Effects of wet N deposition on *Sphagnum capillifolium* in peatland

Sue Owen

Lucy Sheppard
Raul Ochoa-Hueso
Nitrogen deposition – effects on Sphagnum

Isoprene emissions
Pigments
Photosynthesis

(why are we interested?)
Isoprene chemical structure - it’s reactive!

Monoterpenes $\text{C}_{10}\text{H}_{16}$ and sesquiterpenes $\text{C}_{15}\text{H}_{24}$

Isoprene $\text{C}_{5}\text{H}_{8}$
Role of isoprene (and other bVOCs) in plants**

- Thermoprotection
- Photoprotection
- Oxidative damage protection
- Photorespiration role at high temp/low O$_2$
- Antiherbivory
- Antimicrobial
- Pollinator Attractant
- Flowering signal
- Metabolic safety valve
- Allelopathy

AND ... can account for up to 5% photosynthetically fixed C
Role of isoprene and other bVOCs in the troposphere

- Reactive with OH, O$_3$, NO$_3$
- Take part in O$_3$ chemistry
- Can produce particles

(climate change chemistry)
Isoprene emissions: link with pigments

C5-isoprene

C10-monoterpenes

IPP/DMAPP
(Biochemical precursors)

C20-gibberellins, tocopherol, phytanyl side chains

C30-sterols

C40-carotenoids

“Useful, but not essential”

“Essential”

Owen and Penuelas 2005 TIPS
Research questions (many unknowns)

• Are isoprene emissions and pigment content in *S. capillifolium* affected by N deposition?

• Is there any difference in the response of two pigment types of *S. capillifolium* to N deposition?

• Is there any relationship between carotenoid content and isoprene emission, as suggested by the “opportunistic hypothesis”?

• Is there a relationship between photosynthesis and carotenoid concentrations/isoprene emissions?
Study site: Whim Bog

- 9 miles south of CEH, Edinburgh
- Transition between upland blanket bog and lowland raised bog.
- *Sphagnum* sp. and *Eriophorum* sp. are the main peat-forming species
- Treatment + met data since 2002

Globally peat land occupies \(\sim 400\times 10^6\) ha (3\% Earth’s land surface)
Northern hemisphere \(\sim 360\times 10^6\) ha
(cf \(\sim 1000\) million ha forest in Europe and Russia)
Study species: *Sphagnum capillifolium*

- Red type: open spaces, tops of tussocks
- Green type: shade provided by heather (*Calluna vulgaris*)
Automated treatments, linked to rainfall and windspeed

4 treatment blocks of wet deposition
NH$_4$Cl
NaNO$_3$

at 0 (control) and 8, 24, 56 kg N ha$^{-1}$ y$^{-1}$ above ambient (~8 – 11 kg N ha$^{-1}$ y$^{-1}$)

Sampling and laboratory analyses in August 2012
Sphagnum capillifolium samples

High NO3, green

High NO3, red

control, green

control, red
Sampling and measurements from *Sphagnum capillifolium*

- $FvFm$ (Handy PEA, Hansatech)
- Photosynthesis (LICOR LI-6400)
- Isoprene emissions (LICOR LI-6400 and GC-MS)
- Pigment content (HPLC, Waters)
Isoprene emissions and photosynthesis *Sphagnum capillifolium*

5 replicates x 2 moss colours x 2 “treatments”

**Quantitative**

dynamic system, environmental control

Experimental set-up of LICOR and Photograph by Emily Taylor
Sphagnum capillifolium – photosynthesis rates in red and green types

Rate of photosynthesis (μmol CO₂ kg⁻¹ s⁻¹)

Control and high NO₃ together

Control alone: no significant difference between green and red types

High NO₃

P<0.05
Sphagnum capillifolium – effect of high NO3 on photosynthesis rates

Red and Green together: no significant difference between control and high NO3
Sphagnum capillifolium – Fv/Fm and isoprene

<table>
<thead>
<tr>
<th></th>
<th>$F_{v'F_m}$</th>
<th>Isoprene emissions (ng g$^{-1}$ h$^{-1}$-log transformed data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>1,16 0.10</td>
<td>1,16 9.09 &lt;0.01</td>
</tr>
<tr>
<td>Colour</td>
<td>1,16 1.53</td>
<td>1,16 4.48 0.05</td>
</tr>
<tr>
<td>Nitrogen x colour</td>
<td>1,16 1.29</td>
<td>1,16 7.52 0.02</td>
</tr>
</tbody>
</table>
Sphagnum capillifolium – isoprene emissions as a percent of fixed photosynthetic carbon

