# Diversity of the nitrite reductase *(NirK)* gene in an upland pasture system

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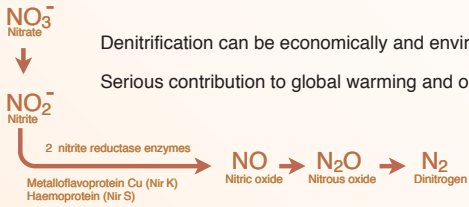
## Introduction




Denitrification can be economically and environmentally undesirable

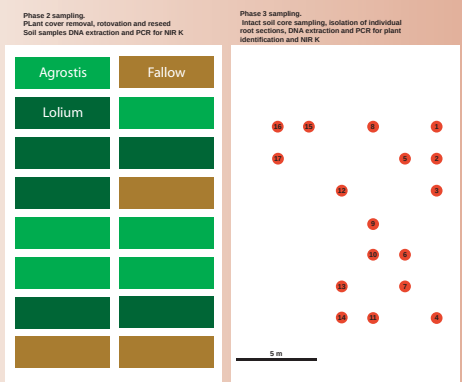
Serious contribution to global warming and ozone depletion

## Materials and methods

Experiment 1

Soil samples were taken from plots which had been rotovated and reseeded with either Lolium perenne or Agrostis capillaris or left fallow (fig 1). Total DNA was extracted and amplified with nirK primers. Clones were screened by RFLP and selected examples were sequenced and placed on a tree (fig 2). Types from this experiment are labelled A-V.

**Fig 1: Field experiment layout**




Experiment 2

Intact soil cores were sampled as shown in fig 1 (red circles), individual root fragments were isolated from the cores, DNA was isolated and plant species identified utilising molecular methods (University of York). From aliquots of the same DNA extract nirK PCR was performed and high throughput sequencing methods were applied. 96 clones from each sample were sequenced. Sequences were analysed and examples with 5 or more clones of each type are displayed in fig 2, types from this experiment are labelled 1-24 and single clones. Percentages of clones representing each sequence type and diversity indices are shown in table 1.Experiment 1 Soil samples were taken from

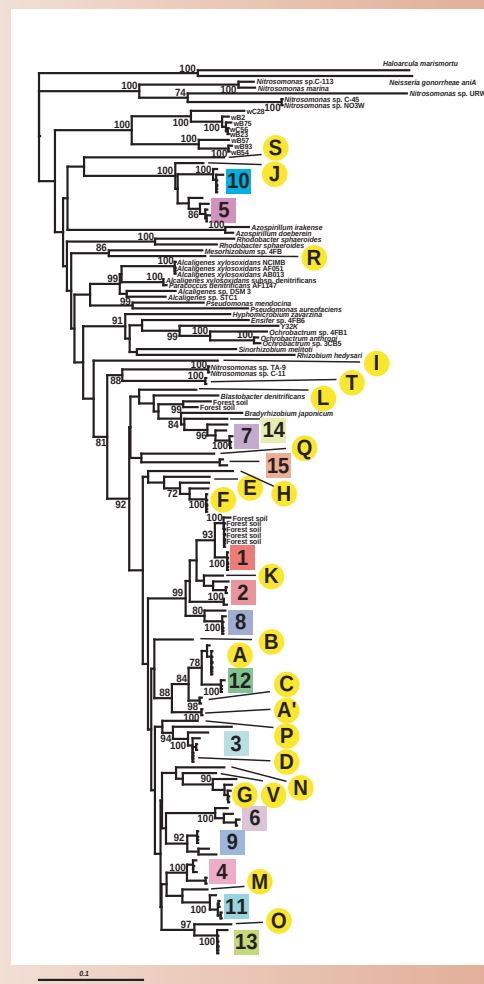



**Fig 2: A Neighbor joining tree of *nirK* sequences. Database sequences are shown in black, sequences from experiment 1 are represented by circles and those from experiment 2 are represented by squares.**
**Sequence groups with a total of less than 5 clones have been removed for clarity. Bootstrap values above 70% are shown.**

