## 1. Objective

Soil aggregate stability test is a rapid assessment of the soil structure behavior under the effect of water, wind and management practices. Soil aggregate stability may be linked to many soil processes such as the composition of soil organic matter, biotic activity, infiltration capacity and resistance to erosion.

## 2. Reference


Herrick, J.E., Whitford, W.G., Soyza, A.G. de, Zee, J.W.V., Havstad, K.M., Seybold, C.A., Walton, M., 2001. Field soil aggregate stability kit for soil quality and rangeland health evaluations. Catena 44, 27–35.


## 3. Hazard and security

None.

## 4. Principle

The principle is based on a visual scoring of aggregate stability after a given time of immersion and dipping cycles in water.

## 5. Material

- 2 plastic plates
- Trowel
- Structural stability box with 18 small sieves (Herrick et al. 2001)
- Tap water

## 6. Operating mode

a) Aggregate sampling

- Collect 6 soil aggregates of about 6-8mm diameter from soil surface (0-2 cm) at each sampling point to have in the end 18 aggregates per plot (6 aggregates x 3 inner-replicates)
- Repeat the same procedure for aggregates of the 2-10 cm depth.
- Spread the aggregates on a plastic plate in order to have only one depth and wait about one hour to air-dry the samples

b) Preparation and score attribution

- Put the 18 aggregates from the surface layer (0-2 cm) into the sieves n°1-18

Preparation of the 18 sieves in the field

- Fill a plastic box with tap water to have around 2 cm water level in the box

• *From $T_0$ to $T_{+5min}$*

During the first 5 min, the samples are rated based on the time to slaking. For each sieve, a score from 0 to 3 is given depending on the aggregate stability (see table below, Herrick et al., 2001).

Immerse one sieve every 15s, starting with sieve n°1.

Immersion of the sieve in the water

Give a score about the structural integrity of the soil aggregate (0-3) according to the table below (Herrick et al., 2001).

• *From $T_{+5min}$*

Lift up and down each sieve 5 times with a 2 sec rhythm (start from sieve n°1 to 18)

Give the score of 4, 5 or 6 depending of the soil remaining in the sieve (table below, Herrick et al., 2001).

Table for the scoring (Herrick et al., 2001)

Criteria for the assignment of crust fragments to stability classes

| Stability class | Criteria for assignment to stability class (for Standard Characterization)[a] |
|---|---|
| 0 | Soil too unstable to sample (falls through sieve)[b] |
| 1 | **50%** of structural integrity lost within **5** s of insertion in water |
| 2 | **50%** of structural integrity lost **5–30** s after insertion |
| 3 | **50%** of structural integrity lost **30–300** s fter insertion **or** < **10%** of soil remains on sieve after five dipping cycles |
| 4 | **10–25%** of soil remains on sieve after five dipping cycles |
| 5 | **25–75%** of soil remains on sieve after five dipping cycles |
| 6 | **75–100%** of soil remains on sieve after five dipping cycles |

- Repeat the “b) Preparation and score attribution part” for the soil aggregate (2-10cm depth)

## 7. Results

**AggSurf** (0-2cm aggregate stability at a sampling point) =
AVERAGE(score of the 6 surface aggregates at the sampling point)

**AggSoil** (2-10cm aggregate stability at a sampling point) =
AVERAGE(score of the 6 soil aggregates at the sampling point)

## 1. Objective

The Beerkan test assesses the soil infiltration rate *in situ*.

## 2. References

Adapted from:
Lassabatère, L. et al., 2006. Beerkan Estimation of Soil Transfer Parameters through Infiltration Experiments—BEST. Soil Science Society of America Journal 70, 521–532.

Also applied in:
Grimaldi, M. et al., 2014. Ecosystem services of regulation and support in Amazonian pioneer fronts: searching for landscape drivers. Landscape Ecol 29, 311–328.

## 3. Hazard and security

None.

## 4. Principle

A defined and fixed volume of water is poured in a single cylinder at the soil surface. The time for each water volume to infiltrate is recorded. The water infiltration rate in ml/minute is then calculated using the slope of the steady state of the infiltration curve.

## 5. Material

- Plastic cylinder; ∅ 20 cm
- Piece of wood
- Hammer
- Scissors
- Pierced plastic bag
- Stopwatch
- Several small plastic bottles marked at 310mL
  *(volume corresponding to 1cm water level in the cylinder)*
- Big filled water tanks

## 6. Operating mode

a) In the field

- Insert the plastic cylinder into the soil (around 1cm depth) with the hammer and the piece of wood
- In the cylinder zone, remove the litter carefully without breaking the casts and cut the cover crops/weeds without removing the roots

Insert the cylinder at 1 cm depth into the soil

- Fill at least two bottles of 310mL with water
- Put a pierced plastic bag in the cylinder zone in order to avoid "splatch" effect when pouring the water
- Pour one bottle in the cylinder and start the stopwatch

