



## Short-term effect of the nitrification inhibitor DMPP on N-turnover and denitrification losses from two agricultural soils in subtropical Australia

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Intense wetting and drying cycles render agricultural soils in the subtropics prone to nitrogen (N) loss via denitrification, with large pulses of the greenhouse gas nitrous oxide ( $N_2O$ ) triggered by rainfall. The nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) proved to be effective under subtropical conditions, demonstrating substantial reductions of  $N_2O$  emitted from cropping soils. However, DMPP has consistently failed to reduce  $N_2O$  emissions from subtropical pasture soils. The aim of this study was therefore to investigate (a) the response of N-transformations and  $N_2O$  emissions from a subtropical pasture and a vegetable soil to DMPP, and (b) if the abundance of *nosZ*, the gene encoding the  $N_2O$  reductase, can explain  $N_2O$  emissions as affected by DMPP.

Soil microcosms were established in centrifuge tubes and fertilised with ammonium nitrate ( $35 \mu\text{g g}^{-1}$  soil) with or without DMPP. Labelling either ammonium ( $\text{NH}_4^+$ ), or nitrate ( $\text{NO}_3^-$ ) with  $^{15}\text{N}$  at 10 atom% excess enabled the quantification of gross N-transformations using  $^{15}\text{N}$  tracer and pool dilution methods. Soil microcosms were incubated at 75% WFPS over two days, and gas samples were taken each day. Gas samples were analysed for  $^{15}\text{N}_2\text{O}$  to split  $N_2O$  production into the ammonia oxidation pathway and denitrification. Soil was extracted before and after the incubation for DNA, quantifying the response of *nosZ* abundance to DMPP.

Denitrification was the main source of  $N_2O$  production in both soils. The pasture soil emitted more than  $1.5 \mu\text{g N-N}_2\text{O g}^{-1}$  soil over two days, exceeding  $N_2O$  emissions of the vegetable soil by a factor of 10. This trend was consistent with the high N-transformation rates in the pasture soil, exceeding those of the vegetable soil by a factor  $>10$ . DMPP reduced gross nitrification by 12 and 60% for the pasture and vegetable soil, respectively. However, DMPP reduced cumulative  $N_2O$  emissions from the vegetable soil only. Fertilisation decreased *nosZ* abundance in the pasture soil, regardless of the treatment. The same trend was observed for the fertiliser only treatment from the vegetable soil. DMPP however increased *nosZ* abundance compared to the fertiliser only treatment in the vegetable soil.

Gross N transformation rates identified the pasture soil as the more productive soil regarding soil mineral N supply and demonstrate the magnitude of  $N_2O$  emissions as a function of N-turnover. The reduction of *nosZ* abundance after fertilisation in both soils reflects the stimulating effect of fertiliser and water addition on N turnover. Increased  $\text{NO}_3^-$  production suppresses *nosZ* activity, limiting further reduction of  $N_2O$  to dinitrogen ( $\text{N}_2$ ). This mechanism was mitigated by DMPP in the vegetable soil, explaining the significant reduction of  $N_2O$  emissions by DMPP. The high N turnover in the pasture soil and the resulting  $\text{NO}_3^-$  concentration is likely to limit the short-term efficacy of DMPP. The relationship between  $N_2O$  emissions and *nosZ* abundance identifies the shift in the  $\text{N}_2:\text{N}_2\text{O}$  ratio to  $\text{N}_2$  as a key mechanism of  $N_2O$  reduction by DMPP. This shift is however driven and limited by soil-intrinsic N-turnover, explaining differences in  $N_2O$  reduction by DMPP observed for different soil types in the field.