Isoprene as %fixed C
(max ~0.34%)

NO3 treatment: P <0.07
Sphagnum capillifolium – pigment content

(red and green types together)
P<0.03

mmol g⁻¹

0 NO₃
56 NO₃

Neox
Lutein
Chlb
Chla
β-car
VAZ

*
*Sphagnum capillifolium* – pigment content

<table>
<thead>
<tr>
<th></th>
<th>Neoxanthin</th>
<th>Lutein</th>
<th>Chlorophyll b</th>
<th>Chlorophyll a</th>
<th>β-carotene</th>
<th>VAZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1,16</td>
<td>7.20</td>
<td>0.02</td>
<td>1,16</td>
<td>4.18</td>
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<tr>
<td>Colour</td>
<td>1,16</td>
<td>6.54</td>
<td>0.02</td>
<td>1,16</td>
<td>6.97</td>
<td>&lt;0.01</td>
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<tr>
<td>Nitrogen x colour</td>
<td>1,16</td>
<td>2.56</td>
<td>0.13</td>
<td>1,16</td>
<td>3.86</td>
<td>0.07</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Chlorophyll a/b</th>
<th>Neox/Chl a+b</th>
<th>Lutein/Chl a+b</th>
<th>β-carot/Chl a+b</th>
<th>VAZ/Chl a+b</th>
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<tr>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
<td>P</td>
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<tr>
<td>Nitrogen</td>
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<td>3.94</td>
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<td>1,16</td>
<td>0.53</td>
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<tr>
<td>Colour</td>
<td>1,16</td>
<td>20.75</td>
<td>&lt;0.01</td>
<td>1,16</td>
<td>8.43</td>
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<tr>
<td>Nitrogen x colour</td>
<td>1,16</td>
<td>2.33</td>
<td>0.15</td>
<td>1,16</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Colour type P<0.05 generally
**Sphagnum capillifolium – isoprene / ∑carotenoids**

**Graphs:**
- **(red and green types together)**
  - P<0.01
  - Isoprene emissions (log) / total carotenoids
  - kg N ha⁻¹ yr⁻¹:
    - 0
    - 56

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Isoprene emissions</th>
<th>Isopre-log/carot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
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</tr>
<tr>
<td>Nitrogen</td>
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<td>9.09</td>
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<td>Colour</td>
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</tr>
<tr>
<td>Nitrogen x colour</td>
<td>1,16</td>
<td>7.52</td>
</tr>
</tbody>
</table>

**Note:**
- Colour type: n.s.
**Sphagnum capillifolium** – isoprene & total carotenoids

**Green Sphagnum**
P = 0.07

**Red Sphagnum**
P = 0.07
**Sphagnum capillifolium – isoprene & carotenoids**

![Graph](image)

**Graph a**
- Neoxanthin / Chlorophylls $a+b$
- Isoprene emissions (ng g$^{-1}$ h$^{-1}$ - log transformed data)
- $R^2 = 0.55; P = 0.01$

**Graph b**
- Neoxanthin (mmol g$^{-1}$)
- Isoprene emissions (ng g$^{-1}$ h$^{-1}$ - log transformed data)
- $R^2 = 0.47; P = 0.03$

*Note: The graphs show the relationship between Neoxanthin / Chlorophylls $a+b$ and isoprene emissions, with statistical significance levels indicated.*
Sphagnum capillifolium – isoprene, carotenoids & photosynthesis

Photosynthesis rate ($\mu$mol kg$^{-1}$ s$^{-1}$)

Isoprene (ng g$^{-1}$ h$^{-1}$)

Total carotenoids (mmol/g)

$y = 252x - 313$

$R^2 = 0.16$

n.s.

$y = 0.02x + 0.2$

$R^2 = 0.2$

P<0.05
<table>
<thead>
<tr>
<th>Photo-synthesis</th>
<th>Isoprene emissions</th>
<th>Pigments</th>
<th>Isoprene/Σcarotenoids</th>
<th>Isoprene vs Σcarotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: RED=GREEN</td>
<td>Control: RED=GREEN</td>
<td>Control: RED&lt;GREEN P&lt;0.05</td>
<td>Control: RED=GREEN</td>
<td>Control: RED&lt;GREEN P&lt;0.05</td>
</tr>
<tr>
<td>NO3: RED&lt;GREEN P&lt;0.05</td>
<td>NO3: RED&lt;GREEN P&lt;0.05</td>
<td>NO3: RED&lt;GREEN P&lt;0.05</td>
<td>NO3: RED&lt;GREEN n.s.</td>
<td>NO3: RED -ve GREEN +ve (P=0.07)*</td>
</tr>
</tbody>
</table>
Summary – NO3 effects on *S. capillofolium*

