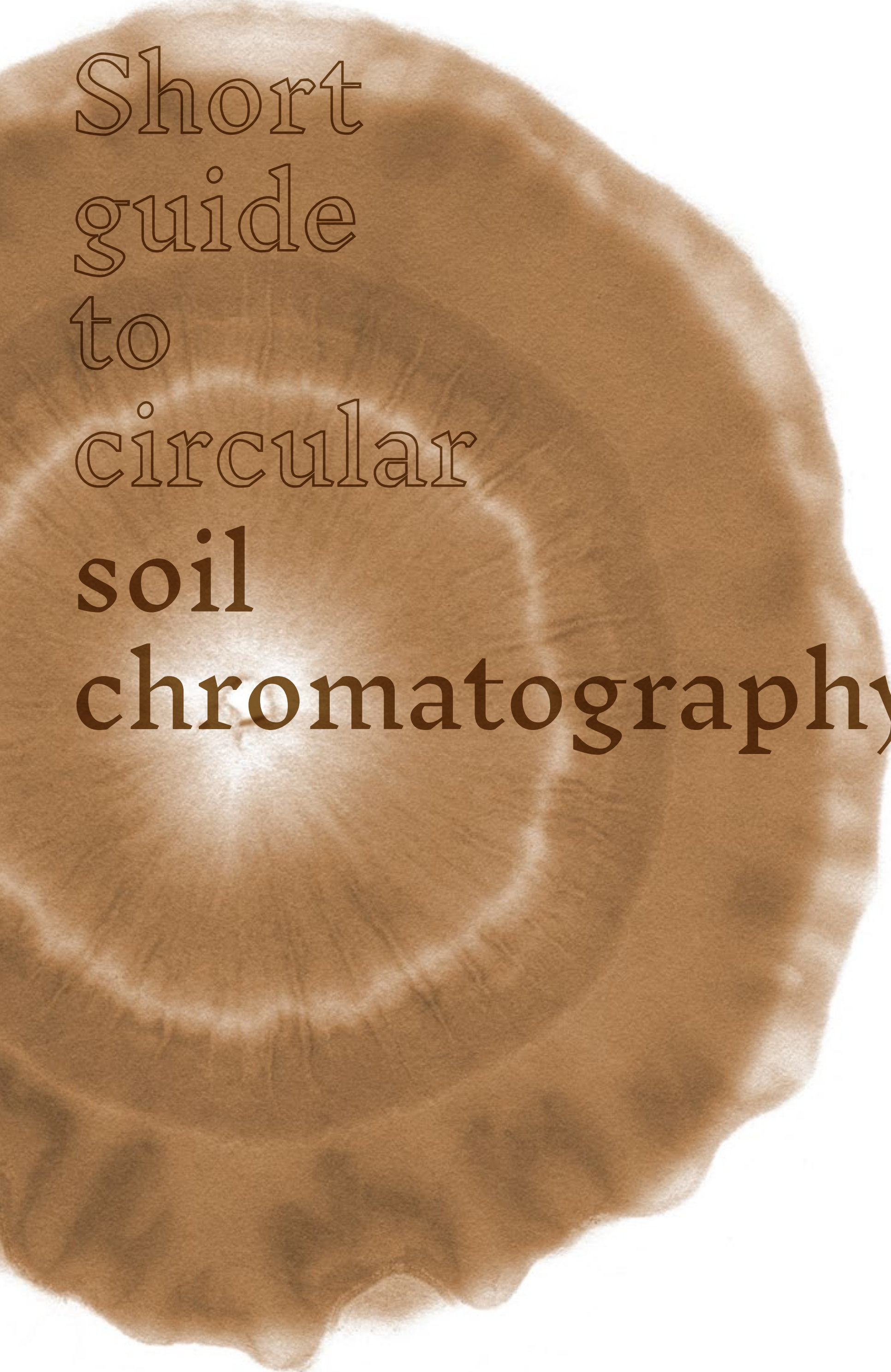
Short guide to circular soil chromatography



Introduction

Soil is an extremely complex substance: minerals, water, air, organic material and a multitude of living beings come together to create a dynamic self-regulating system. For the analysis of soil properties, many different approaches can be useful. Physical characteristics like aggregation and pore structure, can inform us about water retention capacities and possible problems with compaction; the chemical composition informs us about the lack and abundance of certain minerals and the biological diversity allows us certain assumptions concerning the overall function of the soil food web and therefore on the capability of the soil to support plant growth.

