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## Climate change drivers modify N<sub>2</sub>O fluxes via changes in microbial populations in a grassland experiment

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Amélie Cantarel, Juliette Bloor, Thomas Pommier, Nadine Guillaumaud, Jean-François J.-F. Soussana. Climate change drivers modify N<sub>2</sub>O fluxes via changes in microbial populations in a grassland experiment. BES Annual Meeting, Sep 2011, Sheffield, United Kingdom. hal-02804220

**HAL Id: hal-02804220**

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Submitted on 5 Jun 2020

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# *Climate change drivers modify N<sub>2</sub>O fluxes via changes in microbial populations in a grassland experiment*

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<sup>1</sup> Ecologie microbienne Lyon, UMR 5557 Lyon, France

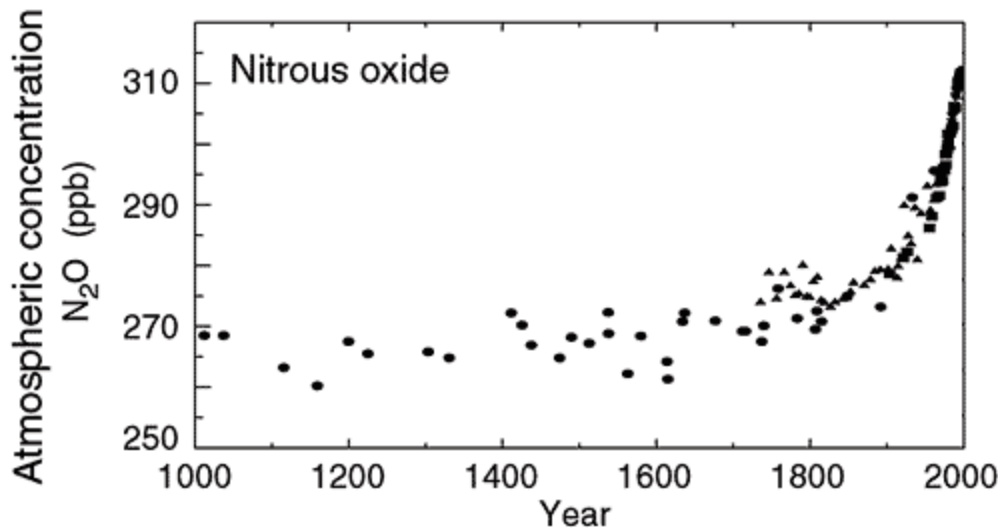
<sup>2</sup> Grassland Ecosystem Research Group (UREP), INRA Clermont-Theix, France

<sup>3</sup> CODIR Environnement, INRA Paris, France

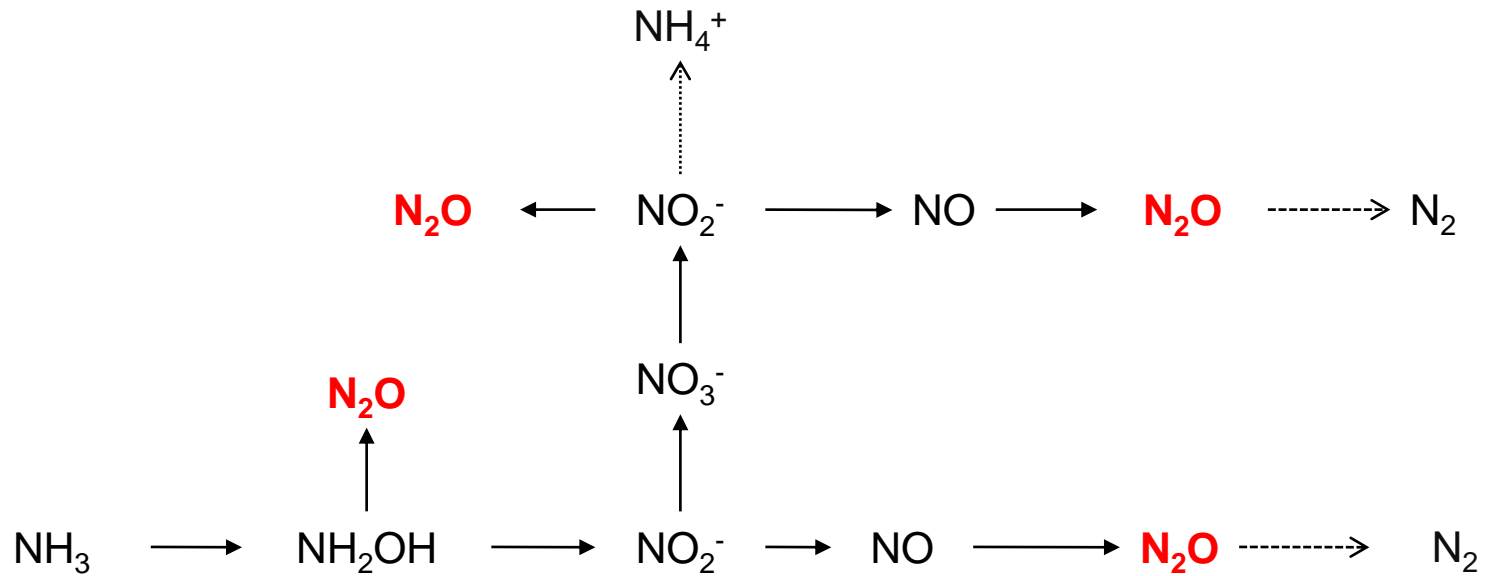
- Current climate models predict (IPCC 2001, 2007)
  - ↑ global air temperatures
  - Changes in regional patterns of rainfall
  - ↑ atmospheric greenhouse gases concentrations (as carbon dioxide, CO<sub>2</sub>)

