

Soil chemistry Lab Course



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We prepared this manual to the best of our knowledge and care, but of course, we cannot exclude errors and misunderstandings. If you find some or have doubts about some of the contents, don't hesitate to contact us and discuss it with us personally.

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1 Introduction

1.1 Soil

Soils are defined as the top layer of loose material formed from weathered rock and other minerals with organic material in various states of decay. It is confined by rocks at the base and the atmosphere, and possibly vegetation cover, at the top.

While some other parts of our ecosystem are visible and their importance thus more obvious soils are often neglected within concerns about environmental issues despite their important regulatory functions within global and regional ecosystems. Soils are storage and filter medium for water. Nitrogen and carbon cycles are partly controlled by soil organisms, e.g. atmospheric nitrogen fixation is restricted to special soil fungi (*Mycorizza*, *rhizobia*) living in symbiosis with plant roots; organic matter is decomposed by soil organisms thus transforming “dead” organic matter back into building blocks of life. Soils also build the habitat for plants and animals, especially decomposers and microorganisms. They are the base for agriculture and therefore the base of modern life. Additionally, soils can be an archive of landscape history. Both natural and cultural history is conserved in soils: soil profiles, for example, can give information about the climate during their formation, and also preserve fossils and manmade objects.

The investigation and understanding of soil processes and disturbances to them is important to preserve soil quality. It is necessary to keep the aim of the investigation in mind during sampling, sample preparation and analysis, as methods are very dependent on the type of analysis conducted.

The aim of the analyzes in this labcourse is the effects that root exudates have on fundamental biogeochemical functions of the rhizosphere soil (e.g., the C- and N-cycle or retention of pollutants) and how these effects differ between native and invasive plants.

The so-called rhizosphere, i.e., the soil directly in contact with plant roots, is a hotspot of microbial activity. Here, most biogeochemical soil processes that are driven by microbial community take place. While plants deliver easy degradable substrates in form of primary metabolites (e.g., carbohydrates, amino acids, and organic acids), they supply the microbial community with nutrients and energy. But the plants may also shape the microbiome by exudates in form of secondary metabolites (e.g., phenolics, flavonoids, auxins) that may act as weapons against pathogens or competitors, or that foster or suppress specific microbial strains and thus affect the root environment for their own benefit.

1.2 Sample site

During the Soil Analysis Labcourse we will investigate samples from a maize cultivated area in Koboko, Kenya. We will sample bulk soil and rhizosphere soil from maize plants



Figure 1: Rhizosphere soil



Figure 2: Bulk soil

<https://www.dairynz.co.nz/feed/crops/maize/>

1.3 Lab course program

The rhizosphere and bulk samples will be characterized for their general characteristics like, water holding capacity (*WHC*), *pH*, electrical conductivity (*EC*). Soil water extract will be further analyzed for Phosphate.

Table 1: Overview over obtained parameters and the respective soil preparation

Preparation	parameter
dest. water extraction	<i>pH</i> , <i>EC</i>
0.01 M CaCl_2 extraction	<i>pH</i>

2 Organization

All important information for the experiments has to be included in a lab journal. If the experimental procedure deviates from the experiment description has to be noted.

3 Soil sampling

3.1 Theory

The aim of sampling in general is to obtain a representative sample. A sample is representative if its composition matches the composition of the sample location in its entirety. Depending on the sample material and location this can be difficult as spatial variations especially in soils can be expected. The compilation of a sampling strategy is necessary depending on the question addressed e.g.

- Detection of hot spots of a contamination
- Temporal or local variations of a contamination
- Risk assessment regarding the future land use

Furthermore, sample area (topography, land use, vegetation and soil type) and the capacities of laboratory (number of samples, investigated parameters, costs) have to be considered to develop an adequate sample strategy for a reduced number of representative pooled samples. A sample strategy includes:

- Question of investigation
- Number and special distribution of sampling points (e.g., grid pattern, Figure 3)
- Sampling depth and amount of samples requested
- Sampling time (e.g., season, weather), transport and storage conditions
- Material of sample tools and transport vessels

In this laboratory class, we have the possibility to analyze 4 (pooled) samples from the rhizosphere of 2 invasive, one native and from a non-rooted soil in an riparian area close to Landau. We will measure the following parameters:

- General parameters (*pH* and electrical conductivity of soil solution, water content, water holding capacity, total carbon and nitrogen content)
- Microbial parameters: Respiration (and potential inhibition by a phenolic compound: resveratrol), enzyme activity, and the potential nitrification rate (and its inhibition by the same phenolic compound: resveratrol)
- Total content and fractions of differing availability of Cu

- Total phenolic compounds (TPC) as markers for root exudates
- Content of resveratrol

With these parameters different questions and hypotheses may be approached: As stressors, Cu (anthropogenically transported from vineyards via flooding to riparian soils) and invasive species may affect microbial rhizosphere community and thus alter important soil functions, including nitrification as an important step in the global N-cycle, respiration and degradation and transformation of organic material or the retention of pollutants, e.g., the availability of Cu. Phenolic compounds are natural compounds found in root exudates and often used from invasive species as weapons against native species. We thus will check: How much phenolic compounds do the different plants exude into their rhizosphere? Do the basal and induced respiration and the nitrification potential of the respective microbial community differ between the plant species? Are microbial activities in the rhizosphere of invasive or native plants more or less resistant against inhibition by phenolic compounds? Is there a relation to the Cu availability between plant species and release of phenolic compounds?

