



# Field-scale evaluation of the oil remediation capacity of the legume *Galega orientalis*

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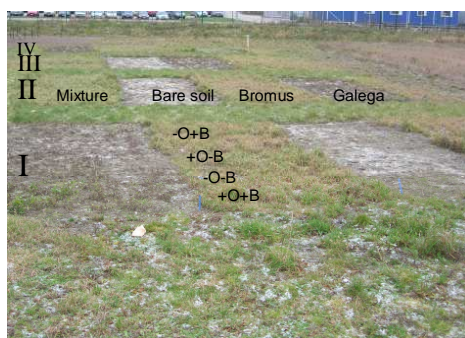
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## Background

- Glasshouse-based experiments have shown:
  - Biological activity associated with *Galega* roots accelerates the degradation of oil contamination
  - Plant growth promoting bacteria (PGPB) improved nodulation and plant yield
- This needed to be confirmed in the field
- Grass-legume mixtures often out-yield their components, and grasses have different root-associated bacteria
- Hence a multi-year trial was prepared using the legume *Galega orientalis* (forage goat's rue) and the grass *Bromus inermis* (smooth brome), singly and in combination, to remediate oil-contaminated soil
- This offered an opportunity to measure N<sub>2</sub>O release

## Aims

- Determine plant responses to the stress
- Follow changes in the bacterial community structure
- Determine the effects of the crops and treatments on N<sub>2</sub>O release



Layout of the experiment, photographed in November 2010. Four replicates I-IV of 4 main plots (Galega, Bromus, Mixture, Bare soil) each of which has 4 subplots of ±O (oil) and ±B (PGPB) in factorial combinations

## Materials and methods

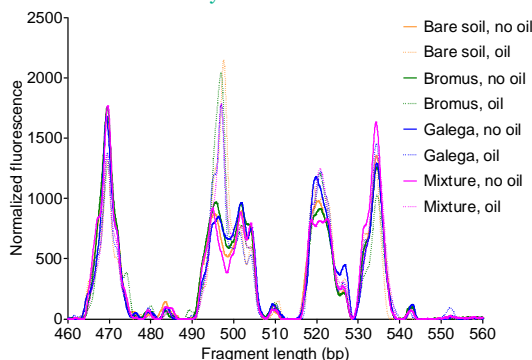
- Plots were established in June 2009
- *Galega orientalis* cv Gale
- *Bromus inermis* cv Lehis
- Singly, in 75 grass:25 legume ratio, or bare soil as main plots in Randomized Complete Block Design
- Subplots ±fuel oil (6 kg per plot, 7000 ppm), ±PGPB, in factorial combinations
- 60 kg/ha N fertilizer was given to the grass plots in 2009 but none subsequently
- Soil samples taken 4 times: A: July 2009, B: May 2010, C: November 2010 and D: May, 2011
- Top soil (0-20 cm) passed through 5 mm sieve and stored in -20 °C freezer before analysis
- DNA extracted using standard techniques
- Soil bacterial community structures studied by length heterogeneity PCR (LH-PCR), using domain specific primers to amplify 16S rDNA (Mikkonen *et al.*, 2011).
- Gas traps were installed in May 2011 on -oil-PGPB plots
- Samples were taken 6 times during 2011
- N<sub>2</sub>O content of the collected gas was determined by GC

## Results

### Plants

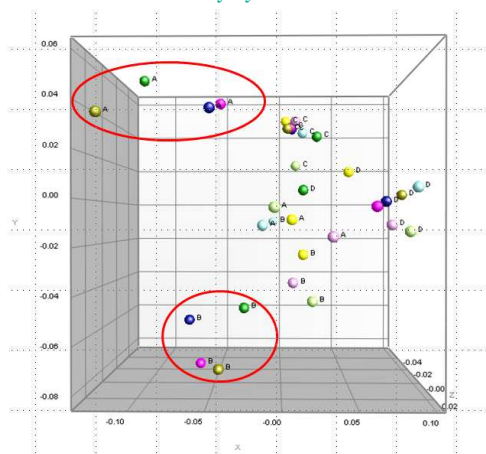
- In 2010, plant dry matter production was greatest in the crop mixture plots and in the +Oil +PGPB treatment
- In 2011, there was no significant difference in plant DM production among the main plot or subplot treatments
- % Galega in the mixtures declined through each growing season but recovered in spring

### Bacterial community structure



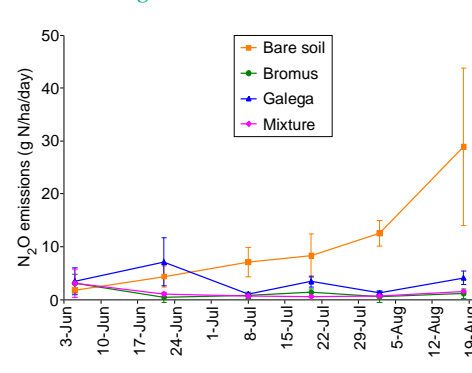
- At the start of the experiment, LH-PCR fragment lengths differed greatly between oil and no-oil plots, but showed few differences between crops

### Bacterial community dynamics



- Multi-dimensional scaling ordination separates the LH-PCR profiles of bare soil (yellow), Bromus (green), galega (blue), and mixture (pink) (non-PGPB treatment only)
- Oil-treated plots at times A and B are clearly separated (red rings) from non-oil plots
- By time C, the oil plots cluster with the non-oil plots
- At time D, all treatments have shifted

### Greenhouse gas release



- N<sub>2</sub>O release was greatest from the bare soil except at the start of the season
- N<sub>2</sub>O release from the Galega plots exceeded that from the grass and mixture plots
- Grass plots were not fertilized in 2011

## Conclusions

- After the first year, yields were not affected by oil or bacteria treatments
- Changes in bacterial community structure followed oil contamination
- These alterations reduced with time
- The soil bacterial community can recover from oil contamination
- N<sub>2</sub>O emission was very high from the fallow plots. Unfertilized grass released little N<sub>2</sub>O
- Harvested biomass can be used for biomethane production

