Updated KCl extraction and ammonium, nitrate and PMN assay protocols for the Yannarell Lab

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KCl extraction:

1. Weigh out 10 +/- 0.05 g ground and dried soil into a 50-mL centrifuge tube. If you are going to measure only ammonium and/or nitrate, weigh out one subsample. If you will also measure PMN, weigh out two subsamples and label one for PMN and the other for ammonium/nitrate. Keep track of labels and weights in your lab notebook.
2. To each sample, add 40 mL 1 M KCl.
3. Shake for one hour at 240 opm (maximum speed).
4. Centrifuge at 2000 rpm for 2 minutes (use centrifuge in Kent Lab). \*Remember to wipe down inside of centrifuge after running samples to clean off any supernatant that may have leaked!
5. Filter out 10-15 mL of supernatant using plastic funnels and Whatman No. 42 filter paper. \*When finished filtering, filters and tubes with KCl solution and soil can be discarded in trash; funnels must be acid washed!
6. Store KCl extracts in freezer until ammonium and nitrate assays are performed.

If measuring potentially mineralizable nitrogen (PMN):

1. Add 10 uL nanopure water to 50 mL tubes containing 10 g soil. Gently shake to ensure soil is uniformly moistened.
2. Purge headspace by introducing He gas (about 20-30 seconds) Use gas canister in Kent Lab. \*Remember to note gas canister level when finished.
3. Place in incubator, set to 37°C. Allow to incubate for 7 days.
4. After incubation, remove samples from incubator and add 30 mL 1.33 M KCl. Cap tightly.
5. Shake for 1 hour at 240 opm.
6. Filter out 10-15 mL of supernatant using plastic funnels and Whatman No. 42 filter paper. \*When finished filtering, filters and tubes with KCl solution and soil can be discarded in trash; funnels must be acid washed!
7. Store KCl extracts in freezer until ammonium assays are performed.

Make the stock and standards:

1. Prepare 100 ppm stock solution for ammonium: 0.23585 g (235.85 mg) ammonium sulfate dissolved in 500 mL ultrapure DI water.
2. Prepare 100 ppm stock solution for nitrate: 0.3609 g (360.9 mg) potassium nitrate dissolved in 500 mL ultrapure DI water.
3. Prepare standards based on high or low concentrations. When you see the term “matrix” in the protocol, use 1 M KCl. Before making standards, stock to 10 ppm. In a 1.5 mL centrifuge tube: add 150 uL stock to 1350 uL matrix (1 M KCl).
4. Use the chart to prepare standards:



1. Note: when I ran this protocol in 01/17, I had problems creating an appropriate curve with the nitrate standards following the high concentration. Therefore, I modified it to range from 0-4 ppm; you may need to make adjustments based on your nitrate levels and the curves you obtain from your plates. If you do make changes, please make sure to update the Gen5 protocol for your plates to reflect different standards.

\*\*I slightly modified how I prepared nitrate stock/standards, and here is what I did:

1. Prepared 100 ppm stock solution the same way as described above.
2. Divided into single-use aliquots of about 10-20 mL (scintillation vials).
3. Purged the headspace with He gas and stored in the freezer in the dark.
4. Prepared solutions fresh each day by diluting to 10 ppm in 1 M KCl and following the protocol above.

\*\*Modifications to ammonium stock/standards:

1. Prepared 100 ppm stock solution the same way as described above.
2. Divided into single-use aliquots of about 10-20 mL (scintillation vials). Stock can be stored in freezer up to 3 days.
3. Prepare standards from 100 ppm stock. Standards can be stored frozen up to 3 months.

Nitrate assay:

1. Remove samples from freezer the night before and place them in the fridge overnight to thaw. Make sure to give the samples a shake before you add them to the plates, as freezing and thawing can alter the concentration of the extracts.
2. If following the LOW concentration protocol, add 100 uL of sample (for “blanks”, add 100 uL 1 M KCl) to plates. After samples are added, use a multi-channel pipette to add 100 uL nitrate reagent.
   1. If following the HIGH concentration protocol, add 10 uL sample (or KCl for blanks) and 160 uL reagent.
3. Wrap in foil and allow to incubate for 5 hours.
4. Read plates at 540 nm using Gen5 software (follow ACY-Nitrate protocol, but remember to save each place as its own file with an informative name). Export data to Excel and save.

Ammonium (and PMN) assay:

1. Remove samples from freezer the night before and place them in the fridge overnight to thaw. Make sure to give the samples a shake before you add them to the plates, as freezing and thawing can alter the concentration of the extracts.
2. Prepare sodium hydroxide and bleach solution fresh daily. (0.2 mL bleach added to 9.8 mL NaOH solution, which is prepared when making all other reagents).
3. If following the LOW concentration protocol, add 80 uL of sample to each well (for “blanks”, add 80 uL 1 M KCl). After samples are added, use a multi-channel pipette to add 60 uL salicylate solution FOLLOWED BY 60 uL bleach solution. Order matters!
   1. If following the HIGH concentration protocol, add 20 uL sample to each well (or KCl for blanks). After sample are added, use a muli-channel pipette to add 90 uL salicylate solution FOLLOWED BY 90 uL bleach solution. Order matters!
4. Plates are incubated for 50 minutes.
5. Read plates at 650 nm using Gen5 software (follow ACY-Ammonia protocol, but remember to save each place as its own file with an informative name). Export data to Excel and save.