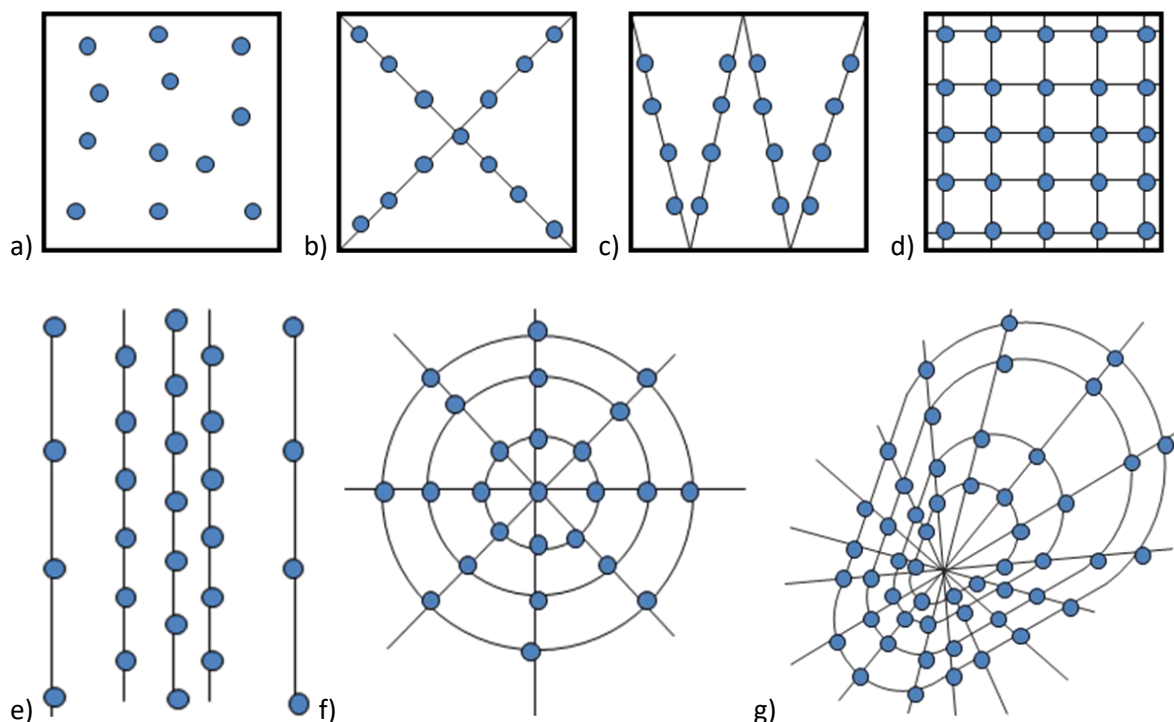


Figure 3: A Randomly, B – C systematically, asymmetrically or D symmetrically distributed sample points for a representative pooled sample for a homogeneous area. E – F Individual samples to describe spatial distribution around a contamination source in form of line (E), point (F) and in dependence of groundwater flow direction (G).

3.2 Sampling procedure

- Define a sampling strategy for representative sampling of an area and assess the mass of soil you will need from each point. (We will discuss this further directly in the field)
- Use a spade or a shovel to take the specified amount of soil. Take care to sample for each point the same amount and the same depth.
- Mix the individual samples in the bucket. Remove stones, soil fauna and large roots.
- Fill the "Soil profile survey forms" (Appendix A) and perform the respective field experiments.
- Fill well mixed samples into labeled PE bags.

4 Field experiments

4.1.1 Determination of pH

- Use a fresh well-mixed soil sample.
- Remove roots, little stones and visible organisms
- Put some soil into a plastic vial
- Add 0.01M CaCl_2 solution to the soil (1 part soil and 2 parts 0.01M CaCl_2 solution), shake the soil suspension, wait 5 minutes for sedimentation of soil particles (Suspended sediments or deeply colored solutions can cause inaccurate readings.)
- Dip indicator stick for several seconds in the solution above the soil for reaction to take place, compare the stick to color chart at the package and read the *pH*.



Figure 4: pH measurement

Table 2: pH classification

Short label	Classification	pH range
a6	extremely alkaline	≥ 10.7
a5	very strongly alkaline	10 - < 10.7
a4	strongly alkaline	9.3 - < 10
a3	moderately alkaline	8.6 - < 9.3
a2	weakly alkaline	7.9 - < 8.6
a1	very weakly alkaline	7.2 - < 7.9
s0	neutral	6.8 - < 7.2
s1	very weakly acidic	6.1 - < 6.8
s2	weakly acidic	5.4 - < 6.1
s3	moderately acidic	4.7 - < 5.4
s4	strongly acidic	4.0 - < 4.7
s5	very strongly acidic	3.3 - < 4.0
s6	extremely acidic	< 3.3

4.1.2 Determination of soil color

For technical description of soil color, Munsell soil color charts, allows separating color into components of hue (relation to red, yellow and blue), value (lightness or darkness) and chroma (paleness or strength).

- Take a bit of fresh well-mixed soil sample onto the top of a knife.
- Compare the soil color with the colors of the color charts.
- Change pages forward and backward to decide on which page the color is most comparable.
- Note the page (hue) / row (value) / line (chroma) into the table.



