



## Can functional gene abundance predict N-fluxes? Examples from a well-studied hydrological flow path in a forested watershed in SW China

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Edaphic, climatic and management factors shape soil microbial communities taxonomically and functionally, resulting in spatial separation of nitrogen (N) oxidation and reduction processes along hydrological flowpaths. In a recent study, we investigated N-cycling processes and N<sub>2</sub>O emissions along a mesic hillslope (HS) and a hydrologically connected groundwater discharge zone (GDZ) in a forested headwater catchment dominated by acid soils (pH 4.0 - 4.5) in subtropical China (Chongqing). The watershed receives 50 kg N ha<sup>-1</sup> a<sup>-1</sup> through atmospheric deposition (2/3 as ammonium), most of which is removed before discharge. Surprisingly, N<sub>2</sub>O emissions were found to be greatest on the well-drained HS, whereas a drop of NO<sub>3</sub><sup>-</sup> concentrations along the flow path indicated that N removal was highest in the moist GDZ. Nitrification was assumed to be none-limiting as the total flux of NO<sub>3</sub><sup>-</sup> leaving the hill slope soils roughly equalled the input of NH<sub>4</sub><sup>+</sup>. To understand watershed N-cycling and removal in more detail, we studied the abundance of functional genes involved in ammonium oxidation (amoA of AOB and AOA), nitrite oxidation (nirB) and denitrification (nirK, nirS, nosZ) in top soils from 8 locations along the flow path spanning from the hilltop to the outlet of the GDZ. 16S rRNA gene abundance was assessed as a general marker for bacterial abundance. All genes showed highest abundance per gram soil in the heavily disturbed GDZ (formerly cultivated terraces), despite lower soil organic carbon content (1-4% w/w as opposed to 10-20% w/w in HS topsoil) and periodically stagnant conditions due to high water tables after monsoonal rainfalls. Ratios of nosZ/nirS+nirK, commonly used to predict denitrification product stoichiometry (N<sub>2</sub>O/N<sub>2</sub>), yielded counterintuitive results with higher values for HS than for GDZ. However, comparing nir gene with 16S rRNA gene abundance revealed that denitrifiers accounted for up to 10% of the bacterial community in the GDZ soils whereas this value was only 1% in HS soils. Even though GDZ soils harbour less nosZ relative to nirS+nirK denitrifiers (i.e. has a lower nos/nir gene copy ratio), the high relative abundance of denitrifiers in the GDZ communities may still provide sufficient N<sub>2</sub>O reducing capacity to explain lower N<sub>2</sub>O emission. High N<sub>2</sub>O reduction capacity in the GDZ is further supported by higher soil pH (4.5 versus 4.0 at the HS) and diffusion limitation in the denser GDZ soil resulting in high dissolved N<sub>2</sub>O concentrations promoting nosZ expression. Archaeal ammonia oxidizers (AOA) were about 5000 times more abundant than bacterial ammonia oxidizers (AOB) which is in line with the low pH of these soils, and amounted to up to 3% of 16S rRNA gene counts. Again, abundances were highest in the GDZ despite periodical waterlogging. Abundance of nitrite oxidizers was similar to that of AOA. Our results show that copy numbers of functional genes in complex landscapes cannot be readily interpreted with respect to ecosystem N fluxes, but need to be analysed in a spatially explicit manner in the context of watershed hydrology.