



The N cycle in Earth subsurface. Reactivity of functional genes to anthropogenic CO₂ injections.

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The Nitrogen cycle has been widely studied in surface ecosystems, due to the importance of this nutrient for the organisms' development, and to the impact in the environment of most of the N forms, many of them being considered pollutants. However, little is known about the importance of the N-related metabolisms in subsurface systems now recognized to host diverse and active microbial life. In this study, we have periodically sampled the subsurface aquifers of the Icelandic pilot site for CO₂ storage associated with the geothermal plant of Hellisheidi (operated by Reykjavik Energy; <http://www.or.is/en/projects/carbfix>). With the aim of understanding the dynamics of N-cycle in the subsurface, and its reactivity to CO₂ injections, we quantified through qPCR the functional genes *amoA* (archaea), *amoA* (bacteria), *nirK*, *nirS*, *nosZ*, *nifH*, and the 16SrRNA genes of the anammox, total archaea and total bacteria.

The 16SrRNA gene quantification provided values of around 10⁷ gene copies/l at non injection periods. CO₂ injection caused first a slight decrease probably due to pH decrease or toxicity by oxygen contamination during the injections. Two months after injection, the copy numbers increased up to 10⁹ gene copies/l, and slowly returned to pre-injection values. The archaeal 16S rDNA copy numbers showed a similar reaction, with higher toxicity effects, and a lower increase afterwards.

Due to the high reactivity of the microbial populations to CO₂ injections, all the N cycle quantifications were related to the total 16S rDNA copies for normalization. Nitrifying genes (*amoA*) were mainly represented by the ammonia oxidizing archaea, and were apparently not affected by CO₂ injections. Anammox bacteria were present in a very low percentage, and the obtained copy numbers tended to decrease after the injection. These results were surprising due to the autotrophic character of ammonia oxidizers, but could be explained by a competitive exclusion. On the contrary, N-fixation (*nifH*) was stimulated by the injections, doubling their relative abundance in relation to bacteria 16S rDNA copy numbers, supplying the N requirements of new biomass formed by autotrophic CO₂ fixation. Finally, denitrifying bacteria (*nirK*, *nirS* and *nosZ*) showed a higher seasonal variation, but were positively stimulated by the CO₂ injections. This process can be autotrophic in some species, using directly the injected CO₂ as C source.

Altogether the results suggest a high response of the N cycle to the CO₂ injections, and its potential contribution to the formation of new biomass and C fixation. We provide evidences for the importance of the N cycle on the subsurface and its reactivity to CO₂ injections, being therefore important the consideration of this cycle in CO₂ storage modelling.