European Mountain lake Ecosystems: Regionalisation, diaGnostics & socio-economic Evaluation

EMERGE

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SOIL SAMPLING AND ANALYSIS

Regionalisation of soil properties relevant to mountain lake water chemistry

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WP-2
Task 2.2: Regionalisation of soil properties relevant to mountain lake water chemistry

Major aims are
(1) to characterize and analyze soils in order to calibrate models in Lake District (LD), where these data are missing (Pyrenees and Tatras) and
(2) (2) to define and characterize the dominant soil types in each LD for WP6 (Remote sensing and GIS).

The major soil characteristics (the 1st level) are as follows: bulk density, cation exchange capacity (CEC), base saturation (BS) and C and N contents. The 2nd level soil analyses involve phosphorus and sulfate sorption capacities, oxalate extractable Al, Fe, and P, and microbiological analyses (C, N and P in soil microbial biomass, nitrification, denitrification and C mineralization potentials).

Selection of sites
- The catchments selected for modeling:

1. Estimation of soil area and its average depth (using soil corer).
2. Sampling of 3 representative cores in each catchment for chemical analyses and bulk density.

- Remote sensing and GIS: Soil classification depends on available data:

1. Classification and chemistry may be obtained from existing maps based on the previous pedological survey. Any type of pedological classification should be transferred by local experts to FAO classification.
2. If not available, soil classification should be done by local experts for each LD and the 1st level chemical analyses should be performed at least in 3 catchments with the characteristic dominant soil types for the LD. These representative sites should be preferably selected in the catchments of validation lakes.

Methods
A) Soil sampling and physical characteristics
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Two types of soil samples will be taken:
- Soil cores for physical, chemical and biological characteristics (1st and 2nd level)
- Integrated samples for biological and chemical analyses (2nd level)

(1) Soil cores - Excavate 0.5 m^2 pit (71×71 cm) or, alternatively, 0.25 m^2 pit (50×50 cm), until the C-horizon. Remove stones >2 cm in a diameter and weight them separately. Give the soil from each horizon into a bucket or plastic bag and weight it in the field. If the pit is not a regular quadrangular prism, estimate the area for individual horizon. Measure the thickness of individual horizons in each corner of the pit and calculate the average value. Number of samples, which are taken from the pit, depends on quality of soil profile:
1. If the individual (both organic and mineral) horizons are visible: Collect the uppermost litter and fermented layers together (O), then the humus horizon (A), and existing mineral horizons.
2. If the mineral profiles are homogenous, take two samples: the uppermost organic horizon and the representative sample of mineral soil layer (along the whole depth of mineral soil from its top to the bedrock).

3. In deep (>20 cm) undeveloped soils without a plant cover, take two samples: the uppermost 10 cm layer and then the rest of the profile to the bedrock.
4. In thin (<20) undeveloped soils without plant cover, take one representative sample along the whole profile.

Samples for chemical analyses - Take a representative ~1 kg sample of each horizon (along its whole thickness) from the walls of the pit into a PE bag and store it in cold (at 4 ^{o}C) and dark until used. In the laboratory, weight each sample for chemical analyses. Remove coarse particles (CP = stones and roots) from the sample (sample of O horizon pass through a 5-mm stailess-steel sieve) and weight them. Then, dry soil sample between two sheets of filter paper for 14-21 days at laboratory temperature (not at direct sunshine), and weight. Sieve the air-dried sample through a stainless-steel 2-mm sieve and weight. The air-dried < 2-mm soil fraction is further referred as AD. Dry the precisely weighted subsample (~1g) of AD in an oven at 105 ^{o}C for 2 h, then let it cool in a desiccator, and weight. The oven-dried < 2-mm soil fraction is further referred as OD. Ignite the weighted OD sample at 550 ^{o}C for 2 hours, let it cool in a desiccator, and weight. Calculate the percent loss on ignition (LOI) using OD weight as 100%.

Note - Soil chemical analyses are conducted using AD samples and the results are reported on an OD basis.

Soil samples for biological analyses – Take a representative ~2 kg sample from each horizon, keep it wet in a closed PE bag at ~4^{o}C. In the laboratory, mix the wet sample, sieve it (5 mm mesh) and store at 4^{o}C up to 5 weeks before biological analyses.

Note - Soil biological analyses are conducted using wet samples and the results are reported on an OD basis.

