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Introduction

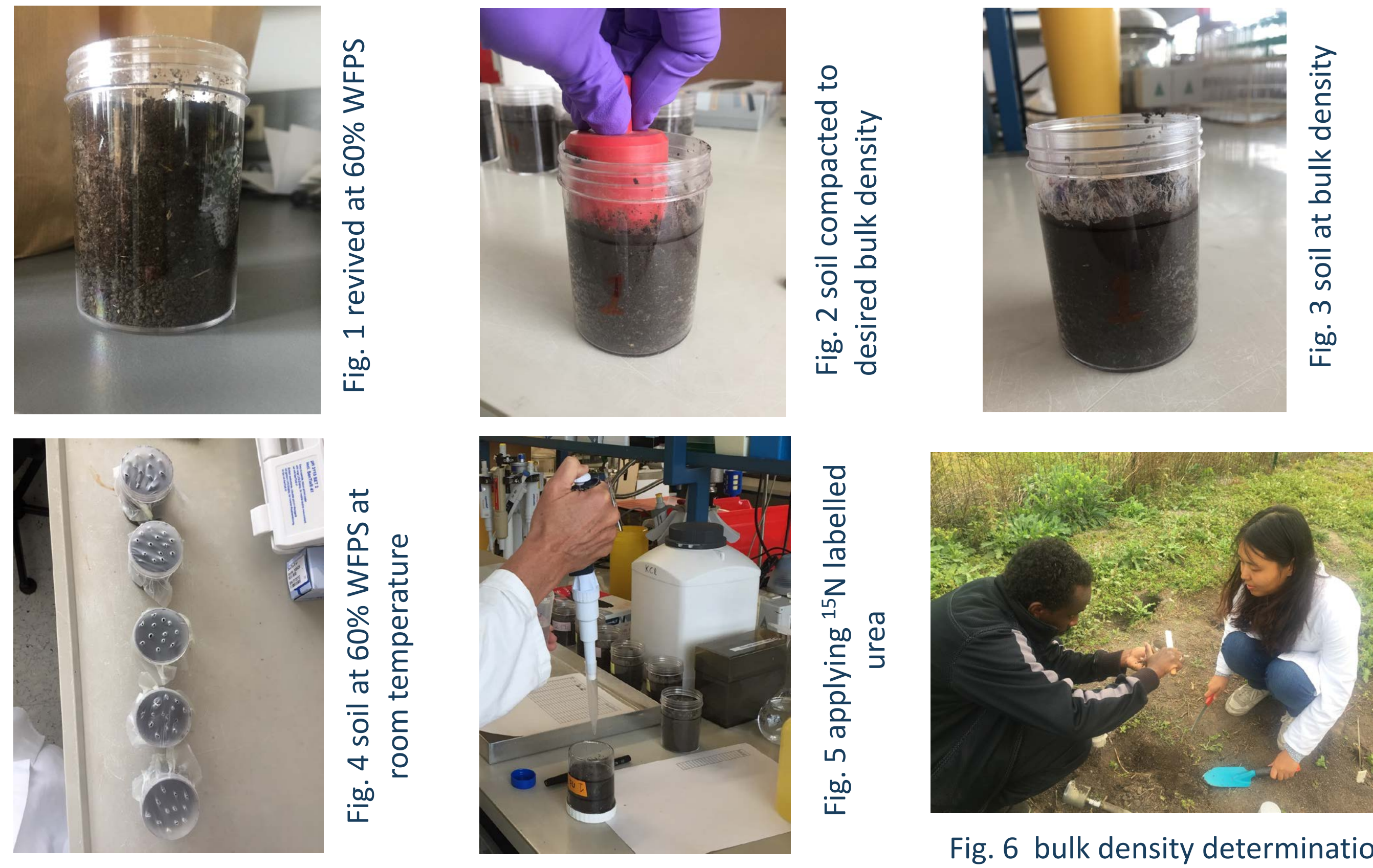
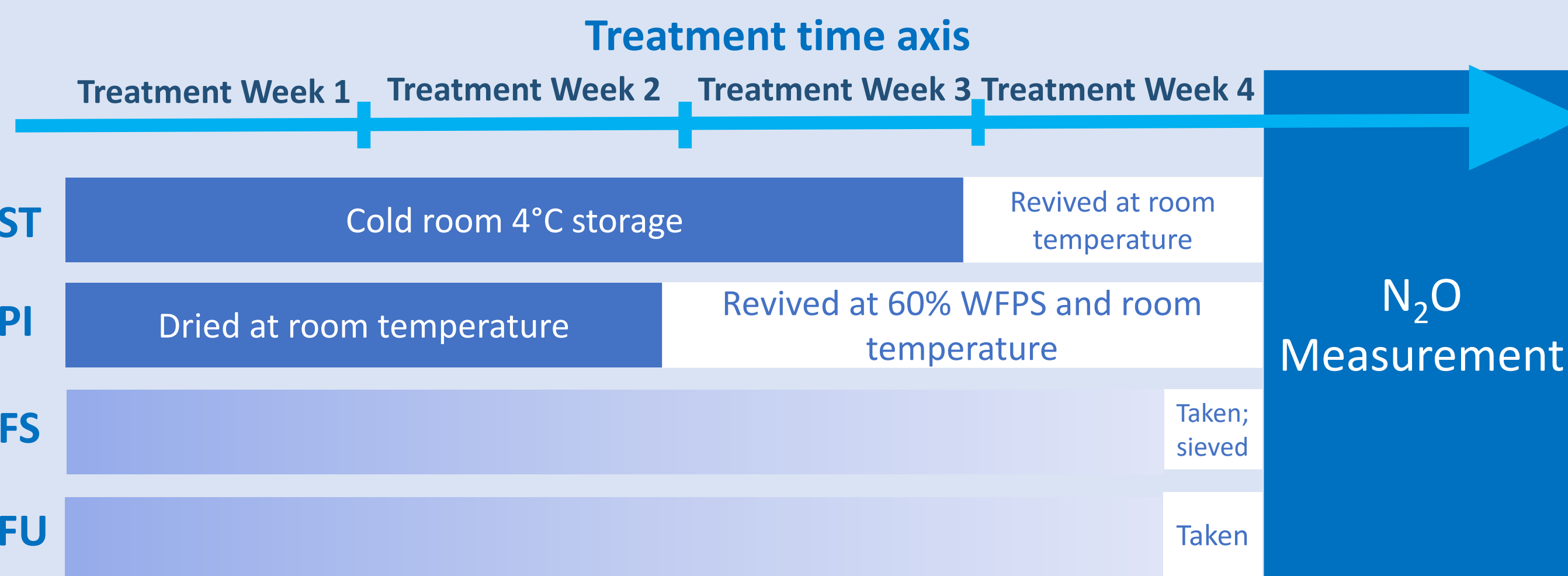
The rapidly changing global climate is due to increased emission of anthropogenic greenhouse gases (GHGs). Among the key GHGs, nitrous oxide (N₂O) is 298 times more powerful than carbon dioxide (CO₂) in its global warming potential. N₂O emissions occur mostly through agricultural and other land-use activities and are associated with the intensification of agricultural and poor agricultural practices. The atmospheric concentration of N₂O has increased by more than 20% from ~271 ppb to 331 ppb since the industrial era (ca. 1750) to 2018 (WMO 2019). To develop climate smart agricultural practices for mitigation of N₂O, isotopic technique of ¹⁵N plays a key role in precise measurement as well as identifying its microbial processes that produce N₂O in soil. Incubation experiments using sieved soils under controlled environment are generally conducted to study microbial processes associated with N₂O emission. In some incubation studies, researchers have used stored soils which are likely to influences the N₂O production due to rewetting of soils. The objective of our study was to investigate the influence of soil storage on N₂O emission and N mineralization rates using ¹⁵N technique.

