Environmental context

Among the main greenhouse gases (GHG), nitrous oxide ($N_2O$) causes a serious environmental problem because of its global warming potential which is 298 times higher than CO$_2$ and because of its lifetime of 114 years. It is well known that microorganisms play an essential role in $N_2O$ emissions (through nitrification and denitrification) and that agricultural soils emit most of this GHG. Thus, characterizing the dynamic of bacterial community and the expression of nitrogen cycle functional genes during a $N_2O$ emission peak is of great interest to understand and anticipate $N_2O$ emissions and improve good agricultural practices recommendations.

Methods

• Automated closed-dynamic-chamber (Fig. 1) system was used to Record $N_2O$ emissions- 1 flux each 30 minutes (Fig. 2).
• Soil samples (10 cm top cm) were collected at strategic time (in triplicates) (green circles).
• Quantitative PCR was used to quantify gene expression during the observed peak (Fig. 3).
• Massive sequencing of 16S rRNA gene (Ion Torrent, MOTHUR) was conducted to assess the bacterial community dynamic (Fig. 4).

Results

Fig. 2 $N_2O$ emissions and soil moisture during the experiment.

(1) Quantitative PCR analysis on N-genes cycle and 16S rRNA genes

### Soil sampling over time (8 dates) and RNA extraction (Fig. 2)

![RNA extraction](image)

16S rRNA: bacterial 16S rRNA gene expression was quantified to report the global bacterial activity during $N_2O$ emission peak.

- **nirS**: Nitrite reductase ($N_2O \rightarrow NO$)
- **nirK**: Nitrite reductase ($N_2O \rightarrow NO$)
- **nifH**: Nitrogenase reductase ($N_2O \rightarrow NH_4^+$)
- **nosZ**: Nitrous oxide reductase ($NH_4^+ \rightarrow NH_3$)
- **amoA**: Ammonia monoxygenase ($NH_3 \rightarrow NH_4OH$)

Quantification of genes (transcript copies number per g DNA)

The transcript quantity of nirS, nirK, nifH, nosZ, and 16S rRNA genes showed no significant changes during the $N_2O$ emission peak. The transcript quantity of amoA gene showed a significant change positively correlated with $N_2O$ emissions. amoA gene encodes for the ammonia monoxygenase which catalyzes during the nitrification the oxidation of ammonium to hydroxylamine ($NH_2OH$). $NH_2OH$ can subsequently be abiotically transformed in $N_2O$ process.

Fig. 3 16S rRNA transcripts abundance evaluation by quantitative PCR

Transcript abundance of OTUs was assessed by quantitative PCR (qPCR) using 16S rRNA genes as a house-keeping gene. Significant changes (20%) were observed for 6 OTUs (Fig. 3). The correlation coefficient of $N_2O$ emissions and the average of $N_2O$ emissions (for each operational taxonomic unit (OTU)) was calculated (0.40 to 0.50).

### Conclusions

- The use of automated closed-dynamic-chamber system allowed the determination of $N_2O$ emissions at a fine scale.
- Denitrification genes expression abundance did not significantly evolve during $N_2O$ emissions.
- Nitrification marker (amoA gene) showed a significant correlation with $N_2O$ emissions. amoA gene expression appeared to be the best proxy to follow $N_2O$ emissions ($R^2 = 0.89$). amoA positive correlation wasn’t explained by an increase of *Nitrosomonas* members and could therefore be the result of a gene induction.
- Bacterial community structure remained globally stable except for 35 OTUs which showed a positive or negative significant correlation with $N_2O$ emissions (including members of the nitrification process).
- Denitrification was expected after the rainfall but results demonstrated that nitrification could be the main driver of $N_2O$ emissions in this agricultural soil.