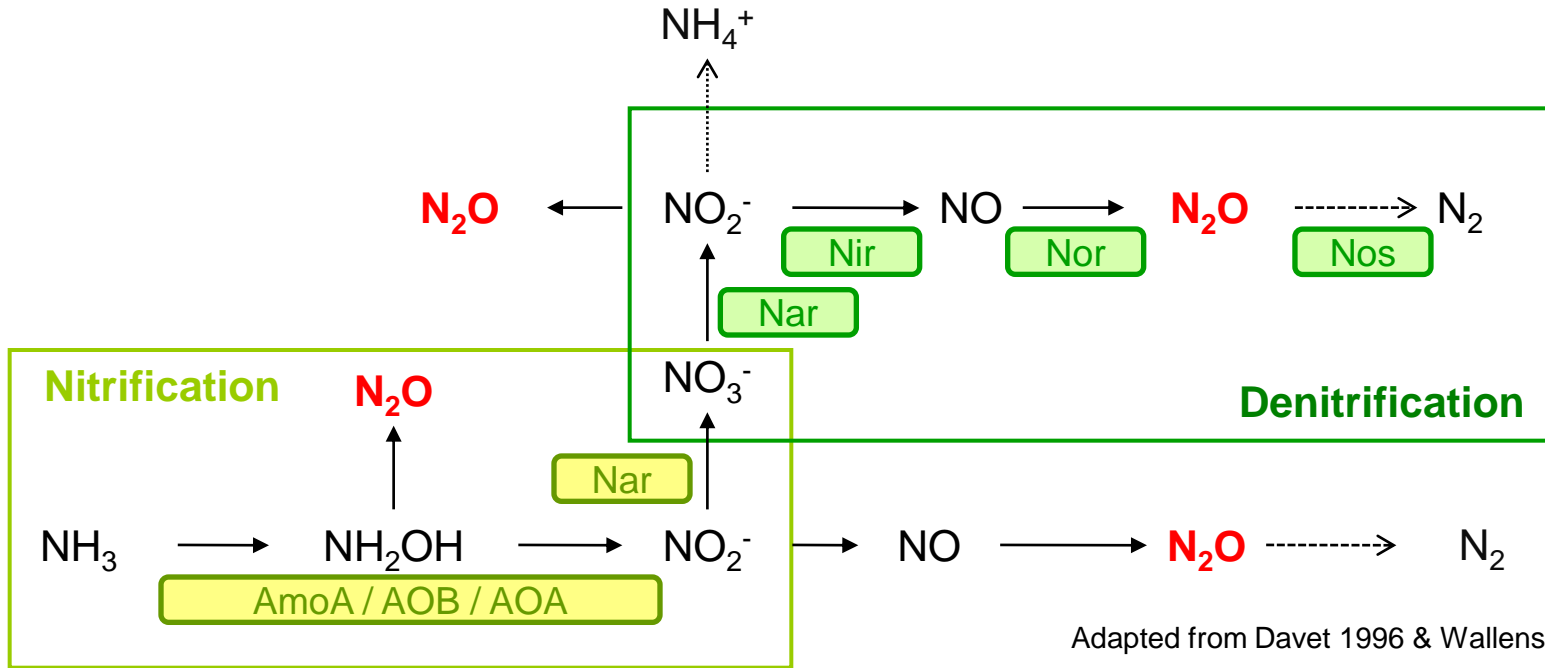
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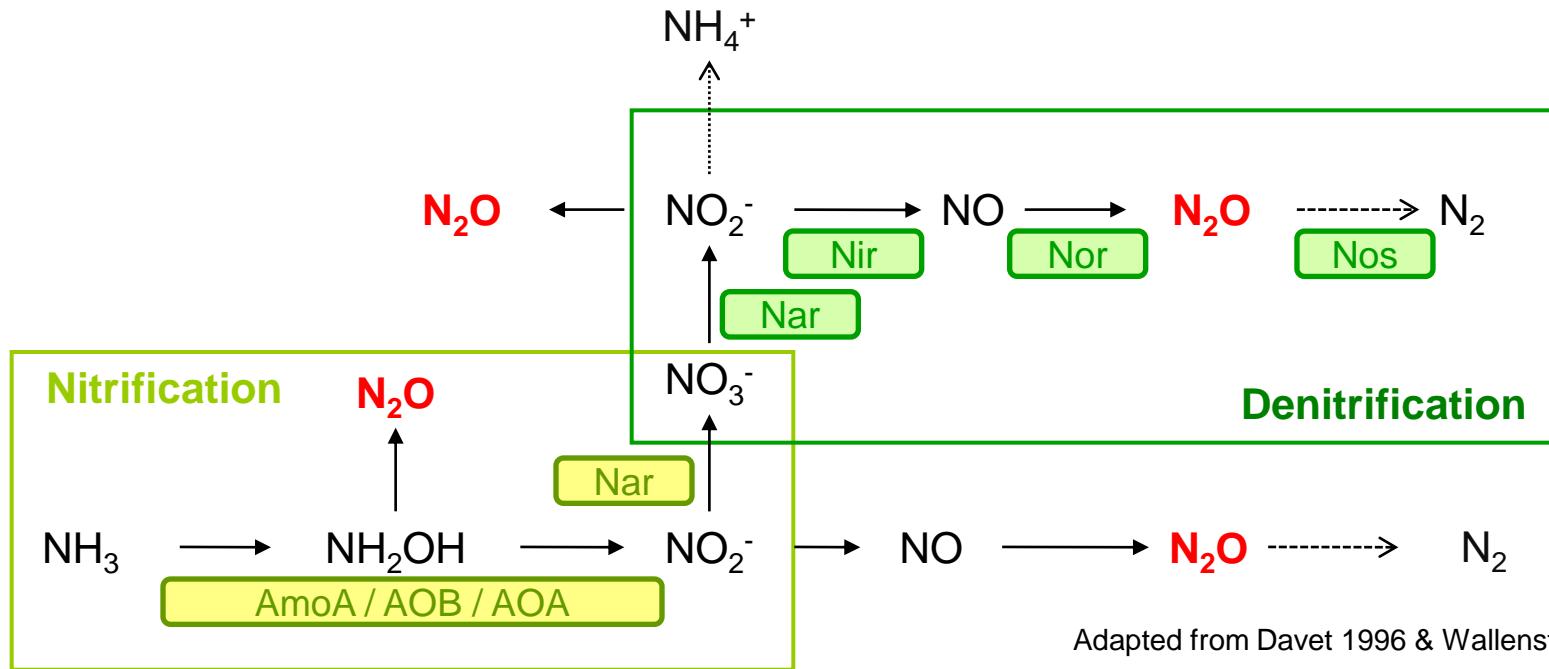


- N<sub>2</sub>O trace gas
  - Third greenhouse gases after carbon dioxide and methane
  - Strong global warming potential (~ 320 > CO<sub>2</sub>)
  - Depletion of the stratospheric ozone layer (Ravishankara *et al.* 2009)





Adapted from Davet 1996 & Wallenstein et al. 2006



- Changes in nitrification and denitrification may be linked to changes in:

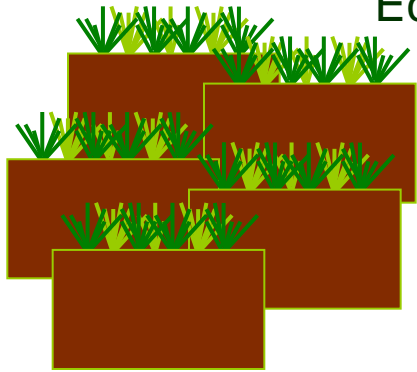
- Microbial population size
- Microbial community structure



- What are the effects of climate change drivers (elevated temperature, drought and elevated atmospheric CO<sub>2</sub> concentrations) on nitrous oxide (N<sub>2</sub>O) fluxes in grasslands?
- How do climate change drivers affect the microbial processes linked to N<sub>2</sub>O fluxes?

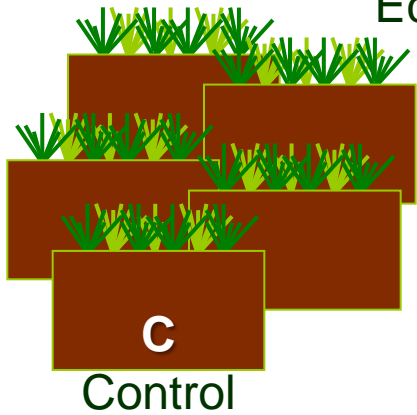
THEIX(850m)