Figure 5: Soil color determination

- Hue (page number) is the color given as letter codes, i.e. Red (R), Yellow-Red (YR), Green (G), Green-Yellow (GY) and so on.
- Value (row number) is the brightness of a color as decreasing numbers from lightest (top) to darkest (bottom).
- Chroma (column number) is the strength of a color as numbers increasing from weak (left) to strong (right).
- Hue is needed to estimate different sesquioxides, value and chroma to estimate the humus content.

Table 3: Sesquioxides related to different soil colors

Color	Munsell color	formula	Mineral
Rust brown	5-7.5YR3-6/4-6	Fe(OH) ₃ ·H ₂ O	Ferrihydrite
(yellow)brown	10YR-2.5YR3-6/4-6	a-FeOOH	Goethite
Orange red brown	2.5-5YR4-6/6-8	g-FeOOH	Lepidocrocite
Red	5-10YR4-6/6-8	a-Fe ₂ O ₃	Haematite
Brown black	5-7YR2-3/2-4	MnO ₂ , -MnOOH ₂	Manganite
Gray-green light blue	5GY-5B2-3/1-3	Fe ^{II} / Fe ^{III}	Green rust
White, after oxidation brown	N7 -> (10YR4/5)	FeCO ₃	Siderite
White, after oxidation blue	N7 -> 5B	Fe ₃ (PO ₄)·8H ₂ O	Vivianite
Blue black (with HCl H ₂ S odour)	5-10B1-2/1-3	FeS (Fe ₃ S ₄)	Iron sulfide
Gold yellow, metallic		FeS ₂	Pyrite

4.1.3 Determination of carbonate content

- Put some soil on a watch glass.
- Add some drops of ~10% HCl to the soil.
- Bubbles are an indicator for calcium carbonate (CaCO₃).
- If no bubbles are visible use a new sample of soil and add some drops of 10% HCl with a second watch glass on top of the other one (with the curvature down).
- The degree of bubbles formed by carbon dioxide gas is indicative for the amount of calcium carbonate present.
- Classes for the reaction of carbonates in the soil matrix are defined Table 4.

Table 4: Determination of carbonate content (Guideline for Soil Description FAO; ftp://ftp.fao.org/agl/agll/docs/guidel_soil_descr.pdf, p. 38)

Observation	Classification	Carbonate content %
No detectable visible or audible effervescence	Non-calcareous	0
Audible effervescence but not visible	Slightly calcareous	0-2
Visible effervescence	Moderately calcareous	2-10
Strong visible effervescence. Bubbles form a low foam	Strongly calcareous	10-25
Extremely strong reaction. Thick foam forms quickly	Extremely calcareous	> 25

4.1.4 Packing density

Take a larger piece of soil out of a profile wall and break it into smaller pieces and / or try to penetrate the profile wall with a knife.

Table 5: Classification of packing density

Estimated packing density			Characteristics	
Label		kg dm ⁻³	Sandy, silty or weakly loamy soils (dry – fresh)	Heavy loam and clay soils (dry – fresh)
Ld1	Very low	< 1.4	Disintegrates already at sampling, many coarse pores visible at profile wall	
Ld2	Low	1.4 - < 1.6	Disintegrates at weak pressure in many pieces or in single particles	Soil breaks at collision into many pieces, further disaggregation possible with low pressure.
Ld3	Mode- rate	1.6 - < 1.8	Knife penetrates soil with weak pressure, soil breaks into a small number of pieces, which can be easily disaggregated into more pieces by hand.	Soil breaks at collision into several pieces, further disaggregation possible with some pressure.
Ld4	High	1.8 - < 2.0	Knife penetrates soil 1 – 2 cm by strong pressure, soil breaks into some pieces hardly disaggre-gable further by hand	Soil hardly breaks at collision, further disaggregation only with strong pressure possible

4.1.5 Determination of soil type by feel and appearance method

Take an amount of moist soil, wet it well and squeeze it in between your thumb and fingers until no liquid water is left. Then follow the next steps in order to determine the soil type:

- 1) Try to form a sausage between your hands (pencil thick).
 - a) not possible: sandy soil continue at 2.
 - b) possible: continue at 4.
- 2) Test the ribbon formation between index finger and thumb
 - a) does not form ribbons, not mouldable: continue at 3.
 - b) slightly mouldable, sticks to fingers slightly: **loamy sand (SI)**
- 3) Rub it between your hands

-
- a) no silty material in lines of hand: **sand (S)**
- b) silty material in lines of hand: **silty sand (Su)**
- 4) Try to form a sausage (thinner than under point 1)
- a) not possible : continue at 5.
- b) possible: continue at 9.
- 5) Test the ribbon formation between index finger and thumb
- a) forms ribbons, sticks well to fingers continue at 6.
- b) no or only slight ribbon formation, few sand grains continue at 7.
- 6) Judge the amount of fine material present in the sample
- a) low fine material concentration: **clayey sand (St)**
- b) high fine material concentration: **strongly sandy loam (Ls4)**
- 7) Test the grittiness
- a) sand grains visible and palpable: **sandy silt (Us)**
- b) sand grains not or only slightly visible/palpable continue at 8.
- 8) Test the ribbon formation between index finger and thumb
- a) not coherent, slick and smooth, only slightly mouldable, ruptures and breaks:
silt (U)
- b) slightly coherent, floury, ruptures and breaks, mouldable: **slilty loamy silt (Uls)**
- 9) Test the grittiness
- a) coherent, sand grains hardly palpable, hardly ruptures and breaks:
strongly loamy silt (UI)
- b) slightly floury, few sand grains, slightly coherent, mouldable:
silty loam (Lu)
- c) not floury: continue at 10.
- 10) Squeeze the sample between index finger and thumb next to your ear

