

Does carbon flow from Ectomycorrhizal fungi stimulate Denitrification in forest soils?

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INTRODUCTION

Ectomycorrhizal (EcM) fungi have key roles in boreal forest nutrient cycling. They are an important route for carbon flow from the host plant into the soil, and the wide range of carbon compounds exuded into the soil by EcM fungi form a rich resource for the wider microbial community.

Denitrification is the step-wise reduction of nitrate (NO_3^-) to nitrous oxide (N_2O) and dinitrogen gas (N_2) undertaken by many heterotrophic bacteria (Fig. 1.). Understanding the factors that control this process is crucial because N_2O is a globally important greenhouse gas. Denitrification occurs in near-anaerobic conditions and is affected by a number of soil properties including carbon availability. However, the final reduction step in denitrification (N_2O to N_2) is limited by the quantity, availability and quality of carbon.

We hypothesise that mycorrhizal carbon flow will be an important factor in forest denitrification. The aim of this study is to investigate if N_2O production from denitrification is stimulated by addition of carbon compounds (glucose, mannitol, oxalic acid) that have been found to be produced by EcM fungi.

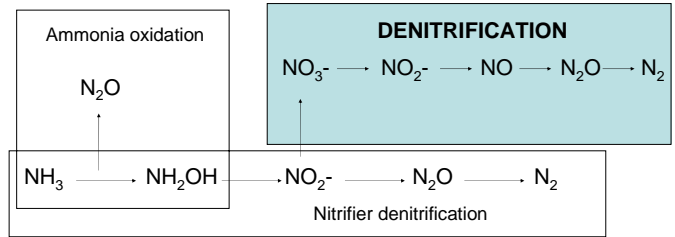


Fig. 1. Pathways of Nitrous oxide (N_2O) production in the Nitrogen cycle

METHODS

Forest soil was collected, and the upper organic horizon was separated from the mineral soil.

- 12 microcosms (100 g organic (Org); 100 g mineral (Min) forest soil) were set up in 500 ml Kilner jars with gas-tight sampling ports.
- To stimulate denitrifying conditions, soil was maintained at 70 % water-filled pore space on a weight basis, and was fertilised with K^{15}NO_3 , 5 g N m^{-2} , 10 at% excess.
- Carbon compounds were mixed into the microcosms in single doses: glucose (Glu), mannitol (Man), oxalic acid (Oxa), at 3.6 g C l^{-1} . The control was fertilised but had no carbon added.
- Gas samples for $^{15}\text{N}_2\text{O}$, $^{15}\text{N}_2$, $^{14+15}\text{N}_2\text{O}$ were taken over a 14 d period on days 1, 3, 5, 7 and 14 for analysis on the SerCon Isotope Ratio Mass Spectrometer.
- Further destructive microcosms were analysed for ammonium and nitrate (KCl extraction) and $\text{pH}_{(\text{water})}$.

CONCLUSIONS

1. Use of stable isotope ^{15}N allowed quantification of $^{15}\text{N}_2\text{O}$ and $^{15}\text{N}_2$ produced from denitrification.
2. Mycorrhizal carbon compounds do affect denitrification, and especially in the organic surface horizon (Fig. 2A).
3. Mannitol is an EcM fungal sugar, which stimulated highest $^{15}\text{N}_2\text{O}$ production (Fig. 2A).
4. Some $^{15}\text{N}_2$ was produced. Contrary to expectations, oxalic acid stimulated highest $^{15}\text{N}_2$ production in the organic horizon (Fig. 2B), suggesting that this carbon compound is preferentially utilised for the final reduction step.

RESULTS

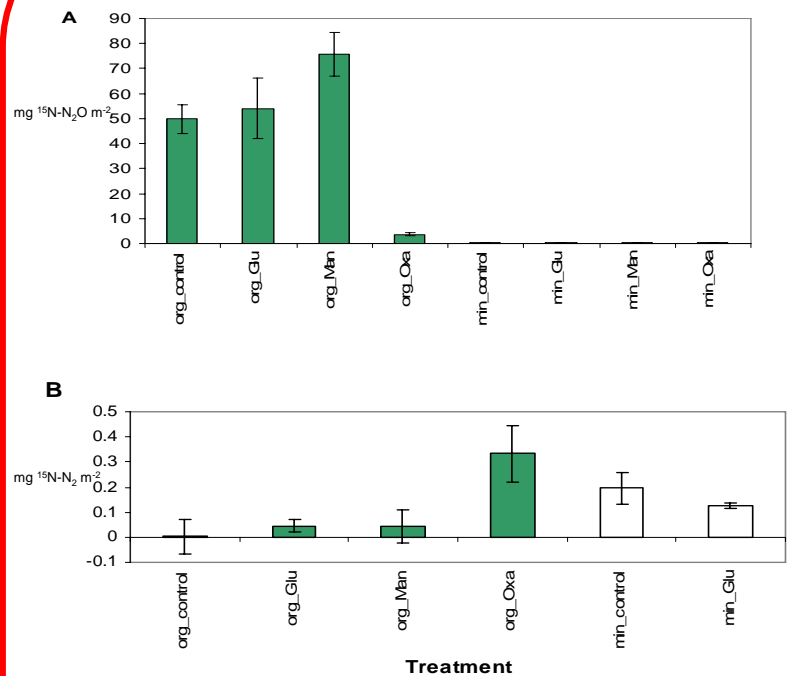


Fig. 2. Total ^{15}N - N_2O (A) and ^{15}N - N_2 (B) produced over 14 days in organic (Org) and mineral (Min) soil after addition of ^{15}N labelled nitrate with no carbon (control), glucose (Glu), mannitol (Man) and oxalic acid (Oxa).

FUTURE WORK

Further experiments are being conducted to investigate the mycorrhizal effect on denitrification.

1. **Denitrification by EcM fungi** will be determined by measuring N_2O from pure culture fungal isolates growing in liquid culture. Fungal denitrification does occur in *Fusarium oxysporum* (Shoun & Tanimoto 1991), but as yet, has not been investigated in EcM fungi. Any fungal contribution to N_2O production will then have to be separated from bacterial denitrification in future experiments.
2. **The effect of the presence of EcM fungi on N_2O production** will be investigated in 2 separate experiments:
 - mesh cores that separate mycorrhizosphere soil from bulk soil (Johnson et al, 2001) will be inserted into pots with young Sitka spruce trees growing in soil with previous N deposition histories. Gas samples from the core headspaces will be taken for N_2O analysis.
 - birch seedlings will be grown in the non-mycorrhizal condition or in the mycorrhizal condition with *Paxillus involutus* in laboratory microcosms. N_2O production in the microcosms will be determined and this will be linked with ^{13}C stable isotope probing of soil patches to trace the flow of fungal carbon into the denitrifying microbial community.