Ecosystem: Acid grassland, no fertilizers



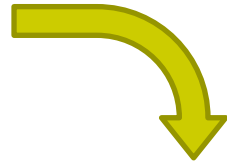
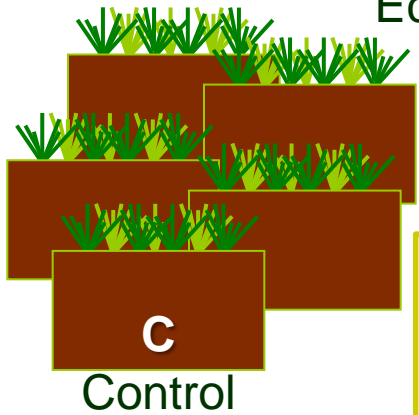
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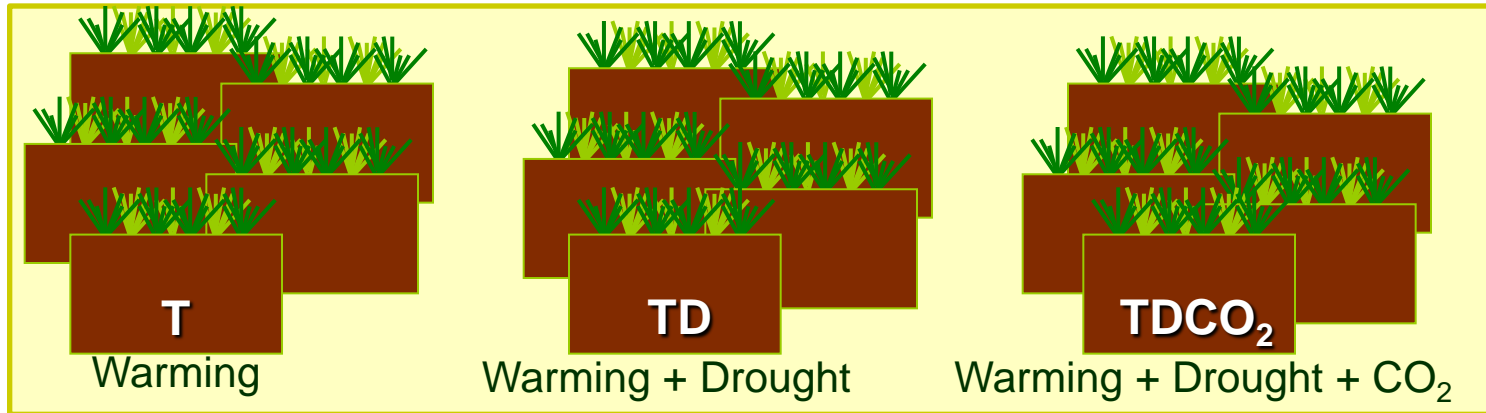


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Ecosystem: Acid grassland, no fertilizers



CLERMONT-FERRAND (350m) + 3.5 °C



Temperature effect

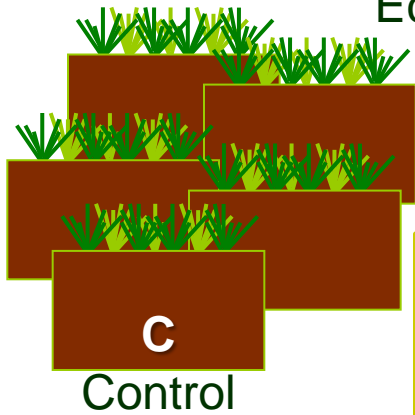
C vs T

Transplantation along an altitudinal gradient

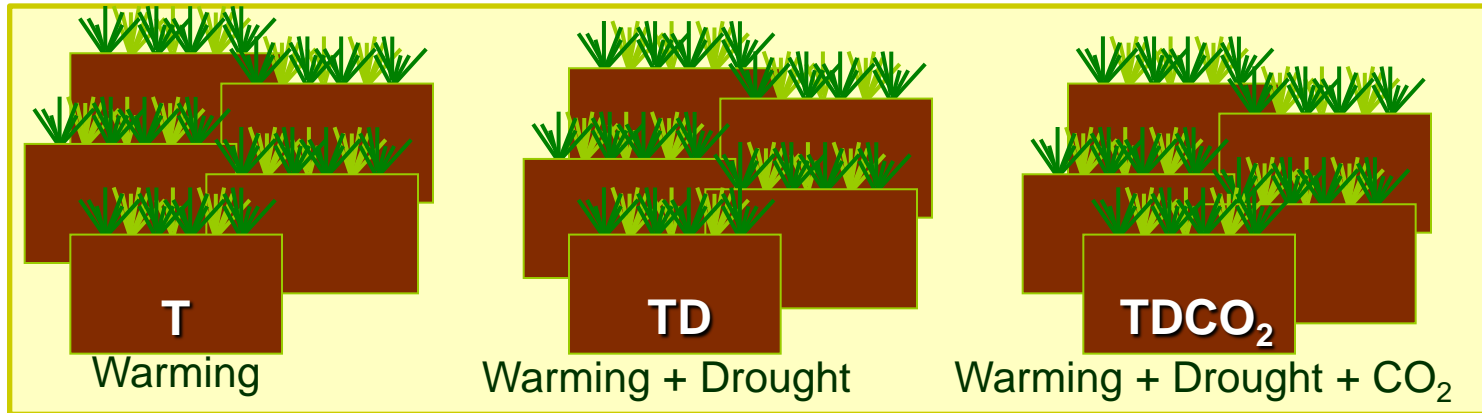
Same watering regime

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Temperature effect

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Drought effect (-20% rainfall)

T vs TD

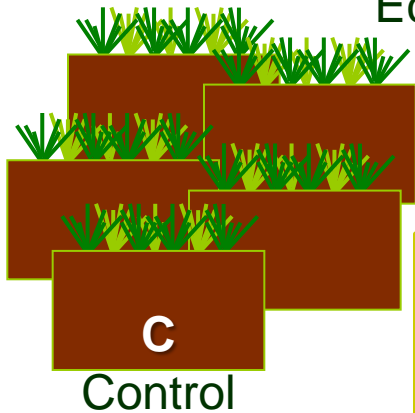
Transplantation along an altitudinal gradient

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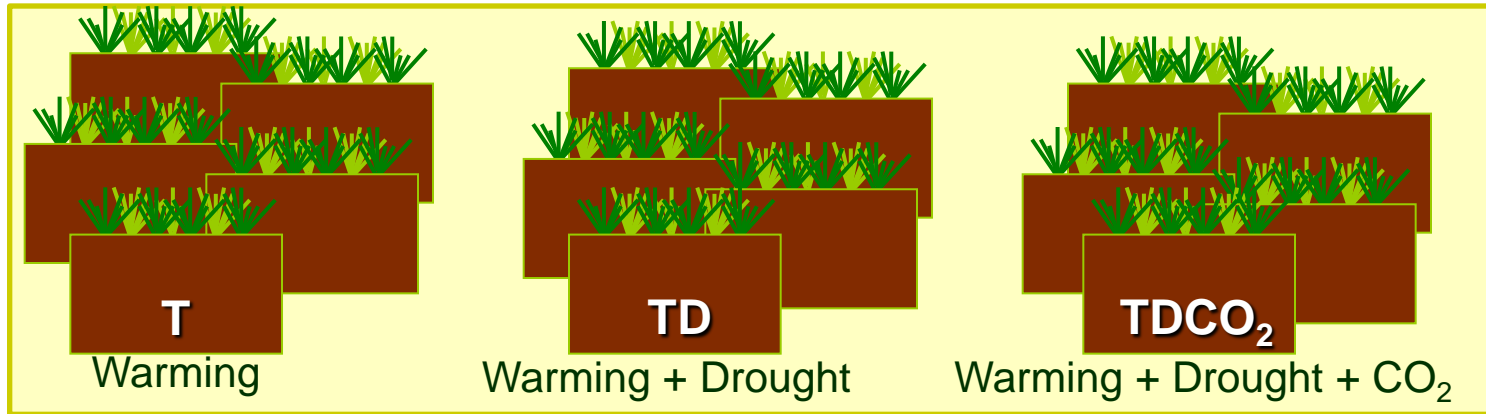
Using rainscreens during June, July and August

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Ecosystem: Acid grassland, no fertilizers



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Temperature effect  
C vs T



Drought effect (-20% rainfall)  
T vs TD



Elevation of atmospheric [CO<sub>2</sub>] + 200ppm  
([CO<sub>2</sub>] = 600 ppm)  
TD vs TDCO<sub>2</sub>