Pouring water on the plastic bag

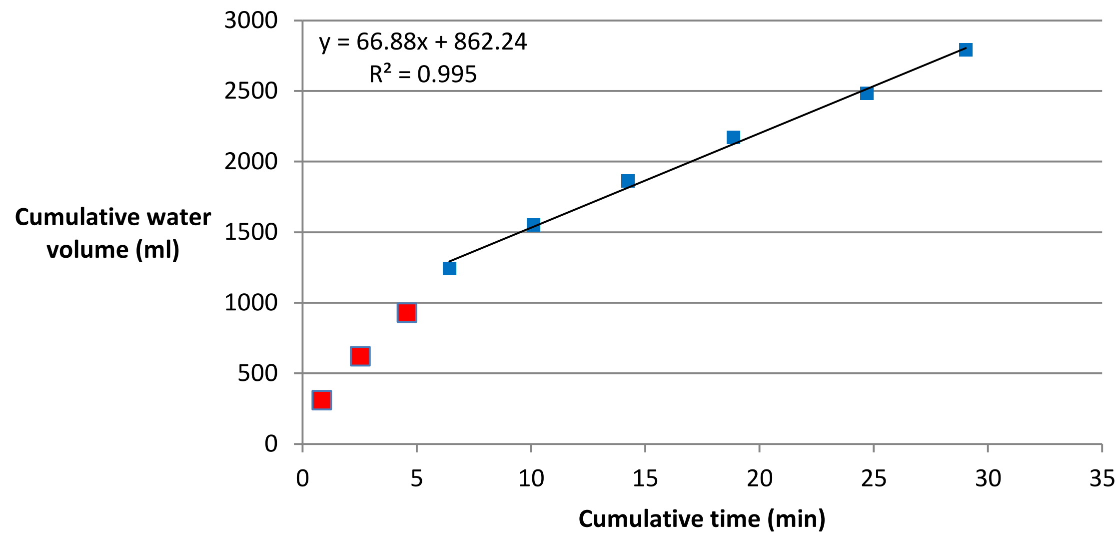
- Note the time when the water volume is completely infiltrated
- Pour the second bottle of water into the zone just after the 1st volume is totally infiltrated

Repeat this procedure until one of this time limit:

- time of the experiment is over 30 minutes
- 10 bottles have been poured

b) Score calculation

- Transform the time in a 2-decimals number expressed in minute (e.g. 2min 30sec = 2.50min)
- Express the cumulated volume as a function of the cumulated time
- Remove visually the transitional phase and keep only the steady state (see the color of the points in the figure below, red points=transitional phase; blue points=steady state).



- The slope of the regression of the permanent phase corresponds to the water infiltration rate in ml/min (66.88 in the example above)
- Sort the results in different classes depending on the slope values:

| Range of water infiltration rate (ml/min) | **Class score** |
|---|---|
| 0-10 | **1** |
| 10-20 | **2** |
| 20-30 | **3** |
| … | **…** |

## 7. Results

**Beerkan** = **class score** values

## 1. Objective

The method aims at quickly assessing the soil structure linked to the biological assemblages in the field, classifying the soil structure of each layer into five scoring classes.

## 2. Reference

Guimarães, R.M.L., Ball, B.C., Tormena, C.A., 2011. Improvements in the visual evaluation of soil structure. Soil Use and Management 27, 395–403.

## 3. Hazard and security

None.

## 4. Principle

The principle is based on the appearance and the feel of a block of soil. Each layer receives a score depending on several parameters (see Table p 2/2; Guimarães et al., 2011). A final score is attributed to the whole block by making a weighted average of the different layers scores.

## 5. Material

- Spade
- Printed chart
- Meter tape or 30cm ruler

## 6. Operating mode

a) Sampling method

- Remove a block of 30x30x25 cm of soil at each sampling point using a spade.
- Distinguish the different soil layers according to the chart (p 2/2).

VESS block in the field

b) Score calculation

- Give a score to each layer following the table on page 2/2.

## 7. Results

$$\text{VESS} = \frac{\sum(\text{Layer score} * \text{Layer depth})}{\text{Total depth of the block}}$$

(depth expressed in cm)

**From Guimarães et al., 2011**

| Structure quality | Size and appearance of aggregates | Visible porosity and Roots | Appearance after break-up: various soils | Appearance after break-up: same soil different tillage | Distinguishing feature | Appearance and description of natural or reduced fragment of ~ 1.5 cm diameter |
|---|---|---|---|---|---|---|
| **Sq1 Friable** Aggregates readily crumble with fingers | Mostly < 6 mm after crumbling | Highly porous Roots throughout the soil | | | Fine aggregates | 1 cm The action of breaking the block is enough to reveal them. Large aggregates are composed of smaller ones, held by roots. |
| **Sq2 Intact** Aggregates easy to break with one hand | A mixture of porous, rounded aggregates from 2mm - 7 cm. No clods present | Most aggregates are porous Roots throughout the soil | | | High aggregate porosity | 1 cm Aggregates when obtained are rounded, very fragile, crumble very easily and are highly porous. |
| **Sq3 Firm** Most aggregates break with one hand | A mixture of porous aggregates from 2mm -10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present | Macropores and cracks present. Porosity and roots both within aggregates. | | | Low aggregate porosity | 1 cm Aggregate fragments are fairly easy to obtain. They have few visible pores and are rounded. Roots usually grow through the aggregates. |
| **Sq4 Compact** Requires considerable effort to break aggregates with one hand | Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm | Few macropores and cracks All roots are clustered in macropores and around aggregates | | | Distinct macropores | 1 cm Aggregate fragments are easy to obtain when soil is wet, in cube shapes which are very sharp-edged and show cracks internally. |
| **Sq5 Very compact** Difficult to break up | Mostly large > 10 cm, very few < 7 cm, angular and non-porous | Very low porosity. Macropores may be present. May contain anaerobic zones. Few roots, if any, and restricted to cracks | | | Grey-blue colour | 1 cm Aggregate fragments are easy to obtain when soil is wet, although considerable force may be needed. No pores or cracks are visible usually. |

0 1 2 3 4 5 10 15 cm

## 1. Objective

The aim of the method is to assess the dynamic of the available nutrients in the soil using an exchangeable membrane that easily adsorb nutrients in a solution.