- | | |
|---|---------------------------|
| a) strong crunching audible: | sandy loam (Ls) |
| b) none or slight crunching: clayey soil | continue at 11. |
| 11) Try to form a ring out of the sausage | |
| (a) hardly mouldable: | sandy clay (Ts) |
| (b) well mouldable: | continue at 12. |
| 12) Judge the hydroplane of the squeezed sample | |
| a) matt: | clayey loam (Lt) |
| b) shiny: | continue at 13. |
| 13) Judge the soil between your teeth (or don't ;)) | |
| a) Crunching: | loamy clay (Tl) |
| b) buttery, no particles: | clay (T) |

The determination of soil type is difficult for soils with high organic content. The organic substance increases soil coherence and mouldability. Therefore, you need to decrease grain size by one or two classes for such soils (especially for sandy soils).



Figure 6: soil type by feel and appearance method

Figure 7: Texture triangle relating soil types to respective clay, silt and sand content**Table 6: Letter codes for different soil types**

U = silt	u = silty	3 = moderately
L = loam	l = loamy	4 = strongly
T = clay	t = clayey	O = point of 50%S, 20% U, 30%T

4.2 Preparation / Evaluation

4.2.1 What you should know

- Calculate how much soil you need for the lab-course to determine all parameters! Consider a *WC* of ~30% and a loss of 20% by collecting out roots and stones.

4.2.2 Evaluation

- Compare *pH* results of laboratory and field assessment.
- Classify the samples regarding their *pH*, carbonate content, texture etc.
- Compare the results regarding the treatments.
- Test possible accordance with results of other analysis, interacting with these parameters.

5 Ecological evaluation

With the following tables, ecologically relevant measures may be estimated from the obtained data:

5.1 Humus content

Use the value of soil color (eventually increased, see footnote under Table 7) and find humus class dependent on moisture state (wet or dry) and soil type.

Table 7: Humus classes for different soil colors depending on moisture state and soil type.

Color	Munsell value ¹⁾	Humus content in classes					
		Wet conditions			Dry conditions		
		Ss	Sl - Ls	L, U, T	Ss	Sl - Ls	L, U, T
Light gray	7	h0	h0	h0	h1	h1	h1
Light gray	6.5	h0	h0	h0	h1	h1	h1 – h2
Gray	6	h0	h0	h0	h1	h1 – h2	h2
Gray	5.5	h0	h0	h1	h2	h2	h3
Gray	5	h1	h1	h1	h2	h3	h3
Dark gray	4.5	h1	h1	h1	h3	h4	h4
Dark gray	4	h1	h1	h1 – h2	h3 – h4	h4 – h5	h4 – h5
Black gray	3.5	h1 – h2	h2	h2 - h3	h4	h5	h5
Black gray	3	h2 - h3	h3	h3 – h4	h5	≥h6	≥h6
Black	2.5	h3 – h4	≥h4	≥h4	≥h5		
black	2	≥h4					

¹⁾ with chroma 3.5 - 6 value increases by 0.5, with chroma > 6, value increases by 1

Table 8: Classification of humus content

Humus (organic substances)		
Short label	Description	Weight %
h0	Humus free	0
h1	Very weak humous	< 1
h2	Weak humous	1 - <2
h3 ¹⁾	Moderate humous	2 - < 4
h4 ¹⁾	Humus rich	4 - <8
h5 ¹⁾	Very humus rich	8 - <15
h6	Extremely humus rich (anmoor)	15 - <30
h7	Organic, peat	> 30

¹⁾ with forest use h3 = 2 – 5; h4 = 5 – 10; h5 = 10 – 15 weight %

5.2 Potential cation exchange capacity CEC_{pot}

CEC_{pot} of mineral soil part can be estimated by the silt and clay content (in %) by Equation 1...

$$CEC_{pot} = 0.5 \cdot \text{clay content} + 0.05 \cdot \text{silt content} \quad (\text{Equation 1})$$

... or by Table 9 using the soil type.

Table 9: Potential cation exchange capacity of the mineral part of soil in dependence of soil type

CEC_{pot} (cmol _c kg ⁻¹)	soil type	CEC_{pot} (cmol _c kg ⁻¹)	soil type
2	Ss, Su2	15	Lu, Ts4
4	Su3, Su4, Sl2	17	Lt2, Tu4
5	Us	19	Lts
6	St2, Sl3, Uu	20	Ts3
9	Slu, Sl4, Ut2, Uls	21	Tu3
11	Ut3, St3	22	Lt3
12	Ls3, Ls4	28	Ts2, Tu2
13	Ls2	29	Tl
14	Ut4	39	Tt

CEC_{pot} of the organic part of soil can be estimated by the humus content (in %) by Equation 2...

$$CEC_{pot} = 2 \cdot \text{humus content} \quad (\text{Equation 2})$$

... or by Table 10 using the humus classes.