Transplantation along an altitudinal gradient  
Same watering regime

Using rainscreens during June, July and August

Using Mini-FACE technology

IPCC scenario for 2080 for Central France



- Nitrous oxide (N<sub>2</sub>O) flux measurements

- 4 dates of N<sub>2</sub>O flux measurements in 2009

- May, July, September and November
    - N<sub>2</sub>O measurements using closed static chambers and a photoacoustic gas analyzer (INNOVA)



- Soil sampling following each flux measurement

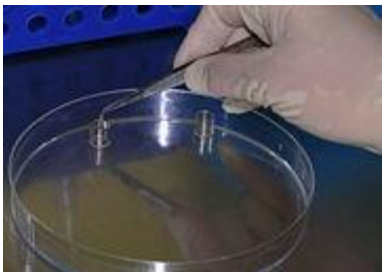
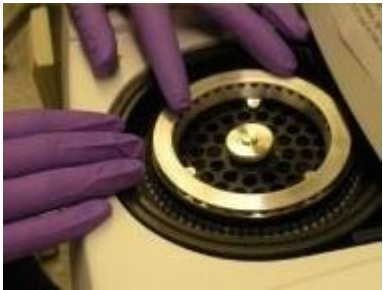
- 3 soil cores (Ø 1.5 cm) from 0-10 cm layer in each monolith

- Sieved at 4 mm

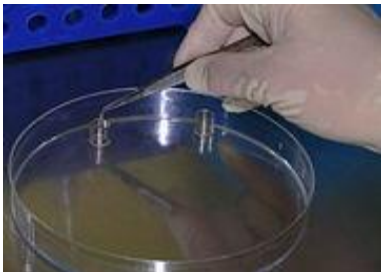
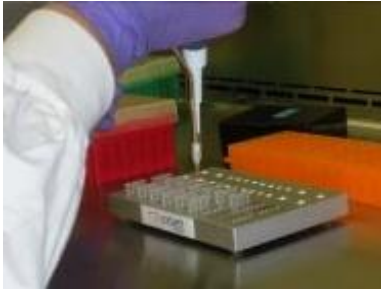
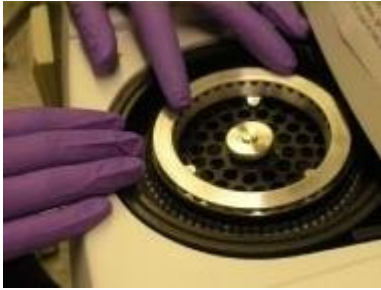




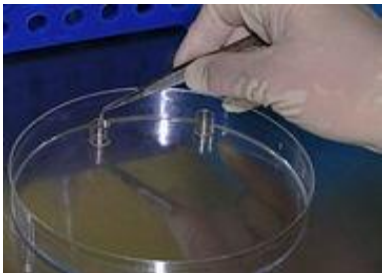
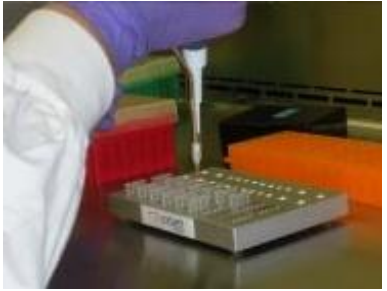
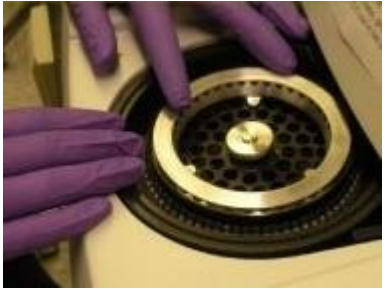
- Nitrifying and denitrifying activities
  - Potential nitrification measured in optimal conditions and analysed by ion chromatography
  
  - Potential N<sub>2</sub>O emissions by denitrification measured by gas chromatography



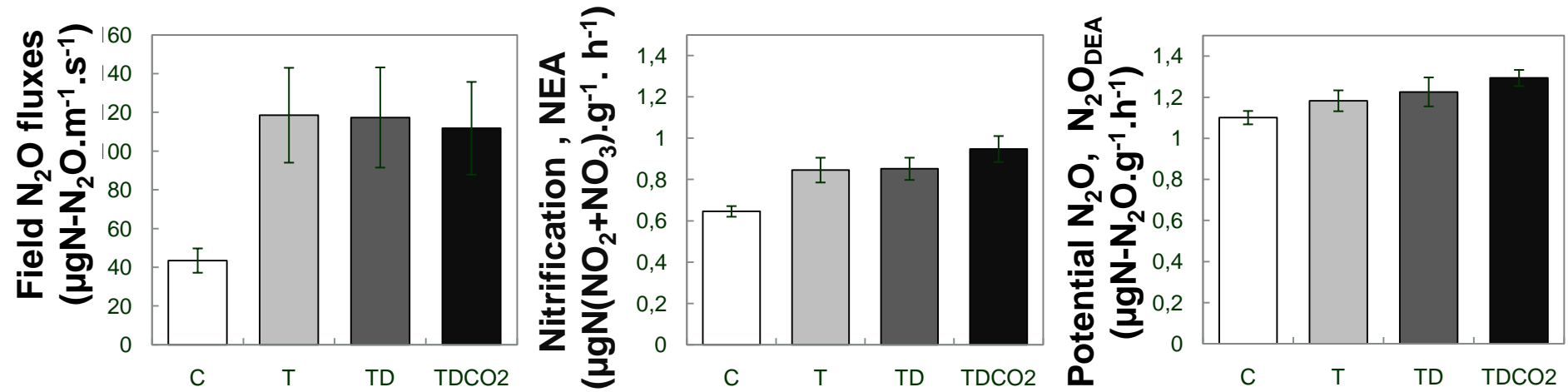


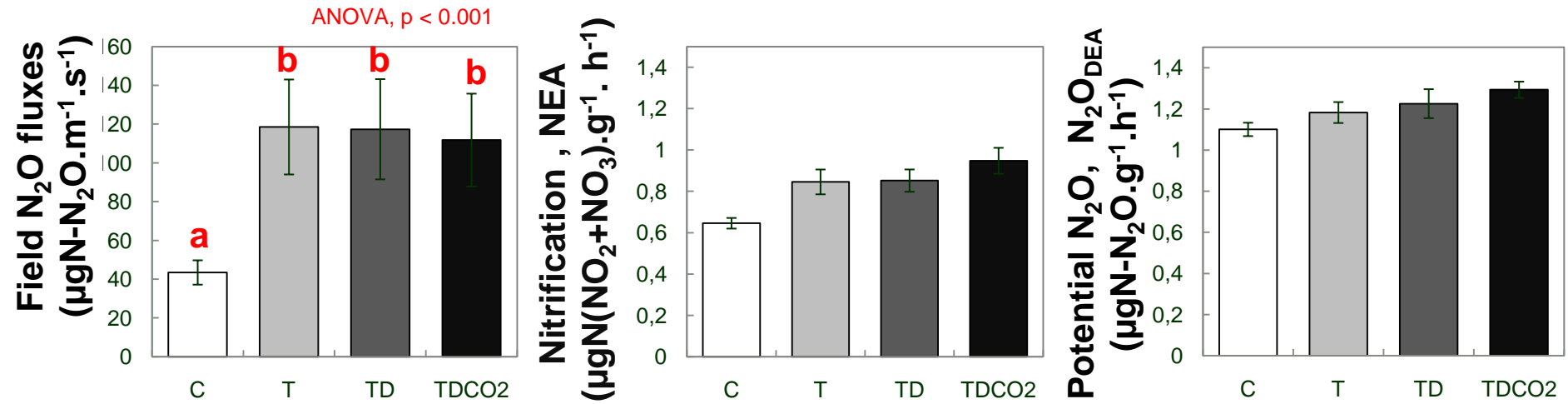


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- Quantification of genes abundances by qPCR
  - Nitrifying population : AOB
  - Denitrifying population : nirK