**Table 1, Percentage of each sequence type (Expt 2) in roots, clone number per root and Shannon-Weiner diversity indices. Colour denotes sequence types shown on tree.**

| Core | Plant species | Replicate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Singles | Clones | Shannon |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Agrostis capillaris | 1 | 80.6 | 9.0 | 4.5 | - | - | 1.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.5 | 1.5 | - | - | 3 | 67 | 0.78 |
| | Agrostis capillaris | 3 | 93.8 | 3.8 | - | 1.3 | - | - | - | 1.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 80 | 0.29 |
| | Agrostis capillaris | 4 | 41.7 | 22.2 | 31.9 | - | - | - | - | - | 1.4 | - | - | - | - | - | - | - | - | - | 2.8 | - | - | - | - | - | - | 72 | 1.22 |
| | Agrostis canina | 1 | 65.9 | 25.6 | 1.2 | - | - | - | - | - | - | - | - | - | - | - | - | 4.9 | - | - | - | - | - | 1.2 | - | 1.2 | - | 82 | 0.93 |
| | Festuca rubra | 1 | 64.0 | - | 3.4 | 3.4 | 6.7 | - | - | - | 5.6 | 11.2 | - | - | - | - | - | - | - | - | - | - | - | - | 2.2 | - | 4.5 | 89 | 1.34 |
| | Festuca rubra | 2 | 96.4 | 1.8 | - | 1.8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 56 | 0.18 |
| | Festuca rubra | 3 | 85.7 | 1.6 | 9.5 | - | - | - | - | 1.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.6 | 63 | 0.55 |
| | Festuca juncifolia | 1 | 60.9 | 4.7 | 3.1 | 6.3 | - | - | - | 6.3 | 1.6 | - | - | 3.1 | 12.5 | - | - | - | - | - | - | - | - | - | - | - | 3.1 | 64 | 1.40 |
| | Festuca juncifolia | 2 | 71.4 | - | - | 11.1 | - | - | - | - | - | - | 15.9 | - | - | - | - | - | - | - | - | - | - | - | - | - | 3.2 | 63 | 0.84 |
| | Festuca juncifolia | 3 | 39.7 | - | 2.7 | 1.4 | 42.5 | - | - | 1.4 | - | 4.1 | - | - | - | - | - | - | - | - | - | 1.4 | - | - | - | - | 5.5 | 73 | 1.43 |
| | Festuca juncifolia | 5 | 71.4 | - | 6.3 | - | - | - | - | 11.1 | 4.8 | - | - | - | - | - | - | - | 4.8 | - | - | - | - | - | - | - | 1.6 | 63 | 1.02 |
| | Poa pratensis | 2 | 33.3 | - | 14.8 | 18.5 | - | - | 3.7 | 18.5 | 9.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 54 | 1.69 |
| | Poa pratensis | 3 | 72.4 | 17.2 | 4.6 | 2.3 | - | - | - | - | 3.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 87 | 0.88 |
| | Poa pratensis | 5 | 56.5 | 17.4 | 5.8 | 1.4 | - | - | 13.0 | - | - | - | - | - | - | - | - | - | 1.4 | - | 1.4 | - | - | - | - | - | - | 69 | 1.36 |
| | Holcus lanatus | 1 | 47.5 | - | 2.5 | - | - | 11.3 | 15.0 | - | 3.8 | - | - | - | - | 8.8 | 6.3 | - | - | 3.8 | - | - | - | - | - | - | 1.3 | 80 | 1.66 |
| | Holcus lanatus | 2 | 68.5 | 22.2 | 1.9 | 1.9 | - | 3.7 | - | 1.9 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 54 | 0.94 |
| | Holcus lanatus | 3 | 50.8 | 6.8 | 5.1 | - | - | 22.0 | 5.1 | - | 1.7 | - | 1.7 | 5.1 | - | - | - | - | - | - | - | - | 1.7 | - | - | - | - | 59 | 1.52 |
| | Deschampsia cespitosa | 1 | 92.2 | 3.9 | 1.3 | - | - | 2.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 77 | 0.35 |
| | Deschampsia cespitosa | 3 | 79.8 | - | 6.7 | 9.0 | - | - | - | - | - | - | - | 3.4 | - | - | - | - | - | - | - | - | - | - | - | 1.1 | - | 89 | 0.74 |

## Results and discussion

Samples from experiment 2 (rhizoplane) are dominated by one sequence type.
This sequence type was not observed in experiment 1.
Very few types are shared between experiment 1 and 2.
This could be due to a number of reasons including:

1. Differences in rhizoplane and bulk soil communities of denitrifiers
2. Spatial differences in community structure
3. Differences in N input (experiment 1 was fenced, experiment 2 was open)
4. Effects of harsh physical disturbance
5. Effects of monoculture

NirK diversity is higher in the rhizoplane of sub-dominant grass species
Samples from

### Acknowledgements

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