Soil samples preparation

- Seibersdorf Soil
- Soil was collected from the International Atomic Energy Agency Laboratories experimental fields in Seibersdorf, Austria (47°58' N, 16°30' E).
 - Haplic Chernozem (IUSS-WRB, 2014), pH = 8.2, total N = 2.27 g kg⁻¹ soil.
 - Top soils from 0 to 10 cm were collected.
 - Bulk density = 1.2 g cm⁻³, soil porosity = 0.55%
- Isotope
- ¹⁵N labelled urea (1 atom%) at the rate of 50 mg N kg⁻¹ soil, at room temperature (23°C).
 - Urea was applied and the N₂O emission was measured on day 0, 1, 2, 3, 4, 7, 8, 9, 11, 14, 17, 21, 23, 25, 28, 30, 32, 35, 37, 39, 42, 44, 46, 49.

- Soil storage/treatment (5 replicates of each treatment)
- **Soil stored at 4°C (ST):** Sieved to 2 mm; stored at 4°C at field moisture (0.123 g g⁻¹ soil) in sealed plastic bags for 3 weeks.
 - **Room temperature dried (PI):** Sieved to 2 mm; stored for 2 weeks dried at room temperature; revived for 2 weeks at 60% WFPS and room temperature.
 - **Fresh sieved (FS):** Taken in the fourth week; sieved to 2 mm.
 - **Fresh undisturbed (FU):** Taken in the fourth week; no treatment; N₂O measured at the same time as the other treatments.

The four treatments were all compacted to the same bulk density as FU and brought to a volumetric water content of 0.330 g cm⁻³ (60% WFPS) during the 49 days.



N₂O measurement

- ¹⁵N amended soil samples, comprised of different treatments, were incubated for 49 days.
 - N₂O measurements were carried out for 49 days using a laser based N₂O analyser (off-axis integrated cavity output spectroscopy (OA-ICOS), Los Gatos Research, California, USA).
 - N₂O fluxes were measured in closed loop mode for 12 min per sample, 3 min cleaning.
 - Cumulative N₂O fluxes are expressed as the integrated area under the curves of the daily fluxes, using the trapezoidal rule.
 - Keeling plot intercepts were calculated to identify the N₂O source
- $$\delta^{15}N_s = \frac{\sum_{i=1}^n \left(\left(\frac{1}{N_i} - \left(\frac{1}{N} \right)_{av} \right) (\delta^{15}N_i - \delta^{15}N_{av}) \right)}{\sum_{i=1}^n \left(\frac{1}{N_i} - \left(\frac{1}{N} \right)_{av} \right)^2}$$

$\delta^{15}N_s$ = the intercept of the Keeling plot, the isotopic mixture of the sources;
 N_i = measured N₂O ppm at time point i;
 $(1/N)_{av}$ = the average value of 1/N₂O ppm over the measurement time;
 $\delta^{15}N_i$ = the isotopic signature at time i;
 $\delta^{15}N_{av}$ = the sources average isotopic signature.



Fig. 7 sample incubated in a gas chamber

- %Ndff and %Ndfs were calculated
- $$\%Ndff = \frac{atom\%excessN_2O}{atom\%excessUrea} \times 100$$
$$\%Ndfs = \left(1 - \frac{atom\%excessN_2O}{atom\%excessUrea} \right) \times 100$$

%Ndff = the percentage nitrogen derived from fertilizer; %Ndfs = the percentage nitrogen derived from soil; atom%excess N₂O = the N atom percent excess of the measured N₂O fluxes; atom%excess Urea = the N atom percent excess of urea (fertilizer).

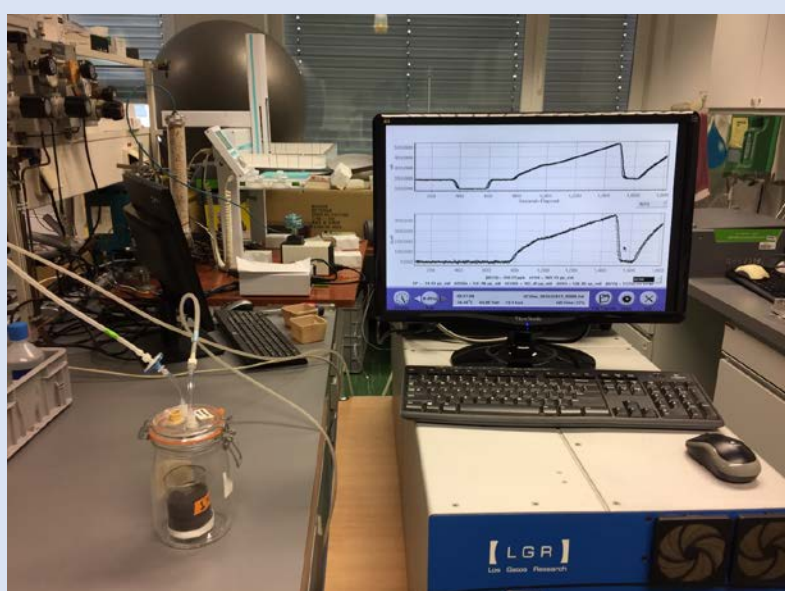


Fig. 8 laser analyser (LGR)



Fig. 9 soil samples, covered with parafilm to keep moisture constant

Results

- **Soil storage had a significant effect on N₂O emission.** Over the 7-week period, ST produced the highest cumulative N₂O emissions (2.70 μg N g⁻¹ soil) and the largest amount of N derived from fertiliser (Ndff) (1.4 μg N g⁻¹ soil).
- FU produced the lowest cumulative N₂O emissions (1.0 μg N g⁻¹ soil) but the largest amount of N derived from soil (Ndfs) (0.6 μg N g⁻¹ soil).
- The daily N₂O fluxes of FS and FU declined rapidly after day 8, PI and ST exhibited several peaks during the measurement.

