The UMB Nitrogen Group (UMBNG) at the Norwegian University of Life Sciences

Website: http://www.umb.no/nitrogengroup/

Group leader team: Lars Bakken, Peter Dörsch, Åsa Frostegård

Contact persons: Åsa Frostegård (E-mail: asa.frostegard@umb.no), Linda Bergaust (E-mail: linda.bergaust@umb.no)

Microbial N-transformations play key roles in ecosystems, and the overarching aim for UMBNG is to improve our understanding of how these transformations are controlled and regulated at scales ranging from the gene through the cell (phenotype) to the ecosystem.

Our strength lies in the combination of detailed phenotypic studies and molecular techniques. The semi-automatic incubation system developed by us (1) allows high-resolution real-time monitoring of gas kinetics (O_2, CO_2, N_2O, N_2, NO, CH_4) in the headspace of pure cultures, extracted microbial communities, soil slurries, or intact soils. By combining this with quantification of denitrification gene transcripts, we determine the denitrification regulatory phenotypes (DRP) of intact soil communities, extracted soil bacteria or bacterial pure cultures in response to environmental controllers (pH; O_2 and NOx levels). Furthermore, a cloning-sequencing study of soil-derived transcripts has been done and a larger 454 pyrosequencing analysis is underway.

Main areas of interest and application of techniques

1. Regulatory biology of denitrification, systems biology

- ✓ Characterization of selected denitrifiers; comparative studies of Denitrification Regulatory Phenotypes (DRP)
- ✓ Effects of environmental controllers such as oxygen levels, pH and concentrations, of nitrate/nitrite on N_2O and NO emission (studies in field, microcosms, extracted cells, pure cultures) (2, 3)
- ✓ The rich data sets obtained are suitable for developing mathematical models to predict phenotypes of bacterial populations/or communities in response to environmental factors

Techniques currently used: Gas measurements (kinetics), DNA/RNA extraction (i.e. from soil), PCR-DGGE, Real-time PCR, 454 sequencing of cDNA derived from soil (external resource)

Techniques to be implemented/on the wish list: Protein quantisation by Western blot and Taqman protein assays, discrimination and sorting of active/inactive cells by flow cytometry

Additional technique: Phospholipid fatty acid analysis (PLFA) for the characterization of soil microbial communities (4)

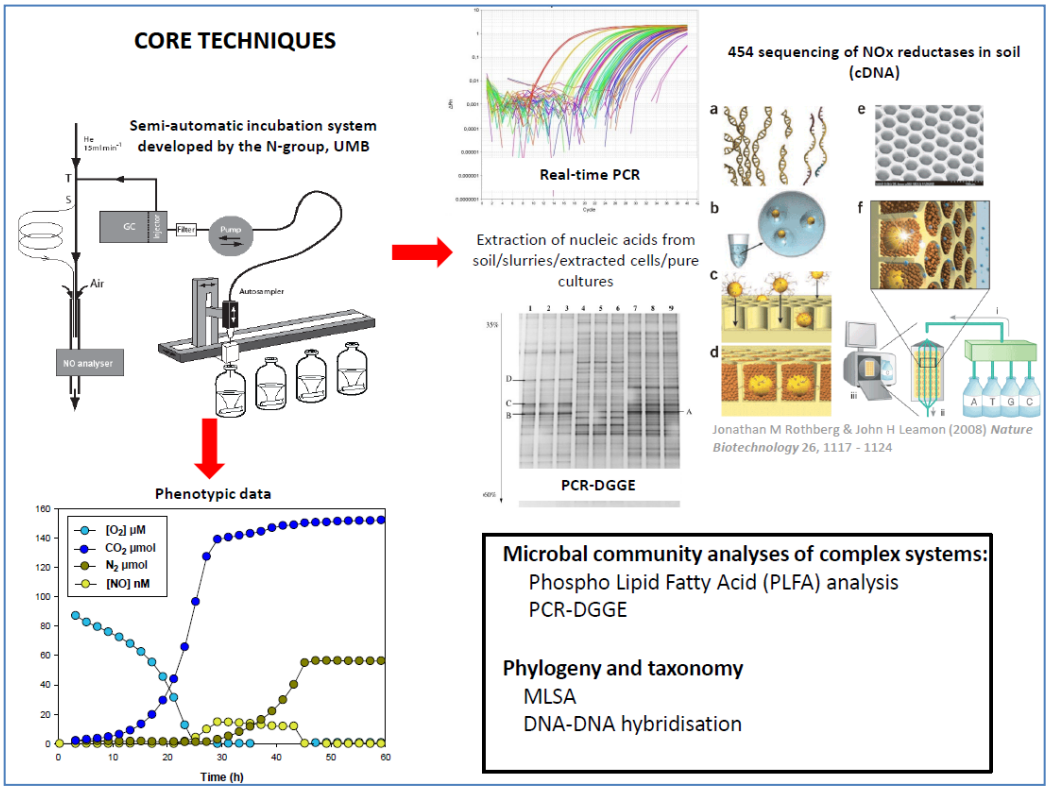

Figure 1: Overview of the techniques most frequently used at UMBNG

2. Biological N fixation

- ✓ A culture collection of rhizobia isolated from tropical, leguminose crops and trees has been established (at present ca 450 isolates)
- ✓ The collection comprises a large metabolic and genomic diversity. Taxonomic and phylogenetic characterizations reveal several novel species
- ✓ Competitiveness as inoculants and effectiveness of N_2-fixation in nodules

Techniques: Isolation of strains, gas measurements, MLSA, Sanger sequencing, (DNA-DNA hybridisation)

Reference List

1. **Molstad L., Dörsch P. and Bakken L.R. 2007. Robotized incubation system for monitoring gases (O_2, NO, N_2O, N_2) in denitrifying cultures. J Microbiol Meth 71:202-211.**
2. **Liu B., Mørkved P.T., Frostegård Å. and Bakken L.R. 2010. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Ecol. Microbiol. 72:407-417.**
3. **Bergaust L., Mao Y., Bakken L., Frostegård Å. 2010. Denitrification response patterns during transition to anoxic respiration and post-transcriptional effects of suboptimal pH on nitrous oxide reductase in *Paracoccus denitrificans*. Appl Environ Microbiol 76:6387-6396.**
4. **Frostegård Å., Tunlid A. and Bååth, E. 2010. Citation classic: The use and misuse of PLFA measurements in soils. Soil Biol. Biochem. In Press.**
5. **Degefu T., Wolde-meskel E. and Frostegård Å. 2010. Multilocus sequence analyses reveal several unnamed *Mesorhizobium* genospecies nodulating *Acacia* species and *Sesbania sesban* trees in Southern regions of Ethiopia. Syst. Appl. Microbiol. In Press.**