SOIL NITROGEN TRANSFORMATION RATES PROTOCOL

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Soil nitrogen transformation rates (potential net N transformation, mineralization, ammonification, nitrification and depolymerisation) (Schimel & Bennett, 2004) are measured by determination of the total K_2SO_4 or KCl extractable nitrogen (TAN), the available mineral N (NH_4^+-N and NO_3^--N) and the dissolved organic N (DON) forms before and after a soil incubation in the laboratory at 80% of water holding capacity (WHC) and 30°C for 14 days (Allen, Grimshaw & Rowland 1986), and extracted with K_2SO_4 0.5M or KCl 1M in a 1:10 ratio. The incubation, extraction and the colorimetric analyses of DON, NH_4^+-N and NO_3^-N in the soil´s extract are made following the procedure describe below. The amount of N forms in the soils is calculated using a linear calibration curve with standards and expressed as mgr N kg^{-1} soil and rates are expressed as mgr N kg^{-1} soil day^{-1}.

Reagents

All reagents used are A.C.S reagent grade. Material used has to be clean with a 6% H_2SO_4 bath and cleared up with deionized (DI) water prior use to avoid N contamination.

- 0.5M K_2SO_4 solution: Dissolve 87.3g K_2SO_4 in 950ml of DI water and adjust to 1L. Prepare all the K_2SO_4 needed for all the samples. Store at room temperature.
- 1M KCl: Dissolve 74.56g KCl in 950ml of DI water and adjust to 1L. Prepare all the K_2SO_4 needed for all the samples. Store at room temperature.
- 0.148M $K_2S_2O_8$: Dissolve 20g low-N $K_2S_2O_8$ in 500ml of DI water. Store at room temperature.
- 3M NaOH: Dissolve 12g NaOH in 90ml DI water, then adjust to 100ml with DI water.
- Devarda´s alloy.
- 0.1M H_2SO_4: Place 0.625ml H2SO4 95-98% in 90ml DI water. Adjust the volume to 100ml with DI water
- Citrate reagent: Dissolve 5g trisodium citrate ($C_6H_5Na_3O_7$ $2H_2O$) and 2 g NaOH pellets in 100ml of DI water. Store at 4°C for no more than one month.
- Salicylate-nitroprusside reagent: Dissolve 7.813 g sodium salicylate ($C_7H_5NaO_3$) and 0.125 g sodium nitroprusside ($Na_2Fe(CN)_5NO$ $2H_2O$) in 100ml of DI water. Store at 4°C for no more than one month.
- Citrate+salycilate reagent: Mix 100 ml citrate with 200 ml salycilate reagent (1citrato:2salicylate relation)
- Hypochlorite reagent: Dissolve 1g Na_3PO_4, 2ml 2M NaOH and 10ml of fresh commercial household bleach (0.7M NaOCl) in 75 ml of DI water and adjust to 100 ml. Prepare the solution daily or weekly and store for no more than a week at 4˚C

- 2M NaOH: Dissolve 8g NaOH in 90ml DI water, then adjust to 100ml with DI water.
- Standard solution 1000 ppm NO_3^--N: Dissolve 7.2221g KNO_3 into 1000ml DI water. Store at 4°C for no more than six months.
- Standard solution 1000 ppm NH_4^+-N: Dissolve 3.8209g NH_4Cl into 1000ml DI water. Store at 4°C for no more than six months.
- NO_3^--N and NH_4^+-N working standard: Dilute 1000ppm standard solution to 100ppm in 0.5 M K_2SO_4. 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12.5 and 15 ppm solutions are prepared by diluting 100ppm standards solutions in 0.5 M K_2SO_4. Store at 4°C for no more than one week.

