

Microbial Biomass-N

1. Weigh out 10g of field moist soil into a specimen cup labeled "MB-N"
2. Add 1ml of chloroform directly into the cup. **NOTE: Do this in the fume hood while wearing lab coat, gloves and safety glasses!**
3. Cap cup and shake vigorously to mix chloroform and soil.
4. Let sit in hood for 18-24 hours.
5. After 18-24 hours, remove cap and let vent for 1-2 hours.
6. Extract with 50ml of 0.5M K_2SO_4 .
7. Hand shake about 20 times.
8. Let sit for 18-24 hours in the lab.
9. Filter through a Whatman GF/A filter using the suc-vac system.
10. Save filtrate in two (set A & B) 20ml plastic scintillation vials.
11. Keep samples frozen until ready to be analyzed.

Microbial Biomass-P

Making Anion resin bags:

1. Anion resin bags were made by placing 5g of wet anions into a 5x10cm concentrate bag (Ankom Technology part #R510) and sewing shut. Note: Try folding the end over before sewing shut to prevent the end from unraveling.
2. Anions can be made a couple weeks in advance and stored at room temperature in a plastic bag to prevent them from drying out.

Mixing 0.5M NaHCO₃:

F.W =84.01g NaHCO₃/M

Multiply F.W. by desired molarity

84.01g NaHCO₃/M x 0.5M NaHCO₃ = 42.005 g NaHCO₃/L DI H₂O

So, weigh out 42.005 g NaHCO₃ per 1 liter DI H₂O

Adjust pH to 8.5

Processing:

1. Weigh out 1g of field moist soil into two 50ml centrifuge tubes. One tube should be labeled "MB-P F" for fumigated and the other should be labeled with "MB-P UF" for unfumigated.
2. Add 30ml of DI H₂O and one resin bag to both the fumigated and unfumigated tubes.
3. Hand shake tubes a couple of times, the put tubes on shaker table at low speed for 16 hours. Tubes should be put in the shaker so that they lay horizontal.
4. After 16 hours, take tubes off shaker and remove the resin bags from the tubes using 1-2ml of DI H₂O to rinse off soil.
5. Save the resin bag from the unfumigated tube and place into a specimen cup for determination of available P.
6. Set the fumigated resin bag aside to be washed, recharged and reused.
7. Centrifuge both the fumigated and the unfumigated tubes on high speed for ten minutes.
8. After centrifuging, pour out the supernate and discard.

For Unfumigated sample:

1. Extract unfumigated sample with 30ml of 0.5M NaHCO₃ and place on shaker at low speed for 16 hours.
2. After 16 hours, remove unfumigated tube from shaker and centrifuge at high speed for 10 minutes.
3. Filter supernate using a Whatman GF/A filter and suc-vac system.
4. Save filtrate into a 20ml plastic scintillation vial labeled "MB-P UF".
5. Store sample in refrigerator until ready to process.

For Fumigated samples:

1. For fumigated sample, add 1ml of chloroform directly into the tube.
NOTE: Do this in the fume hood while wearing lab coat, gloves and safety glasses!
2. Cap the tube and let sit in the hood for 16 hours.
3. After 16 hours, uncap tubes and let vent for 1-2 hours.
4. After venting, extract samples in the same manor as the unfumigated samples.
5. Save filtrate in a plastic 20ml scintillation vial labeled "MB-P F".

Available P

1. Take resin bag that was saved from unfumigated tube and extract with 100ml of 0.5M HCl and let sit for 1 hour, loosely capped (to allow for out gassing).
2. After 1 hour, filter samples using Whatman GF/A filters and suc-vac system.
3. Save filtrate in two 5ml plastic test tubes labeled "AV-P A" and "AV-P B".
4. Store samples in the refrigerator until they can be processed.

Diluting 0.5M HCl:

Start with 12M of concentrated HCl

$$(C1)(V1) = (C2)(V2)$$

$$(0.5M\ HCl)(1,000ml) = (12M\ HCl)(V2)$$

$$\frac{(0.5M\ HCl)(1,000ml)}{(12M\ HCl)} = 41.67\ ml\ 12M\ HCl$$

Dilute 41.67 ml of 12M HCl with DI H₂O into a 1,000ml volumetric flask.

Recharging Anion Resins

NOTE: Do not charge or recharge resins more than 24 hours before intended use. Resins must be charged before they are used!

1. Rinse anion resin bags well with DI H₂O. (a colander works well for this)
2. Place resins and 0.5M NaHCO₃ in a 1 liter nalgene bottle.
3. Cap and place on the shaker at low speed for 10 minutes.
4. After shaking, pour out NaHCO₃ and rinse bags well with DI H₂O (use colander)
5. Repeat this entire process 2 more times.
6. After charging, seal resins in a plastic bag until they are needed.

Mixing 0.5M NaHCO₃:

$$F.W = 84.01\ g\ NaHCO_3/M$$

Multiply F.W. by desired molarity

$$84.01\ g\ NaHCO_3/M \times 0.5M\ NaHCO_3 = 42.005\ g\ NaHCO_3/L\ DI\ H_2O$$

So, weigh out 42.005 g NaHCO₃ per 1 liter DI H₂O