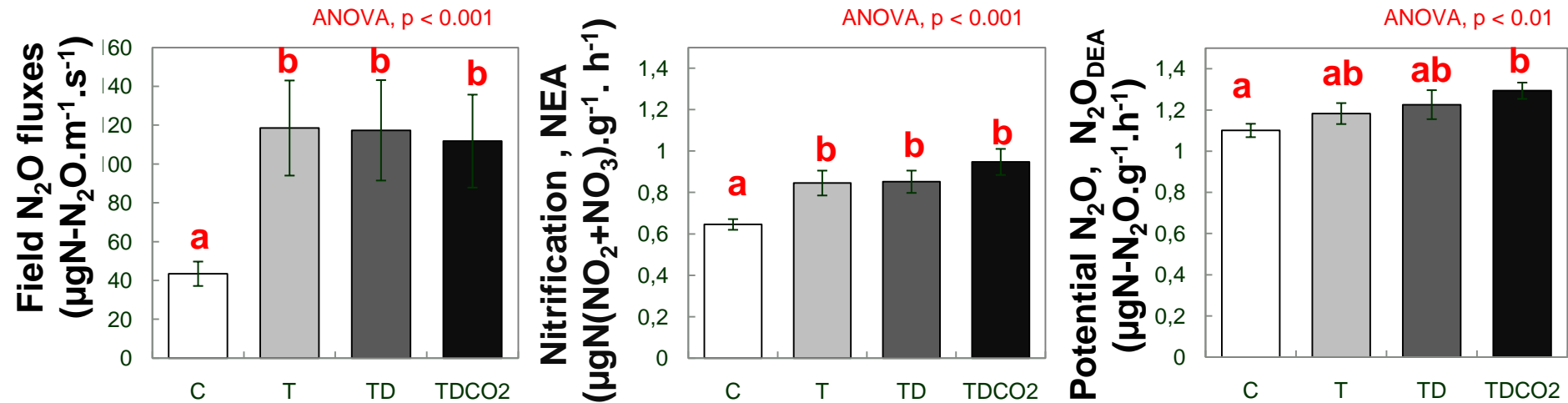


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- Quantification of genes abundances by qPCR
  - Nitrifying population : AOB
  - Denitrifying population : nirK
- Characterization of denitrifying communities (*nirK*) by cloning-sequencing





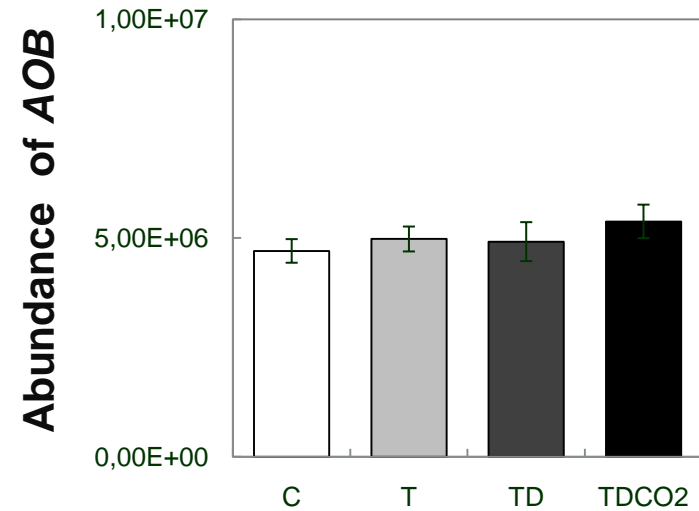
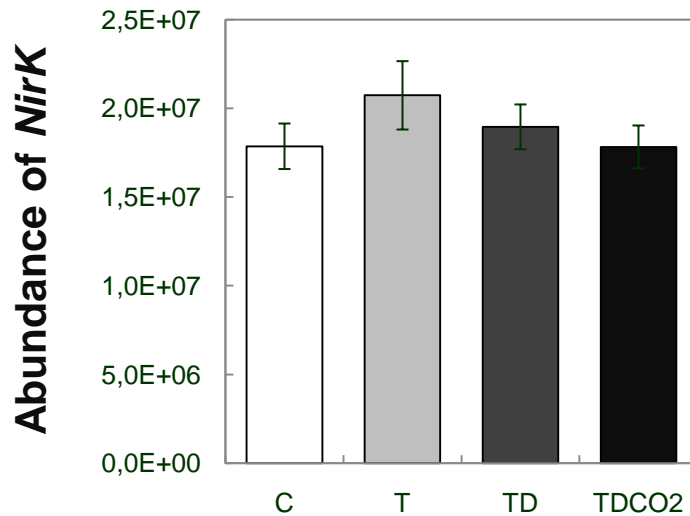
- Positive effect of air warming on N<sub>2</sub>O emissions (C vs T)



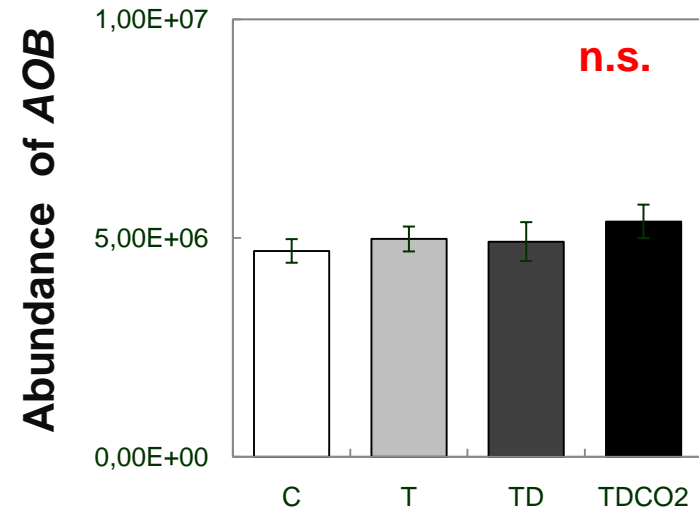
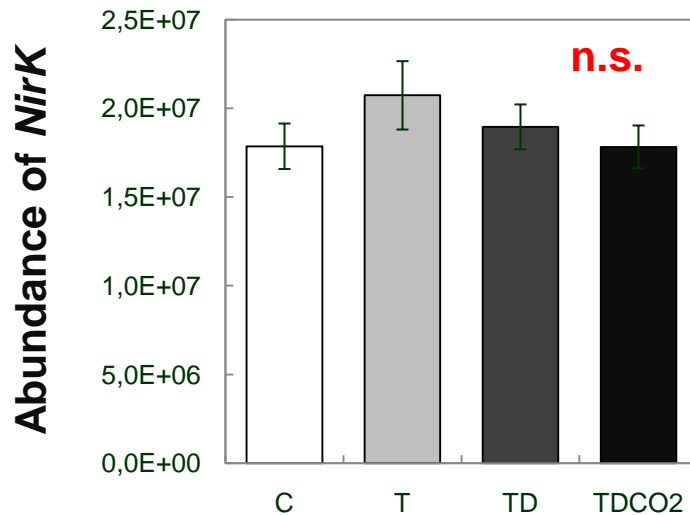
- Positive effect of air warming on N<sub>2</sub>O emissions (C vs T)
- Effects of climate change on nitrification and denitrification activities mirrored changes in N<sub>2</sub>O fluxes

