Monitoring the dynamic of bacterial community and nitrogen cycle functional genes expression during a N_2O emission peak.

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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.

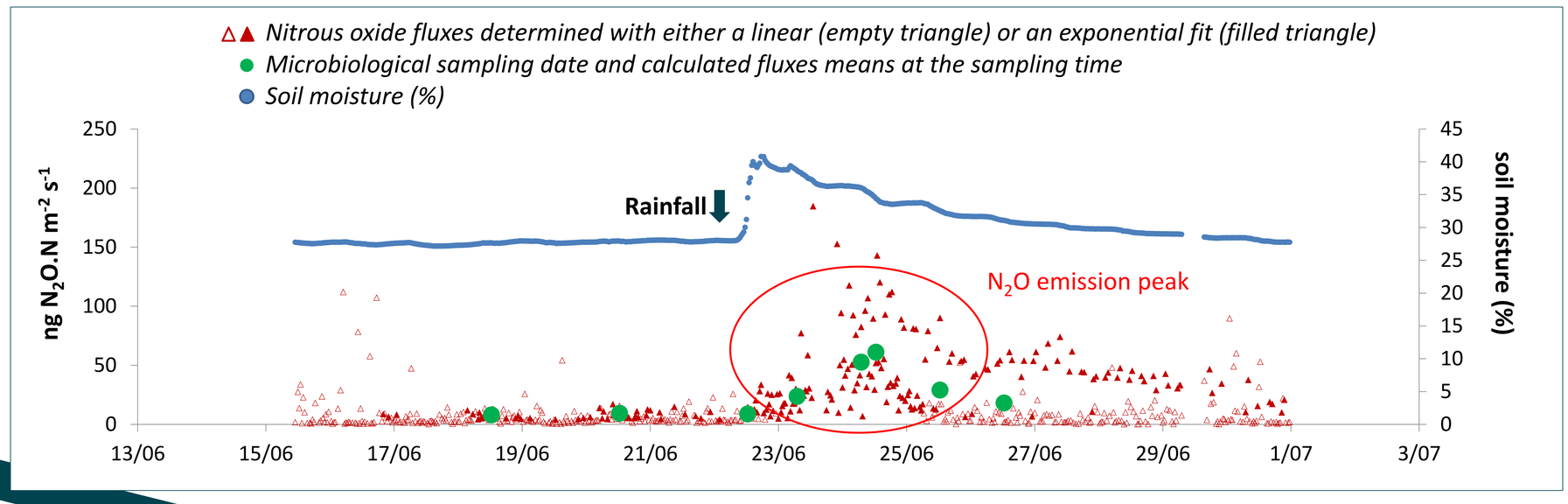
Methods

- **Automated closed-dynamic-chamber** (Fig. 1) system was used to Record N_2O emissions- 1 flux each 30 minutes (Fig. 2).
- **Soil samples** (10 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).

Fig. 1 Automated closed-dynamic-chamber system on the field

Results

Fig. 2 N_2O emissions and soil moisture during the experiment



Soil sampling over time (8 dates) and **RNA** extraction (fig. 2)

