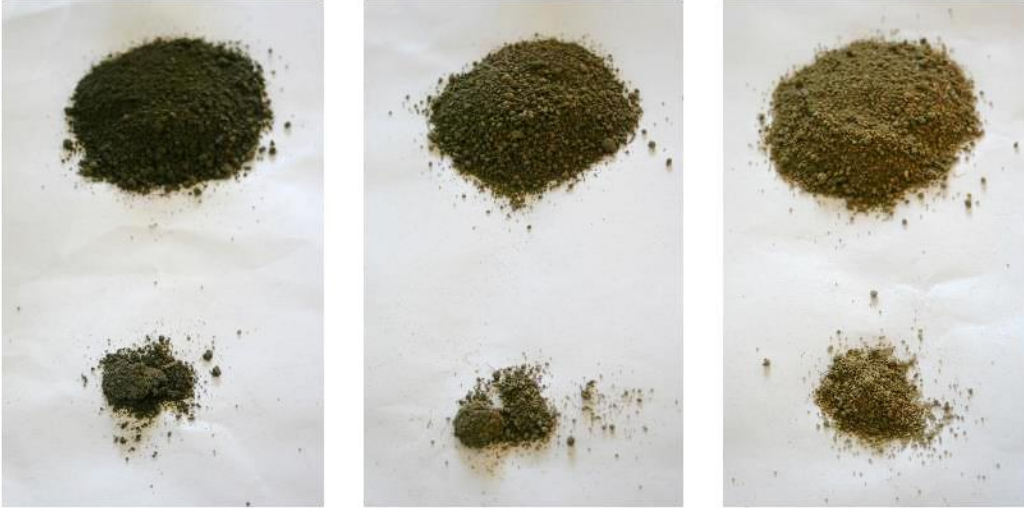
III. **Most crumbs moist**. The loamy sand no longer adheres when pressed, while the clay loam and high organic matter soil still adhere a little bit, about half sticks together. Sieving is now relatively easy.

IV. **Most crumbs dry**: Aggregates are still moist inside. When pressed together, there is no adhesion between sieved crumbs. Sieving is now very easy without soil clogging the sieve.

V. **Air dry**: Samples have dried until they appear completely dry, though many days or weeks have not passed, and the soil has not been dried at higher temperatures.

VI. **Air dried, many days or heated**: The soil has been in dry conditions for many days to months, or has been heated for drying, at 40 to 45 degrees C for example. Usually no adjustment is made for moisture.

Addition for high organic matter soils: for soils that are visible very high in organic matter, with a notable dark color or organic matter higher than 5%, the amounts given in the table based on texture should be augmented by a few percentage points, as shown in the last line of the table.

Pictures of the different stages:

High-organic matter silty clay loam	Silty Clay Loam	Loamy Sand
I. Very moist:		

Wet clump (5-15mm) from handling and soil sticking together   Loose (spooned)

Pressed with fingers and thumb

II. Moist

Loose (spooned)

Pressed with fingers and thumb

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III.   Most crumbs moist

IV.   Most crumbs dry
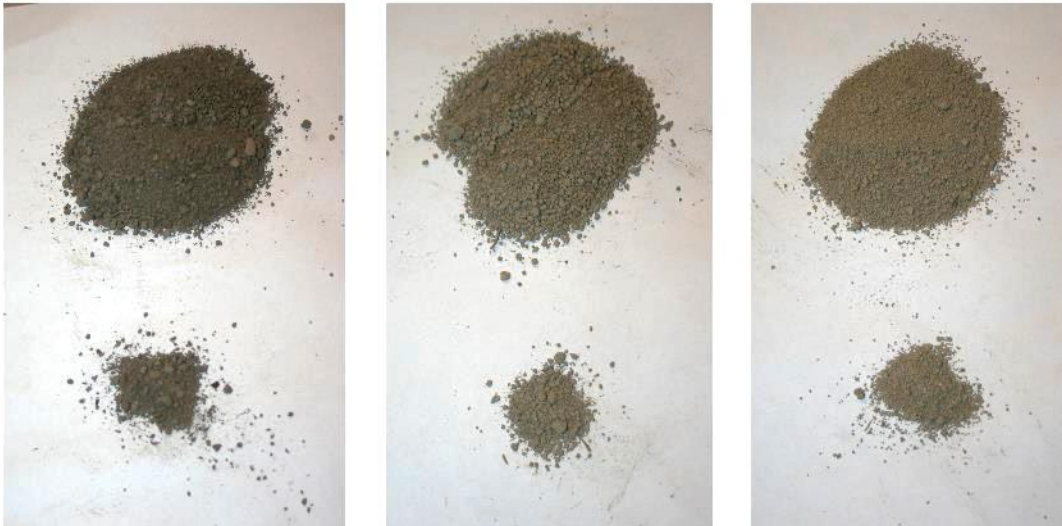
V.   Air dry:

Appendix B: Simplified Key to Macrofauna.