## 2. References


Qian, P., Schoenau, J.J., 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. Canadian Journal of Soil Science 82, 9–21.

Saggar et al., 1990. A simplified resin membrane technique for extracting phosphorus from soils. Fertilizer Research 24, 173–180.


## 3. Hazard and security

Wear nitrile gloves during the laboratory steps. In case of the use of a biocide, safety glasses must be worn. The biocide must be prepared under a chemical fume hood (fume cupboard) and handling protection should be adapted to the hazard.

## 4. Principle

The principle is to charge a membrane with an elution step, before burying it into the soil **(1).** The membrane exchanges its ions with the ions from the soil solution **(2).** When the contact has been enough established, the membrane is removed from the soil **(3)** and desorbed within a strongly charged solution **(4).** The ions targeted ($NO_3^-$ in the example of the figure 1) are analyzed quantitatively **(5).**

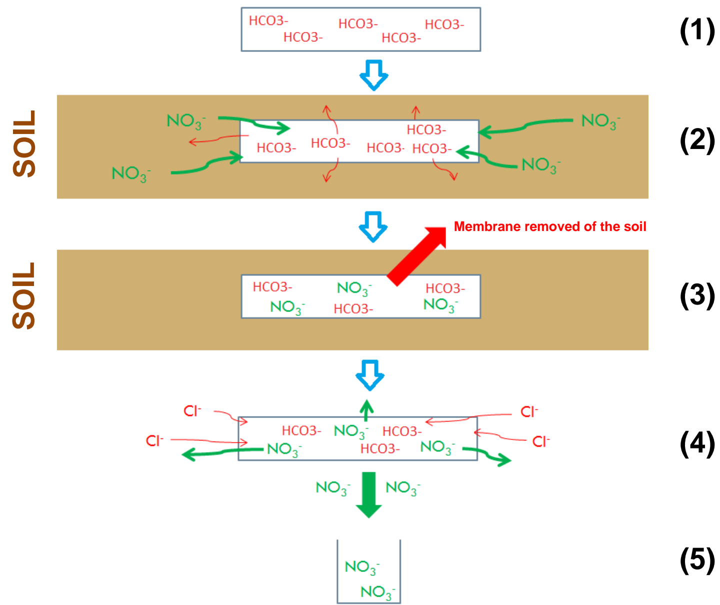



Figure 1: Principle of the Ion Exchange Membrane with the example of an anion membrane charged with $NaHCO_3$ solution and eluted with KCL solution to target $NO_3^-$ anions

## 5. Material

| Before the field, in the **laboratory**<br>(for a set of 72 anion exchange membranes) | In the **field** | After the field, in the **laboratory**<br>(for a set of 72 anion exchange membranes) |
|---|---|---|
| **A- Membrane preparation and elution**<br>- Sodium Bicarbonate ($NaHCO_3$; FW=84.00 g.mol$^{-1}$; n° CAS : 144-55-8)<br>- 1 pack of Anion Exchange Membrane leaves (VWR – 6x125mmx125mm)<br>- ≈ 9L of distilled water<br>- 2 glass slabs<br>- 8L solution container *(can be split in smaller volumes)*<br>- Clean graduated ruler<br>- Cutter with clean blade<br>- Nitrile gloves<br>- Thin indelible felt pen<br>- Thin fishing line<br>- Needle<br>- Scissors<br>- 72 zip bags (around 8x4cm)<br>- Cool box with ice packs<br>- Aluminum foil<br><br>**B- Extraction solution preparation**<br>- 72 Plastic conical tubes (50mL)<br>- Potassium Chloride (KCl; FW=74.55 g.mol$^{-1}$; n° CAS : 7447-40-7)<br>- ≈4L distilled water<br>- Laboratory glassware to prepare solutions | - 1 Trowel<br>- 72 colorful stakes<br>- 1 Thin tweezer<br>- PVC Cylinder (Ø≈10cm)<br>- Hammer<br>- Piece of wood<br>- ≈10L distilled water *(74x3cm water level of the PVC cylinder)*<br>- 72 plastic tube with KCl solution **(B)**<br>- Scissors<br>- 1 Tooth brush | **C- Membrane extraction**<br>- Ruler<br>- 72x10mL syringes<br>- Sterile needles<br>- 72x0,2µm diameter filters (*or filter paper*)<br>- 72x10mL vacutainer tubes without additives<br>- Nitrile gloves<br>- Automatic horizontal shaker |

## 6. Operating mode

a) In the laboratory, membrane preparation and elution **(A)**

*i. Prepare the elution solutions*

- Prepare a 0.5M $NaHCO_3$ solution in the solution containers (around 2L of solution for 20 membranes)

*ii. Cut the membrane leaves*

(Wear nitrile gloves, to avoid any contact with the membrane)
- Put the leaf on a glass slab and water it with distilled water
- Mark zones with a felt pen and the ruler to have 60x20mm membranes
- Cut the membrane leaf with the cutter. To make straight cutting, use the second glass slab.
- Cut 30cm piece of fishing line
- Pierce on top of the membrane with the needle and tie the fishing line to the membrane

