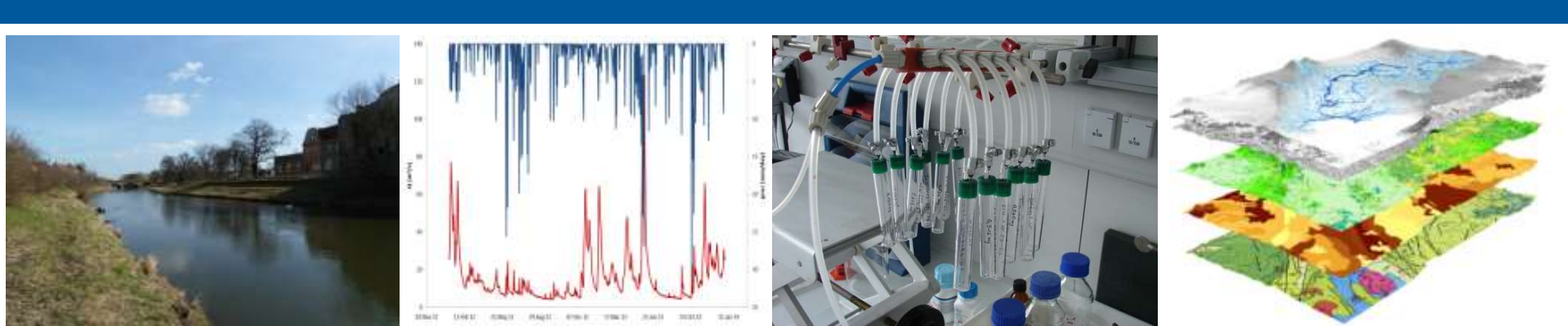


Denitrifier Method

Analysis of nitrate stable
isotope signatures



TU Wien
10th August 2016

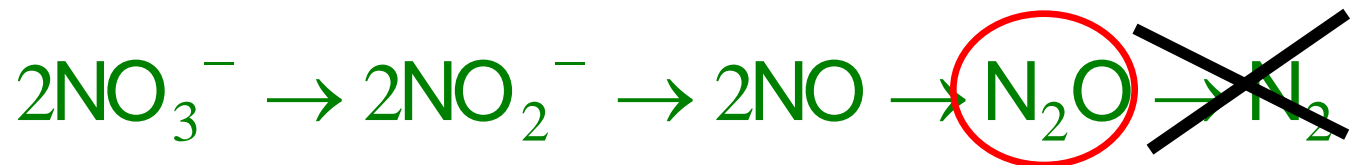
Christin Müller, Martina Neuber, Kay Knöller

Isotope composition of dissolved nitrate

Denitrifier method

basic principle :

- defined bacterial reduction of dissolved nitrate to gaseous N_2O in headspace vials
- Bacteria: denitrifiers lacking an active N_2O reductase



- measurement of mass ratios 45/44 and 46/44 of N_2O for calculation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of precursor nitrate

Isotope composition of dissolved nitrate

Denitrifier method

Sample preparation (I)

Preparation of bacterial working cultures

- bacterial strain: *Pseudomonas chlororaphis* (ATCC #13985)
- culture medium: tryptic soy broth amended with KNO_3 , NH_4Cl , KH_2PO_4
- incubation time: 7-16 days room temperature (regular shaking)
- test smell and appearance
- after incubation 3 fold concentration of the culture by centrifugation
- venting with nitrogen gas stream for 2 h



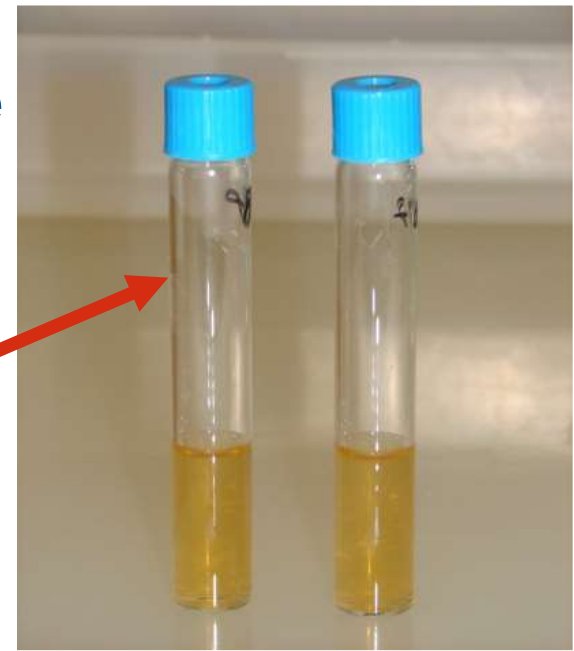
Isotope composition of dissolved nitrate