Adapted from the FAO/IRD macrofauna field manual, check the link for the original: http://ftp.Fao.org/docrep/fao/011/i0211e/i0211e.pdf

The key Identifies most macrofauna to the level of order:

WITHOUT LEGS:
1. **WITHOUT LEGS, NON-SEGMENTED**, clear head with antennae; **MOLLUSCA**
    a) With Shell: **Snails** (Fig. 1)
    b) Without a shell: **Slugs** (Fig. 2)

Fig. 1. Snails   Fig. 2. Slugs

2. **WITHOUT LEGS, SEGMENTED**
    a) *WORM - LIKE*,
        ➢ More than 15 body segments, pigmented:
            Earthworms (most >20 mm long) – (Fig. 3)
        ➢ Suckers at both ends of a flattened body:
            Hirudínea (leeches) (Fig. 4)

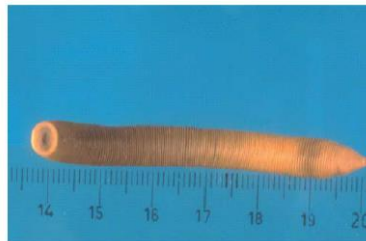   Fig.3: Earthworm   Fig.4: Hirudinea (leeches)

    b) *Not worm-like*, fewer than 15 segments
        ➢ Beetle Larvae (**Coleoptera**), generally with strongly developed head capsule (well developed coronal structure). Often U-shaped and more or less swollen . (Fig. 5)
        ➢ Fly larvae (**Diptera**), often without strongly developed head capsule. Long and thin, not U-shaped (Fig. 6)

Fig.5: Beetle Larvae (coleóptera)

Fig.6: Fly Larvae (diptera)

1. **WITHOUT WINGS:**
   3 Pairs of Legs (INSECTS).
   A. Caterpillar-Like, Soft bodied
   ➢ With Pseudo-legs (four pairs or fewer):
   Larvae of **Lepidoptera** (larvae of butterflies and moths, Fig. 7) these lepidopteran caterpillars have both true legs found on the thorax and pseudolegs (prolegs) found on the abdominal segments:
   ➢ Without pseudo-legs, and 3 pairs of legs on thorax:
   Larvae of **Coleoptera** (beetles; frequently U-shaped "grubs") - (Fig. 8)

Fig.7: Lepidoptera Larvae (butterflies and moths)   **Fig.8**: Beetle Larvae, "grubs"

   B. Abdomen > 6 segments and > 4 segmented antennae
   ➢ Conspicuous pronotum (the upper dorsal plate of the first segment of thorax):
      i. Pronotum saddle-shaped, not projecting forward:

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Orthoptera (grasshoppers) (Fig. 9)
ii.   Pronotum not saddle-shaped, projecting forward over head :
Blattaria (cockroaches) (Fig. 10)

Fig.9: Orthoptera

Fig.10: Blattaria

➢ Pronotum not conspicuous (the upper dorsal plate of the first segment of thorax):

i. Mouthparts formed into sucking tube held under body, no palps: **Hemiptera** (such as lace bugs, aphids and woodlice (Fig. 11)

Fig.11: Hemiptera, note sucking tube

ii. No sucking tube, palps: Abdomen ends in a **certain number of cerci** (paired appendages on the rear-most segments of many arthropods) Cerci either:

a) **2 cerci**

❖ Curved into pincers: **Dermaptera** (earwigs) (Fig.12)
❖ Long and thin, at least 1/3 length of abdomen, projecting from tip, Antenna short: < 2 x head width: **Coleoptera larvae** (Fig. 13)

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Fig. 12. Dermaptera (earwigs)

