

Hofmockel Lab Protocol

INORGANIC NITROGEN ANALYSIS – MICROPLATE FORMAT

Modified from: Allison, Treseder, Suding, and Castellano Lab protocols; detection limit <0.05 ppm

Based on:

* Hood-Nowotny et al. 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. SSSAJ 74(3):1018-1027

* Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry

Materials

- Solutions (listed below)
- 96-well microplates (Fisher #08-772-5) – only use new, not acid washed, and UV sterilize before use
- Solutions boats – only use new, not acid washed
- Single channel & multichannel pipettes and tips – only use new tips, not acid washed
- 25 mL volumetric flasks (x5)
- Dust mask

Plate Layouts: Run a standard curve and blanks with each plate; 3 reps per sample.

For extractable inorganic N determination, run ammonium and nitrate protocols on un-fumigated, un-oxidized samples. For total N determination, run nitrate protocol on persulfate oxidized samples. For microbial biomass N determination, run nitrate protocol on fumigated and un-fumigated persulfate oxidized samples.

AMMONIUM DETERMINATION

Solutions

1) Matrix

2M KCL or 0.5M K₂SO₄ (87.13 g in 500 ml) made with deionized **ultrapure** water

2) Sodium salicylate solution – (*prepare fresh daily*)

(dissolve in ~ 50 ml **ultrapure** water and bring to 100 ml in a volumetric flask)

100 ml = 10 plates

6.8 g sodium salicylate (Fisher # S395-500) – phenolic compound for formation of color

5.0 g sodium citrate (Fisher # BP327-1)

5.0 g sodium tartrate (Fisher # BP352-500)

0.025 g sodium nitroprusside (Fisher # S350-100) – catalyst

3) 1.5M NaOH (Fisher # S318-1) – (*store on lab bench – stored in cupboard above pH meter*)

Used for solution 4

Add 60 g to volumetric flask and bring to 1000 ml with **ultrapure** H₂O.

4) Bleach/NaOH solution – (*mix fresh every day*)

100 mL = 10 plates

2.0 mL bleach in 100 ml volumetric

98 mL 1.5M NaOH

5) Ammonium Stock Solution, 100 ppm – (*store at 4°C for several weeks*)

Bring to 500 ml in a volumetric flask

0.23585 g ammonium sulfate (Fisher # A938-500)

500 ml ultrapure water

NITRATE DETERMINATION**Solutions****1) Matrix**

2M KCL or 0.5M K₂SO₄ made with deionized ultrapure water, or oxidized 0.5M K₂SO₄ for microbial biomass N

2) 0.5M HCl – (store on bench)

In a 250 mL volumetric, add

10.4 mL concentrated HCl and bring to

250 mL with ultrapure H₂O (always add acid to water!)

3) Vanadium (III) reagent – (store at 4C for up to 2 weeks, or until color change is noticeable; stable longer if frozen) *Work quickly and wear dust mask!*

In a 500 mL beaker:

Pour 200 mL 0.5M HCl (use graduated cylinder to measure volume)

Weigh 0.5g VCl₃, and add to HCl in hood; briefly shake to mix particles

Add 0.2 g sulfanilamide

Add 0.01 g N-1(1-naphthyl)ethylenediamine dihydrochloride

Transfer to amber bottle

Store at 4C until ready to use

Nitrate Stock Solution (100 ppm) – (store at 4°C for several weeks)

Bring to 500 ml in a volumetric flask

0.3609 g potassium nitrate (Fisher # P38100)

500 ml ultrapure water

PROTOCOL**1. Preparation:**

- a. Set incubator (for 1 hour version of nitrate protocol only)
- b. Sterilize plates in UV hood
- c. Spin plates and let warm to room temperature
- d. Let stock warm to room temperature
- e. Make reagents
- f. Design plate layout

2. Make standards: see below**3. Load plate**

In each well: 200 uL reagent (100 uL each of salicylate and bleach solutions; 200 uL of vanadium chloride reagent) + 40 uL [sample/standard + matrix]

To determine the amount of sample/standard to transfer per well: 100/highest calibration standard (ppm).

For example:

<u>Highest calibration standard</u>	<u>Sample/standard</u>	<u>Matrix</u>
15 ppm	6	34
10 ppm	8	32
1 ppm	40	0

Notes:

- Use manual multichannel pipettes to load plates
- Dispense sample/standards first, into bottom of well; change tips every row!
- Dispense reagents over wells; use same tips if tips do not touch plates.

4. Incubate in the dark

- a. Ammonium: 50 minutes at room temperature
- b. Nitrate: 5 hours at room temperature or 1 hour at 37°C

5. Read plates

- a. Ammonium: 650 nm
- b. Nitrate: 540 nm

STANDARDS

Final ppm	uL of 100 ppm stock to add to 25 mL volumetric	Standard Curve
15	3750	High
10	2500	High
5	1250	High
2	500	High
1	250	Low/high
0.5	125	Low/high
0.2	50	Low
0.1	25	Low
0.05	12.5	Low

Ammonium analysis notes: The samples will turn a blue green color; however, if the concentration of ammonium is too high, the reaction will go too far and the sample will turn yellow. If this happens the sample should be diluted and ran again. (Common dilution for sample: 100 µl sample + 900 µl matrix). If you are diluting samples before running, remember to run a control (a sample that did not need to be diluted) with your diluted samples to check that your dilutions worked.