Treseder Lab Protocol
Nutrient analyses on microplate reader

Ammonium analysis

**Sodium salicylate solution (bring to 100 ml with DI water)**
6.8 g sodium salicylate
5 g sodium citrate
5 g sodium tartrate
0.025 g sodium nitroprusside

**Bleach solution (bring to 100 ml with DI water)**
6 g sodium hydroxide
Add 2% bleach fresh each day (20 µl bleach + 980 µl solution)

For resin bag extracts, the matrix is 0.1 M HCl/2.0 M NaCl. Extracts from unfertilized plots should follow the protocol for low concentrations and use the low standard curve. Extracts from fertilized plots should be diluted in matrix and run using the high protocol.

For low concentrations (0-5 ppm):
Add the following to each well:
80 µl sample
60 µl salicylate solution (add using multichannel pipet)
60 µl bleach solution (add using multichannel pipet)

For high concentrations (1-10 ppm):
20 µl sample
90 µl salicylate solution (add using multichannel pipet)
90 µl bleach solution (add using multichannel pipet)

Standard curves: Dilute the 100 ppm stock solution to 10 ppm in a 1.5 ml centrifuge tube (150 µl stock:1350 µl matrix). Create the following standard curves in 1.5 ml centrifuge tubes.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>High 10 ppm</th>
<th>High matrix</th>
<th>Low 1 ppm</th>
<th>Low matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>50</td>
<td>950</td>
<td>0.05</td>
<td>50</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>100</td>
<td>900</td>
<td>0.10</td>
<td>100</td>
</tr>
<tr>
<td>2.0 ppm</td>
<td>200</td>
<td>800</td>
<td>0.20</td>
<td>200</td>
</tr>
<tr>
<td>5.0 ppm</td>
<td>500</td>
<td>500</td>
<td>0.50</td>
<td>500</td>
</tr>
<tr>
<td>10.0 ppm</td>
<td>1000</td>
<td>0</td>
<td>1.00</td>
<td>1000</td>
</tr>
</tbody>
</table>

Pipet up and down to mix well, incubate 50 min and read plate at 650 nm.
Detection limit <0.05 ppm
Stock ammonium solution: 0.23585 g ammonium sulfate in 500 ml ultrafiltered DI water
Nitrate analysis

**Vanadium solution**
50 ml saturated vanadium (III) chloride in 1 M HCl (0.35 g/50 ml, filtered)
CAUTION: the vanadium chloride powder is very reactive with air! Work quickly.
3.3 ml 2 % w/v sulfanilamide in 1 M HCl
3.3 ml 0.2 % w/v N-(1-naphthyl)-ethylenediamine dihydrochloride in DI water
400 ml DI water
Purge with nitrogen or helium and store for up to 3 months frozen

For resin bag extracts, the matrix is 0.1 M HCl/2.0 M NaCl. Extracts from unfertilized plots should follow the protocol for low concentrations and use the low standard curve. Extracts from fertilized plots should be diluted in matrix and run using the high protocol.

For low samples, combine 100 µl sample and 100 µl reagent. For high samples combine 10 µl sample and 160 µl reagent. Tap microplate corner to mix well, incubate 5 h or overnight, and read plate at 540 nm.

Standard curves: Dilute the 100 ppm stock solution to 10 ppm in a 1.5 ml centrifuge tube (150 µl stock:1350 µl matrix). Create the following standard curves in 1.5 ml centrifuge tubes.

<table>
<thead>
<tr>
<th>Concentration (µl)</th>
<th>10 ppm</th>
<th>Matrix</th>
<th>Concentration (µl)</th>
<th>1 ppm</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>50</td>
<td>950</td>
<td>0.05</td>
<td>50</td>
<td>950</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>900</td>
<td>0.10</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>2.0</td>
<td>200</td>
<td>800</td>
<td>0.20</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>5.0</td>
<td>500</td>
<td>500</td>
<td>0.50</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>10.0</td>
<td>1000</td>
<td>0</td>
<td>1.00</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

1 M HCl = 84 ml concentrated HCl per liter.

Detection limit <0.05 ppm
Stock nitrate solution: 0.3609 g potassium nitrate in 500 ml ultrafiltered DI water
**Inorganic phosphorus analysis**

**AMP solution**
Add 250 ml DI water to a 500 ml volumetric flask.
Add 53 ml concentrated sulfuric acid.
Dissolve 8.775 g ammonium para-molybdate and bring to volume.

**Malachite green solution**
Heat 400 ml DI water to 80°C in an Erlenmeyer flask with stir bar.
Add 1.75 g polyvinyl alcohol and stir to dissolve.
Add 0.175 g malachite green and stir to dissolve.
Cool and bring to 500 ml volume in a volumetric flask.

For resin bag extracts, the matrix is 0.1 M HCl/2.0 M NaCl.

Add 30 µl AMP solution to 150 µl sample and tap corner of microplate to mix. After 10 min, add 30 µl malachite green solution and pipet up and down mix. Do not place plate on shaker to mix- a nasty precipitate will form. Read microplate at 630 nm after 30 min.

Standard curves: Dilute the 50 ppm stock solution to 1 ppm in a 1.5 ml centrifuge tube (30 µl stock:1470 µl matrix). Create the following standard curve in 1.5 ml centrifuge tubes.

<table>
<thead>
<tr>
<th>Concen ppm</th>
<th>µl 1 ppm</th>
<th>µl matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>0.02</td>
<td>20</td>
<td>980</td>
</tr>
<tr>
<td>0.05</td>
<td>50</td>
<td>950</td>
</tr>
<tr>
<td>0.10</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>0.20</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>0.50</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

Detection limit <0.02 ppm
Stock phosphate solution: 0.2195 g oven-dried KH$_2$PO$_4$ in 1000 ml ultrafiltered DI water