Electronic Supplementary Information (ESI)

Journal: Applied Science

Paper: Gross ammonification and nitrification rates in soil amended with natural and NH₄-enriched chabazite zeolites and nitrification inhibitor DMPP

Authors: Giacomo Ferretti^(1*), Giulio Galamini⁽¹⁾, Evi Deltedesco⁽²⁾, Markus Gorfer⁽³⁾, Jennifer Fritz⁽³⁾, Barbara Faccini⁽¹⁾,

Axel Mentler⁽²⁾, Sophie Zechmeister-Boltenstern⁽²⁾, Massimo Coltorti⁽¹⁾, Katharina Maria Keiblinger⁽²⁾

(1) Department of Physics and Earth Science, University of Ferrara, Via Saragat 1, Ferrara 44122 (Italy)

(2) University of Natural Resources and Life Sciences Vienna (BOKU), Peter Jordan Strasse 82, Vienna 1190 (Austria),

(3) Austrian Institute of Technology (AIT), Konrad-Lorenz-Straße 24, 3430 Tulln (Austria)

Corresponding author contacts: katharina.keiblinger@boku.ac.at

Gene	Primer	Sequence	Amplicon size [bp]	Reference
archaeal <i>amoA</i>	amo19F CrenamoA616r48x	ATGGTCTGGCTWAGACG GCCATCCABCKRTANGTCCA	624	Leininger et al. (2006) Schauss et al. (2009)
bacterial <i>amoA</i>	amoA1F amoA2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	500	Rotthauwe et al. (1997)
nirS	nirSC1F nirSC1R	ATCGTCAACGTCaargaracvgg TTCGGGTGCGTCttsabgaasag	410	Wei et al. (2015)
nosZ	nosZ2F nosZ2R	CGCRACGGCAASAAGGTSMSSGT CAKRTGCAKSGCRTGGCAGAA	267	Henry et al. (2006)

Table ESI 1 Primers used for quantitative PCR.

Reference gene fragments from soil used in this study were amplified with the same primers as used for qPCR (Table ESI 1). Amplicons of each gene were cloned into pJet1.2 Blunt Cloning Vector (ThermoFisher Scientific) according to manufacturer's instructions. Sequencing primers, pJet1.2 forward and reverse were used to amplify and sequence the inserts of the genes to confirm the amplification of respective genes. Further, the appropriate PCR products were purified (Qiaquick PCR Purification Kit - Qiagen, Venlo, Netherlands) and quantified (iQuant Broad Range dsDNA Quantitation Kit - GeneCopoeia, MD, USA). As a result, dilution series of gene copies were used for the standard curve.

Target gene	Thermal profile	
archaeal amoA	1: 95 °C – 3 min	
	95 °C − 15 sec	
	55 °C – 45 sec	
	72 °C – 45 sec	
	65 °C – 5 sec	
	95 °C – 5 sec	
	39 cycles	
bacterial amoA	95 °C – 3 min	
	95 °C – 15 sec	
	55 °C – 45 sec	
	72 °C – 45 sec	
	65 °C – 5 sec	
	95 °C – 5 sec	
	39 cycles	
nirS	95 °C – 3 min	
	95 °C – 15 sec	
	56 °C – 45 sec	
	72 °C – 45 sec	
	65 °C – 5 sec	
	95 °C – 5 sec	
	39 cycles	
nosZ	95 °C – 3 min	
	95 °C – 15 sec	
	60 °C – 45 sec	
	72 °C – 45 sec	
	65 °C – 5 sec	
	95 °C – 5 sec	
	39 cycles	

Table ESI 2 Quantitative PCR thermal profiles for the different target genes

References

Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology 72, 5181-5189.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806-809.

Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology 63, 4704-4712.

Schauss, K., Focks, A., Leininger, S., Kotzerke, A., Heuer, H., Thiele-Bruhn, S., Sharma, S., Wilke, B.M., Matthies, M., Smalla, K., Munch, J.C., Amelung, W., Kaupenjohann, M., Schloter, M., Schleper, C., 2009. Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environmental Microbiology 11, 446-456.

Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S., Senoo, K., 2015. Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. The ISME journal 9, 1954.