- Warming effects on microbial activities may be related to changes in microbial population size or community structure

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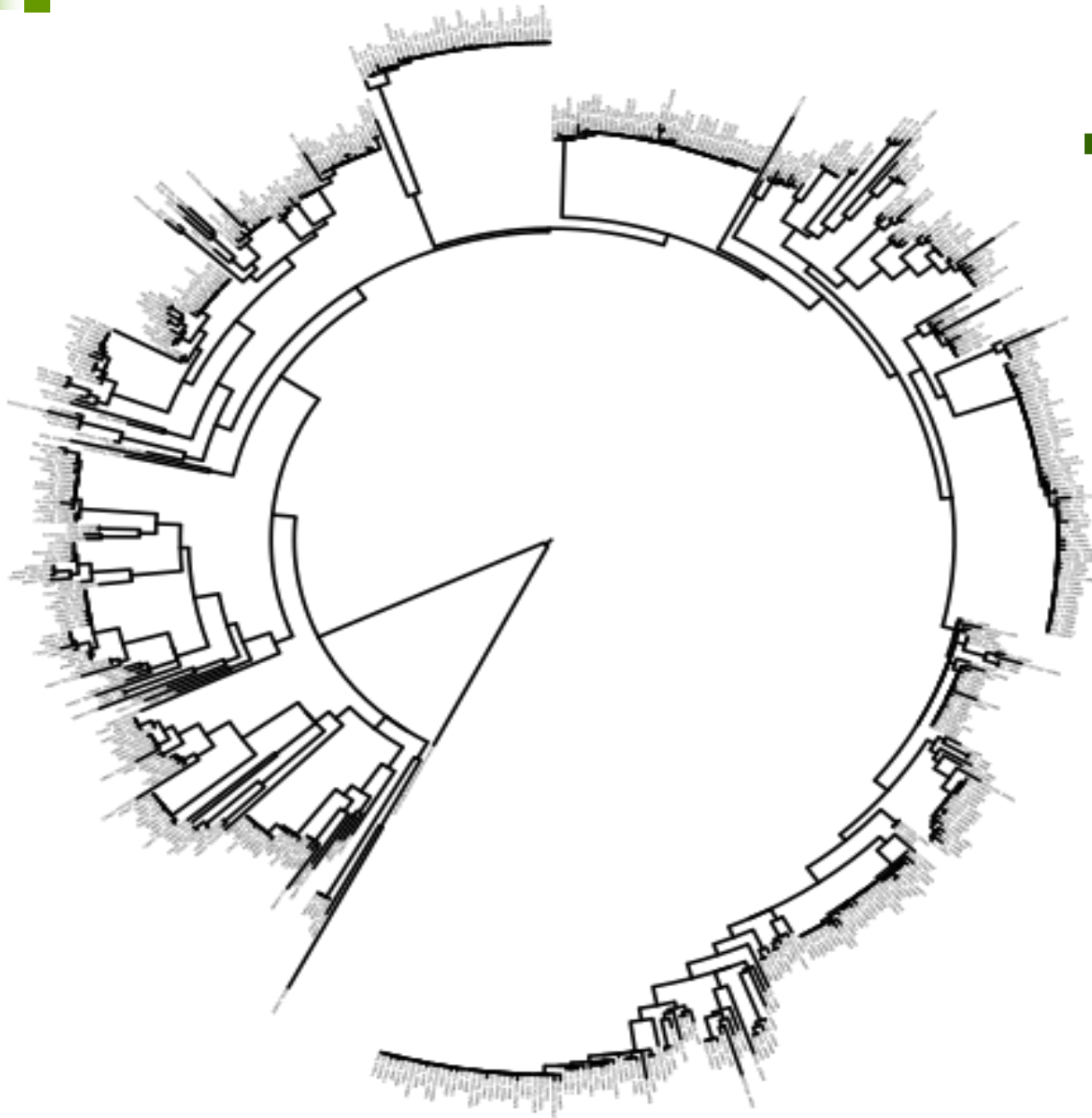


- Warming effects on microbial activities may be related to changes in microbial population size or community structure



- No significant climate effects on size of denitrifying bacterial populations (*NirK*) or on nitrifying bacterial populations (AOB) but tendency for increased *nirK* abundance in response to warming





0.1

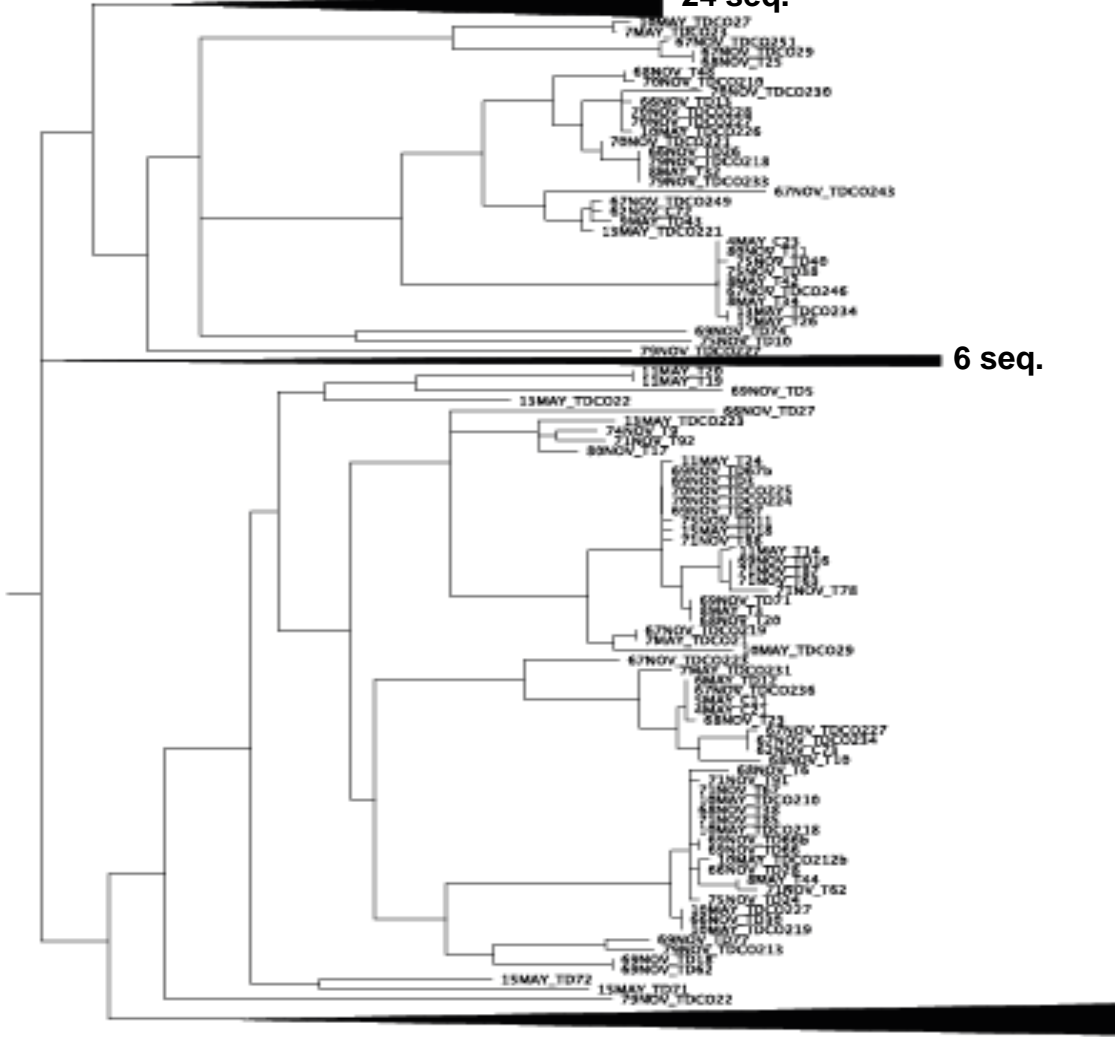
- Phylogenetic tree of *nirK* communities
  - on 600 sequences greater than 250 bp
  - Statistical analyses with Unifrac software

24 seq.

