

## Oxygen supply and demand as controls of denitrification at the microscale in repacked soil

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## Understanding microscopic processes controlling microbial denitrification in terms of $\text{N}_2\text{O}$ and $\text{N}_2$ production in soil through a combination of soil incubation with microstructure analysis

Combining measurements of **nitrous oxide ( $\text{N}_2\text{O}$ ) and ( $\text{N}_2\text{O}+\text{N}_2$ ) fluxes** from biotic **denitrification in soil** with 3D soil structural properties measured by **X-ray computed tomography** (X-ray CT) to explore controlling factors of the complete denitrification process including  $\text{N}_2$  formation

## Denitrification in soil



- Proximal controlling factors:  $\text{O}_2$ , N and C  
affected by
- Distal controlling factors: Main **physical** controlling factors for microbial denitrification at the bulk scale are **water content** and **soil structure**
- Influence on microscale processes  
→ unaddressed with bulk measurements of soil respiration and soil diffusivity

**Which processes govern complete denitrification in soil?**

**How to substitute microscale information by bulk properties?**

# Experimental setup

## Pretreatment of the soil

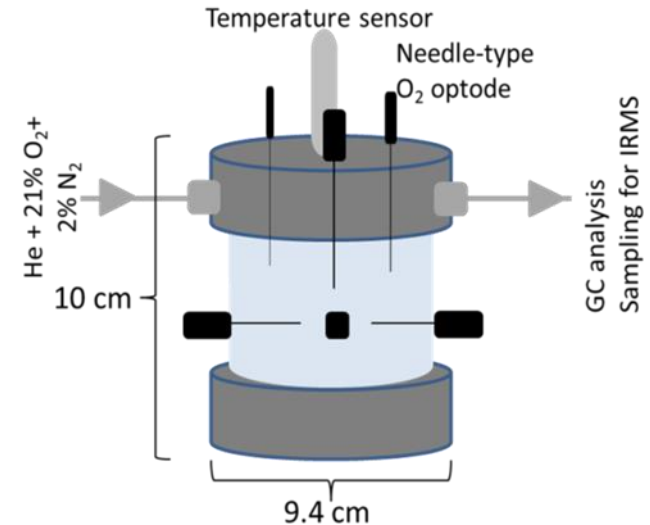
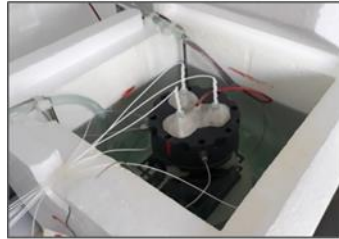
- 2 soils differing in organic carbon content: Rotthalmünster (RM): 2.0%  
Gießen (GI): 4.1%
- Air-dried and sieved for two aggregate sizes: 2-4mm & 4-8mm
- Preincubation at 50% WFPS (water filled pore space) for 2 weeks
- Electron acceptor:  $^{15}\text{N}$  labelled  $\text{NO}_3^-$  ( $50\text{mg kg}^{-1}$ ) was supplied during adjustment to ~65%WFPS
- Repacking:
  - Oxygen content controlled by 3 different saturation:  $\approx 65, 78, 85\%$  WFPS was adjusted during repacking the aggregates in 2cm intervals
  - target bulk density: RM  $1.3\text{g/cm}^3$   
GI  $1.0\text{g/cm}^3$