<table>
<thead>
<tr>
<th>Photo-synthesis</th>
<th>Isoprene emissions</th>
<th>pigments</th>
<th>Isoprene/Σ carotenoids</th>
<th>Isoprene vs Σ carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>RED: ↓ n.s.</td>
<td>RED: ↓ P&lt;0.02</td>
<td>RED: ↑ n.s.</td>
<td>RED: ↓ P&lt;0.05</td>
<td>RED: –ve curves (P=0.07)*</td>
</tr>
<tr>
<td>GREEN: no effect</td>
<td>GREEN: no effect</td>
<td>GREEN: ↑ P&lt;0.05</td>
<td>GREEN: ↓ P&lt;0.05</td>
<td>GREEN: +ve curves (P=0.07)*</td>
</tr>
</tbody>
</table>
Research questions

• Are isoprene emissions and pigment content in *S. capillifolium* affected by N deposition? **YES**

• Is there any difference in the response of two pigment types of *S. capillifolium* to N deposition? **YES**

• Is there any relationship between carotenoid content and isoprene emission, as suggested by the “opportunistic hypothesis”? **YES**

• Is there a relationship between photosynthesis, carotenoids and isoprene emissions? **YES**
Thank you! any questions? (and any job vacancies for Raúl?)
Summary

• N deposition ↑ pigment content in *S. capillifolium* (P<0.05 green; n.s. red)
  - increased demand for carotenoid photoprotection? (successful because Fv/Fm not sig diff)
  - need more chlorophyll to sustain level of p/s?

• N deposition ↓ isoprene emission and photosynthesis in red *S. capillifolium* (P < 0.05)
  - reduced substrate availability for all products of isoprenoid pathway (IPP, DMAPP), carotenoids more important so resources diverted there?

• Some degree of support for the “Opportunistic hypothesis” in *S. capillifolium*
Thank you – any questions?
Nitrogen deposition – biodiversity loss

Sala et al., 2000 Science 287: 1770-1774
Sphagnum capillifolium – isoprene, carotenoids & photosynthesis

Photosynthesis rate (umol kg⁻¹ s⁻¹)

Total carotenoids (mmol/g)

- Control (green type)
- High NO3 (red type)

Isoprene emissions (ng g⁻¹ h⁻¹)
<table>
<thead>
<tr>
<th>Source</th>
<th>Ecosystem</th>
<th>Isoprene emission rate $\mu$g m$^{-2}$ h$^{-1}$</th>
<th>Chamber T °C</th>
<th>Chamber PAR $\mu$mol m$^{-2}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiiva et al 2007</td>
<td>Subarctic peatland</td>
<td>71</td>
<td>26</td>
<td>628</td>
</tr>
<tr>
<td>Janson et al 1999</td>
<td>Sphagnum fen June</td>
<td>62</td>
<td>15 – 18</td>
<td>cloudy</td>
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<tr>
<td>Janson et al 1999</td>
<td>Sphagnum fen August</td>
<td>459</td>
<td>26</td>
<td>sunny</td>
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<tr>
<td>Holst et al 2010</td>
<td>high latitude wetland site</td>
<td>373</td>
<td>20</td>
<td>1000</td>
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<tr>
<td>Faubert et al 2010</td>
<td>Subarctic peatland growing season mean emission</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiiva et al 2008</td>
<td>Subarctic heath growing season 1 mean emission</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subarctic heath growing season 2 mean emission</td>
<td>36</td>
<td></td>
<td></td>
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<tr>
<td>Ekberg et al 2011</td>
<td>Northern Swedish mire: Average peak growing season wet</td>
<td>120</td>
<td>20</td>
<td>1000</td>
</tr>
<tr>
<td>Ekberg et al 2011</td>
<td>Northern Swedish mire: Average peak growing season dry</td>
<td>84</td>
<td>20</td>
<td>1000</td>
</tr>
<tr>
<td>THIS STUDY</td>
<td>Ombrotrophic bog, Scotland</td>
<td>0.1- 518</td>
<td>12-14</td>
<td>300 – 2300(?)</td>
</tr>
<tr>
<td>Laffineur et al 2011</td>
<td>Mixed forest Europe</td>
<td>3276</td>
<td>30</td>
<td>1000</td>
</tr>
<tr>
<td>Owen 1998</td>
<td>Mediterranean forest</td>
<td>~800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart et al 2003</td>
<td>South Edinburgh “hot spot”</td>
<td>20-80</td>
<td>Cool-hot</td>
<td>Cloudy-sunny</td>
</tr>
</tbody>
</table>
Nitrogen deposition

