

NITROGEN FRACTIONATION IN SOILS

Objective: To determine the amounts of nitrogen fractions in the soil by extraction.

Extractable Fractions:

Inorganic N:

a) Exchangeable nitrate and ammonium (KCl extraction)

Total N:

a) Total microbial biomass N (extraction with potassium sulfate + persulfate digestion)

b) Total microbial biomass N (chloroform fumigation, extraction with potassium sulfate, persulfate digestion)

c) Total organic N (analyzed by combustion on Perkin Elmer CHNS/O Analyzer)

Safety Information:

Follow all laboratory safety procedures when working with acids, bases, chloroform, and potassium persulfate.

Field Sampling and Storage:

Using Chris Craft's personalized core sampler, collect a 30 cm soil core.

Excessive contact with oxygen, mineralization of organic matter, and changes in microbial biomass will affect the results. Squeeze air out of bags before sealing. Store soil on ice while in the field, and transfer to cold room in the lab. All soil must be kept cold at 3°C except when extracting or centrifuging.

Extracts should be frozen if analyses will not be performed within one week.

Labeling of Samples:

Three 50 mL centrifuge tubes are need for each sample. Label the first set of tubes with A and sample name. The A tubes will be treated with KCl only. Label the second set of tubes with B and sample name. This set will be extracted with potassium sulfate, and digested with potassium persulfate. Label the third set of tubes with C and sample name. This set will be fumigated with chloroform, extracted with potassium sulfate, and digested with potassium persulfate.

Soil Wet/Dry Weight Conversion:

Record wet weight of soil core. Weigh 10 g wet soil into aluminum weigh boats. Allow soil to air dry (approximately 1 week), record air dried weight, then oven dry soil for 24 hours at 105°C, and record oven dry weight.

Convert wet/dry soil weight by:

$$\text{Bulk Density} = \frac{\text{wet core wt} * \frac{(\text{oven dry wt}_{\text{subsample}})}{(\text{wet wt}_{\text{subsample}})}}{\text{vol of core}}$$

INORGANIC N**KCl Extraction:**

Weigh 20 g wet soil into each “A” tube. Pipette 30 mL 2M potassium chloride (149.12 g KCl in 1L DI water) into each tube using a repipette dispenser. Extract samples on the shaker for 1 hour at 180 shakes per minute. Centrifuge for 1 hour at 3400 rpm (Fisher Centric Centrifuge, dial set to 6.4). While centrifuging, remember to refrigerate the remaining samples. The supernatant (liquid extract) should become clear. If there are plant particles floating in the supernatant, remove by filtering supernatant with Whatman #42 filter paper. Then, pour off about 15 mL into scintillation vials and store in freezer or cold room. Be careful not to lose any solids.

Analyze for nitrate on Lachat AE, using “QuikChem Method 12-107-04-1-B, Nitrate in 2M KCl Soil Extracts,” and for ammonium using “QuikChem Method 12-107-06-1-B, Ammonia (Phenolate) in Soils.” For details of modified procedures, see Central Lab’s Lachat Procedure, “NNSOILS/SOILS.”

TOTAL N**Total Microbial Biomass N (Part a):**

Weigh 20 g wet soil into each “B” tube. Pipette 30 mL 0.5M potassium sulfate (87.13 g K₂SO₄ in 1L DI water) into each tube using a repipette dispenser. Extract samples on the shaker for 1 hour at 180 shakes per minute. Centrifuge for 1 hour at 3400 rpm (Fisher Centric Centrifuge, dial set to 6.4). While centrifuging, remember to refrigerate the remaining samples. The supernatant (liquid extract) should become clear. If there are plant particles floating in the supernatant, remove by filtering supernatant with Whatman #42 filter paper. Then, pour off about 15 mL into scintillation vials and store in freezer or cold room. Be careful not to lose any solids.

Before using the CEM Microwave, obtain proper training from Central Lab personnel.

Digest extracts in CEM Microwave in Central Analytical Lab. Reagent is 15 g NaOH and 50 mL low nitrogen potassium persulfate dissolved in 1L DI water (modified from Cabrera and Beare,

1993). Procedure is named “TNSOIL,” pressure = 21 psi, TAP = 30 minutes, power = 80%, temperature = 120°C (optional).

Use a 1:1 sample:reagent ratio, e.g. 5 mL of sample (K_2SO_4 extract) to 5 ml persulfate. In each tray, remember to digest a 5 ppm urea standard and a blank. Also digest 2 L of 0.5M K_2SO_4 for the Lachat carrier. See details for using the microwave in the CEM Microwave Procedures book located next to the microwave.

Analyze digested extracts for nitrate on Lachat AE, using “QuikChem Method 10-107-04-01-B, Nitrate/Nitrite in Water,” also listed as “TNSOIL” in Central Lab’s Lachat Procedure binder. NO_3 -N numbers given by Lachat have to be multiplied by 2 because of the dilution factor - 5 mL sample in 5 mL reagent.

Total Microbial Biomass N (Part b):

Weigh 20 g wet soil into each “C” tube. In the hood, add 2.8 ml chloroform to each tube. Shake to mix soil and chloroform, being sure the soil does not clump. Fumigate for 1 hour. Uncap the tubes and allow the chloroform to evaporate for 48 hours, or until there is no more chloroform smell. Lay moist paper towels under the tubes to prevent soil from drying out. Check the samples periodically to ensure that a crust has not formed on the soil surface. If so, the crust can be broken up with a spatula.

After fumigation, extract with potassium sulfate, and digest in microwave as above.

Analyze digested extracts for nitrate on Lachat AE, using “QuikChem Method 10-107-04-01-B, Nitrate/Nitrite in Water,” also listed as “TNSOIL” in Central Lab’s Lachat Procedure binder. NO_3 -N numbers given by Lachat have to be multiplied by 2 because of the dilution factor - 5 mL sample in 5 mL reagent.

Total Organic N:

Follow the “CHN Analysis” procedure in this binder for performing this step.

NOTES FOR CHEMICAL ANALYSIS ON LACHAT AE

Note: Results provided by Lachat are NO_3 -N and NH_4 -N.

Correction for soil moisture content: Corrected value = lachat value * (oven wt / wet wt)

Total Microbial N is: amount N extracted from fumigation (part b) less that extracted from non-fumigated soils (part a).

REFERENCES

Cabrera, M.L. and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil. Sci. Soc. Am. J.* 57:1007-1012.

CEM Microwave Procedure Manual. Compiled by Central Lab.

CHN Procedure Manual. Compiled by Central Lab.

Fisher Centric Centrifuge Model 225. IEC. Needham Heights, MA.

Lachat AE Procedure Manual. Compiled by Central Lab.

Perkin Elmer Corporation. PE2400 Series II CHNS/O Analyzer. Norwalk, CT.

QuikChem AE Automated Ion Analyzer. Lachat Instruments. Milwaukee, WI.