*iii. Membrane charging up - elution*

- Put the membrane with the line in the elution solutions.
- Leave the membranes during at least 24h
- Check as often as needed that the membranes are not aggregating by mixing the solution

b) In the laboratory, extraction solution preparation (B)

- Prepare 1M KCl solution
- Pour 35mL of KCl solution in the plastic conical tube[1]. Prepare as many conical tubes as membrane buried in the soil

c) Membrane preparation before going to the field (maximum 24h before putting it in the field)

- Put the membrane into individual zip bag, labelled with the sampling zone
- Keep the membranes cold by putting the zip bag into a cool box with iced surrounded by aluminum foil to avoid direct contact between the ice and the membrane
- The membrane should not be kept in the zip bag for a long time, plan the field burying the day after maximum

d) Membrane drop-off in the field

- Dig a hole at 8cm depth
- Take the membrane from the zip bag with the tweezer

Example of anion membrane, ready to be buried in the soil

[1] To prevent bacterial degradation of nitrate prior to analysis, a biocide was added to the solution. Cold storage or microfiltration could also be used at this step depending on field conditions and resource availability.

- Put the membrane horizontally into the hole.
- Close the hole gently with soil
- Tie the fishing line to the stake
- Insert the plastic cylinder into the soil (around 1cm depth) with the hammer and the piece of wood
- Pour 3cm of water level in the cylinder
- Wait around two weeks (on wet season)

e) Membrane get back from the field

- Take the membrane out following the fishing line – avoid membrane disturbances as much as possible
- Try to remove the bigger soil particles by shaking the membrane or brush it gently with the tooth brush
- Put the membrane into the prepared KCl solution tube **(B)**
- Cut the fishing line with the scissors

f) Membrane extraction in the laboratory **(C)**

- Shake the conical tube using an automatic shaker, during 16h at 100 rpm at 30°C
- Take small volume of supernatant using a syringe and threw it away in order to clean the syringe
- Adapt the 0.2µm filter to the syringe
- Take 10mL of supernatant with the syringe.
- Inject the solution in the vacutainer tube through the septum with the needle

- Measure the remaining membrane surface after extraction step

g) Ions analysis

Analyze in the laboratory, the anions nutrients targeted in the solutions

## 7. Results

$$\textbf{AEMNO3}\ (\mu gN\text{-}NO_3^{-}.cm^{-2}.d^{-1}) = \frac{a \times b}{c \times d}$$

a = result of the laboratory quantitative analysis ($\mu g.L^{-1}$)
b = volume of extraction solution (L); b=0.035L in our case
c = surface of the exchange membrane
d = incubation time in the soil (days)

## 1. Objective

The aim of this analysis is to measure the soil available nitrogen per mass of soil.

## 2. Reference

Maynard, D.G., Kalra, Y.P., 1993. Nitrate and Exchangeable Ammonium Nitrogen. In: Carter, M.R., Ed., Soil Sampling and Methods of Analysis, Lewis Publishers, Boca Raton.

## 3. Hazard and security

Wear nitrile gloves during the laboratory steps. When a biocide is added, safety glasses must be worn. The biocide has to be prepared under a chemical fume hood (fume cupboard) and protection adapted to the hazard must be worn.

## 4. Principle

The principle is to measure available nitrogen through a soil nutrient extraction. The extraction is directly done in the field with a 1M KCl solution. Nutrient quantitative analysis of the solution is done in the laboratory.

## 5. Material

| In the **laboratory**<br>(for one sampling point) | In the **field** |
|---|---|
| - Potassium Chloride<br>(KCl; FW=74.55 $g.mol^{-1}$)<br>- Distilled water<br>- 1x250mL bottles<br>- 1x10mL syringes<br>- 1xSterile needles<br>- 1x300mL aluminium box<br>- 1x0.2µm diameter filters or filter paper<br>- 1x10mL vacutainer tubes without additives<br>- Laboratory glassware to prepare solutions<br>- Horizontal shaker<br>- 0.01 decimal scale<br>- 105°C oven<br>- Nutrient analyser | - Soil sampling cylinder<br>- 5mm sieve<br>- 0.01 dec. portable scale<br>- Funnel<br>- Aluminum box |

## 6. Operating mode

a) Preparation in the laboratory

- Weigh a 250mL plastic bottle with the lid (**P0**) and number it
- Add 180mL of 1M KCl solution [1]
- Cover and weigh again (**P1**)

[1] To prevent bacterial degradation of nitrate and ammonium prior to analysis, a biocide was added to the solution. Cold storage or microfiltration could also be used at this step depending on field conditions and resource availability.

b) In the field

- Collect soil sample at a depth of 0-10cm using a soil sample cylinder
- Sieve the soil at 5mm
- Take around 50g of sieved soil, record the exact mass, and put the soil in the 250mL bottle with the solution using the funnel

c) Extraction in the laboratory

- Weigh the bottle containing the KCl and the soil (**P2**)
- Shake the bottle for 1 hour with an automatic horizontal shaker (≈150rpm)
- Open the bottle and let soil settle for at least 1h
- Take small volume of supernatant using a 10mL syringe and throw it away in order to clean the syringe
- Adapt the 0.2µm filter to the syringe
- Take 10mL of supernatant with the syringe
- Inject the solution in the vacutainer tube through the septum with the needle

d) Ions analysis

Analyse in the laboratory, the anions or cations targeted in the solutions. In this case, NminSoil is the addition of $NO_3^-$ and $NH_4^+$ ions.