(2) Integrated samples (for 2nd level). Dig a 15×15 cm pit until the 1st mineral horizon at *n* (≥5) representative sites in each catchment and collect the whole samples of organic (O and A) horizons in a PE bag. Measure the depth of each pit. Calculate the total area (0.15×0.15×*n*; m^2) and average depth (cm) of the sampled soil. Mix the sample, remove coarse (>1 cm) particles and weight. Then, take a ~2 kg subsample for analyses in to a PE bag and store it at 4^{o}C in dark until used. In laboratory, remove grass, roots, and CP and pass wet samples through a stainless-steel 5-mm sieve. Then continue as described above.

Bulk density of soil

The aim is to estimate amount of soils (wet, AD or OD) in the individual horizons per m^2 of a catchment.

Soil content of each component (M; g m^{-2}) is estimated from equation (1):

$$M = \sum C_i A_i \qquad (1)$$

where C_i is concentration of an element (mol per kg of oven-dried <2-mm soil fraction) in the horizon and/or layer (i) and A_i is the amount of the OD soil fraction in the horizon and/or layer. A_i value is obtained from the amount of a horizon in the field (kg m^{-2}) and the mass ratio of OD soil fraction in the wet sample.

B. Soil chemical analyses

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1st level

pH

pH is determined in the water (pH_w) and calcium chloride (pH_s) extracts:

- pH_W (Deionized water; 1:5; 2.5 h): Shake 4 g of AD sample with 20 ml of deionized water for 2.5 h, then measure pH with electrode directly in the suspension.
- pH_S (0.01*M* $CaCl_2$; 1:5; 2.5 h): Shake 4 g of AD sample with 20 ml of 0.01*M* $CaCl_2$ for 2.5 h, then measure pH with electrode directly in the suspension.

C and N

Determination: CHN-analyzer; AD samples should be ground to pass through a 100-μm sieve and homogenized.

Exchangeable acidity **(EA)**

Potassium chloride method (neutral pH) refers to the existing neutral salt-exchangeable acidity of soils.

Procedure - (1*M* KCl extraction; 1:20; 17 h in 3 steps): 1st step - Add 20 ml of 1*M* KCl to 2.5 g of AD soil in a 50-ml PE centrifuge tube, stopper, and shake for 1 hour. Place the tube in a centrifuge and spin at ~2 500 min^{-1} for 10 min. Pour the supernatant to a 100-ml PE bottle. 2nd step - Repeat with an additional 15 ml of 1*M* KCl and add the supernatant to that from the 1st step. 3rd step - Repeat with an additional 15 ml of 1*M* KCl but shake overnight (~15 h). Total volume of extraction solution is 50 ml. Combine supernatants from all the 3 steps and filter (paper or membrane filter). Total EA and exchangeable Al are determined by NaOH and HCl (after KF addition) titration, respectively, according to Thomas (1982). Exchangeable H^+ is the difference between EA and exchangeable Al.

Exchangeable cations (Richter et al. 1992)

Method is based on displacing soil cations with 1*M* NH_4Cl and analyzing filtrates with AAS or ICP techniques for Ca, Mg, Na, K, and Al concentrations.

Procedure – (1*M* NH_4Cl extraction; 1:20; 17 h in 3 steps): 1st step - Add 20 ml of 1*M* NH_4Cl to 2.5 g of AD soil in a 50-ml PE centrifuge tube, stopper, and shake for 1 hour. Place the tube in a centrifuge and spin at ~2 500 min^{-1} for 10 min. Pour the supernatant to a 100-ml PE bottle. 2nd step - Repeat with an additional 15 ml of 1*M* NH_4Cl and add the supernatant to that from the 1st step. 3rd step - Repeat with an additional 15 ml of 1*M* NH_4Cl but shake overnight (~15 h). Total volume of extraction solution is 50 ml. Combine supernatants from all the 3 steps and filter (paper or membrane filter). Analyze concentration of Ca, Mg, Na, K, and Al in the extract (mg l^{-1}) by ICP or AAS and recalculate the results to mg per g of AD.

- Effective cation exchangeable capacity (CEC_e) = sum of 1*M* NH_4Cl extractable Ca, Mg, Na, K, and Al.
- Cation exchangeable capacity (CEC) = sum of 1*M* NH_4Cl extractable Ca, Mg, Na, and K and EA (1*M* KCl extractable H and Al).
- Exchangeable base cations (EBC) = sum of 1*M* NH_4Cl extractable Ca, Mg, Na, and K.
- Base saturation (BS) = percent of EBC in CEC.

2nd level (Tatras and Pyrenees)

Total phosphorus (P)

Total content of P in AD soil is determined using finely ground subsample (that for C and N analyses) with nitric-perchloric acid digestion, followed by phosphomolybdate blue method (e.g. Kopáček and Hejzlar, 1995).

