



Introduction

Nitrate pollution is one of the main concerns for water management in the Osona region (NE Spain, Fig. 1), as high nitrate concentrations are frequently found in groundwater, reaching up to 500 mg NO₃⁻/L. The main source of nitrate in the Osona aquifers is slurry and manure applied to crops as fertilizers. Nitrate can be reduced by dissimilatory nitrate reduction to ammonia (DNRA) or by denitrification, being the later the unique process that ensures nitrogen removal in groundwater. Although several studies have observed natural groundwater denitrification in Osona area, mainly related to pyrite oxidation, little or no attention has been made on bacterial functional marker genes involved in nitrate reduction. In this work, functional gene abundances together with hydrochemical and isotopic characteristics (by means of δ¹⁵N_{NO3} and δ¹⁸O_{NO3} isotopic signatures of NO₃⁻) were used to characterize nitrate reduction conditions from an ecological approach.

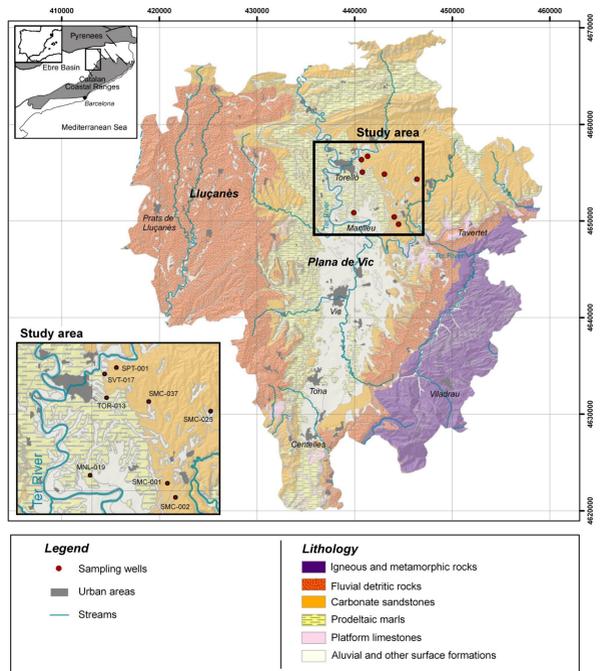


Fig. 1: Geographic and geological setting of the studied area (modified from Boy-Roura et al. (2013)).

Methodology

Samples were taken from eight wells in similar geological formations in the Osona area (Fig. 1), that is wells exploiting carbonate sandstones and prodeltaic marls. Physicochemical parameters, major ions, nutrients, and water and nitrate stable isotopes were measured in all of them. Moreover, abundances of the genes implied in the two key steps of denitrification (*nirK*, *nirS*, *nosZI* and *nosZII*) were determined by qPCR.

Analysis of 16S rRNA gene community was carried out by sequencing using Illumina MiSeq.

Results

Hydrochemical and isotopic data

Most of the hydrochemical parameters increased following the groundwater flowpath. This is not only the case of Ca²⁺, Mg²⁺, SO₄²⁻ and Cl⁻, but also of NO₃⁻, which showed lower values in NE wells (ranging 16.6-26.3 mg/L) and tended to increase in SW wells (with concentrations of 54.6-89.17 mg/L).

According to the δ¹⁵N and δ¹⁸O results, all the samples were in the range of manure and/or sewage values, most of them showing an enrichment caused by denitrification (Fig. 2). According to the O enrichment factor, samples SPT-001, TOR-013 and SMC-001 could present a degree of denitrification lower than 10%, in SMC-002 is approximately of 15%, and in SMC-037, SMC-025 and MNL-019 the degree of denitrification could be higher than 25%.

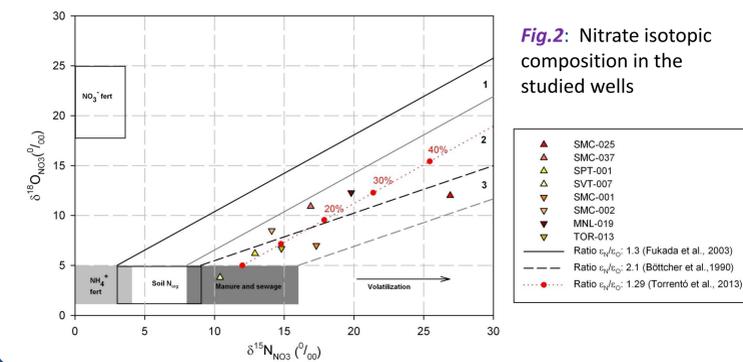


Fig. 2: Nitrate isotopic composition in the studied wells

The groundwater microbiome

Microbial communities detected in groundwater samples did not cluster entirely according to potential denitrification. Other environmental variables did seem to have an effect (Fig. 3).