Procedure

- **INCUBATION**

1. Place twice 2.5g of air-dry soils into two 50 ml centrifuge tube. Soil samples should be thoroughly mixed or sieved at <2mm before, to provide a representative sub-sample. Label first tube as **T_0** (pre-incubation tube) and the second one **T_{14}** (post-incubation tube). *(Note I: The amount of soil could be change if needed, just keeping the ratio 1:10 soil: K_2SO_4 in the extraction step 5)*. *(Note II: the relation 1:10 could be change if very low or high amount of N form are expected).*
2. Reserve **T_0** tubes and add DI water to each sample into **T_{14}** tubes to reach 80%WHC.
3. Cover **T_{14}** tubes with gas-permeable thin film plastic that allows the exchange of gases but not water, with the help of a tape that holds the plastic to the tube, and place all **T_{14}** tubes into an incubation chamber with 30°C and 50% air-moisture in dark conditions (see picture 1). Incubate then for 14 days.

Picture 1: T_{14} tubes inside the incubation chamber

- **EXTRACTION**

4. **T_0** could be processing while **T_{14}** are into the incubation chamber, or they could be analysed all together once **T_{14}** had finished the incubation period. Once **T_{14}** tubes are ready (14 days after the beginning), take them out of the chamber and remove the film.
5. Add 25ml of 0.5M K_2SO_4 (or KCl 1M) solution to each tube (**T_0** and **T_{14}**) *(Note: Just before K_2SO_4 addition, add the water necessary to get 80%WHC to **T_0** tubes)* and close.
6. Shake soils extract in an orbital shaker at 200rpm for 1h at 20°C and filtered to pass a 0.45μm Millipore filter. Extract could be centrifuged instead filtered to separate extract from soils and pass the supernatant to another tube. The filtered or centrifuge extracts could be kept at 4°C until colorimetric analyses are conducted, at most once week, or could be frozen for months (*Note: **T_0** and **T_{14}** should be processed in the same way*). Extract could be centrifuged instead filtered to separate extract from soils and pass the supernatant to another tube.

- **DETERMINATION OF NITROGEN FORMS**

<u>Conversion of DON to NO_3^-</u> (Sollins *et al.*, 1999)

DON in the extract is first oxidized to NO_3^--N with $K_2S_2O_8$ in an autoclave at 121°C for 55 min.

7. Add 900 μl of extract to a 2ml microtube with screw-cap (picture 2).

Picture 2. Microtubes

8. Add 900 μl of standards NO_3^--N solution to another 2 ml microtube with screw-cap
9. Add 300 μl of 0.148M $K_2S_2O_8$ and 15 μl of 3M NaOH to samples and standards microtubes
10. Close the microtubes, shake and autoclave at 121°C for 55 min
11. Kept the microtubes at room temperature overnight (Note: it is important to wait until next day before continue next step)

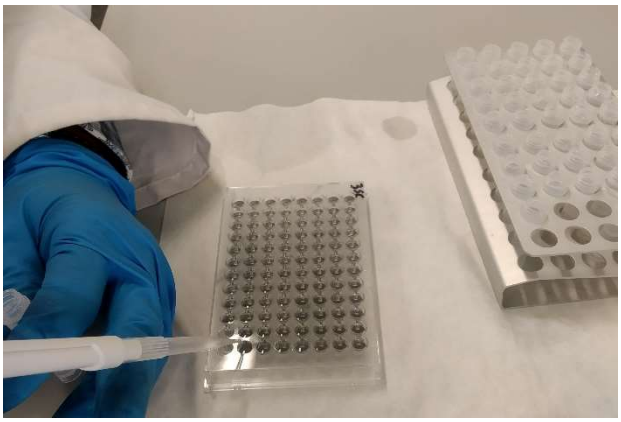
<u>Conversion of NO_3^- to NH_4^+ (Sims *et al.*, 1995)</u>

NO_3^--N in the extracts and in the digestion microtubes are reduced to with Devarda´s alloy as follow:

12. Add triplicate aliquots (250μl) of soil extract, NO_3^--N NH_4^+-N standards and digestion of samples and standards into three wells of 96 well plates containing 15-25mg of

Devarda´s alloy. To avoid volatilization loss of NH_3, add 25 µl of 0.1M H_2SO_4 to each well after samples (picture 3).