Table 10: Potential cation exchange capacity of the mineral part of soil in dependence of humus classes

Humus		CEC_{pot}
short label	weight %	cmol _c kg ⁻¹
h 1	< 1	< 2
h 2	1 - < 2	2 - < 43
h 3	2 - < 4	4 - < 8
h 4	4 - < 8	8 - < 16
h 5	8 - < 15	16 - < 30
h 6	15 - 30	30 - 60

The total CEC_{pot} is the sum of the soil type depending and the humus depending CEC_{pot} .

5.3 Effective cation exchange capacity CEC_{eff}

CEC_{eff} can be estimated by a pH depending factor. Except for allophones, for the mineral part effective CEC is quite identical with the potential CEC. Therefore only the CEC_{pot} of the organic part has to be corrected using Table 11 by multiplying with the conversion factor.

Table 11: Conversion factor for effective CEC from the potential CEC of organic part of soil

pH value	≥ 7.5	$< 7.5 - 6.5$	$< 6.5 - 5.5$	$< 5.5 - 4.5$	$< 4.5 - 3.5$	< 3.5
Conversion factor	1	0.8	0.6	0.4	0.25	0.15

The total CEC_{eff} is the sum of the effective CEC of humus and of the potential CEC_{pot} of the mineral part of soil.

Table 12: Classification of potential CEC

short lable	class	CEC_{pot} (cmol _c kg ⁻¹)
CEC1	very low	< 4
CEC2	low	$4 - < 8$
CEC3	moderate	$8 - < 12$
CEC4	high	$12 - < 20$
CEC5	very high	$20 - < 30$
CEC6	extremely high	≥ 30

5.4 Air capacity (AC), available water capacity (AWC), and field capacity (FC)

The water and air balance of a soil can be described by field capacity (FC), the percentage of water hold against the gravity of the total pore volume (TPV), the air capacity (AC), the percentage of volume of large pores filled with air at field capacity and the available water capacity (AWC), the volume of water available for plants which equals FC reduced by the hygroscopic water (HW).

$$TPV = AC + FC \quad (\text{Equation 3})$$

$$FC = AWC + HW \quad (\text{Equation 4})$$

Therefore, packing density, soil type and humus content, which determine the porosity of a soil are used to estimate AC, AWC and FC. This assessment needs 3 steps using Table 13-Table 15. Don't forget the third step!!

1. Estimate oven-dry density by soil type or clay content.

Table 13: Oven-dry soil density (r_t) depending on soil type and packing density

short label soil type	clay content weight %	classes of oven-dry density r_t				
		Effective packing density				
		Ld1	Ld2	Ld3	Ld4	Ld5
Ss	3	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
SI2	7	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
SI3	10	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
SI4	15	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Slu	13	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
St2	11	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
St3	21	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Su2	3	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Su3	4	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Su4	4	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Ls2	21	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Ls3	21	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Ls4	21	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Lt2	30	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Lt3 ²⁾	40	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Lts ²⁾	35	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Lu	24	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Uu	4	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Uls	13	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Us	4	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Ut2	10	r_{t2}	r_{t3}	r_{t4}	r_{t5}	r_{t5}
Ut3	14	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Ut4	21	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Tt ²⁾	75	r_{t1}	r_{t1}	r_{t1}	r_{t2}	r_{t3}
Tl ²⁾	55	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Tu2 ²⁾	52	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Tu3 ²⁾	36	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Tu4	28	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}
Ts2 ²⁾	55	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Ts3 ²⁾	40	r_{t1}	r_{t1}	r_{t2}	r_{t3}	r_{t4}
Ts4	30	r_{t1}	r_{t2}	r_{t3}	r_{t4}	r_{t5}

2. Estimate AC, AWC, FC and HW by r_t and soil type.

Table 14: Uncorrected air, available water, field capacity and hygroscopic water dependent of r_t

Pores / pF Soil type	Air capacity			Available water			Field capacity			Hygroscopic		
	$< 50 \mu\text{m} / < 1.8$			$0.2 - 0 \mu\text{m} / 4.2 - 1.8$			$\leq 50 \mu\text{m} / \geq 1.8$			$\leq 0.2 \mu\text{m} / \geq 4.2$		
	r_{t1+2}	r_{t3}	r_{t4+5}	r_{t1+2}	r_{t3}	r_{t4+5}	r_{t1+2}	r_{t3}	r_{t4+5}	r_{t1+2}	r_{t3}	r_{t4+5}
Ss ¹⁾	36	32	27	9	7	7	14	11	10	5	4	3
SI2	23	28	13	20	18	17	28	25	23	8	7	7