Circular chromatography of soil extracts is a method of analysis that was developed in the mid- to late 20th century and is still used by “biodynamic” farmers all over the world. Although it’s scientific validity is being disputed, the procedure follows a strict protocol and yields highly reproducible results. Similar to other qualitative approaches, personal experience is the key to obtain valuable information and with some practice this method might allow insights that reach beyond classical physical and chemical analysis. Due to it’s simplicity and the aesthetic value of the chromas, circular soil chromatography is also highly suited for education and as a tool to reconnect farmers, gardeners and the general public to the soil on which their life depends.

How it works

Liquids travel through filter paper, drawn by capillary forces. The individual components of mixed samples 'migrate' faster or slower according to their size and physical/chemical properties. This specific way of separation is called chromatography.

In circular soil chromatography, the extracts are made with sodium hydroxide - a substance widely used to extract organic matter from soil and compost samples. It reacts actively, breaking down rigid, solid substances, long and complex molecules, making them smaller and more mobile. Before applying the soil extracts, the filter papers are soaked with a very diluted solution of silver nitrate, which is known for its extreme sensitivity to light. The soil components that are being separated by the filter paper create specific patterns and when they react with silver nitrate, some of them also develop characteristic colors.

Similar substances share similar characteristic patterns and colors, which means that soil samples from 'conventional' industrial agriculture are similar to each other but very different from rich organic soils or composts. Over the last decades, some efforts have been made to quantify and objectify the results of soil chromatography, with interesting results (see chapter X & further reading) but it's main strengths may lie precisely in it's subjective nature.

Tools

- scale (at least 0.1g precision) high precision is not needed if you have the $AgNO_3$ as a solution
- measuring glass (~50-100ml)
- glass jars (min. 100ml)
- petridishes, lids of jars, or something similar
- pipette (~2-10 ml)
- rubbergloves
- scissors

Materials (for 20 chromas)

- silver nitrate ($AgNO_3$) → 0,2 g
- sodium hydroxide (NaOH) → 10 g
- distilled water → ~1.5 l
- 22 filter papers (15 cm diameter) 2 of them will be used for the "wicks" (see step 3)

Plan in advance:
$AgNO_3$ and suitable filter papers may have 1-2 weeks delivery time.
The other materials are usually available in any drug store.
Filter papers: we had good experiences with retention rates of 5-8 µm - but others may work fine as well.

The workflow

If you do chromatography for the first time, we recommend to start with only few samples to get a feeling for the process and concentrate on adapting the workflow to your local conditions. Try a few very different soils, to get a glimpse on the variety of shapes/colors and compare the same sample in two different dilutions. Take your time to observe and get some practice with handling the material. Whenever you are ready for a more systematic approach, begin by formulating a precise research question: e.g. is there a difference between the part of the garden that you covered with mulch and the part that you left exposed?

Recommendations:

- make a time-plan - when to take samples, prepare the filter-paper, etc.
- find a place where you can improvise a darkroom (see step 3)
- take at least 2 samples of each plot that you want to compare
- make a "blank" chroma with pure extraction solution (1% NaOH)
- be very precise in the preparation – treat all samples exactly the same

STEP 1 –
Preparing the solutions

0.5 % $AgNO_3$ solution ↗ minimize light exposure when handling $AgNO_3$!!

e.g. 0.5g $AgNO_3$ in 100ml of distilled water

you will need 2 ml per chroma, so this would be enough for about 50 chromas - you can store the solution in a lightproof bottle (use aluminum foil to improvise)

