Where Does Denitrification Occur in the Rhizosphere?

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Aims

To determine the effect of root-derived C on the location of denitrification in the rhizosphere.

Initially by:-
Determining if the quantity & quality of C affects denitrification, through looking at changes in the production of N_2O and N_2 gas along a carbon gradient.

Introduction

N_2O is a green house gas with a global warming potential around 300 times greater than that of CO_2. Atmospheric N_2O concentrations have been steadily increasing over the last 100 years (Fig 1).

Denitrification from soils is an important source of N_2O to the atmosphere. An improved understanding of the factors that drive denitrification may help to create better mitigation strategies for N_2O .

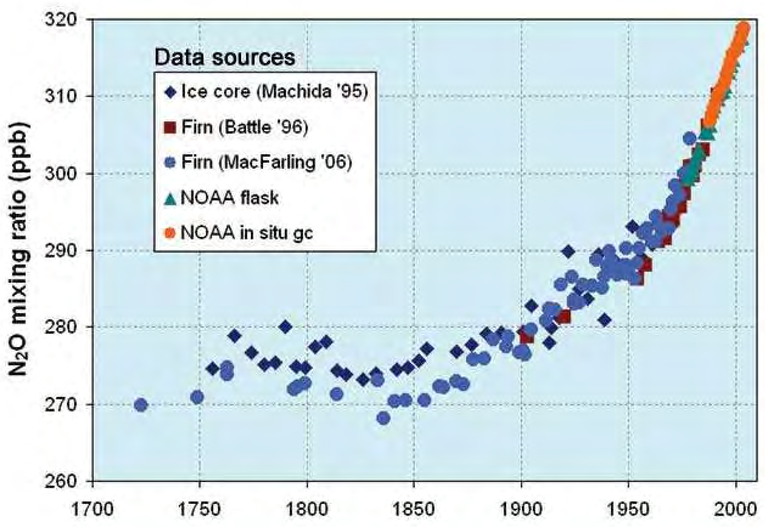

Figure 1 – Atmospheric N_2O concentrations (IPCC 2007)

A number of factors affect denitrification. One potentially important factor is that of carbon availability. C can act as an energy source for the heterotrophic bacteria involved in denitrification.

Root-derived C is an important source of carbon to the rhizosphere but quantity and quality of this C vary spatially. Root exudates are one form of root-derived C and are an important source of readily useable carbon. Different C compounds and quantities are produced in different areas of the root (Fig 2). Root tips are believed to be the area of greatest exudate production, followed by areas where roots branch.

This could potentially create a complex pattern of C availability within the rhizosphere, leading to spatial variation of denitrifying bacteria and therefore denitrification rates.

Different quantities of C have been found to be associated with increases in rates of denitrification, while C quality (compound) is hypothesised to also affect the rates and gaseous products (N_2O:N_2) of denitrification.

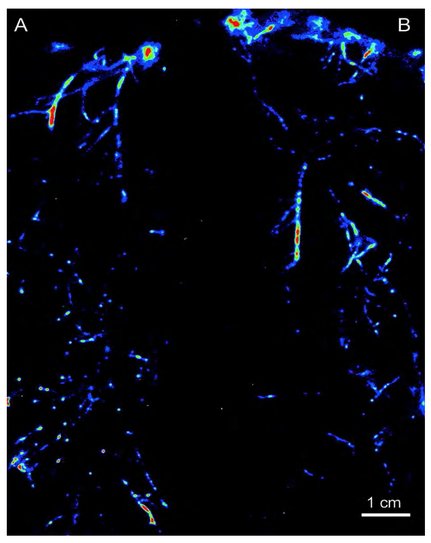

Figure 2- Bioluminescence response of the biosensor *Pseudomonas fluorescens* 10586 pUCD607 to root exudation from *Hordeum vulgare* (Paterson *et al* 2006).

Methods

Initially methods aim to determine if it is possible to create and detect a carbon gradient in sterile soil.

A known quantity of single and mixed C compounds will be added to soil through a Rhizon sampler at a steady rate, using a peristaltic pump.

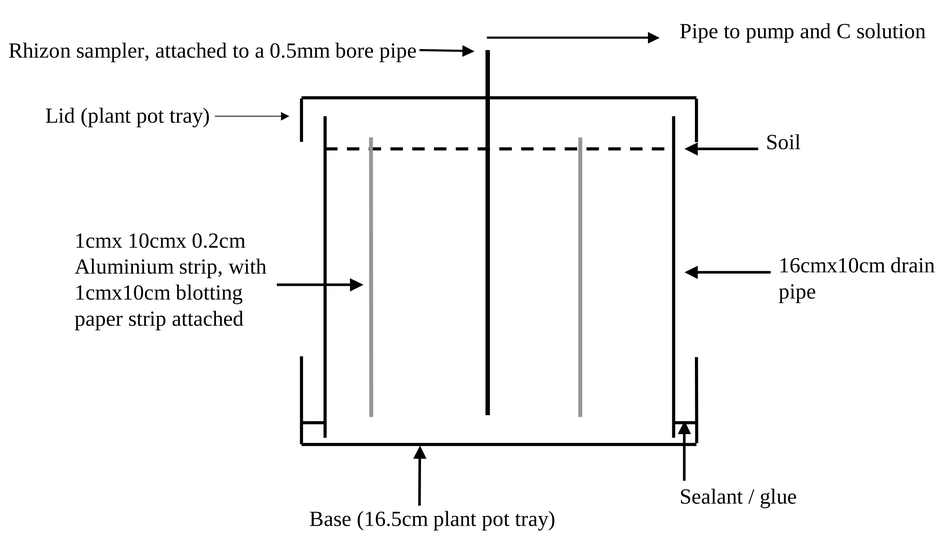

Figure 3- Microcosm set up

Methods Continued

The microcosm set up is shown in figures 3 and 4.