SI3	18	15	10	22	18	17	34	27	25	12	9	8
SI4	18	12	8	22	18	15	36	30	26	14	12	11
Slu	14	10	7	23	21	19	38	33	30	15	12	11
St2	24	20	15	18	16	13	26	22	18	8	6	5
St3	18	14	9	18	15	12	35	30	26	17	15	14
Su2	24	21	15	20	18	17	26	23	21	6	5	4
Su3	17	14	10	25	21	20	35	29	26	10	8	6
Su4	14	11	8	27	23	21	39	32	28	12	9	7
Ls2	13	9	6	21	16	14	40	34	31	19	18	17
Ls3	15	9	6	21	16	14	39	33	30	18	17	16
Ls4	15	11	7	20	16	13	39	32	28	19	16	15
Lt2	11	7	5	18	14	11	42	36	32	24	22	21
Lt3 ²⁾	10/6	5	3	20/14	12	10	49/43	39	35	29/29	27	25
Lts ²⁾	11/9	6	5	21/17	14	11	47/42	37	31	26/25	23	20
Lu	12	7	4	21	17	15	41	36	33	20	19	18
Uu	10	7	3	30	26	23	43	38	35	13	12	12
Uls	13	8	5	24	22	21	39	35	33	15	13	12
Us	11	9	4	28	25	22	41	35	32	13	10	10
Ut2	10	6	3	28	26	23	40	37	35	12	11	12
Ut3	11	6	3	26	25	23	39	37	35	13	12	12
Ut4	12	7	3	23	21	19	39	37	35	16	16	16
Tt ²⁾	8/4	3	2	20/15	13	12	56/48	43	35	36/33	30	23
Tl ²⁾	9/5	4	3	19/14	13	11	54/47	41	35	35/33	28	24
Tu2 ²⁾	7/5	4	3	20/15	12	10	53/46	42	36	33/31	30	26
Tu3 ²⁾	10/8	6	3	22/16	13	10	50/42	38	35	28/26	25	25
Tu4	10	6	3		17	16	41	37	35	22	20	19
Ts2 ²⁾	10/5	4	3	18/15	13	12	51/46	39	34	33/31	26	22
Ts3 ²⁾	15/7	6	5	17/16	13	11	50/44	37	32	33/28	24	21
Ts4	13	10	6		14	11	43	32	30	26	18	19
fS, fSms, fSgs ¹⁾	34	31	23	10	9	8	16	14	12	6	5	4
mS, mSfs, mSgs ¹⁾	36	32	26	9	6	5	14	10	8	5	4	3
gS ¹⁾	38	33	29	8	5	4	12	8	6	4	3	2

¹⁾lower limit for pF for pure sands <2.5 ²⁾for high clay content separate values for r₁ & 2

3. Correct results by adding or reducing values of the following table according to humus content and soil type..

Table 15: Correction of air, available water, and field capacity for the effect of humus

soil type	Air capacity				Available water capacity				Field capacity			
	Humus content classes											
short label	h2	h3	h4	h5	h2	h3	h4	h5	h2	h3	h4	h5
Ss	0	-1	-2	-3	1	3	4	5	3	6	9	12
SI2	0	1	2	3	2	3	4	6	3	6	9	13
SI3	1	2	3	4	1	3	4	6	3	5	9	13
SI4	2	2	3	4	2	4	5	6	3	7	11	14
Slu	2	3	4	6	1	2	4	6	2	5	8	11

St2	0	0	1	1	3	4	5	7	5	7	11	15
St3	1	2	3	4	2	4	6	9	2	5	10	14
Su2	0	0	-1	-2	2	3	4	6	3	6	9	13
Su3	1	1	2	2	1	3	3	4	2	6	8	11
Su4	2	3	4	6	1	2	3	4	2	4	8	11
Ls2	2	3	4	5	1	3	5	8	3	6	11	14
Ls3	1	2	3	4	1	3	5	8	3	6	11	14
Ls4	1	2	3	3	2	4	6	8	4	6	12	15
Lt2	2	3	5	6	3	5	8	10	5	8	13	15
Lt3	1	2	4	7	2	4	8	11	5	6	12	15
Lts	1	2	5	6	3	5	7	9	3	7	13	15
Lu	2	3	6	7	3	5	7	8	6	7	13	15
Uu	2	3	5	9	1	2	3	4	2	4	8	11
Uls	2	3	4	8	3	4	4	7	4	7	10	15
Us	2	3	5	8	1	2	3	4	2	4	7	10
Ut2	2	4	6	8	1	1	2	4	2	4	7	12
Ut3	2	4	6	8	1	1	2	4	2	3	8	12
Ut4	2	4	6	7	2	3	4	6	4	6	9	13
Tt	1	2	4	8	2	4	5	7	5	6	9	11
Tl	1	2	3	7	2	4	6	8	5	6	11	13
Tu2	1	2	3	7	1	3	5	8	5	6	10	13
Tu3	2	2	3	6	2	4	7	9	6	8	12	14
Tu4	1	3	4	6	3	5	6	8	5	8	11	15
Ts2	1	2	3	7	2	4	6	8	6	7	12	14
Ts3	2	3	4	5	2	5	7	9	5	6	12	14
Ts4	2	3	4	5	2	4	7	9	4	6	11	14

Table 16: Classification of total pore volume (TPV), and air (AC) and field capacity (FC), available water capacity (AWC) and hygroscopic water (HW)

Label	Classes	TPV	AC	FC	AWC	HW
very low	1	< 30	< 2	< 21	< 6	< 4
low	2	30 - < 38	2 - < 5	21 - < 30	6 - < 14	4 - < 10
moderate	3	38 - < 46	5 - < 13	30 - < 39	14 - < 22	10 - < 22
high	4	46 - < 54	13 - < 26	39 - < 48	22 - < 30	22 - < 34
very high	5	≥ 54	≥ 26	≥ 48	≥ 30	≥ 34

5.5 Effective root penetration depth

Effective root penetration depth depends on both, soil type and packing density and can be estimated by Table 17 (consider also the note at the end of the table):

Table 17: Effective root penetration depth in dependence of soil type and packing density class.