13. Seals plates and incubate until reduction is completed overnight (Note: it is important to wait until next day before continue next step)

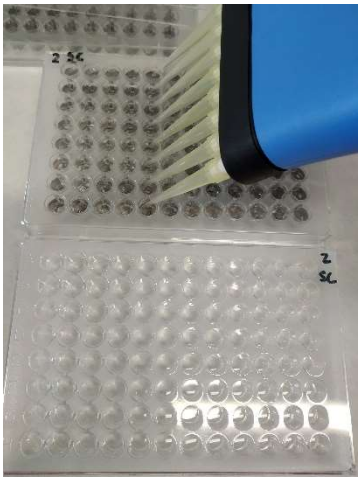


Picture 3. Example of NH_4^+-N $_{NO3}$ plate

<u>Determination of NH_4^+ by the indophenol blue method (Sims *et al.*, 1995)</u>

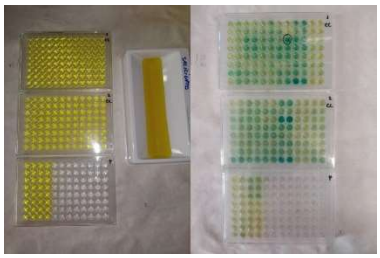
NH_4^+-N in the extract, in the well plates with the extract reduction (NH_4^+-N $_{NO3}$) and in the well plates with digestion reduction (NH_4^+-N $_{NO3+DON}$) will be analysed by colorimetric assay using the indophenol blue method as follow:

14. Add triplicate aliquots (75µl) of extract and NH_4^+-N standards into three wells of a 96 well plate. Note this well plate as NH_4^+-N plate.
15. Transfer 50 µl of each plate with devarda´s alloy to another plate. Note this plate as NH_4^+-N $_{NO3}$ and NH_4^+-N $_{NO3+DON}$ plates (picture 4)



Picture 4. Example of transfering sample´s step

16. Add 100 µl of deionized water to each well into NH_4^+-N plate and 125 µl into NH_4^+-N $_{NO3}$ and NH_4^+-N $_{NO3+DON}$ plates to get 175 µl volume in all the wells.
17. Add 75 µl of citrate+salicylate reagent and 25 µl of hypochlorite reagent.



Picture 5. Example of colour development after reagents addition

18. After 45 minutes, read with a spectrophotometer at 650 nm (picture 5).
19. Use the absorbance of standards to obtain a linear calibration curve of ppm-absorbance (one linear calibration curve per: NH_4^+-N, NH_4^+-N $_{NO3}$ and NH_4^+-N $_{NO3+DON}$ plates). Average data obtained from the three well of each standard are used to develop a calibration curve for the tested soil.
20. Calculate ppm of your samples with the linear curve.
21. Transform ppm value to mg N kg-1 soil:
    mg N kg-1 soil = (ppm x 25ml K_2SO_4) / 5 gr soil

N-forms calculation

- **TAN** is calculated with the results of the T0 NH_4^+-N $_{NO3+DON}$ plates
- **Available mineral N** is calculated with the results of T0 NH_4^+-N $_{NO3}$ plates
- **N-NH_4** is calculated with the results of T0 NH_4^+-N plates
- **N-NO_3^-** is calculated with the results of T0 NH_4^+-N $_{NO3}$ plates – T0 N- NH_4^+-N plates
- **DON** is calculated with the results of T0 NH_4^+-N $_{NO3+DON}$ plates – T0 NH_4^+-N $_{NO3}$ plates

Rates calculation

Rates units: mg N kg-1 soil day-1

Mineralization: The N transformation from organic N to inorganic N (T14 Available Mineral N – T0 Available Mineral N)/14 days
Ammonification: The N transformation from organic N to NH_4 (T14 NH4 – T0 NH4)/ 14 davs
Nitrification: The N transformation from NH_4 to NO_3 (T14 NO3 – T0 NO3)/14 davs
Depolymerization: The N transformation from soil and litter polymers into dissolved organic N (T14 DON – T0 DON)/ 14 days

Potential N transformation: The N transformation

$(T14_{TAN} - T0_{TAN})/14$ days

References

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