Denitrifier method

Sample preparation (II)

Incubation of samples

- 2 ml of concentrated culture in each sample vial tightly closed with septum
- 15 min He flushing of each sample vial
- injection of 2ml sample solution
 - necessary amount of nitrate 2-5 μ g (=1 - 2.5 mg/l)
 - higher original concentration: dilution of sample
 - lower original concentration: nitrate enrichment by freeze drying
- incubation for 2-3 days at room temp.
- end of incubation: injection of 0.5ml 10N NaOH (kill bacteria) and 15 min ultrasonic bath



Isotope composition of dissolved nitrate

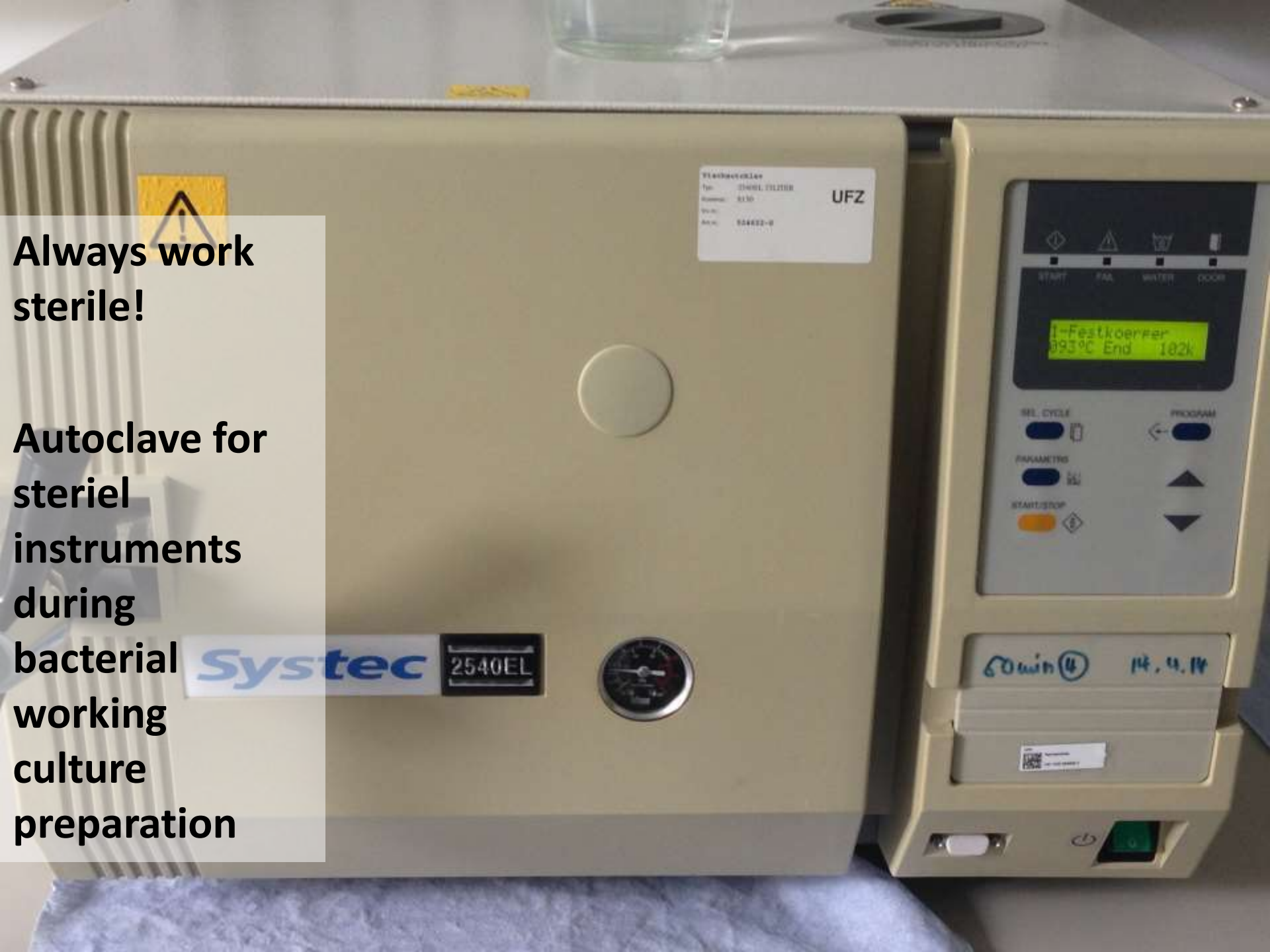
Denitrifier method

measurement procedure:

- Load mode: sample headspace N_2O is carried by He-flow (50 ml/min) to cold trap
- Inject mode: N_2O is flushed out of the cold trap to the mass spec (conflow-IRMS) by He-flow (5 ml/min), simultaneous back flush of sample capillary
- Calibration and normalization to the AIR and VSMOW scale by reference materials (RM) that are treated as samples (USGS 34, USGS 35, IAEA-NO3)
- analytical precision: $\delta^{15}\text{N}$: $\pm 0.2 \text{ ‰}$ $\delta^{18}\text{O}$: $\pm 0.5 \text{ ‰}$
- sample throughput: ca. 150 samples per week

**Always work
sterile!**

**Autoclave for
steriel
instruments
during
bacterial
working
culture
preparation**



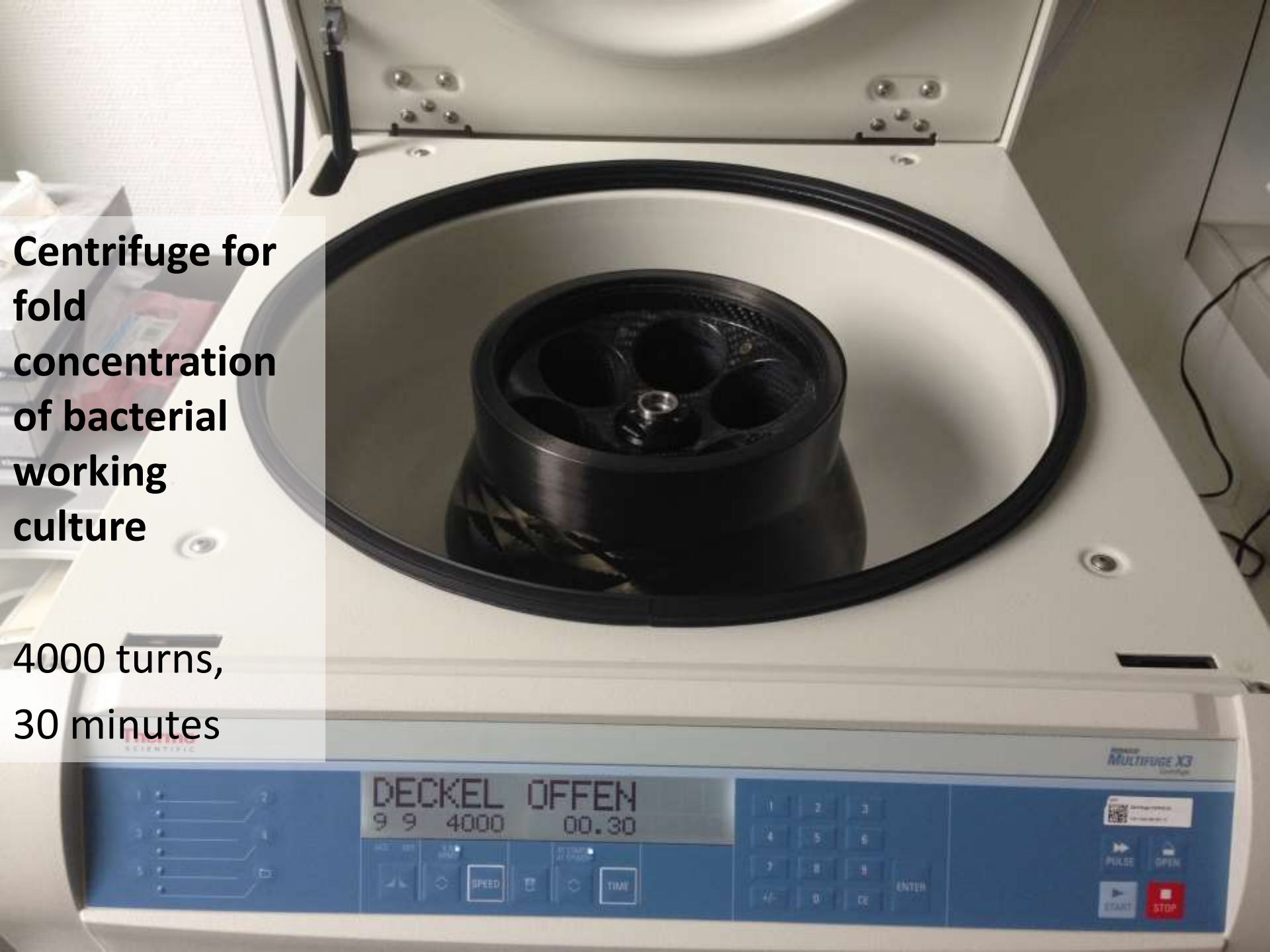
**Culture
medium with
bacterial
strain**

during
incubation
time



**Centrifuge for
fold
concentration
of bacterial
working
culture**

**4000 turns,
30 minutes**



**Venting with
nitrogen gas
stream for
two hours**

cleaning
process