• annually:
  anthropogenic fixed N \approx naturally fixed N (\sim 120 \text{ Tg N})

• predicted to increase

• associated with uncontrolled human activities
  (e.g., energy use and food production)

Galloway et al., 2003 BioScience 53: 341-356
IPP/DMAPP – precursors of all isoprenoids

2 isoprenoid biosynthesis pathways:

(1) Mevalonate pathway (cytosol)
begins with 2 molecules of acetyl coenzyme A (acetyl coA)

(2) MEP pathway (chloroplast)
begins with 1 molecule each of pyruvate and glyceraldehyde-3-phosphate (G-3-P)

These compounds are derived from products of respiration and photosynthesis biochemistry
Effect of N-deposition and pigmentation on isoprene emissions from *Sphagnum capillifolium* – a laboratory study (with Lucy Sheppard and Raul Ochoa-Hueso)
**Sphagnum capillifolium** – isoprene, carotenoids & photosynthesis

### Total Carotenoids (mmol/g)

- **Control (o)**
  - $y = 0.05x + 0.04$
  - $R^2 = 0.5$
  - Slope $P=0.03$

- **NO3 ($\Delta$)**
  - $y = 0.02x + 0.25$
  - $R^2 = 0.3$
  - Slope $P=0.1$

### Isoprene Emissions (ng g$^{-1}$ h$^{-1}$)

- **Control (o)**
  - $y = 765x - 2431$
  - $R^2 = 0.35$
  - Slope $P=0.07$

- **NO3 ($\Delta$)**
  - $y = 85x + 8.4$
  - $R^2 = 0.3$
  - Slope $P=0.1$

### Photosynthesis Rate (umol kg$^{-1}$ s$^{-1}$)

- **Control (o)**
  - $y = 0.05x + 0.04$
  - $R^2 = 0.5$
  - Slope $P=0.03$

- **NO3 ($\Delta$)**
  - $y = 0.02x + 0.25$
  - $R^2 = 0.3$
  - Slope $P=0.1$
Isoprenoid precursors: link with respiration

Glycolysis

- Sucrose
- G-3-P
- Pyruvate
- Acetyl co-A

Tricarboxylic acid cycle

MEP pathway for isoprenoid production

Mevalonate pathway for isoprenoid production
Isoprenoid precursors: link with photosynthesis:

- **Ribulose 1,5 biphosphate**

  - **Carboxylation**
    - CO₂ + H₂O

  - **Reduction**
    - 3-phosphoglycerate

  - **Triose phosphate**

  - **Regeneration**

Sucrose, starch
Controls on isoprene emissions

(Many)
Temperature
PAR (photosynthetic active radiation)

Plant species-specific

Important to know if/how N deposition affects emissions
Whim Bog Treatments automated, linked to rainfall and windspeed

Background N depn 8-11 kg N ha\(^{-1}\) y\(^{-1}\)

Plot number 1 - 44

Block 1

NH\(_4\) Cl
56 kg N ha\(^{-1}\) y\(^{-1}\)
+ PK (K\(_2\)HPO\(_4\))

Block 2

Na NO\(_3\)
24 kg N ha\(^{-1}\) y\(^{-1}\)

Block 3

NH\(_4\) Cl
8 kg N ha\(^{-1}\) y\(^{-1}\)

Block 4

Lucy Sheppard
What does isoprene do?

- it confers protection to the producing plant against oxidative/thermal stress
- monoterpenes can attract pollinators/repel herbivores
- it can be important in O₃ and aerosol chemistry
- it can account for up to 5% photosynthetically fixed C

-Large literature in general (~1800 “isoprene and (emissions or flux)” titles)
-Paucity of work in peatlands (~33 relevant to peatland/bog)
### Existing work on isoprene from peatlands

<table>
<thead>
<tr>
<th>Web of Science search</th>
<th># hits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Topic:</strong> (isoprene AND (emission* or flux*))</td>
<td>1649</td>
</tr>
<tr>
<td>(isoprene AND (peat* or bog*))</td>
<td>22 relevant*</td>
</tr>
<tr>
<td><strong>Title:</strong> (isoprene or VOC* or bVOC* or volatile) and (peat* or bog* or mire* or wetland*)</td>
<td>12</td>
</tr>
</tbody>
</table>

*Tiva et al (Finland)*

Faubert et al (Finland)
Ekberg et al (Sweden)
Backstrand et al (Sweden)