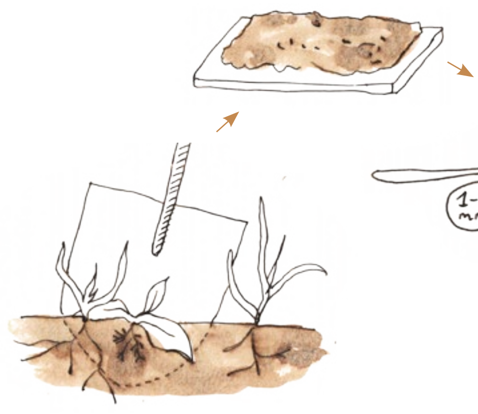
1 % NaOH solution

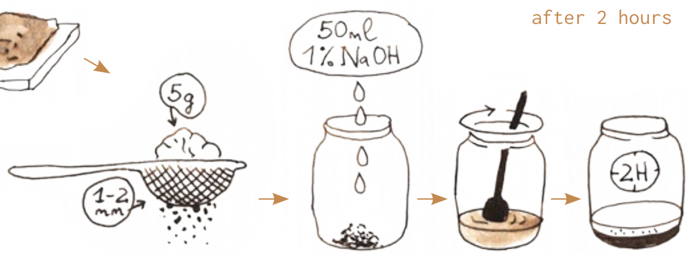
e.g. 10g NaOH in 1 l of distilled water

you will need 50 ml per chroma, so this is enough for 20 chromas - no special storage conditions needed

STEP 2 –
Making soil extracts

- **collect a handful of soil (without big stones, roots, plants, etc.)**
- **if you want to compare locations, take at least 2 samples from each place**
- **spread the soil on a table/cardboard/etc. to let it dry** → tidak langsung di bawah matahari
- **sieve 5 g of dry soil (~1-2 mm holes)** → some protocols use 10 g, but for us 5 g worked better
- **mix the soil with 50ml 1% NaOH**
- **gently shake or stir the solution several times during the next 2-3 hours** → e.g. at the beginning, after 15min, after 1 hour, after 2 hours
- **let the sample settle/ sediment for 2 h before chromatography**





If the sample is rich in organic matter it will take longer to settle

Ideally the sedimentation happens in your 'darkroom', so you don't have to move them again when doing the chromatography

STEP 3 –
Soaking the filter paper with $AgNO_3$

* this step should be done with gloves and in relative darkness

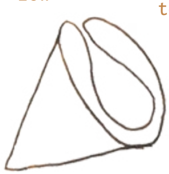
* $AgNO_3$ is a strong stain and can cause skin irritations

* Keep the filter papers clean

· improvise a darkroom

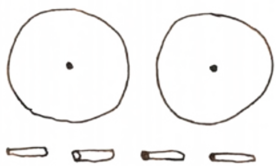
close the curtains, hang some blankets,… the darker the better but don't panic about it. You can also wait for sunset and use red flashlights or any other low intensity lightsource.

· make a hole in the middle of each filter paper

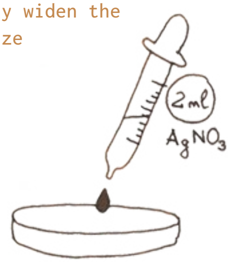


to find the middle, you can fold it, mark the center with a short line and fold it again in the other direction - then you can stack the filters and punch a hole through all of them with scissors or a knife; gently widen the hole to 3-5 mm size

· make twice as many wicks as you have filters (you will need them for step 4)

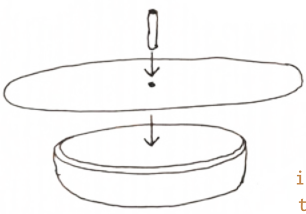


cut one filter paper into ~2x2 cm squares and roll them into cylinders



· pipette 2 ml of AgNO3 solution onto the petridish / jar cap

· insert a wick into the hole of the filter paper and place it onto the dish

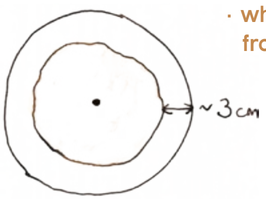


make sure the wick touches the solution - it will immediately start to soak into the filter paper