❖ Short cerci and may be located forward of the tip of the abdomen:
- o Antennae long, 8 segments:
  Isoptera (changed to Blattaria, recently**)** blind poorly pigmented, sometimes with large mandibles [soldiers], legs fully developed, tropics and subtropics) (Fig. 14)
- o **Antennae short**, <6 segments: beetle larvae, flat, short antennae (<8 segments) (Fig. 15)

Fig.13: Coleoptera Larvae

Fig.14: Isoptera (now Blattaria)

Fig.15: Coleóptera larvae – cerci (beetles)

b. **Without cerci**; with a certain number of antennal segments: (see number of segments below):

❖ <6 antennal segments, with 3 clear thoracic segments : Larvae of coleoptera (beetles; Fig. 16)

Fig.16: Coleoptera (beetle) thorax (three clearly defined segments behind the head)

❖ More than 10 segments in antennae, with a wasp-type waist (very narrow):

❖ Waist with 1 to 2 petioles: **Ants** (Fig. 17)
❖ Waist without petioles: other **Hymenoptera** (bees y wasps)

❖ More than 10 segments in the antennae, without narrow wasp-type waist

❖ Long and thin: **Phasmida** (walking sticks and leaf insects) (Fig. 18)
❖ Small insects, with relatively long antennae: **Psocoptera** (bark lice) (Fig. 19)
❖ With short antennae: beetle larvae or wingless adults (Fig.20)

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Fig.17: Ants; at right, one or two petioles connect the thorax and abdomen

Fig.18: Phasmida

Fig.19: Psocoptera

Fig.20: Beetle larvae or wingless adults

4 pairs of legs: Arachnida

(sometimes pedipalps –the second pair of appendages of the head and thorax section– look like an extra pair of legs)

A. Thorax and abdomen separated by a constricted waist, pedipalps without claws:
Spiders (Fig. 21)

B. Thorax and abdomen fused into one, without pedipalps-
  ➢ Body clearly segmented, with ocularium (eye-area tubercle):
    Opilions (very similar to spiders) (Fig. 22)
  ➢ Body not segmented, without ocularium: **Acarina** (mites and ticks) (Fig. 23)

Fig.21: Spiders

Fig.22: Opilions

Fig.23: Acarina (mites y ticks)

C. Pedipalps with claws or pincers
   ➢ Large claws, telson (sting): **Scorpions** (Fig. 24)
   ➢ Small claws, without telson (sting): Pseudoscorpions (Fig. 25)

Fig.24: Scorpions

Fig.25: Pseudoscorpions

6 or 7 pairs of legs: Isopoda (Fig. 26)

Fig.26: Isopoda

More than 15 pairs of legs:

A. One leg pair per body segment: **Chilopoda** (centipedes, generally flattened body) (Fig. 27)

Fig.27: Chilopoda

B. Two leg pairs per body segment: **Diplopoda** (**millipedes**), generally the body is more round than flat, usually >30 pairs of legs (Fig. 28)

Fig.28: Diplopoda

A. Only two wings, no appendage: Diptera adults (flies)

(with halteres –small knobbed paired structures near wings) (Fig. 29)

Fig.29: Diptera

B. 4 wings

➢ Mouthparts modified into sucking tube, no palps: **Hemiptera** (Fig. 30)

Fig.30: Hemiptera

➢ Biting mouthparts, palps:
❖ Forewings hardened to form a wing case:
o Hind legs long:
i. Hind legs modified for jumping, head not partially covered by pronotum: **Orthoptera** (Fig. 31)
ii. Hind legs not modified for jumping, head partially covering pronotum: **Blattaria** (Fig. 32)

Fig.31 Orthoptera

Fig.32: Blattaria

o Hind legs short:
   i. Abdomen with terminal pincers : **Dermaptera** (Fig. 33)
   ii. Without terminal pincers: **Coleoptera** (beetles) (Fig. 34)

Fig.33: Dermaptera

Fig.34: Coleoptera (both left and right image))

❖ Forewings not hardened - hind legs modified for jumping ; pronotum saddle shaped : **Orthoptera** (Fig. 31)

➢ Other winged groups are rarely found in hand-sorted soil samples, but example is shown: **Hymenoptera**: bees and wasps (Fig. 35) and **Lepidoptera** (moths and butterflies).

Fig.**35**: Hymenoptera (Not commonly found in soils)