Ecology of microbes causing N loss and retention in arable soils

Sara Hallin
Nitrate is lost or retained

[Diagram showing the cycle of nitrogen and nitrate conversion]

- Org-N
- NH₄⁺
- NO₂⁻
- NO₃⁻
- N₂O
- NO
- N₂
Respiratory ammonification (rather than DNRA)

\[ \text{Org-N} \rightarrow \text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow N_2 \rightarrow N_2O \rightarrow NO \rightarrow NO_2^- \rightarrow \text{Org-N} \]
Carbon:nitrate ratio
Nitrate:nitrite ratio
Generation time

(Kraft et al. 2014 Science
Yoon et al., 2015 ISMEJ)
Respiratory ammonification bacteria in arable soils?

Säby (Sweden)

<table>
<thead>
<tr>
<th></th>
<th>Annual crops</th>
<th>Perennial crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.38</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td>4.29</td>
<td>4.48</td>
</tr>
<tr>
<td>SOM [%dw]</td>
<td>6.37</td>
<td>8.41</td>
</tr>
<tr>
<td></td>
<td>6.79</td>
<td>8.33</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>3.98</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>4.57</td>
<td>4.35</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>Total N %</td>
<td>0.20</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>Total C %</td>
<td>2.14</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>2.28</td>
<td>3.02</td>
</tr>
<tr>
<td>C:NO$_3^-$</td>
<td>0.55</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.70</td>
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</tbody>
</table>

(Schleusner et al, manuscript)
Fertilization promotes denitrifiers

(Schleusner at al, manuscript)
Denitrification pathway is modular

$\text{NO}_3^-$ \xrightarrow{\text{NirS}} \text{NO}_2^- \xrightarrow{\text{NirK}} \text{NO} \xrightarrow{\text{NOR}} \text{N}_2\text{O} \xrightarrow{\text{NosZ}} \text{N}_2
Niche differentiation between nirS and nirK types

(Enwall et al. 2010 Appl Environ Microbiol)

(Graf et al. 2014 PLOS ONE)
Production of N$_2$O at different amounts directly in soil by inoculating serial dilution of *A. tumefaciens* into arable soils:

*Agrobacterium tumefaciens* C58

(Philippot et al 2011 *Global Change Biology*)
Causal effects of community changes on $\text{N}_2\text{O}$ production

Denitrification (ng N g$^{-1}$ dw soil min$^{-1}$)

N$_2$O/(Tot denitrification)

Dilution rate of *A. tumefaciens* C58 inocula

(Philippot et al. 2011 *Global Change Biology*)
652 genomes

(Graf et al, 2014 *PLOS ONE*)
Nearly 46% of 652 genomes are potential N$_2$O reducers.

- 30% have *nosZ* without *nir* and 24% have only *nosZ*, and are potential N$_2$O sinks.
- 70% of *nosZ* genomes have a denitrification pathway.

(Graf et al, 2014 *PLOS ONE*)
The new clade of the N$_2$O reductase a N$_2$O sink?

(Jones et al. 2013 *ISME J*)

(Graf et al, 2014 *PLOS ONE*)
... and it is abundant in soils and other systems

(Jones et al. 2013 *ISME J*)
Range of potential soil N$_2$O sink capacity across 47 soils

Capacity to consume N$_2$O:

(Jones et al, 2014 *Nature Climate Change*)

Poster 158 by Laurent Philippot
Network analysis of N$_2$O reducing communities

Co-occurring nosZ genotypes that were significant indicators of the soil N$_2$O sink capacity.

(Jones et al, 2014 Nature Climate Change)
Ranking of parameters that predict soil N$_2$O sink capacity

- C:N ratio
- nirS/nirK ratio
- pH
- $#nosZ$ clade II
- PD $nosZ$ clade II
- PD $nosZ$ clade I

(Jones et al, 2014 *Nature Climate Change*)
Factors underlying soil N$_2$O source or sink

(Jones et al, 2014 *Nature Climate Change*)
Causal effects of community changes on $\text{N}_2\text{O}$ reduction

Reduction of $\text{N}_2\text{O}$ produced by inherent soil microbes by inoculating *Dyadobacter fermentans* into arable soils:

- *nosZ II*
- not capable of denitrification
- not capable of $\text{N}_2\text{O}$ production

(Domeignoz-Horta et al, manuscript)
Addition of the nosZII strain can decrease soil \( N_2O \) production

First evidence that non-denitrifying \( N_2O \)-reducers contribute to soil \( N_2O \) reduction

(Domeignoz-Horta et al, manuscript)
### Effects of N-fertilization on abundance of \( \text{N}_2\text{O} \) reducers

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>nirK</td>
<td>0.25*</td>
</tr>
<tr>
<td>nirS</td>
<td>0.31**</td>
</tr>
<tr>
<td>nosZI</td>
<td>0.55***</td>
</tr>
<tr>
<td>nosZII</td>
<td>0.34**</td>
</tr>
</tbody>
</table>

* \( p<0.05 \) ** \( p<0.01 \) *** \( p<0.001 \)

**High nosZ I** →
less \( \text{N}_2\text{O} \) produced relative to \( \text{N}_2 \) in fertilized soils

**High nosZ/nir ratio** →
less \( \text{N}_2\text{O} \) produced relative to \( \text{N}_2 \)
N-fertilization affects Clade II N$_2$O reducing communities

<table>
<thead>
<tr>
<th></th>
<th>nosZI</th>
<th>nosZII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>site</td>
<td>fertilization</td>
</tr>
<tr>
<td>Species richness</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Evenness</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Phylogenetic diversity</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Community structure</td>
<td>✓</td>
<td>✗</td>
</tr>
</tbody>
</table>

Phylogenetic diversity correlated with N$_2$O/[N$_2$O+N$_2$]

(Putz et al, manuscript)
After 3 weeks: Roots are selecting *nosZ* Clade I

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(Graf et al, 2016 *Soil Biol Biochem*)
After 3 weeks: Roots are selecting *nosZ* Clade I, soil Clade II

(Graf et al, 2016 *Soil Biol Biochem*)
Soil type rather than plant determines activity

(Graf et al, 2016 Soil Biol Biochem)
Community composition on roots mainly depending on soil type

- sunflower root
- barley root
- sunflower soil
- barley soil
- unplanted soil

(Graf et al, manuscript)
Conclusions

- Fate of nitrate affected by fertilization; promotes denitrifiers over bacteria capable of reparatory ammonification

- Modularity of the denitrification pathway underpin the importance of community structure for N₂O emissions

- *nosZ* Clade II organisms important for regulating N₂O emissions

- Co-occurring *nosZ* genotypes as indicators of soil N₂O sink capacity

- Niche differentiation between *nosZ* Clade I and II organisms:
  - affected by different edaphic factors, N-fertilization, plants
  - Clade I associated with roots, Clade II with soil
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