

Assessing the Effectiveness of 3, 4-dimethylpyrazole phosphate (DMPP) Inhibitor in Mitigating N₂O Emissions from Contrasting Cd-Contaminated Soils

Muhammad Laeq Ur Rehman Hashmi^a, Yasir Hamid^a, Muhammad Usman^b, Jipeng Luo^a, Sangar Khan^c, Tang Sheng^a, Nabila Bano^{a,d}, Talha Tufail Bhatti^e, Tingqiang, Li^{a*}

^a Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

^b Université de Rennes, Ecole Nationale Supérieure de Chimie de Rennes, CNRS, ISCR-UMR 6226, F-35000, Rennes, France

^c Department of Geography and Spatial Information Techniques, Ningbo University, Ningbo 315211, China

^d Pakistan Tobacco Board, Ministry of National Food Security and Research, Islamabad, Pakistan

^e Institute of Regional Studies, Islamabad, Pakistan

For correspondence: litq@zju.edu.cn .

Supporting Material

Table S1. Primers and conditions used in the manuscript.

Table. S2 (a-e). Simple linear regression analysis of selected genes with selected parameters in paddy soil.

Table. S3 (a-e). Simple linear regression analysis of selected genes with selected parameters in red soil.

Figure S1. Pearson correlation between N₂O emission and different parameters across Two soils (Paddy and Red) receiving four treatments during the experimental incubation.

Table. S1. Primers used for PCR and qPCR in the manuscript

Target	primers	Sequence (5'-3')	Fragment Size (bp)	Annealing Temp (°C)	Reference
AOA- <i>amoA</i>	Arch-amoAF Arch-amoAR	STAATGGTCTGGCTTAG ACG GCGGCCATCCATCTGTA TGT	635	53	Zhang et al. (2012) Tourna et al. (2008) Cui et al. (2016)
AOB- <i>amoA</i>	amoA-1F amoA-2R	GGGGTTTCTACTGGTGG T CCCCTCGGCAAAGCCTT CTTC	491	56	(Rotthauwe et al., 1997)
<i>nirS</i>	cd3aF R3cd	GTSAACGTSAAAGGARAC SGG GASTTCGGRTGSGTCTTG A	425	59	(Michotey et al., 2000) [Throback et al., 2004)
<i>nirK</i>	F1aCu R3Cu	ATCATGGTSCTGCCGCG GCCTCGATCAGRTTGTG GTT	473	59	[Hallin & Lindgren 1999]
<i>nosZ</i>	nosZ2F nosZ2R	CGCRACGGCAASAAGGT SMSSGT CAKRTGCAKSGCRTGGC AGAA	267	59	[Henry et al., 2006]