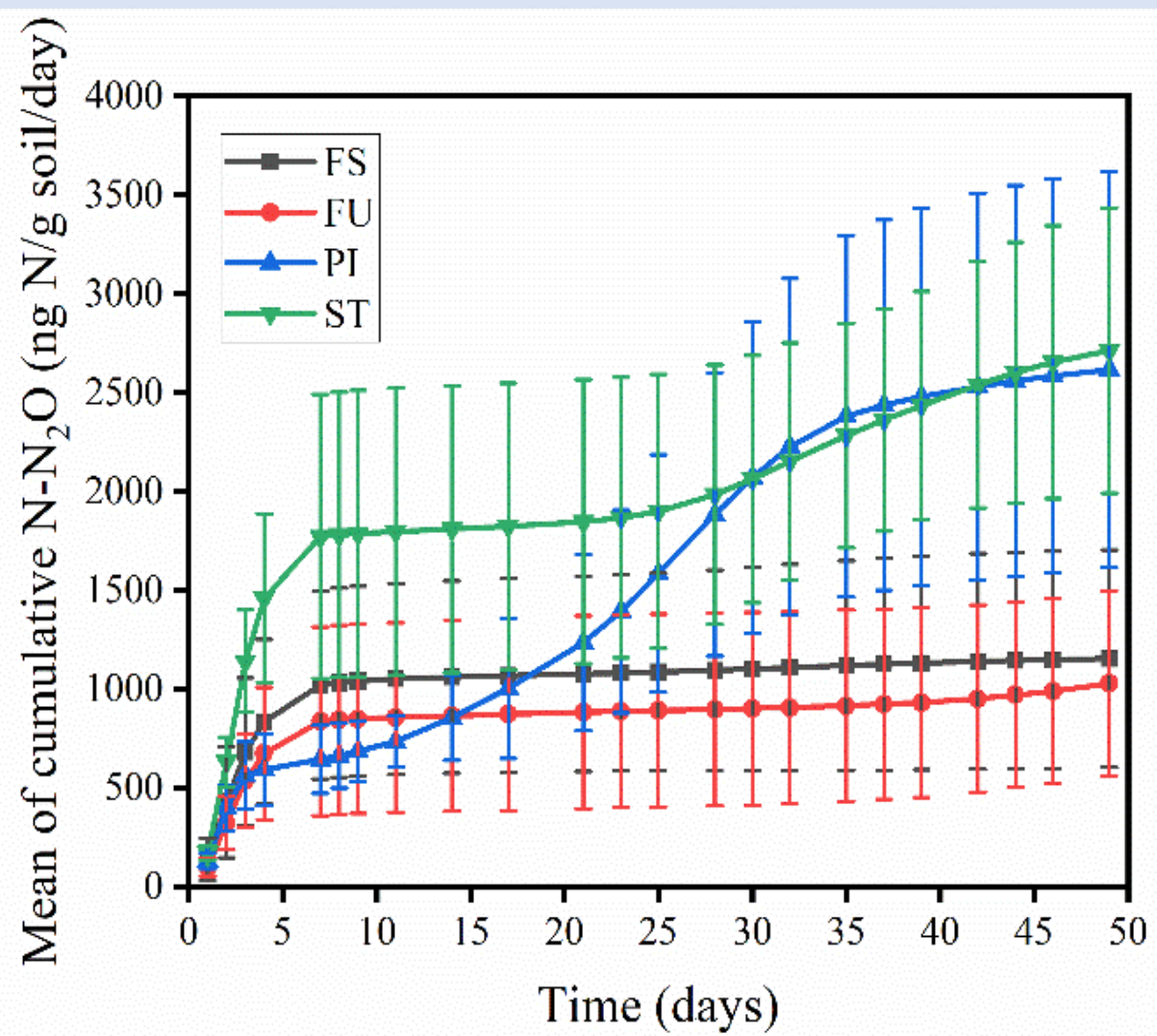


Fig. 10 effect of soil storage on N₂O emission

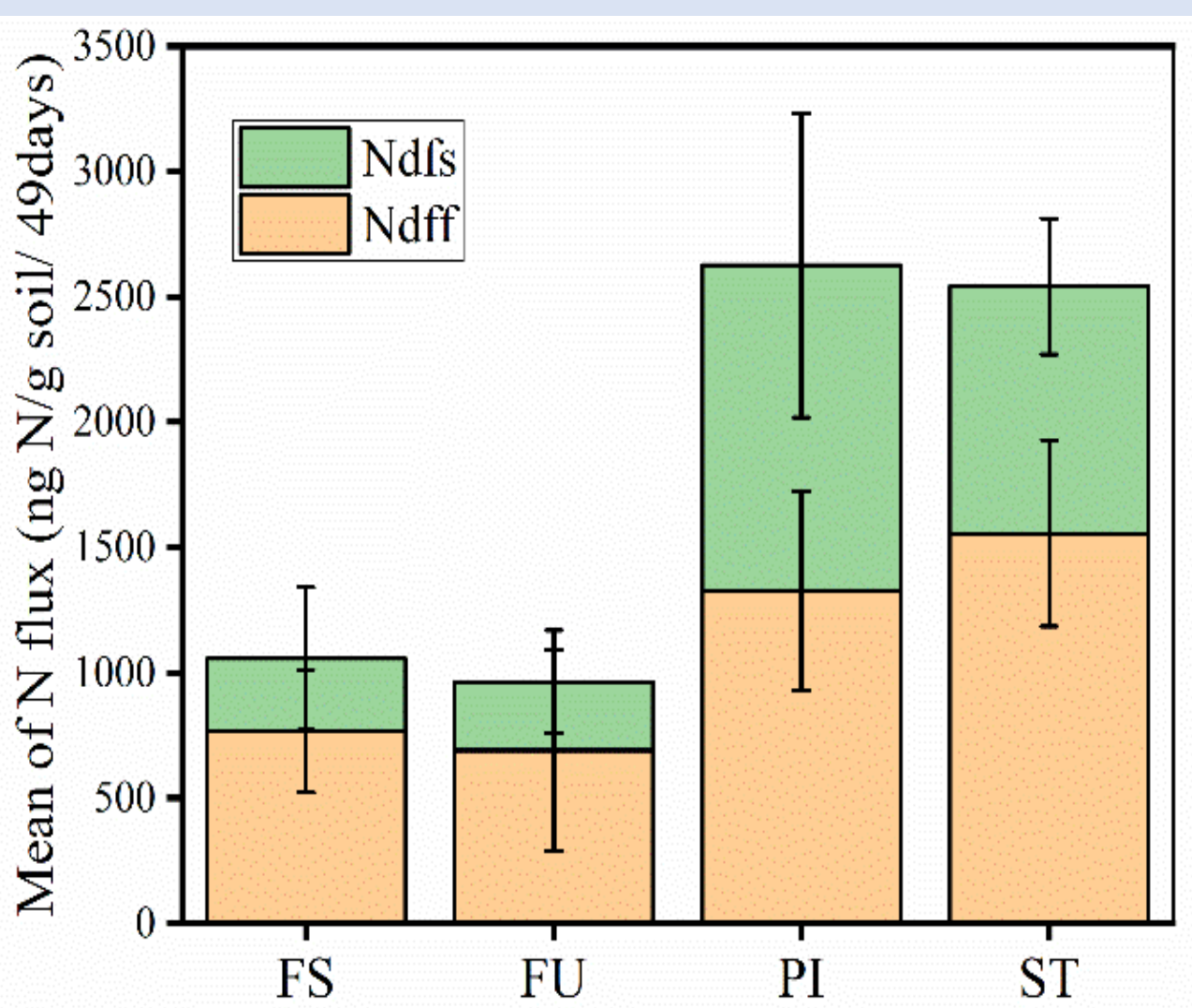


Fig. 11 N sources of N₂O emission for different soil storage

Fresh sieved (FS), Fresh undisturbed (FU), Room temperature dried (PI), and Soil stored at 4°C (ST)

Conclusions

These results indicate that soil storage affects microbial processes and therefore N₂O emissions. Our results suggest using fresh soil to avoid storage effects. If this is not possible, the effect of soil storage should be considered before the experiment.