## 7. Results

**NminSoil** (mgN.kg$^{-1}$ soil) = Results of the lab analysis, adjusted with soil moisture

........................................................................................................................

**Soil humidity rate needs to complementary be measured**

**Shortly:**

a) In the field:
- take a 0-10cm soil sample using the soil sampling cylinder
- put the soil in the aluminum box and weigh the fresh mass (**$M_0$**)

b) In the laboratory:
- put the soil sample in the oven for 48h at 105°C
- take the mass of the sample again (**$M_1$**)

$$\mathbf{H}\ (\%) = \left(\frac{M0 - M1}{M1}\right) \times 100$$

## 1. Objective

The aim of the method is first to achieve a morpho-functional diagnosis describing the state of degradation of the litter at the soil surface and then to quantify the density of earthworm casts.

## 2. References

Adapted from:

Loranger, G., Ponge, J.-F., Imbert, D., Lavelle, P., 2002. Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. Biol Fertil Soils 35, 247–252.

Ponge, J.-F., Chevalier, R., Loussot, P., 2002. Humus Index : an integrated tool for the assessment of forest floor and topsoil properties. Soil Science Society of America Journal 66, 1996.

Thomas, F., Rossi, J.-P., Decaëns, T., Grimaldi, M., Lavelle, P., Fernando da Silva Martins, P., Garnier-Zarli, E., 2008. Comparative analysis of Andiodrilus pachoensis casts in forests and pastures of South-Eastern Amazon (Brazil). European Journal of Soil Biology, Special Section of the 7th International Apterygota Seminar 44, 545–553.

Zanella, A., et al., 2017. Humusica 1, article 4: Terrestrial humus systems and forms—Specific terms and diagnostic horizons. Applied Soil Ecology, HUMUSICA 1 - Natural terrestrial humus systems 122, 56–74.

## 3. Hazard and security

None.

## 4. Principle

The mass of different degradation state of the litter, linked to different biotic assemblage activity is evaluated. The earthworm cast density at the soil surface is also quantified, as a proxy of earthworm activity.

## 5. Material

| In the **field**<br>(for 1 sampling point) | In the **laboratory** |
|---|---|
| - 4 envelopes labeled as “entire”, “skeleton”, “fragment”, “cast”.<br>- 25x25 cm frame<br>- Trowel<br>- Scissors/cutter<br>- 1 big plastic plates or tray | - 0.01 dec. scale<br>- 60°C oven |

## 6. Operating mode

a) In the field

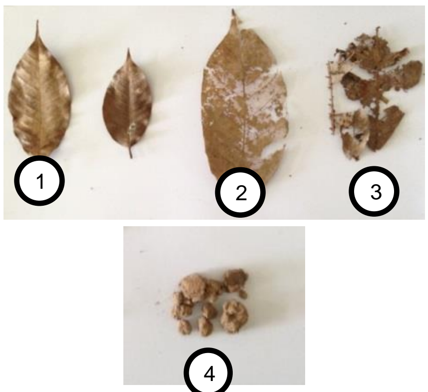
- Put the frame at the soil surface
- Clear the litter around the frame with the trowel to isolate the studied zone
- Remove the frame and cut the above ground fresh living biomass

Studied zone isolated litter is ready to be sorted on the tray

- Put all the litter layers on the plastic plate or tray
- Sort the litter into 3 envelopes following the categories below:

  **(1)** "Entire": between 50 to 100% of leaf area remaining, including the one artificially cut during sampling.
  **(2)** "Skeleton": >50% of the leaf area (the entire leaf, or just a part if it is fragmented) is skeletonized.
  **(3)** "Fragment": <50% of leaf area remaining.

- Sample all the cast at the soil surface and put it in the 4th envelope
  **(4)** "Cast"




b) In the laboratory

- Dry the samples at 60°C during at least 48h
- Weigh each category ("Entire", "Skeleton", "Fragment", "Cast") individually with the decimal scale and record the weight.

## 7. Results

$$\textbf{Cast}\ (\text{g.m}^{-2}) = \frac{\text{Weight of dry casts } (\mathbf{4})}{\text{Surface sampled}}$$

$$\textbf{Fragment}\ (\text{score}) = \frac{\text{Fragment leaves } (\mathbf{3})}{\text{Total leaves } (\mathbf{1+2+3})}$$

$$\textbf{Skeleton}\ (\text{score}) = \frac{\text{Skeleton leaves } (\mathbf{2})}{\text{Total leaves } (\mathbf{1+2+3})}$$

## 1. Objective

The bait lamina test aims at assessing the soil biological activity through the decomposition of a substrate in contact with the soil.

## 2. References


Gestel, C.A.M. van, Kruidenier, M., Berg, M.P., 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. Biol Fertil Soils 37, 115–123.

von Törne, E., 1990. Assessing feeding activities of soil-living animals. I: Bait lamina- tests. Pedobiologia 34, 89–101.


## 3. Hazard and security

Thermal gloves and safety glasses when using the heater.

## 4. Principle

The bait lamina is a strip with 16 small holes filled with a substrate. The strips are vertically inserted in the soil. After a time of incubation, the baits are removed and a score is given based on the remaining substrate. The decomposition of the substrate in the holes is directly associated to the feeding activity of soil biota.