· repeat this process for all filter papers

you can do all of them in parallel - depending on the paper it will take 20-30min to soak

· when the solution reaches ~3 cm from the edge, remove the wick and let them dry



place them on some toilet paper or cardboard and leave them in the dark

STEP 4 -
Soaking the filter paper with your soil extracts

✱ this step should be done with gloves
and in relative darkness ◐

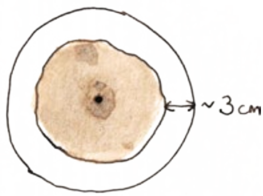
- mark the edge of each filter papers (soaked in $AgNO_3$) with the sample name (e.g. numbers)

- clean the petridishes / jar-lids and use fresh wicks

- pipette 2 ml of the supernatnat (above the sedimented soil) without mixing the liquid

- repeat the same process as for soaking with $AgNO_3$

- stop the chromatography when the solution reaches ~3 cm from the edge,
  or when the image does not change anymore
  depending on your sample and paper, this can take up to 1h



- let your chromas dry and then expose them to indirect sunlight for 2-3 hours
  for more controlled conditions, you can use artificial light in a darkened environment

Troubleshooting

- if the solution travels less than ~1/2 the distance to the edge, consider using a higher dilution for your next experiments and/or a longer time for sedimentation

- if all chromas appear pale and without clear patterns, try a higher concentration of soil (e.g. 10 g)

Interpretation & evaluation

An image is worth more than a thousand numbers

Chromas are unique, beautiful images that reveal some of the complexity and integrity of the soils they originate from. We like the method because it is simple enough to be performed at home or in a workshop and it allows us to capture some of the properties of a soil sample onto a piece of paper that we can admire, share and archive (e.g. on the refrigerator). Most importantly, the image is not created by us, but by the chemical and physical composition of the soil and by the billions of microorganisms that inhabit it. The patterns and colors directly emerge from the soil's living system - we can only assist in their manifestation and development.

Within the framework of biodynamic agriculture, circular chromatography is not only considered a reliable method for analyzing soil composition, but the chromas are also interpreted in regards of the 'energetic' properties of the soil and their ability to support plant life. On the following pages, we want to share the insights from two of our experiments, but we will not dive deeper into the art of 'reading' a soil chroma. If you want to learn more about how to interpret the results, please consult scientific publications on the topic (see "further reading") and/or contact your local biodynamic farming association.

People with great experience have standardized the reading by separating the chroma into 3-5 concentric rings (zones) and other features, like spikes and channels. The presence and colors of those zones and their relation to the radial features has allowed them to distinguish and characterize properties like: the maturity of a compost (Binton, 2010), the presence of industrial practices (e.g. use of chemicals & heavy machinery) and the effectiveness of using compost (Restrepo and Pinheiro. 2011).

It is evident that for a better interpretations of a chroma we must conjugate a certain amount of experience with the method, a basic knowledge of the chemistry and physics of the process involved but most importantly, we need to appeal to our experience and knowledge of the samples, its smell, texture, history: where is it from? what was grown here? what kind of treatment did it receive over the years?

Some examples
Sand vs. soil

The evaluation of soil quality is a highly complex problem and circular chromatography might help to understand certain aspects, but since we are no experts on this technique (yet), we do not want to claim that the following interpretations are correct. We generally recommend a combination of several methods, including visual & haptic (e.g. as described by Graham Shepherd) and microbiological approaches (see also our "Short guide to soil microscopy").

100% sand

75% sand / 25% garden soil

50% sand / 50% garden soil

The 3 images on the side show how circular chromatography reacts on different ratios of sand and garden soil. For the experiment we used sand from a construction site and rich, dark brown soil from a mulched garden bed. The results were highly reproducible and consistent with our previous experiences concerning color saturation and zone formation in soils with different humus content.