Oxalate-extractable Fe, Al and P

Oxalate-extractable Fe (Fe_o), Al_o, total phosphorus TP_o, and soluble reactive P (SRP_o) are

determined according to Cappo et al . (1987) by extraction of 0.5 g soil with 50 ml of acid ammonium oxalate solution (0.2 *M* $H_2C_2O_4$ + 0.2 *M* $(NH_4)_2C_2O_4$ at pH 3) in 3 steps. *Procedure* –1st step - Add 20 ml of oxalate solution to 0.5 g of AD soil in a 50-ml PE centrifuge tube, stopper, and shake for 1 hour. Place the tube in a centrifuge and spin at ~2 500 min^{-1} for 10 min. Pour the supernatant to a 100-ml PE bottle. 2nd step - Repeat with an additional 15 ml of oxalate solution and add the supernatant to that from the 1st step. 3rd step - Repeat with an additional 15 ml of oxalate solution but shake overnight (~15 h). Total volume of extraction solution is 50 ml. Combine supernatants from all the 3 steps and filter (glass filter). Analyze concentration of Al_o and Fe_o in the extract (mg l^{-1}) by ICP or AAS, TP_o with nirtic-perchloric acid digestion (Kopáček and Hejzlar 1995) and SRP_o colorimetrically (Wolf and Baker, 1990). Recalculate all the results to mg per g of AD soil.

P -sorption studies (Yuan and Lavkulich 1994)
P sorption maximum (X_m): 8 samples (containing 1.0 g of AD soil) are shaken for 24 h at ~25 ^oC with 25 ml of 0.002 M $CaCl_2$ containing between 0 and 100 mg l^{-1} P as KH_2PO_4 . Since sorption is pH dependent, sorption experiments should be conducted at the original pH values of the samples. After shaking, the suspensions are centrifuged at ~1000 g for 15 minutes and filtered (0.4 μm, glass filters). The SRP concentration in the supernatants is determined by the method of Murphy and Riley (1962). The difference between SRP concentration before and after the procedure is used to calculate the quantity of P sorbed by the soil sample. The sorption data are fitted to the linear form of the Langmuir equation and the sorption maximum (X_m,) is calculated for each sample.
Total sorption capacity (TSC) is:

$$TSC = X_m + SRP_o$$

where SRP_o represents P originally adsorbed on soil (determined by oxalate extraction).

C. Soil biological analyses (the 2nd level only)
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All biological analyses are performed in four replicates.

C and N in soil microbial biomass (C_{mic} , N_{mic})
Method - Chloroform fumigation extraction method
Principle - Moist soil is exposed to ethanol free chloroform vapor for 24 hour and fumigant is removed by repeated evacuation. Microbial cells are disrupted with chloroform vapor during fumigation and an amount of C, N, P released from cells into soil solution is determined.
Procedure – A 20-g sample of moist soil is split into two subsamples (10 g each).
(1) The unfumigated control is placed into a 100-ml bottle, extracted with 40 ml of 0.5 M K_2SO_4 (soil : extractant ratio 1: 4, w/v) in a overhead shaker (40 rev min^{-1}) for 45 min (or oscilating shaker 200 rev min^{-1} for 30 min). Then extract is filtered through a paper filter (Whatman No 42).
(2) The fumigation is carried out in a 50-ml glass vial placed in a desiccator. The desiccator contains wet tissue paper, a vial with soda lime (or NaOH to trap CO_2), and a beaker with 25 ml of ethanol free chloroform and few boiling chips. Then, the desiccator is evacuated until the $CHCl_3$ boils vigorously for 2 min. Then, the desiccator is incubated in dark at 25^oC for 24 hours. After fumigation, $CHCl_3$ is removed by repeated (6 –8 fold) evacuations and the soil is transferred to a 100-ml bottle and extracted in the same way as control soil.

All extracts should be stored at -15 ^{o}C prior to analysis.
Note: In soils containing more than 20% of organic matter (OM), the soil to extractant ratio of 1: 4 must be increased by 1 unit per each 5% of OM (e.g. when soil contains 25% of OM the ratio is 1:5; for the litter layers with ~95% of OM, the ratio is 1:20).

In the soil extracts, carbon content is analyzed by dichromate oxidation according to Vance et al. (1987), or by ultraviolet persulphate oxidation (Wu et al. 1990). Concentration of nitrogen is analyzed as total N after kjeldahlisation (Pruden et al. 1985) or after alkaline persulphate oxidation of extracted N to nitrate (Cabrera, Beare 1993). The last method can be used when automated continuous flow analysing systems are available.