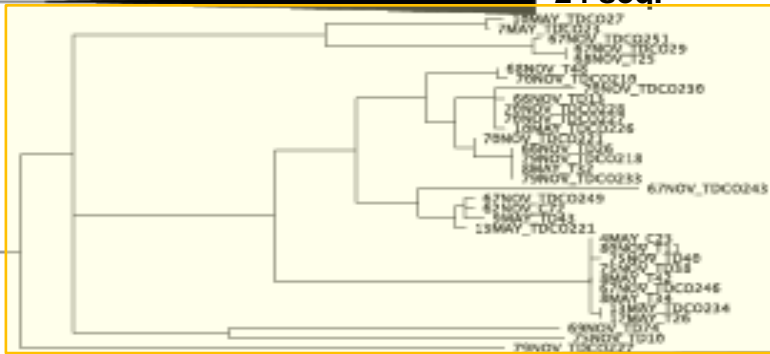
6 seq.

472 seq.

0.1



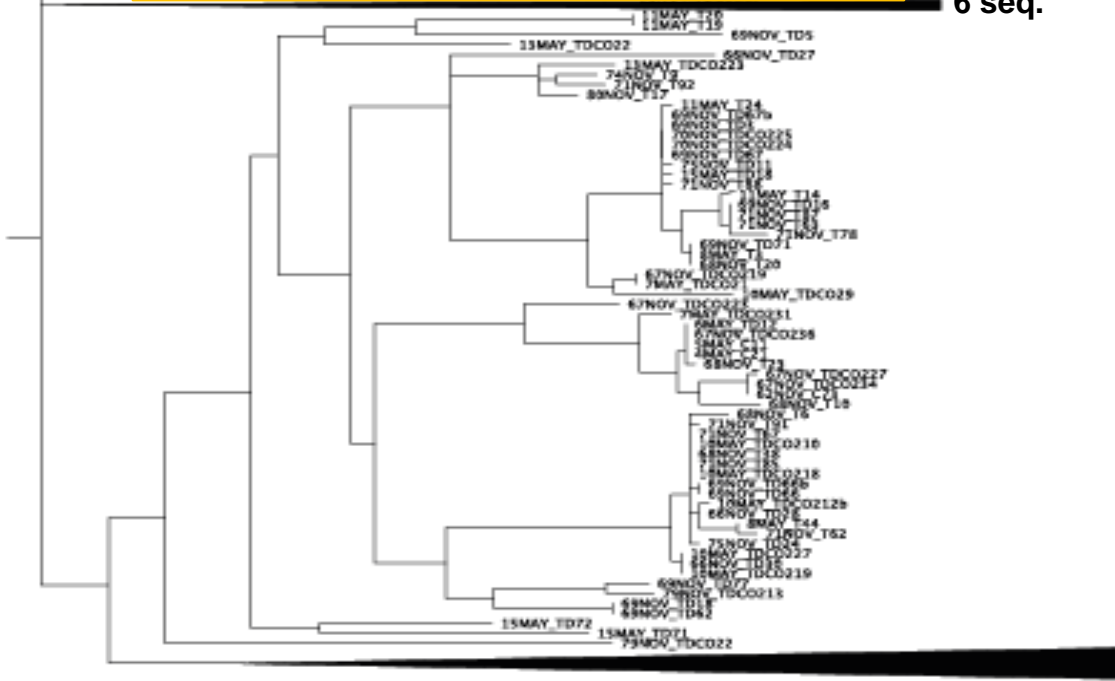
24 seq.



= 34 sequences  
6 seq.



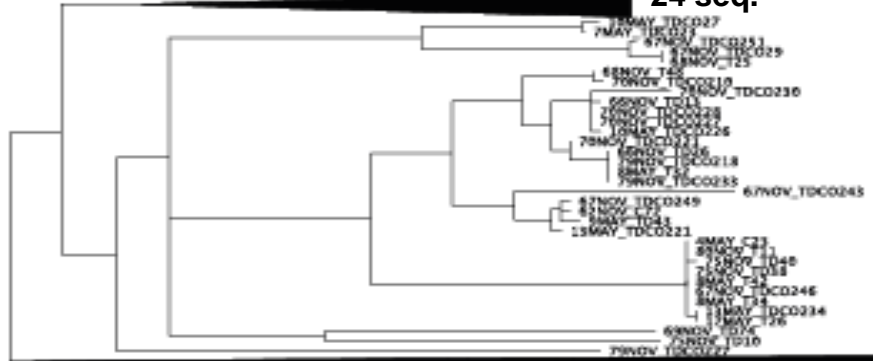
- C
- T
- TD
- TDCO<sub>2</sub>



0.1

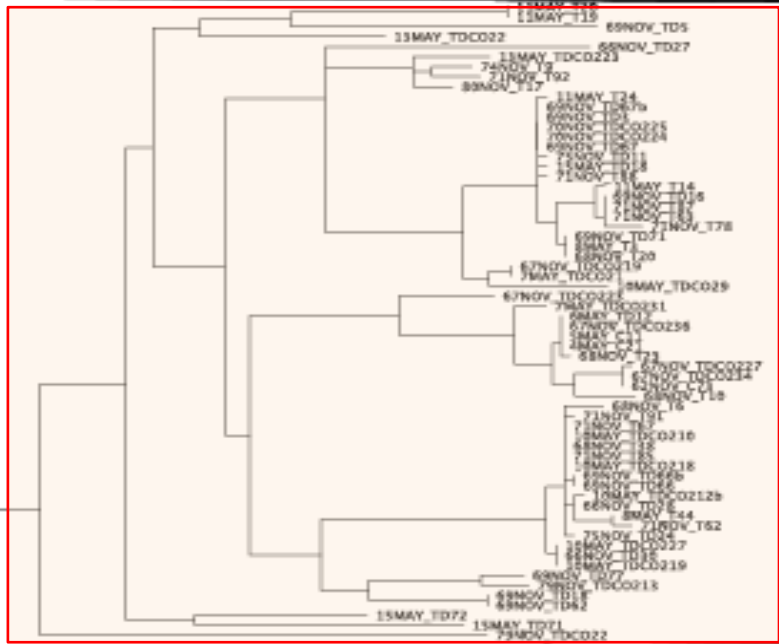
472 seq.

24 seq.



6 seq.

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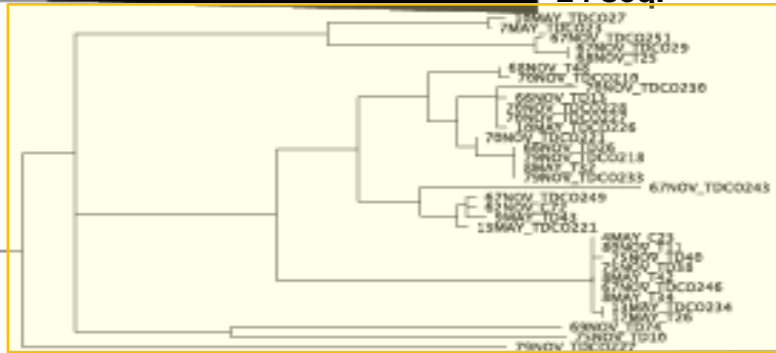
= 64 sequences



472 seq.