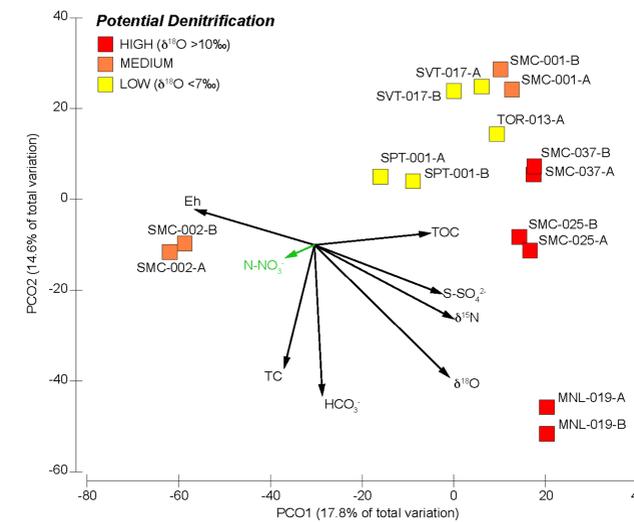


Fig. 3: PCoA analysis of groundwater microbiomes (Weighted Unifrac similarity of an OUT based definition of bacterial communities). Environmental variables are shown as vectors correlating to PCoA axes (Spearman's correlation, R>0.6, NO₃⁻ R<0.2).

The most represented classes in the groundwater microbiome were *Alphaproteobacteria*, *Nitrospira*, *Clostridia*, *Parcubacteria*, *Acidobacteriia* and *Sphingobacteriia* (Fig. 4), which could include some denitrifier members.

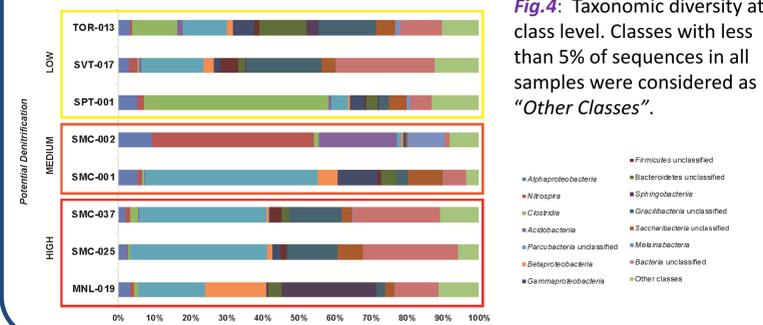


Fig. 4: Taxonomic diversity at class level. Classes with less than 5% of sequences in all samples were considered as "Other Classes".

Quantification of denitrification genes

Genes for a complete denitrification pathway were found in all the studied samples (Fig. 5). Low concentrations of *nir* and *nos* genes were found in wells showing low potential denitrification. In wells with a medium potential denitrification activity, *nir* genes increased significantly, indicating a potential accumulation of N₂O. Wells with the highest potential denitrification showed a high variability in gene abundance.

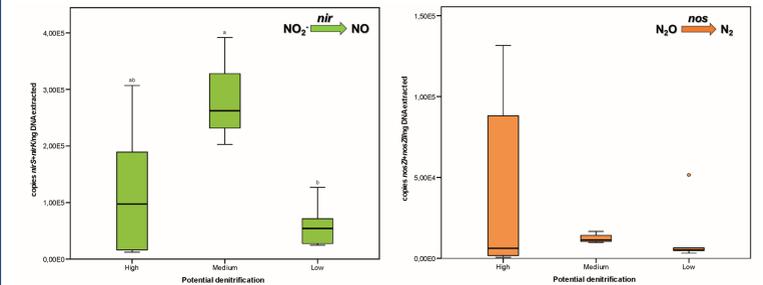


Fig. 5: Abundances of genes implied in the key steps of denitrification. Letters above boxes indicates significant differences in genes abundances.

Conclusions

✓ Denitrification was detected in all the sampled wells nearly half of them showing denitrification higher than 25%.

✓ Denitrifying genes were detected in all the studied wells, confirming the capability to reduce completely NO₃⁻ to N₂ in the polluted areas.

✓ The *nosZ* gene encodes the final step of denitrification, consequently denitrifier communities with low *nosZ* to *nir* gene ratios can result in N₂O accumulation. This situation occurred in wells showing a medium denitrification potential.

✓ Changes in the microbial community in nitrate polluted groundwater was not entirely determined by denitrification potential, other physicochemical variables, such as the redox potential, sulfate concentration and TOC, seemed to have an equivalent similar effect.

Rererences

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