Soil type	Effective root penetration depth (dm)		
	r_{t1+2} Ld1+2	r_{t3+2} Ld3	r_{t4+5} Ld4+5
gS	7	5	5
Ss, mS, mSfs, mSgs, fs, fSms, fSgs	8	6	6
Sl2, Su2, Su3, Su4	9	7	6
Sl3, St2	10	8	7
Sl4, St3, Slu	13	9	8
Ls2, Ls3, Ls4, Lt2, Lt3, Lts, Uu, Us, Tu2, Tl, Tt	13	10	8
Uls, Ut2, Ut3, Ut4, Lu, Tu3, Tu4	13	11	9

For grassland reduce by 2 dm, for coniferous wood multiply by 1.5, (for Ts2 and Ts4 no data available)

Table 18: Classification of available water in the root zone

Label	Classes	AWC in the root zone (mm/dm)
very low	1	< 50
low	2	50 - < 90
moderate	3	90 - < 140
high	4	140 - < 200
very high	5	200 - < 270
extremely high	6	≥ 270

Available water capacity within the root zone is the product of the AWC and effective root penetration depth and is the potential amount of water plants can use without addition from groundwater and capillary rise during a dry period.

6 Sample preparation

6.1 Homogenizing

First preparation step is homogenizing and a thoroughly mixing of each soil sample in order to get subsamples for different measurements which are identical in composition. Thereby, aggregates have to be destroyed and larger pieces of plant material picked out manually. Prepare an own concept, how much sample is needed for which analysis and has to be pre-treated in which way. After a good mixing and homogenizing, split each soil sample into 3 (pseudo)replicates that are further well homogenized and separated in single plastic bags. By this way, we can later directly relate the results of replicate 1 of one parameter to all other parameters obtained from replicate 1. Important is a very well homogenizing and mixing. From the moisture state of the soil after sampling, we will decide whether a sieving can be done already in field fresh state or we have to air dry the soil before.

→ Use the spoon/rod to mix the soil samples in the bucket



Figure 8: Sample homogenization in a bucket

6.2 Drying

Different sample drying is necessary for some experiments: For the determination of total carbon and nitrogen content (CN_{tot}), samples have to be water free, i.e. dried at 105 °C (for this, samples from the water content determination can be used). For the sequential extraction and for the MicroResp™ method air dried soil is needed. The drying process should be done as soon as possible after sampling in order to minimize microbial activity. Drying can be facilitated by exposing as much surface of the soil to air as possible. If possible, the drying temperature should not exceed 40°C, as significant changes in the physico-chemical properties of the soil may occur at higher drying temperatures.

6.3 Water content determination

- Label the container with sample name and determine the tare of the container.
- Weigh ~15 g of sample into the container (3 replicates à ~ 5 g).
- Place sample in the oven at 105 °C and dry the samples for ~36 hours.
- Remove sample from the oven and place them in an exicator in order to cool down to room temperature.
- Reweigh the samples and calculate the water content (mass basis) as a fraction of the mass of dry soil as follows:

$$WC = \frac{(m_{moist+tin} - m_{dry+tin})}{(m_{dry+tin} - m_{tin})} \quad (\text{Equation 5})$$

WC	water content	$m_{dry+tin}$	mass of tin with dry soil (g)
$m_{moist+tin}$	mass of tin with moist soil (g)	m_{tin}	mass of empty tin (g)

Do NOT dispose the soil samples from the water content determination. These samples are also needed for the microwave extraction and determination of CN_{tot} .

6.3.1 Sieving and determination of coarse fraction

(If we can sieve the samples already in field fresh state, we can skip the determination of coarse fraction because we will only use the fine fraction).

All dried samples have to be sieved in order to keep only the “fine fraction” (with particles smaller 2 mm) for the following analysis and to determine the “coarse fraction” (with particles larger 2 mm).

- Weigh the unsieved sample.
- Sieve the air dried and the 105°C dried samples separately over a sieve with a mesh width of 2 mm.
- All aggregates in the sieving residue (the part that not passes the meshes) consisting of smaller particles have to be crushed (using a pestil) until only single particles larger 2 mm remain on top of the sieve.
- Weigh the sieving residue (coarse fraction) and the respective empty vessel.
- Store the sieved samples (fine fraction) in labeled glass vials. The coarse fraction may be discarded.

The coarse fraction is calculated by the following equation as a fraction of the unsieved soil mass:

$$F_c = \frac{m_{2mm+tin} - m_{tin}}{m_{unsieved+tin} - m_{tin}} \quad (\text{Equation 6})$$

F_c	coarse fraction	$m_{unsieved+tin}$	mass of tin + unsieved soil (g)
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$m_{>2mm+tin}$ mass of tin + sieve residue (g) m_{tin} mass of empty tin (g)

Results of each analysis performed with unsieved samples which are related to the mass of the soil used, this mass has to be corrected for its actual WC and F_c :

$$m_{dry,unsieved} = \frac{m_{moist,unsieved}}{(WC + 1)} \quad \text{(Equation 7)}$$

$$m_{<2mm} = m_{unsieved} - m_{>2mm} \quad \text{(Equation 8)}$$

$$m_{dry,<2mm} = m_{dry,unsieved}(1 - F_c) \quad \text{(Equation 9)}$$

$$m_{dry,<2mm} = \frac{m_{moist,unsieved}}{(WC + 1)}(1 - F_c) \quad \text{(Equation 10)}$$

$m_{dry, unsieved}$ mass of used soil corrected by WC (g)

$m_{moist, unsieved}$ uncorrected mass of used soil (g)

$m_{<2mm}$ mass of fine fraction (g)

$m_{dry, <2mm}$ mass corrected by F_c and WC (g)

6.4 Grinding

For CN_{tot} determination and the microwave extraction, 105°C dried and sieved samples have to be ground to smaller size in order to increase the sample surface and thereby increase the contact area for the extraction liquid or for the heat flow.