Soil organic matter is removed to ensure that the primary source of C to the system is that added through the Rhizon sampler.

The soil will be held at 70% WFPS, the optimal water content for denitrification.

The rhizon sampler through which C will be added, is placed at the centre of the pot. Blotting paper is inserted into the soil at 1cm increments from this central point (Fig 5).

The system will be run for 24 hours. The blotting paper will sample the fluid passing through it including the C. The quantity of C at each distance will be measured and C compounds identified using High performance Liquid Chromatography.

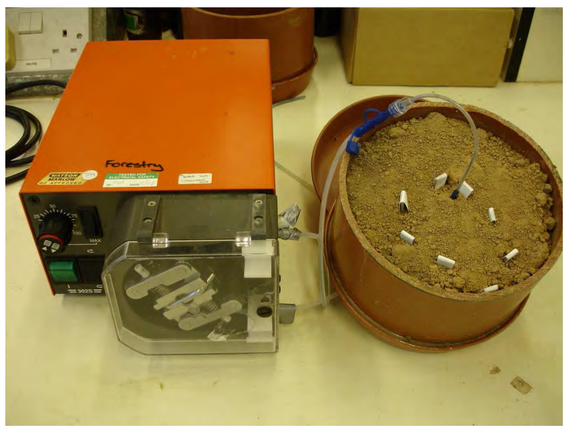

Figure 4- Microcosm and pump set up

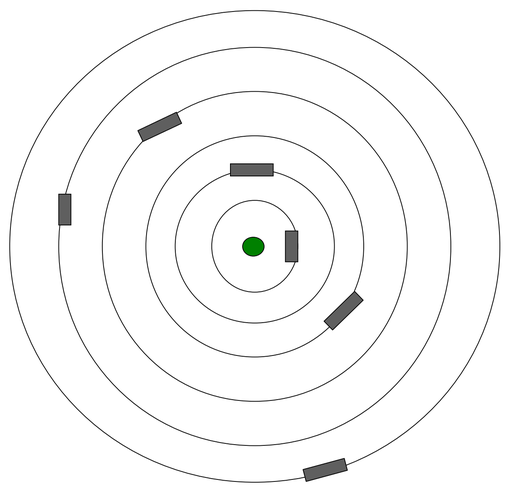

Figure 5- Arial view of microcosm set up. Grey rectangles represent the blotting paper on which carbon will be captured. The blotting paper is attached to metal strips to keep them upright. The green dot represents the Rhizon sampler through which the carbon is pumped.

Results

The system was tested using methylene blue allowing a visual indication of liquid spread through soil. A weak gradient in colour on the blotting paper was found (Fig 6), indicating that blotting paper closest to the Rhizon sampler picked up the most dye and blotting paper at the greatest distance the least. This suggests that, with optimisation, it is possible to set up C gradients with this microcosm design.

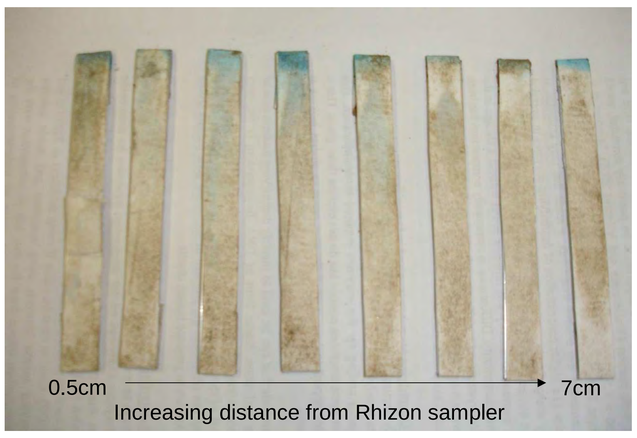

Figure 6- Blotting paper removed from microcosm after 12 hours of running dye through the soil system. Blotting paper on the left was closest to the Rhizon sampler.

Future Experiments

Once its established that it is possible to create and detect a C gradient, with a variety of C compounds, the system will be made more complex in order to measure a number of other factors.

A known species of denitrifier will be added to the soil system from this it should be possible to measure:-

- N_2O and N_2 produced along the carbon gradient. This will be achieved by sampling pore water using Rhizon samplers and measuring the N_2O and N_2 dissolved within it.
- Population size of denitrifying bacteria along the carbon gradient using real time PCR and/or culturing.

A number of different denitrifiers will be used in order to investigate the different responses by denitrifiers to carbon gradients.

The addition of ^{15}N, as nitrate, to the system will allow ^{15}N-N_2O sampling to be performed in order to gain a measure of N_2O produced by denitrification.

The importance of carbon relative to other factors that control denitrification (O_2, pH, NO_3^-) will be investigated in a series of factorial experiments.