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Higher diversity and abundance of denitrifying microorganisms in environments than considered previously

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Denitrification is an important process in the global nitrogen cycle. The genes encoding NirK and NirS (*nirK* and nirS), which catalyze the reduction of nitrite to nitric oxide, have been used as marker genes to study the ecological behavior of denitrifiers in environments. However, conventional polymerase chain reaction (PCR) primers can only detect a limited range of the phylogenetically diverse *nirK* and *nirS*. Thus, we developed new PCR primers covering the diverse nirK and nirS. Clone library and qPCR analysis using the primers showed that nirK and nirS in terrestrial environments are more phylogenetically diverse and 2-6 times more abundant than those revealed with the conventional primers. RNA- and culture-based analyses using a cropland soil also suggested that microorganisms with previously unconsidered *nirK* or *nirS* are responsible for denitrification in the soil. PCR techniques still have a greater capacity for the deep analysis of target genes than PCR-independent methods including metagenome analysis, although efforts are needed to minimize the PCR biases. The methodology and the insights obtained here should allow us to achieve a more precise understanding of the ecological behavior of denitrifiers and facilitate more precise estimate of denitrification in environments.

Keywords: denitrification, nitrite reductase gene, nirS, nirK