# Experimental setup

## Incubation experiment

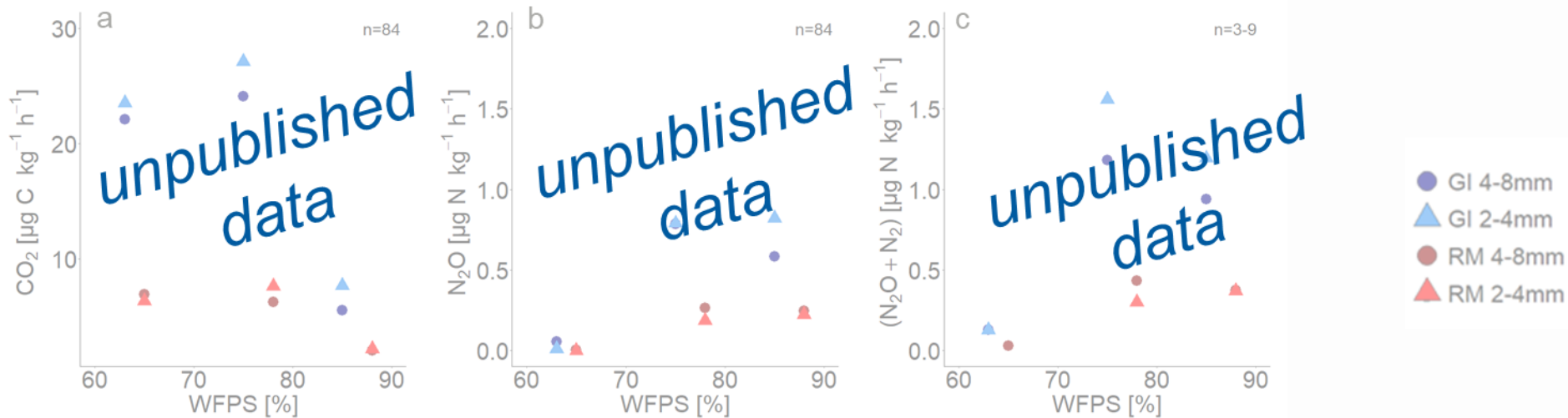
- Closed tightly and flushing with  $O_2$  in He ( $5\text{ mL min}^{-1}$ )
- Monitoring:
  - pressure and temperature
  - Gas production ( $CO_2$ ,  $N_2O$  and  $N_2$ ) by GC and IRMS
  - $O_2$  distribution with needle-type sensors
- Incubation time: 8 days



- X-ray tomography and image analysis after incubation
- Simulation of diffusivity (DiffuDict module in the GeoDict 2019 Software (Math2Market GmbH, Kaiserslautern, Germany))
- Calculation of product ratio ( $N_2O/(N_2O+N_2)$ ) as a measure of denitrification completeness

# Fluxes from denitrification

- CO<sub>2</sub> fluxes higher with GI soil than with RM soil; lowest values with highest water saturation (a)
- Substantial N<sub>2</sub>O (b) and (N<sub>2</sub>O+N<sub>2</sub>) (c) emissions for saturations ≥75% WFPS (again approx. 3 times higher in GI soil than in RM soil)



Average values as a function of water saturation for RM and GI soil and two aggregate sizes (2-4 and 4-8 mm) of 3 replicates with n=84 (a and b) or n=3-9 (c) values of each treatment.

# Anaerobic soil volume fraction

- Image analysis enables to differentiate between air volume and solid fraction (a)
- Air connected with the headspace can be detected (b)
- volume fraction of air distance larger than a certain threshold is regarded as the **anaerobic soil volume fraction** of the soil core (c)



Are you interested in the results on  
anaerobic soil volume fraction derived  
from image analysis?

*Please contact us!*

# Controlling factors of $\text{N}_2\text{O}$ or $\text{N}_2\text{O}+\text{N}_2$ fluxes

- **$\text{CO}_2$  production:** 3 times higher with GI soil than with RM soil and it was lowest with highest water saturation for both soils
- **$\text{O}_2$  saturation:** lowest with highest water saturation and roughly the same for saturations  $<80\%$ WFPS
- **Product ratio:** similar course as a function of water saturation like  $\text{N}_2\text{O}$  release with a plateau for saturations  $\geq 75\%$  WFPS at 0.6 and a lower, but somewhat more erratic product ratio for the lowest saturation due to a generally low  $^{15}\text{N}$  gas release
- **Anaerobic soil volume fraction:** escalates in the wet range and amounts to 50-90% of the sample volume
- **Connected air content:** decreasing with water saturation; substantial amount of air is trapped with higher water saturation
- **Diffusivity:** reduction by five orders of magnitude with increasing water saturation. At high saturations it fell below the oxygen diffusion coefficient in pure water due to the tortuosity of the pore system
- aggregate size did not affect  $\text{CO}_2$  production,  $\text{O}_2$  saturation, product ratio, anaerobic soil volume fraction, connected air content or diffusivity



# What controls microbial denitrification?

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Main controlling factors for the interplay of oxygen supply and demand:

- formation of **anaerobic soil volume fraction** as an imprint of the spatial distribution of connected air or diffusivity to estimate oxygen supply
- **CO<sub>2</sub> production (respiration)** to estimate oxygen demand
- O<sub>2</sub> concentration measured by microsensors was a poor predictor -> variability in O<sub>2</sub> at short scales combined with the small measurement volume of the microsensors.
- Substitution of predictors by independent, readily available proxies for O<sub>2</sub> supply (diffusivity) and O<sub>2</sub> demand (SOM) reduced the predictive power

**Many thanks**  
**to your interest in our experimental results!**

**If you would like to get more information or if you  
have any comments, please contact us:**

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