## 5. Material

| In the **laboratory** | In the **field**<br>(for 1 sampling point) |
|---|---|
| - Agar<br>- Cellulose (CAS n° : *9004-34-6)*<br>- Bait lamina sticks<br>- Container to prepare the paste (e.g. petri dish)<br>- Tap water<br>- 250mL beaker<br>- Heating magnetic stirrer<br>- Thermometer<br>- 2 decimals scale<br>- Oven 30-40°C | - 8 baits lamina sticks filled with the substrate<br>- Knife |

## 6. Operating mode

a) <u>In the laboratory, filling the lamina baits</u>

- Boil 100mL of tap water with 1g of agar in a 250mL beaker (100°C)
- Turn down the heating to reach a temperature of 60°C
- Put some cellulose in a petri dish
- Add the agar solution drop by drop
- Try to make a homogenous paste
- Fill the holes with the paste by pulling the strips between the thumb and finger with the paste
- Dry the first layer one night at 30-40°C in the oven

- Fill in a second layer of paste and dry it again one night
- Repeat this procedure several times (3-5 layers) to fill completely the holes
  *(to check that the lamina is well filled, try to see if a ray light can go through the supposed filled holes).*

b) In the field

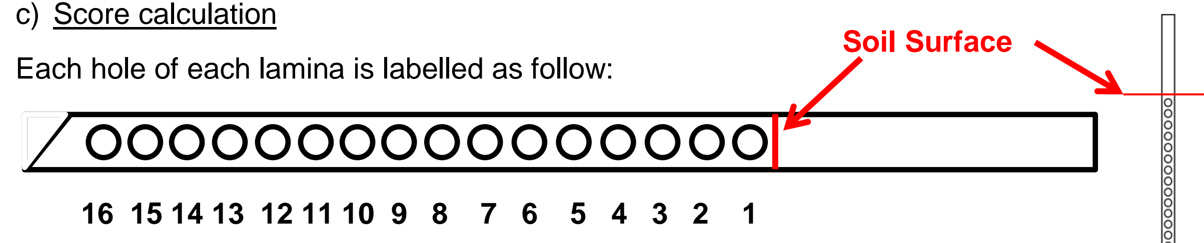
- Dig a crack in the soil with the knife
- At each sampling point, insert 8 bait lamina following a straight line with 30cm space between two sticks in the soil until the last hole is buried

Bait lamina in straight line buried in the soil

- Wait until ≈80% of the holes of one lamina is degraded
  *(about 2 weeks, depending on the pedo-climatic conditions and substrate)*
- At this date, remove all the laminas from the soil
- Attribute a score according to the substrate degradation.

c) Score calculation

Each hole of each lamina is labelled as follow:




A score is attributed to each hole depending of their decomposition state:

| State of the degradation | No degradation | Partial degradation | Full degradation | Soil aggregated |
|---|---|---|---|---|
| Score for analyses | **0** | **0.5** | **1** | **S** (NA in stats) |

## 7. Results

$$\text{Score for each lamina (a) } (\%\text{deg.d}^{-1}) = \frac{\text{AVERAGE(16 holes of each lamina)}}{\text{Incubation time (day)}}$$

**Lamina (%deg.d$^{-1}$)** = AVERAGE (8 scores for the 8 lamina (a) per sampling point)

## 1. Objective

The aim of this method is to estimate the labile fraction of the soil organic carbon.

## 2. References


Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012a. Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. Soil Science Society of America Journal 76, 494–504.

Weil, R.R., Islam, I.R., Stine, M.A., Gruver, J.B., Samson-liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. American Journal of Alternative Agriculture 3–17.


## 3. Hazard and security

Wear nitrile gloves and safety glasses during the laboratory steps, and when manipulating the $KMnO_4$ solution.

## 4. Principle

Weil et al. (2003) developed a simple method of estimating changes in POXC with a $KMnO_4$ solution. The dilute $KMnO_4$ reacts with the most readily oxidizable forms of active C, converting Mn(VII) to Mn(II), and proportionally lowering absorbance. The reduction in absorbance is proportional to the labile fraction of carbon (POXC) in soil. The lower the absorbance is, the higher the amount of soil labile carbon there is.

**The protocol proposed by Weil et al. (2003) to measure the permanganate oxidizable carbon was adapted to conditions with low soil organic carbon (table 2 of the paper).**

**See the adjusted procedure in the field, p.3 of the protocol***

## 5. Material

| In the **laboratory** | In the **field** (for 18 sampling points) |
|---|---|
| - Potassium Permanganate ($KMnO_4$; FW=158.03g.mol$^{-1}$; CAS n°: 7722-64-7)<br>- Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$; FW=147.01g.mol$^{-1}$; CAS n°:10035-04-8)<br>- Sodium Hydroxyde (NaOH, FW=40g.mol$^{-1}$; CAS n°: 1310-73-2)<br>- 0.5mL micropipette with 50 cones<br>- 5mL pipette<br>- 20 plastic 4.5ml spectrophotometer macro-cuvettes<br>- 40 graduated conical tubes (50ml)<br>- Distilled water<br>- 0.01 dec. portable scale<br>- Portable spectrophotometer linked to a laptop or a datalogger with 550nm wave length filter (e.g. Vernier-SpectroVis)<br>- pH meter<br>- Magnetic stir plate and stir bars<br>- Laboratory glassware to prepare solution | - 10 containers to dry the soil<br>- 100 ml of 0.2*M* $KMnO_4$ solution<br>- 3 containers to weigh 2.5g of soil<br>- 1 plastic disposable 10mL syringues<br>- 1 plastic disposable 2 ml syringes<br>- 18 plastic disposable 0.5 ml syringes<br>- 1.5L distilled water<br>- 36 plastic conical tubes (50ml)<br>- 2mm sieve<br>- Soil sample cylinder<br>- Air dryer (12V to be plugged to the car)<br>- Portable spectrophotometer linked to a laptop or a datalogger with 550nm wave length filter (e.g. Vernier-SpectroVis)<br>- 19 plastic 4.5ml spectrophotometer macro-cuvettes (one for blanc)<br>- 0.01 dec. portable scale |