P in soil microbial biomass
Method - Chloroform fumigation extraction method.
Principle - See the earlier method.
Procedure - For description of fumigation see the earlier method. A 30-g sample of moist soil is split into 3 subsamples (10 g each).
(1) The control subsample is extracted with 200 ml of 0.5*M* $NaHCO_3$ (soil to extractant ratio of 1: 20, w/v) in an overhead shaker(40 rev min^{-1}) for 45 min (or oscilating shaker 200 rev min^{-1} for 30 min). The resulting suspension is filtered through a paper filter (Whatman No 42).
(2) The subsample for the P recovery test is extracted with 0.5*M* $NaHCO_3$ as described above but with the addition of 1 ml of KH_2PO_4 standard solution (250 mg l^{-1}).
(3) The fumigated subsample is after fumigation extracted with 0.5*M* $NaHCO_3$ as the control subsample. Concentration of phosphate in the extracts is determined by phosphomolybdate blue method (Brookes et al. 1982) and the amount of P released from microbial biomass is calculated (Brookes et al. 1982)

Potential denitrification
Method - Denitrifying enzyme activity (DEA) by Smith and Tiedje (1979)
Principle - Soil in the form of slurry is amended with glucose and KNO_3 and incubated up to several hours; N_2O evolved is determined using gas chromatography.
Procedure - Soil slurry is prepared in a 120-ml serum bottle by mixing of 25 g of the field-moist soil with 25 ml of mixing solution (1 m*M* glucose, 1 m*M* KNO_3, and 1 g l^{-1} chloramphenicol). Bottles are sealed with butyl rubber stoppers, evacuated and flushed four times with 99.99 % He. Each evacuation (up to -0.01 MPa) and/or flushing (up to 0.11 MPa) lasts for 2 min and lack of oxygen in the internal atmosphere is occasionally confirmed by gas chromatography. Then, 10 ml of pure acetylene is added with a syringe and internal pressure is equilibrated to atmospheric pressure. Immediately, the soil slurry is shaken on an end-to-end shaker at 25 ^{o}C. After 30 and 60 minutes from the addition of acetylene, 1-ml samples of headspace atmosphere are taken with a gas-tight syringe and N_2O is measured on a gas chromatograph with an electron capture detector. DEA is calculated from the N_2O increase during the half-hour incubation (between the 30th and 60th minute). Alternatively, headspace is sampled for the N_2O determination several times within 3 hours of incubation (this procedure yields a curve of N_2O evolution).

Potential nitrification
Method - Short-term nitrification assay (nitrifying enzyme activity, NEA) by Schmidt and Belser (1982)
Principle - Soil in the form of slurry is amended with NH_4^+ and incubated under oxic

conditions for 5-10 hours; $NaClO_3$ is used to inhibit nitrite oxidation; then, the only product of nitrification is NO_2^-, which is determined.
Procedure - Soil slurry is prepared in a 300-ml serum bottle by mixing of 20 g of the field-moist soil with 100 ml of phosphate buffer (20 ml 0.2*M* K_2HPO_4 [3.48 g in 100 ml H_2O] and 80 mL 0.2*M* KH_2PO_4 [2.72 g in 100 ml H_2O]). Then, 0.2 ml of 0.25*M* $(NH_4)_2SO_4$ and 1 ml of 1*M* $NaClO_3$ are added. Immediately, the soil slurry is shaken on an end-to-end shaker at 25 ^oC. After 5 and 10 hours, 10-ml subsamples of slurry are taken and NO_2^- is determined as follows: 10 ml of 4*M* KCl is added to the subsample, suspension is shaken for 1 hour and filtered (Whatman No 42). In the filtrate, nitrite is determined (e.g., Griess-Ilosvay technique according to Forster, 1995). Potential nitrification is calculated as a difference in NO_2^- concentration between the 1st and 2nd sampling.

Carbon mineralization
Method - Estimation of soil respiration
Principle - **Air dried soil** is incubated under controlled conditions for about 20 days and soil respiration (CO_2 production) is measured 7 times during this period . Values of measured CO_2 (C_T) are fitted in two component model (Šantrůčková et al. 1993). An amount of C, that is mineralized from from soil organic C pool (C_S) and labile fraction (C_0), and rate of mineralization of labile C is estimated (k).
Description –
Air dried soil is rewetted to 50% WHC. A 10-g sample of moist soil is placed in sealed 100-ml bottle and respiration is measured after 1,2,4,7,11,15 and 21 days of incubation at 25^oC. Evolved CO_2 is trapped in sodium hydroxide: vial with 2 ml of 0.5 N NaOH is placed on the surface of soil sample, the excess NaOH is titrated by 0.05 N HCl after addition of 12% $BaCl_2$ and phenolftalein. Cumulative respiration is fitted in two component model:

$$C_T = C_S + C_0 (1-e^{-kt})$$

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