



Impact of pea genetic variability on the control of N₂O reduction by soil-microorganisms-plant systems

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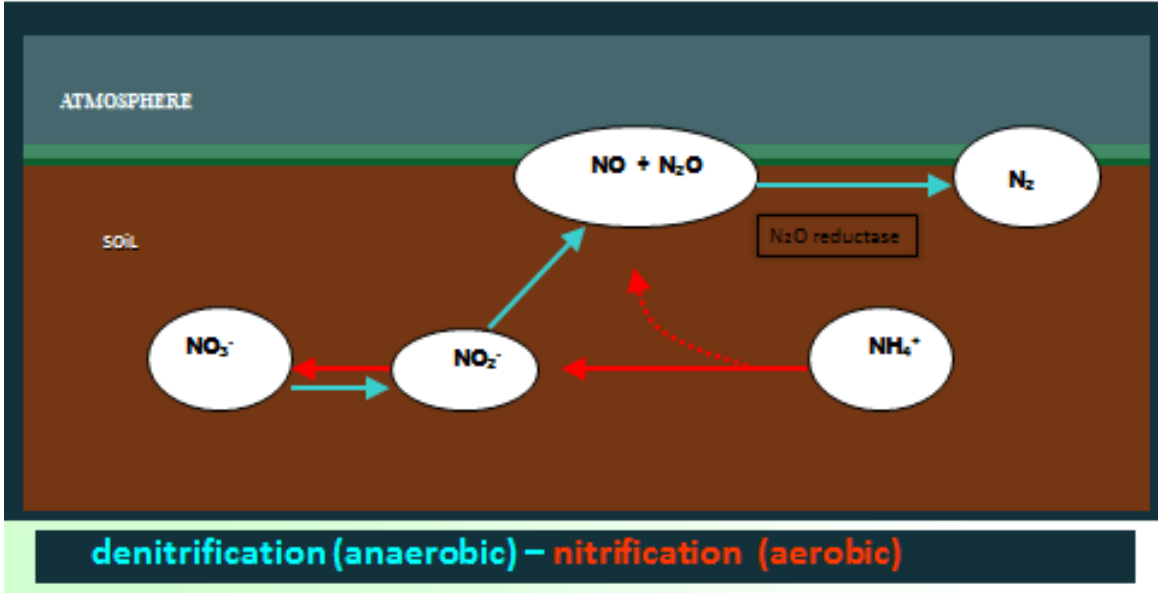
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1. Introduction

- Agriculture, through soil emissions, is an important anthropogenic source of the greenhouse gas N₂O that has a Global Warming Potential 300 times higher than CO₂ on a molar basis (UNEP, 2013).
- In soils, N₂O is mainly produced through the microbial processes of denitrification and nitrification. The last step of the denitrification process is currently the only known pathway for the terrestrial removal of N₂O. N₂O reduction is catalyzed by the N₂O reductase enzyme encoded by the *nosZ* gene (illustr 1).
- Strategies to mitigate N₂O emissions from agricultural soils could be based on the promotion of the N₂O reduction, for example by inoculating crops with microorganisms able to reduce N₂O. Previous studies have demonstrated that soybean roots nodulated by *Rhizobia* strains carrying the *nosZ* gene are able to reduce the greenhouse gas N₂O (Sameshima-Saito *et al.*, 2006; Hénault and Revellin, 2011).
- Legume crops play a major role in sustainable agricultural systems as their nitrogen demand is mainly satisfied by the root endo-symbiosis they establish with nitrogen-fixing bacteria (*Rhizobia*) from the soil. Pea is an important legume crop (illustr 2). However, from our knowledge, the *nosZ* gene has not yet been observed in any isolated strain of *Rhizobium leguminosarum* biovar *viciae*.
- The aim of this study was to assess the ability of reducing N₂O of different systems involving pea genotypes varying for their nodulation abilities.



Illustr 1 : Mechanisms involved in the N₂O budget in



Illustr 2 : A field of pea, at flowering

2. Materials and Methods

- Five different pea genotypes were chosen from their varying nodulation abilities. They were (i) the old cultivar Frisson and (ii) the more recent one Austin ; hypernodulating mutants, respectively (iii) Frisson-sym29 (Bourion *et al.*, 2007) and (iv) Austin-sym29, and (v) P2 a non-nodulating mutant of Frisson. All mutants were obtained from ethyl methane sulfonate mutagenesis.
- All these five genotypes (illustr 3) plus a control (bare soil) were cultivated in a greenhouse (6 pots per genotypes, 3 plants per pot) on a soil known as unable to reduce N₂O. No inoculations were made. Nodosities were established with soil indigenous strains.
- At the beginning of seed filling, pots were incubated for 48 h in airtight chambers. The N₂O concentration in chambers was periodically analyzed using a GC (electron capture detector) to evaluate N₂O consumption/reduction rate (illustr 4).

Illustr 3 : Pictures of the different pea genotypes



Illustr 4 : Incubation of peas during N₂O consumption measurement

3. Results

- Both the number and the mass of nodosities were higher for hypernodulating genotypes.
- Variations in N₂O concentration in the airtight chambers after the 48 hours of incubation were observed between the pea genotypes.
- No change of the N₂O concentration in chambers was observed on both the bare soil control and the system with Austin.
- A low increase of N₂O concentration was observed for the system with the non-nodulating P2 genotype.
- Conversely, the hypernodulating Austin-sym29 genotype clearly allowed a N₂O consumption.
- The pea Austin-sym29 genotype has probably enhanced the development of strains having the *nosZ* gene and able to reduce N₂O.

		Bare Soil	P2	Frisson	Frisson sym29	Austin	Austin sym29
Number of nodosities per root			0 ^C	232 ^B	288 ^B	195 ^B	535 ^A
Dry weight of nodosities per root (mg)			0 ^D	0.102 ^{BC}	0.126 ^{AB}	0.082 ^C	0.146 ^A
R e p l i c a t e s	R1	Not detected	0.027	-0.017	Not detected	Not detected	-0.016
	R2	Not detected	0.012	Not detected	-0.0126	Not detected	-0.019
	R3	Not detected	0.027	Not detected	Not detected	Not detected	-0.018
	R4	Not detected	Not detected	-0.029	Not detected	Not detected	-0.026
	R5	Not detected	0.02	-0.011	Not detected	Not detected	-0.033
	R6	Not detected	0.018	Not detected	Not detected	Not detected	-0.021

Table 1 : Characteristics of nodosities and Net Flux of N₂O (μg N-N₂O h⁻¹ and per pot) by the soil planted with different genotypes of peas. Positive values indicate a production of N₂O while negative values indicate N₂O consumption.

4. Conclusions and Perspectives

- The use of hypernodulating pea genotypes has allowed to create systems (soil-plant-microorganisms) able to reduce the greenhouse gas N₂O
- The next step of this study is to investigate whether if the increase of nodosities for the Austin-sym29 genotype would explain the detected N₂O consumption or if specific strains of *Rhizobium leguminosarum* biovar *viciae* with the functional *nosZ* gene has been preferentially selected by the pea Austin-sym29
- Therefore field tests both with pea inoculation with isolated strains or with hypernodulating Austin sym 29 genotype would be necessary to quantify the N₂O reduction by this process at the field scale.
- At the end, this study suggests that pea breeding and its interactions with soil microbial activities could contribute to promote the N₂O reduction in soils, as a strategy for mitigating greenhouse gas emission by agriculture.

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