## 6. Operating mode

See Culman et al., 2012b, Procedure for the Determination of Permanganate Oxidizable Carbon, KBS POXC Protocol.

a) In the laboratory: preparation of the $KMnO_4$ stock solution

*For 1L of $KMnO_4$ stock solution (0.2M)*

- Weigh 147g of $CaCl_2.2H_2O$ and add 900mL of distilled water into a 1L beaker
- Stir until $CaCl_2.2H_2O$ is completely dissolved
- Transfer to a 1L volumetric flask or graduated cylinder
- Bring to 1L volume with distilled water **(1)**
- Weigh 31.60g of $KMnO_4$ and add 900mL of the $CaCl_2$ **(1)** solution into a 1000mL beaker
- Heat gently and stir until dissolved completely **(2)**
- Keep the **(1)** $CaCl_2$ remaining solution
- Prepare a 0.1M NaOH solution by dissolving 0.4g of NaOH in 100 mL distilled water
- Adjust the pH of the $KMnO_4$ solution **(2)** to 7.2 by adding 0.1M NaOH solution
- Once the pH is adjusted, pour the solution into a 1000mL volumetric flask or graduated cylinder and bring the volume to 1000mL with the $CaCl_2$ (1) solution.
- Transfer to a dark glass bottles in order to avoid solution degradation.

b) In the laboratory: preparation of the standard curve

**First dilution[3]**

Fill 17 conical tubes with 10mL of solution following the volume proportion of $KMnO_4$ and distilled water (Table 1[3]).

| Tube label number | $KMnO_4$ stock solution volume (ml) | Water volume (ml) | **Concentration (M)** |
|---|---|---|---|
| 1 | 0,100 | 9,900 | **0,002** |
| 2 | 0,125 | 9,875 | **0,0025** |
| 3 | 0,200 | 9,800 | **0,004** |
| 4 | 0,250 | 9,750 | **0,005** |
| 5 | 0,300 | 9,700 | **0,006** |
| 6 | 0,375 | 9,625 | **0,0075** |
| 7 | 0,450 | 9,550 | **0,009** |
| 8 | 0,500 | 9,500 | **0,01** |
| 9 | 0,550 | 9,450 | **0,011** |
| 10 | 0,625 | 9,375 | **0,0125** |
| 11 | 0,700 | 9,300 | **0,014** |
| 12 | 0,750 | 9,250 | **0,015** |
| 13 | 0,800 | 9,200 | **0,016** |
| 14 | 0,875 | 9,125 | **0,0175** |
| 15 | 1,000 | 9,000 | **0,02** |
| 16 | 1,500 | 8,500 | **0,03** |
| 17 | 2,000 | 8,000 | **0,04** |

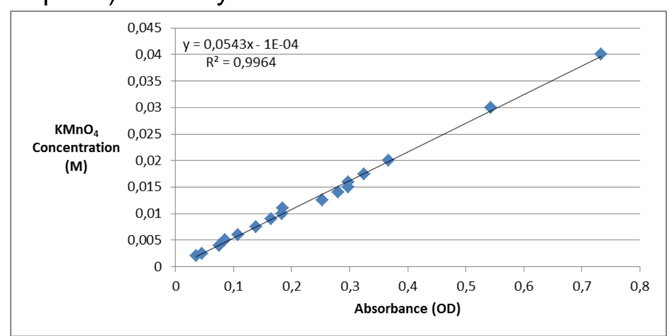
Table 1: Volume chart of the first dilution

**Second dilution**

- Pour about 40mL of distilled water in 17 conical tubes
- Add 0.50mL from each of the 10mL solutions prepared during the first dilution
- Add distilled water in the conical tubes to reach 50mL of solution
- Pour the solutions in 17 plastic macro-cuvettes
- Make the blanc of the spectrophotometer with a cuvette with distilled water
- Record the absorbance of the solution with the spectrophotometer at 550nm

[3] This number of solution may not be needed; this number of solutions was rather used for a training objective. Follow Culman et al. 2012b protocol for reduced number of solution for the calibration.

- Construct a standard curve with absorbance on the x-axis and concentration (of the last column of the Table 1 p 2/3) on the y-axis and extract the calibration equation as follow:




c) <u>In the field</u>

- Collect soil at 0-10cm depth, homogenize and sieve it at 2mm
- Put the soil in a container and dry it using a portable air-dryer
- Weigh 2.5g of dry soil using the portable scale
- Insert the soil into a 50mL plastic conical tube
- Add **first*** 2mL of $KMnO_4$ stock solution with the 2mL syringe in each tube **and then*** 18 mL of distilled water with the 10mL syringe. The water should be introduced 10 sec. max after the $KMnO_4$ was introduced.
- Close the tube and shake by hand for 2min following a strict and reproducible movement at a tempo of one shake per second
- Leave the tube for 10min
- Take 0.5mL of the supernatant solution with the 0.5mL syringe and transfer into a second 50mL conical tube
- Add distilled water until 50mL of solution
- Homogenize by slowly shaking twice
- Insert the solution in a cuvette with a 10mL syringe
- Make the blanc of the spectrophotometer with a cuvette with distilled water
- Measure the absorbance of the solution using the portable spectrophotometer at 550nm