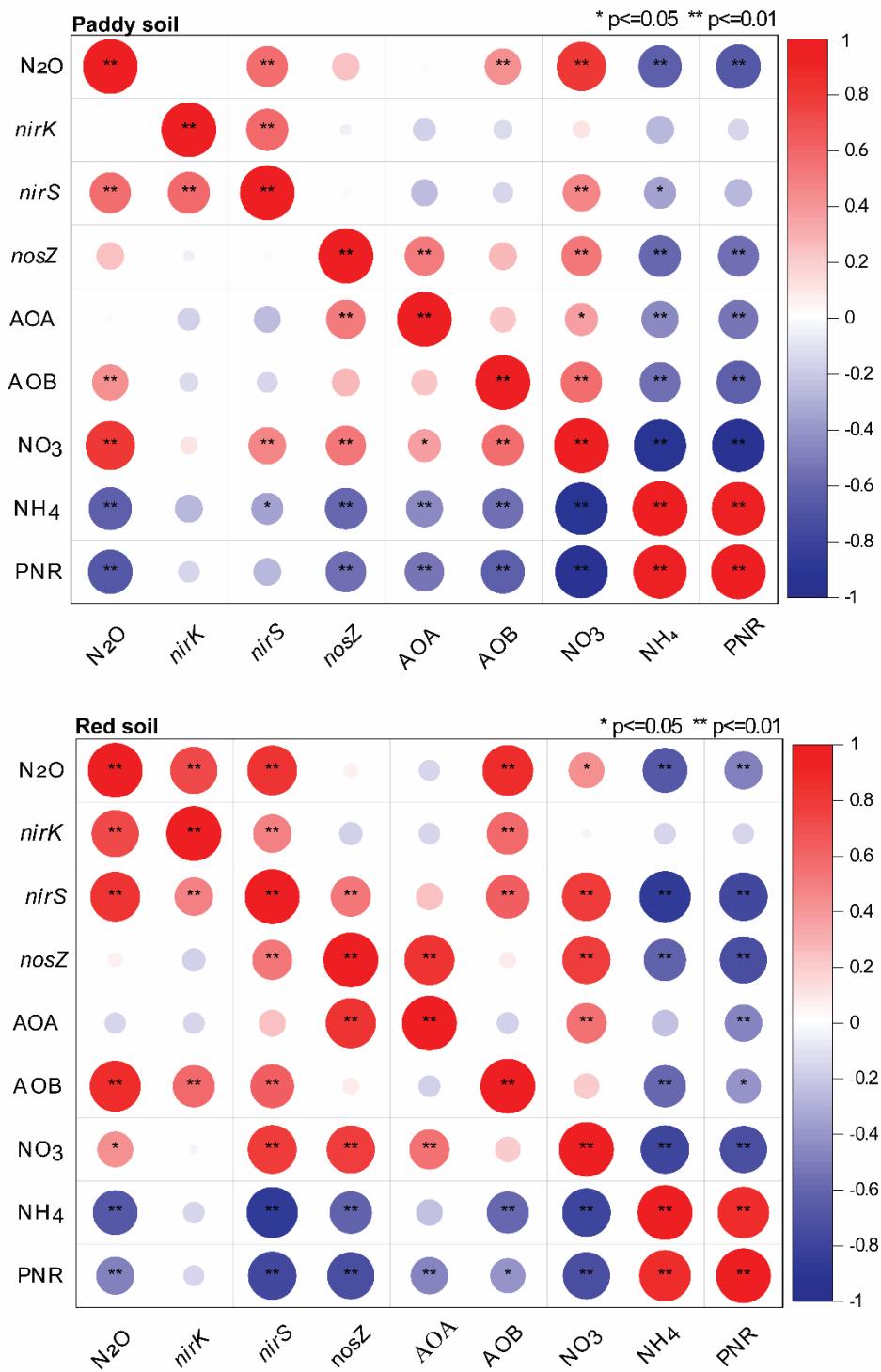


Fig. S1. Pearson correlation between N_2O emission and different parameters across Two soils (Paddy and Red) receiving four treatments during the experimental incubation.

Table. S2a. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Paddy soil	<i>NirK</i>	CK	N ₂ O	y = -3E-06x + 59.0	R ² = 0.98	P<0.05
			NO ₃ -N	y = 2E-06x - 7.2	R ² = 0.89	P<0.05
			NH ₄ -N	y = -3E-07x + 5.7	R ² = 0.78	P<0.05
			PNR	y = -4E-07x + 6.9	R ² = 0.70	P<0.05
		Cd	N ₂ O	y = -1E-07x + 18.2	R ² = 0.03	P<0.05
			NO ₃ -N	y = 3E-07x + 12.3	R ² = 0.49	P<0.05
			NH ₄ -N	y = -2E-08x + 2.1	R ² = 0.35	P<0.05
			PNR	y = -5E-08x + 3.45	R ² = 0.44	P<0.05
		DMPP	N ₂ O	y = 6E-07x + 2.82	R ² = 0.87	P<0.05
			NO ₃ -N	y = 4E-06x - 16.3	R ² = 0.53	P<0.05
			NH ₄ -N	y = -4E-07x + 4.9	R ² = 0.42	P<0.05
			PNR	y = -5E-07x + 5.5	R ² = 0.36	P<0.05
		DMPP + Cd	N ₂ O	y = 9E-06x - 38.12	R ² = 0.96	P<0.05
			NO ₃ -N	y = 2E-06x + 7.2	R ² = 0.038	P<0.05
			NH ₄ -N	y = 2E-07x + 1.2	R ² = 0.017	P<0.05
			PNR	y = 6E-07x - 1.12	R ² = 0.157	P<0.05