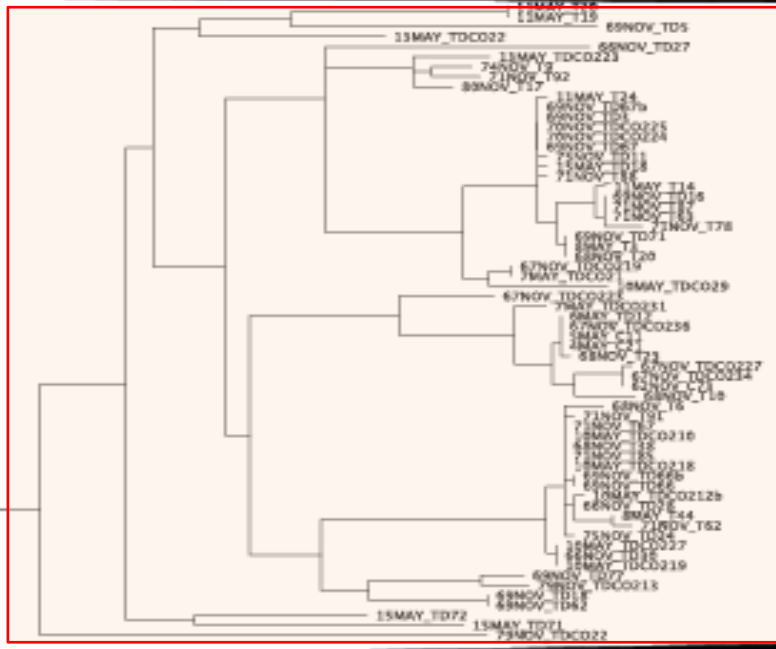
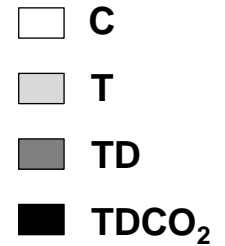
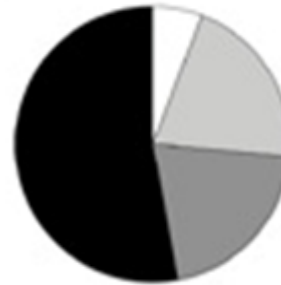
0.1

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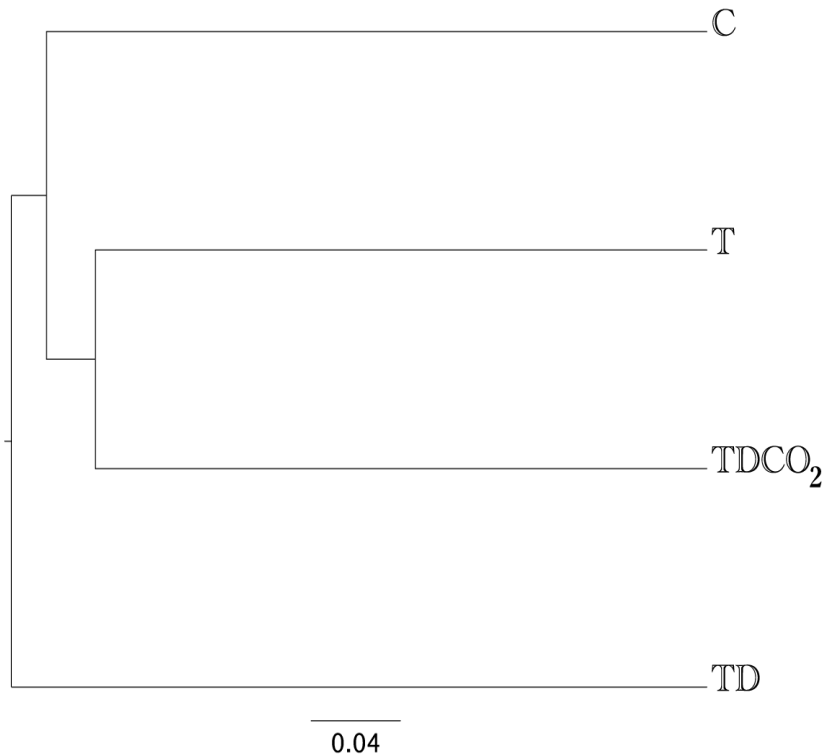
= 64 sequences



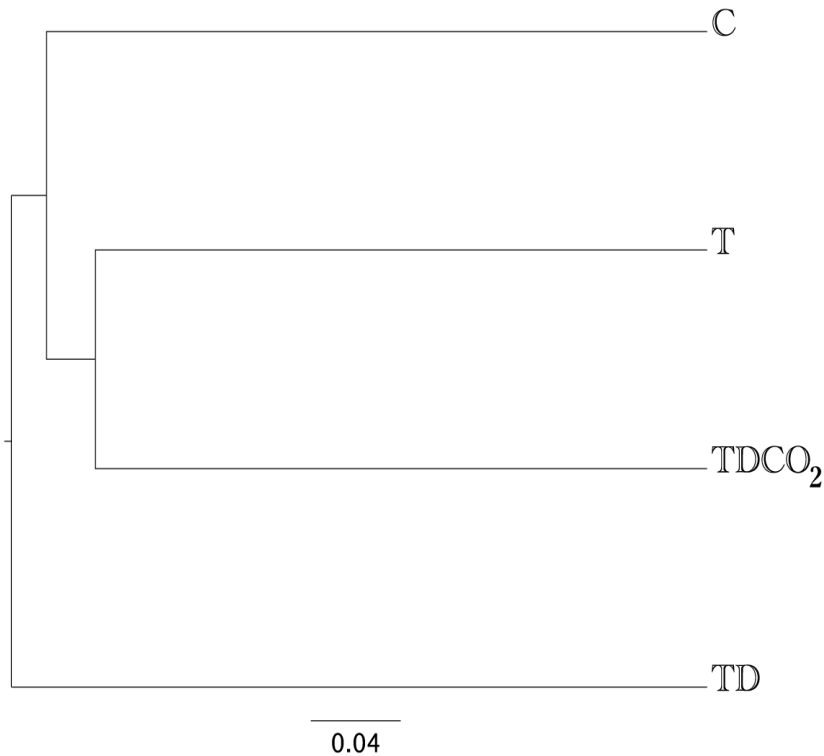
472 seq.

0.1

- Five years of climate change has selected specific lineages of *nirK* denitrifiers (two deeply branched lineages respond differently to warming and elevated CO<sub>2</sub>)



- Climate treatments show significantly different communities (Jackknife Environment Clusters,  $p < 0.001$ )



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- *NirK* community structure in warmed, dry conditions is an outgroup compared to the other climate treatments
  - Greater selective effect of drought on denitrifier community structure?

- Warming is a key driver of climate change for field nitrous oxide ( $\text{N}_2\text{O}$ ) fluxes and microbial processes in our study system



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- Warming effects on N<sub>2</sub>O fluxes are strongly linked to microbial activities
  - Microbial population sizes do not show strong climate treatment effects
  - *nirK* community structure show significant responses to climate treatment after five years
  - Further work is needed to test effects of climate on microbial enzyme upregulation

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  - *nirK* community structure show significant responses to climate treatment after five years
  - Further work is needed to test effects of climate on microbial enzyme upregulation
- We find evidence for specific *nirK* lineages in response to climate change



# Thank you for your attention !

Thanks to Deltroy Nicolas, Pichon Patrick, Gaumy Laurent, Tardif Antoine and Jouve Amandine