Nutrient Analyses on Microplate Reader

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Adapted from Miranda et al., 2001, Nitric Oxide - Biology and Chemistry
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Nitrate & Nitrite Analysis

Reagents
Vanadium solution (VCl₃)
In 100 ml volumetric flask
0.80 g vanadium (III) chloride in 50 ml of 1 M HCl
CAUTION: Work quickly because Cl₃V powder will react with air!
Flush with N₂, store in the dark at 4°C, or freeze, should be blue.

2.0 % (w/v) Sulfanilamide solution (SULF)
In 100 ml volumetric flask
2 g Sulfanilamide
Bring to volume with 5% (v/v) HCl
Flush with N₂, may be stored in the dark at 4°C for several months, and discard if colored

0.1% (w/v) NEDD solution
In 100 ml volumetric flask
100 mg N-(1-naphthyl)-ethylenediamine dihydrochloride
Bring to volume with DI water
Flush with N₂, may be stored in the dark at 4°C for several months, and discard if colored

Premix solutions just prior to analysis to a ratio of 2:1:1 (20 ml VCl₃ : 10 ml SULF : 10 ml NEDD)

Standard (40 ppm NO₃⁻)
In 100 ml volumetric flask
4 ml 1,000 ppm nitrate (NO₃⁻) standard
Bring to volume in 1 M KCl

Procedure
Soil solution extraction
10 g Oven-dried equivalent field fresh, sieved soil
20 ml 1M KCl
Shake for 1 hr and centrifuge (10 min @ 3000 rpm)

Ambient (initial):
Add 100 μl of reagent to 100 μl of sample, or standard in clear 96-wellplate.
Incubated (or farm soils):
Add 50 μl KCl and 100 μl of reagent to 50 μl of sample, or standard in clear 96-wellplate.

Incubate at 37°C and measure when color develops. Measure absorbance at 540 nm. Samples should turn pink, if dark magenta (OVRFLW), redo by adding only 10 μl of sample with 90 μl of KCl and 100 μl of reagent.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Standard (ml)</th>
<th>1 M KCl (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
<td>9.75</td>
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<tr>
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</tr>
<tr>
<td>24</td>
<td>1.0</td>
<td>9.0</td>
</tr>
<tr>
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<td>8.0</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
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<tr>
<td></td>
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<td>4.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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