Table. S2b. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Paddy soil	<i>NirS</i>	CK	N ₂ O	y = 1E-06x - 25.4	R ² = 0.90	P<0.05
			NO ₃ -N	y = 3E-07x + 3.4	R ² = 0.35	P<0.05
			NH ₄ -N	y = -4E-08x + 3.3	R ² = 0.08	P<0.05
			PNR	y = -3E-08x + 3.8	R ² = 0.04	P<0.05
		Cd	N ₂ O	y = 8E-07x - 10.9	R ² = 0.96	P<0.05
			NO ₃ -N	y = 5E-07x + 4.03	R ² = 0.42	P<0.05
			NH ₄ -N	y = -4E-08x + 3.2	R ² = 0.44	P<0.05
			PNR	y = -1E-07x + 7.28	R ² = 0.27	P<0.05
		DMPP	N ₂ O	y = 2E-06x - 51.5	R ² = 0.99	P<0.05
			NO ₃ -N	y = 1E-06x - 11.03	R ² = 0.33	P<0.05
			NH ₄ -N	y = -9E-08x + 4.16	R ² = 0.26	P<0.05
			PNR	y = -1E-07x + 4.36	R ² = 0.13	P<0.05
		DMPP + Cd	N ₂ O	y = 1E-06x - 17.3	R ² = 0.88	P<0.05
			NO ₃ -N	y = 9E-07x - 6.74	R ² = 0.42	P<0.05
			NH ₄ -N	y = -7E-08x + 4.4	R ² = 0.13	P<0.05
			PNR	y = -2E-08x + 2.9	R ² = 0.016	P<0.05

Table. S2c. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value	
Paddy soil	<i>NosZ</i>	CK	N ₂ O	y = 3E-08x - 9.28	R ² = 0.30	P<0.05	
			NO ₃ -N	y = 2E-08x - 10.4	R ² = 0.91	P<0.05	
			NH ₄ -N	y = -5E-09x + 6.8	R ² = 0.94	P<0.05	
			PNR	y = -6E-09x + 9.13	R ² = 0.89	P<0.05	
		Cd	N ₂ O	y = 7E-09x + 8.45	R ² = 0.12	P<0.05	
		NH ₄ -N	NO ₃ -N	y = 2E-08x - 4.20	R ² = 0.93	P<0.05	
			NH ₄ -N	y = -2E-09x + 3.4	R ² = 0.94	P<0.05	
			PNR	y = -3E-09x + 5.5	R ² = 0.98	P<0.05	
	DMPP	N ₂ O	DMPP	N ₂ O	y = 3E-09x + 9.75	R ² = 0.079	P<0.05
			NO ₃ -N	y = 8E-09x + 3.12	R ² = 0.94	P<0.05	
			NH ₄ -N	y = -9E-10x + 3.2	R ² = 0.98	P<0.05	
			PNR	y = -1E-09x + 3.62	R ² = 0.96	P<0.05	
		DMPP + Cd	N ₂ O	y = -3E-10x + 12.5	R ² = 0.0013	P<0.05	
		NH ₄ -N	NO ₃ -N	y = -3E-10x + 12.5	R ² = 0.0013	P<0.05	
			NH ₄ -N	y = -2E-10x + 2.79	R ² = 0.02	P<0.05	
			PNR	y = -9E-11x + 2.5	R ² = 0.0036	P<0.05	

Table. S2d. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Paddy soil	AOA	CK	N ₂ O	y = 4E-06x - 18.8	R ² = 0.97	P<0.05
			NO ₃ -N	y = 1E-06x + 1.46	R ² = 0.43	P<0.05
			NH ₄ -N	y = -2E-07x + 3.9	R ² = 0.35	P<0.05
			PNR	y = -2E-07x + 4.6	R ² = 0.23	P<0.05
		Cd	N ₂ O	y = 2E-06x - 8.11	R ² = 0.54	P<0.05
			NO ₃ -N	y = 3E-06x - 12.3	R ² = 0.83	P<0.05
			NH ₄ -N	y = -2E-07x + 3.9	R ² = 0.79	P<0.05
			PNR	y = -4E-07x + 6.2	R ² = 0.72	P<0.05
		DMPP	N ₂ O	y = -6E-08x + 19.4	R ² = 0.26	P<0.05
			NO ₃ -N	y = 6E-08x + 12.2	R ² = 0.32	P<0.05
			NH ₄ -N	y = -7E-09x + 2.2	R ² = 0.42	P<0.05
			PNR	y = -1E-08x + 2.41	R ² = 0.48	P<0.05
		DMPP + Cd	N ₂ O	y = -1E-07x + 17.16	R ² = 0.38	P<0.05
			NO ₃ -N	y = 1E-07x + 14.06	R ² = 0.32	P<0.05
			NH ₄ -N	y = -1E-08x + 2.06	R ² = 0.34	P<0.05
			PNR	y = -2E-08x + 3.13	R ² = 0.54	P<0.05