cDNA

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

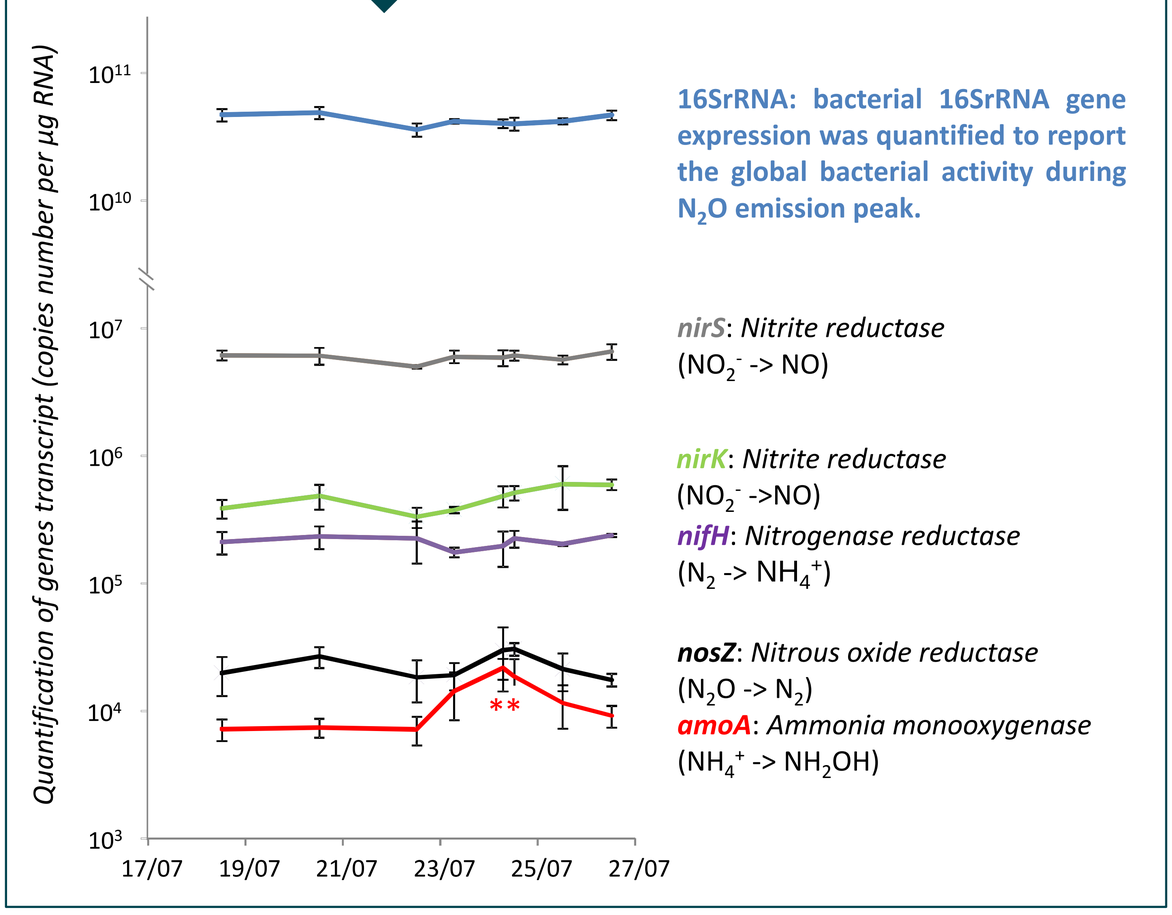


Fig.3 Gene transcripts abundance evaluation by quantitative PCR
Significant differences are marked with *

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

nirS: *Nitrite reductase* (NO_2^- -> NO)

nirK: *Nitrite reductase* (NO_2^- ->NO)
nifH: *Nitrogenase reductase* (N_2 -> NH_4^+)

nosZ: *Nitrous oxide reductase* (N_2O -> N_2)
amoA: *Ammonia monooxygenase* (NH_4^+ -> NH_2OH)

(2) Microbial communities dynamic was assessed using **massive sequencing (Ion Torrent)**

Relationship between **16SrRNA transcripts abundance** and the average of **N_2O emissions** (for each operational taxonomic unit (OTU)

phylum	class	order	family	genus	coefficient correlation
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	-0,41
Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified	-0,41
Proteobacteria	**Betaproteobacteria**	**Nitrosomonadales**	**Nitrosomonadaceae**	**unclassified**	**-0,41**
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Albidiferax	-0,42
Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	Sandaracinus	-0,42
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified	-0,42
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	-0,42
Proteobacteria	Betaproteobacteria	unclassified	unclassified	unclassified	-0,43
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	-0,44
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified	-0,55
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Rhizomicrobium	0,54
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0,53
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	Nordella	0,53
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	0,52
Proteobacteria	Alphaproteobacteria	Rhodospirillales	DA111	unclassified	0,50
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	unclassified	0,50
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	0,49
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	0,49
Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	unclassified	0,49
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	unclassified	0,48
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	0,47
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	unclassified	0,47
Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis	0,47
Nitrospirae	**Nitrospira**	**Nitrospirales**	**Nitrospiraceae**	**Nitrospira**	**0,46**
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0,46
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	0,45
Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	0,45
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	0,44
Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Roseiflexus	0,44
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Flavisolibacter	0,44
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	unclassified	0,43
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0,42
Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Streptosporangium	0,42
Nitrospirae	**Nitrospira**	**Nitrospirales**	**Nitrospiraceae**	**Nitrospira**	**0,42**
Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	0,41
Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Roseiflexus	0,40

Fig. 4 List of OTU positively and negatively correlated with nitrous oxide

Transcript quantity of ***nirS***, ***nirK***, ***nifH***, ***nosZ***, and **16srRNA** 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 monooxygenase which catalyzes during the nitrification the oxidation of ammonium to hydroxylamine (NH_2OH). NH_2OH can subsequently be abiotically transformed in N_2O process.

On the 14267 OTUs identified in this experiment, only **36 showed an activity** significantly **correlated with N_2O** emission (>0,40, <-0,40, n=24). Among them, 2 OTUs positively correlated were identified as members of ***Nitrospira* genus** which is responsible of the nitrification. One OTU affiliated to the family ***Nitrosomonadaceae*** was **negatively correlated** with N_2O emissions. Members of this family have generally the gene *amoA*.

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 *Nitrosomonadaceae* members and could therefore be the result of a gene **induction** .
- **Bacterial community** structure remained globally stable except for **36 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.