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Sequential Microbial Biomass and Nitrogen Extraction

In 1 collection

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ABSTRACT

The method is modified from the Hofmockel lab at PNNL, modified from Suding Lab protocol, modified from S. E. Hobbie, 5 May 1998.

MATERIALS

- [M] 0.5 Molarity (M) K2SO4 (87.13 g or 8.71 g K2SO4 per 1 L extractant made in ultrapure water)
  Always test background concentrations of new lots of K2SO4!
- Buchner Funnel
- Ethanol cleaned forceps
- Whatman No.42 filter paper, pre-leached 3x with K2SO4 ([M] 0.5 Molarity (M) or [M] 0.05 Molarity (M) depending on sample extractant) and 1x with ultrapure water; (Whatman Part#1442-047)
  See Rinsing Filters section below
- Vacuum Pump
- 50 mL conical tubes with V-bottom (new sterile or acid washed)-label with Sample_ID, date, initials; 1 per sample + blanks
- 2-gallon glass jar with bottle-top repeat dispenser, calibrate to 24 mL
- Filter supports
- Acid washed funnels
- 20 mL glass scintillation vials (acid washed)- 2 per sample + blanks- label with Cat#, date, and either “background” or “fumigated”
- Ethanol-free chloroform (for analysis of carbon) (VWR # BJ049-1L)
- Viton/Butyl Gloves
- Phosphoric acid for preservation
- Dropper for acid
- Manual 2 mL pipet pump
- Data sheets

Subsample soil

1 Subsample (~ 8 g) place in V- bottomed conical tube labeled with Cat# and weight. Label caps with Cat#

2 Gravimetric water content will also be essential for final calculations on a dry weight basis.

Data to Collect

3 Data to Collect

3.1 Weight of soil and Cat# for each sample on the data sheet

3.2 Time onto and off the shaker 1st extraction (by batch)

3.3 Time of chloroform addition (by batch)

3.4 Time of chloroform evacuation (by batch)

3.5 Time onto and off the shaker 2nd extraction (by batch)

Rinsing Filters

4 Gather the vacuum pump, Erlenmeyer filter flask, number of filters needed, K2SO4, Nano pure water, Buchner funnel, and tweezers.

5 Set up a tray with bench paper. Wipe the plastic side of the bench paper with ethanol.

6 Set up vacuum, Erlenmeyer flask, and Buchner funnel.

7 Place a stack of 5-8 Filters in the funnel.

8 to cover the filters. Turn on the vacuum pump.

9 Once the K2SO4 has been pulled through, repeat with K2SO4 two more times.

10 Wait a few seconds to ensure all K2SO4 is pulled thought (you will hear the vacuum noise change) Then add ultrapure water until filters are completely covered.

11 Wait a few seconds to vacuum off as much liquid as possible then turn off the vacuum and use tweezers to remove the filters.

12 Spread the filters out on the tray with the clean bench paper. Place the tray in the hood overnight to dry.

Extraction

13 Work in batches (when possible randomized experimental treatments among batches, but do not mix isotopically labelled and natural abundance samples in order to prevent cross contamination)

14 Include multiple (minimum 3 per day) empty 50 mL conical tubes. Carry these through the entire process as blanks.

15 Add 24 mL K2SO4 to non-fumigated subsample using repeat dispenser. Cap well.

16 Place on shaker for 200 rpm, 02:00:00 or low setting at room temp (20 °C)

17 Balance samples and centrifuge @ 4000 rpm, 00:20:00 . 20m

18 Place funnels in support racks and insert a pre-leached filter in each funnel.

19 Remove sample from centrifuge and place scintillation vial with matching Cat# below funnel.

20 Pour supernatant into funnel, take care to check that filtrate is dripping directly into the vial.

21 Leave at least 1cm head space in vials to prevent bursting (collect excess in waste container)

22 Using ethanol clean forceps transfer filter paper back into conical tube with soil pellet.

23 In the fume hood- add 2 drops of phosphoric acid to the extract (for preservation for TOC analysis).

24 Store extracts at -20 °C .

25 Using ethanol clean forceps transfer filter paper back into conical tube with soil pellet.

26 Rinse and acid wash funnels for next extraction.

FUMIGATION

3d

27 **FUMIGATIONS MUST BE DONE IN THE FUME HOOD**

CHLOROFORM IS A KNOWN CARCINOGEN

Wear Viton/Butyl gloves with disposable nitrile gloves inside during steps 28-31. Always wear a lab coat and safety glasses with side guards when working with chloroform.

28 Add 2 mL chloroform to soil pellet, plus filter.

29 Add additional labeling to tube and cap if needed, chloroform will cause ink to run if it comes in contact with ink

30 Let sit in the dark in the hood for 24:00:00 : cover the tube racks with a black garbage bag (darkness prevents the chloroform from breaking down). 1d

31 Remove garbage bags and caps and allow to vent 48:00:00 . Be sure to keep track of which cap goes to each tube. 2d

31.1 Note: Be sure to place notice on the fume hood that chloroform containing samples are inside

32 Extract the sample as above: as per steps #13-24.

33 As an added precaution against any residual chloroform, perform the K2SO4 addition, filtration, and phosphoric acid addition steps inside the hood

34 The filter from the second extraction can be discarded.

34.1 For all experiments leveraging isotopic labeling the leftover soil pellets should be archived at 20 °C. This includes 1abeled samples and the unlabeled controls.

34.2 For experiments not requiring the pellet: At the end of the extraction open all tubes inside the fume hood and leave to dry completely to remove any trace of chloroform before disposing of tubes containing filter and soil.