Circular chromatography is highly sensitive: with 50% garden soil (2,5 g in 50 ml NaOH) the chroma was fully saturated. A further increase of concentration did not produce any change in color, zoning or radial features (images not shown). The samples with pure sand were pale, showed a gradient instead of zones and had a fuzzy edge. According to literature, this can be attributed to the very low amount of organic compounds and weak microbial activity. Even small amounts of garden soil lead to a clear separation of zone borders, a defined edge without spikes and strong radial features („channels") that penetrated most of the zones. Except for the color intensity, there are two main differences between 25 % and 50 % garden soil: 1) the thin light rim on the outer edge of the chroma, which was only visible in the lower concentrations; 2) the number, shape & size of the channels, which were fewer but broader and reaching into the central zone, only in the higher concentrations.

We conclude from these experiments, that the optimal dilution for chromatography has to be determined individually for each sample. Otherwise it might happen that important differences get lost due to saturation or other concentration dependent effects in pattern formation.

Some examples
Agricultural soils

Non cultivated soil
(next to grape farm)

Conventional grape farm

Regenetative grape farm
(3 years after transition)

The 3 images on the side show the chromas of samples that were taken in close proximity to each other in the wine growing region of Mendoza, Argentina. The first represents non-cultivated soil; the second a conventional grape farm that uses chemical fertilizers and pesticides; the third a "regenerative" organic grape farm 3 years after transition.

The non-cultivated soil sample shows a very light central zone, clearly separated concentric rings and a slightly fuzzy outer edge. The sample from the conventional grape farm also shows a clear separation of the zones, an even more fuzzy edge, but a much darker coloring in the central zone. The chroma of the regenerative grape farm is quite different from the first two, as it shows blurred zone borders and a defined outer edge with a dark rim. All chromas lack radial features.

The main differences between the 3 chromas are:
1) coloring; 2) zone separation; 3) outer edge.
1) Dark brown colors are usually associated with humus content. The soil from both grape farms shows a higher amount of organic compounds than the non-cultivated soil, but their distribution is different. The regenerative farms sample seems to lack certain large organic molecules (light patch in the central zone) but has a higher amount of small organic molecules (very dark outer zones).
2) We could not find any conclusive explanation for the blurry zone borders in the regenerative farms sample. It could be speculated that a complex soil-food-web creates a high diversity of organic and inorganic compounds, which then leads to a more diffuse appearance on the chroma, but we have no evidence to support this idea.
3) A defined outer edge is usually considered a sign of high soil fertility. It is associated with strong microbial activity and the presence of small organic molecules.

Further readings

Pfeiffer, E.E. (1960) “**Qualitative chromatographic method for the determination of biological factors**” Biodynamics 50, 2-15.

Hassild-Piezunka (2003) **Eignung des Chroma-Boden-Tests zur Bestimmung von Kompostqualität und Rottegrad**. Ph.D. Dissertation, Carl-von-Ossietzky University Oldenburg

William F Brinton “**Assessing Compost & Humus Condition by Circular Chromatography**” (2010) Journal of the woods end research Lab Vol 1:1

Restrepo Rivera, J. R; Pinheiro, S. (2011) “**Cromatografía - Imágenes de vida y destrucción del suelo**” Juquira Candiru Satyagraha

Maria Olga Kokornaczyk, Fabio Primavera, Roberto Luneia, Stephan Baumgartner & Lucietta Betti. (2016) “**Analysis of soils by means of Pfeiffer’s circular chromatography test and comparison to chemical analysis results**” Biological Agriculture & Horticulture.

Ford, B., Cook, B., Tunbridge, D., and Tilbrook, P. (2019) “**Using paper chromatography for assessing soil health in southwestern Australia**” Centre of Excellence in Natural Resource Management, University of Western Australia.

Benjamin M. Ford, Barbara A. Stewart, David J. Tunbridge, Pip Tilbrook, (2021) “**Paper chromatography: An inconsistent tool for assessing soil health**” Geoderma, Volume 383,

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Links

(1) hackteria.org/wiki/UROS
(2) mikrobiomik.org/humussapiens
(3) openhardware.science
(4) instagram.com/ayllucoope
(5) zavodrizoma.si
(6) kons-platforma.org

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