

**ESR 5 (WP2): Linda Hink ([linda.hink@uniabd.ac.uk](mailto:linda.hink@uniabd.ac.uk)) University of Aberdeen, UK**

**Local supervisory team: Professor James Prosser, Dr Graeme Nicol**

Project: "Microcosm-driven identification and isolation of key nitrous oxide generators"

Linda Hink's research within work package 2 (systems) will involve the determination of "key strains" (key N<sub>2</sub>O-producing strains) by performing controlled microcosm-experiments under different environmental conditions. This will include investigations under different O<sub>2</sub>-concentrations, substrate levels (ammonia and nitrate) and pH. Inhibitors and isotopes will be used to discriminate between nitrous oxide producers. Community abundance and composition will be determined using qPCR and high-throughput sequencing (HTS) of denitrifier and ammonia oxidiser functional genes and gene transcripts. The investigations will lead to a development and improvement of molecular tools for measuring genes/gene transcripts involved in nitrous oxide production. Under certain environmental conditions key N<sub>2</sub>O generators will be enriched and, if possible, isolated and further characterized with regards to N<sub>2</sub>O emissions under various environmental conditions (pH, O<sub>2</sub> and substrate levels).

Specific tasks (first year):

- To perform a preliminary experiment determining the capability of soils from Craibstone Experimental Farm to produce N<sub>2</sub>O through activities of ammonia oxidisers and heterotrophic denitrifiers. Microcosms will be constructed containing soils from this site at pH 6.6 and incubated under oxic conditions with either NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> as substrate. Differential inhibitors will be used to distinguish between ammonia oxidiser and denitrifier N<sub>2</sub>O-production. Transcriptional activity of ammonia oxidizing bacteria and archaea and denitrifying bacteria will be analyzed by quantification of functional genes and gene transcripts.
- A large scale microcosm experiment will be performed to test hypotheses based on assumptions regarding the environmental factors differentiating N<sub>2</sub>O production by denitrifiers (heterotrophic denitrification) or nitrifiers (nitrifier denitrification, nitrification-coupled denitrification and N<sub>2</sub>O production as byproduct of ammonia oxidation) or chemodenitrification. The microcosms will be prepared with soils from Craibstone Experimental Farm maintaining a gradient of pH values and at controlled O<sub>2</sub> and substrate concentrations. The different organisms generating N<sub>2</sub>O will be discriminated using inhibitors and/or dual <sup>15</sup>N and <sup>18</sup>O isotope methods. Applied analyses and skills are mainly the same as in the preliminary experiment. Molecular analyses will be performed for selected samples. Additional analysis related to isotope analysis will also be performed.