- Put the achat grinding balls into the grinding vessel of the same material and add ~10 mL of 105°C dried and sieved samples into the grinding vessel.
- Follow the description and the instructions of the tutors and grind the samples.
- Store the ground samples in labeled glass vials.
- Clean vessels and balls first with a brush and finally rinse intensively with dest water.

6.5 Electrical conductivity and pH

6.5.1 Theory

Conductivity is a measure of how well a solution conducts electricity. Water with absolutely no impurities (which in reality hardly exists) would conduct electricity poorly. In real life, the impurities (dissolved material, salts and ions) in water increase its conductivity. EC is measured in units called Siemens per unit length (e.g. mS/cm), and the higher the dissolved material in a water or soil sample, the higher the *EC* will be in that material.

The *pH* is a measure of the acidity of soil based on its hydrogen ion concentration and is mathematically defined as the negative logarithm of the hydrogen ion concentration, or

$$\text{pH} = -\log [\text{H}^+] \quad \text{(Equation 11)}$$

where the brackets around the H^+ symbolize "concentration". The pH of a material ranges on a logarithmic scale from 1-14, where pH 1-6 are acidic, pH 7 is neutral, and pH 8-14 are basic. Lower pH corresponds with higher $[\text{H}^+]$, while higher pH is associated with lower $[\text{H}^+]$.

Soil *pH* is an indication of the soil's chemistry and fertility. The *pH* affects the chemical activity of the species in the soil, as well as many of the soil properties. Different plants grow best at different pH values (e.g. spinach at *pH* 6.0, wheat *pH* 5.5 and apple *pH* 5.0). Soil *pH* is an important parameter for soil characteristics. It is influenced by the parental material, the humus content and humus type, soil organisms, soil water, climate and age of the soil. Effects of soil *pH* are both direct and indirect. Direct effects, while not numerous, can be critical. In the case of a soil that is too acid or too alkaline, there can be toxic effects on the plants themselves, and an unfavorable balance between acid and alkaline elements needed by plants. The soil *pH* indirectly influences the chemical weathering of mineral soil constituents, the soil texture, the air and water household of soil and also the availability of essential elements, activity of soil micro organisms and solubility, mobility and potency of toxic elements (like heavy metal or aluminum ions).

The results for the *pH* measurements with CaCl_2 solution are usually half *pH* unit lower than the *pH* measured in water for soils with a net negative charge and vice versa for soils with a net positive charge (mainly tropical soils). The results in CaCl_2 solution are more reproducible and better represent the conditions in soil environment than pH values measured in water, but the difference between both offer additional information about the net charge of the soil.

6.5.2 Procedure

Electrical conductivity (*EC*) and *pH* are determined in a suspension of soil in dest water and for the *pH* additionally in 0.01M CaCl₂-solution. As both extractions are performed for total phenolic compounds determination (dest. Water) and for mobile Cu (0.01M CaCl₂ solution), you will measure *pH* and *EC* during these experiments.

- Insert the *EC* meter into the water suspension and move it gently in the soil-water extract and note the reading after ~30-60 seconds or after the reading has stabilized.
- Measurement of *pH* will be obtained in water- and in 0.01M CaCl₂-solution-soil suspensions.
- Calibrate the *pH*-probe using 2 buffer solutions with *pH* in the range of the expected *pH* of the soil sample.
- Stir 0.01 M CaCl₂ solution again gently before taking the *pH* measurement (not the soil, only solution above the soil is stirred).
- Insert the *pH* meter into the beaker and move it gently around in the soil-water extract.
- Take the reading after approximately 30-60 seconds or after the *pH* reading has stabilized.

The *pH* like the electrical conductivity depends on temperature! Take care that a correction for the temperature is made or standards and samples are at the same temperature. Note: Please clean the electrode after each measurement with distilled water and dry softly with a tissue and keep the *pH* electrode in a solution between the measurements (e.g., rinsing dest. Water).

6.5.3 Preparation / Evaluation

What you should know

- Definition of *pH* and *EC*
- Functioning of *pH* and *EC* electrodes
- Temperature dependency of *pH* and *EC*
- Environmental relevance of *pH* and *EC* in soil

Evaluation

- Classify the samples regarding their *pH* and *EC* and test possible accordance with results of other analysis, which may interact with these parameters.

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Soil profile survey form

(simplified version after Ad-hoc-Ag Boden, 2005)

Profile number: _____ Date: _____ Operator: _____ Weather: _____

Geogr. length: _____ Geogr. width: _____ Height: _____ Site name: _____

Slope: _____ Exposition: _____ Relief form: _____ Vegetation / Use: _____

Anthropogenic changes: _____ Parent material: _____ Ground / Stagnant water: _____

Nr:	depth (cm)	Soil color	Packing density	Carbonate content	pH	Soil type	Ecological assessment				
							Humus [%]	CEC [cmol _c /kg]	AC [vol%]	AWC [vol%]	FC [vol%]
1											
2											
3											
4											
5											
6											
7											
8											
9											