## 7. Results

$$\mathbf{POXC = ([KMnO_4] - (\alpha + \beta * Abs)) \times C_{ox} \times (Vol_{KMnO4}/M_{soil})}$$

<u>Where:</u>

POXC ($mg.kg^{-1}$soil): soil labile carbon
[KMnO4] ($mol.L^{-1}$): concentration of the initial $KmNO_4$ solution *(0.02)*
$C_{ox}$ ($mg\ C.mol^{-1}$): amount of C oxidized by 1 mole of $MnO_4$ *(9000)*
$Vol_{KMnO4}$ (L): volume of the $KMnO_4$ solution reacted *(0.02)*
$M_{soil}$ (kg): the mass of soil *(0.0025)*
α: intercept of the standard curve
β: slope of the standard curve
Abs: absorbance read

## 1. Objective

SituResp® method assesses the soil biological activity through the carbon dioxide release from a fresh soil sample.

## 2. Reference

Thoumazeau, A., Gay, F., Alonso, P., Suvannang, N., Phongjinda, A., Panklang, P., Chevallier, T., Bessou, C., Brauman, A., 2017. SituResp®: A time- and cost-effective method to assess basal soil respiration in the field. Applied Soil Ecology 121, 223–230.

## 3. Hazard and security

Wear nitrile gloves and safety glasses during the laboratory steps. Add thermal gloves when handling the heated solutions.

## 4. Principle

The SituResp method is based on a 24h-incubation of a fresh soil sample with a pH sensitive color gel filled in a spectrophotometer macro-cuvette. The color of the gel changes along the incubation process as the result of the reaction of the $CO_2$ by the bicarbonate it contains. The change in the color of the gel can be measured with a spectrophotometer at 570nm.

## 5. Material

| In the **laboratory** | In the **field** |
|---|---|
| - Cresol Red<br>($C_{21}H_{18}O_5S$; FW=382.43 $g.mol^{-1}$; CAS n°: 1733-12-6)<br>- Potassium Chloride<br>(KCl; FW=74.55 $g.mol^{-1}$; CAS n°: 7447-40-7)<br>- Sodium Bicarbonate<br>($NaHCO_3$; FW=84.00 $g.mol^{-1}$; CAS n°: 144-55-8)<br>- Soda Lime or Sodium Hydroxyde<br>(NaOH; FW=40 $g.mol^{-1}$; CAS n°: 1310-73-2)<br>- Purified Agar for microbiology<br>(e.g. Sigma A1296; CAS n°: 9002-18-0)<br>- Distilled water<br>- Optical 4.5ml spectrophotometer plastic macro-cuvettes (1cmx1cmx4.5cm)<br>- Hot plate with magnetic stirrer<br>- Thermometer<br>- Laboratory precision scale (0.0001 dec.)<br>- Pipette able to pour 1.5mL<br>- Laboratory glassware to prepare solution<br>- Desiccator or 1L air tight jar | - 5mm sieve<br>- Trowel or soil sampling cylinder<br>- Paper, tissue or small open jar to prevent cuvettes surfaces from soil contact<br>- Airtight jar (around 250mL)<br>- 0.01 dec. portable scale<br>- Portable spectrophotometer linked to a laptop with 570mn length wave filter (SpectroVis)<br>- Plastic cube (1cmx1cmx1cm) to raise the cuvette in the spectrophotometer rack |

## 6. Operating mode

a) In the laboratory, one week before the experiment:

**Indicator solution (to prepare 1L of solution)**

- Using a precision scale weigh 16.77g of KCl, 0.315g of $NaHCO_3$ and 0.0187g of Cresol Red
- Put into a 1L volumetric flask and fill up to 1L with distilled water

*(Use this solution immediately or keep it maximum 2 month at 4°C)*

**Gel preparation (for 100 cuvettes)**

- Using a hotplate with a magnetic stirrer heat and stir 1.5g of purified agar in 50mL of water solution until full ebullition.

*(solution must reach at least 90°C, agar must be completely in fusion)*

- Dilute the agar solution with 100mL of the indicator solution and keep the solution at 60-65°C
- Fill the spectrophotometer plastic cuvettes with 1.5ml of gel solution at 60-65°C
- Let the cuvette cool-down at room temperature around 2h on a bench

Keep the cuvette in a desiccator in the dark at room temperature with excess of soda lime (or 200mL 1M NaOH) and water for one week. The color of the gel must turn from red to purple.

b) In the field:

- Collect around 150g of soil using a trowel or a soil sampling cylinder
- Sieve the soil at 5mm
- Put 100g of the sieved soil in the jar
- Make the blanc of the spectrophotometer with a cuvette with distilled water
- Read the absorbance (**$Abs_{T0}$**) of a cuvette at 570 nm

*(once removed from the desiccator the cuvette must be read immediately)*

- After reading $A_{T0}$, protect the reading surface of the cuvette with tissue, paper or insert it in a small open jar.
- Put the cuvette immediately in the air tight jar and close the jar
- Keep the jar 24h at ambient temperature.
- After 24h, read the absorbance (**$Abs_{T24}$**) of the cuvette with the spectrophotometer at 570 nm.

## 7. Results

$$\textbf{SituResp} = Abs_{T0} - Abs_{T24}$$