Table. S2e. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Paddy soil	AOB	CK	N ₂ O	y = 1E-08x + 18.5	R ² = 0.06	P<0.05
			NO ₃ -N	y = 1E-08x + 6.1	R ² = 0.72	P<0.05
			NH ₄ -N	y = -3E-09x + 3.51	R ² = 0.82	P<0.05
			PNR	y = -4E-09x + 4.4	R ² = 0.89	P<0.05
		Cd	N ₂ O	y = -4E-09x + 19.4	R ² = 0.087	P<0.05
			NO ₃ -N	y = 1E-08x + 13.57	R ² = 0.39	P<0.05
			NH ₄ -N	y = -7E-10x + 2.23	R ² = 0.44	P<0.05
		DMPP	PNR	y = -2E-09x + 3.16	R ² = 0.56	P<0.05
			N ₂ O	y = -2E-08x + 20.4	R ² = 0.28	P<0.05
			NO ₃ -N	y = 2E-08x + 10.2	R ² = 0.35	P<0.05
			NH ₄ -N	y = -3E-09x + 2.48	R ² = 0.47	P<0.05
		DMPP + Cd	PNR	y = -4E-09x + 2.75	R ² = 0.53	P<0.05
			N ₂ O	y = -2E-08x + 24.08	R ² = 0.35	P<0.05
			NO ₃ -N	y = 3E-08x + 11.2	R ² = 0.38	P<0.05
			NH ₄ -N	y = -2E-09x + 2.2	R ² = 0.4	P<0.05
			PNR	y = -4E-09x + 3.59	R ² = 0.60	P<0.05

Table. S3a. Simple linear regression analysis of selected genes with selected parameters

Soil	Genes	Treatments	Selected parameters	Equation	R ²	P value
Red soil	<i>NirK</i>	CK	N ₂ O	y = 1E-06x + 5.1	R ² = 0.90	P < 0.05
			NO ₃ -N	y = 5E-07x + 4.29	R ² = 0.17	P < 0.05
			NH ₄ -N	y = -2E-07x + 7.2	R ² = 0.31	P < 0.05
			PNR	y = -9E-08x + 2.06	R ² = 0.15	P < 0.05
		Cd	N ₂ O	y = 3E-06x + 3.6	R ² = 0.70	P < 0.05
			NO ₃ -N	y = 1E-06x + 8.7	R ² = 0.06	P < 0.05
			NH ₄ -N	y = -3E-07x + 6.7	R ² = 0.112	P < 0.05
			PNR	y = -9E-08x + 1.76	R ² = 0.03	P < 0.05
		DMPP	N ₂ O	y = 4E-07x + 7.84	R ² = 0.64	P < 0.05
			NO ₃ -N	y = 3E-07x + 12.5	R ² = 0.016	P < 0.05
			NH ₄ -N	y = 5E-08x + 5.9	R ² = 0.02	P < 0.05
			PNR	y = -1E-08x + 0.9	R ² = 0.004	P < 0.05
		DMPP + Cd	N ₂ O	y = 1E-06x + 6.3507	R ² = 0.65	P < 0.05
			NO ₃ -N	y = 8E-07x + 12.158	R ² = 0.05	P < 0.05
			NH ₄ -N	y = -1E-07x + 5.6705	R ² = 0.07	P < 0.05
			PNR	y = -2E-08x + 0.98	R ² = 0.01	P < 0.05

Table. S3b. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Red soil	<i>NirS</i>	CK	N ₂ O	y = 6E-07x + 3.2129	R ² = 0.69	P<0.05
			NO ₃ -N	y = 4E-07x + 0.6272	R ² = 0.36	P<0.05
			NH ₄ -N	y = -2E-07x + 9.0919	R ² = 0.86	P<0.05
			PNR	y = -1E-07x + 3.4725	R ² = 0.88	P<0.05
		Cd	N ₂ O	y = 5E-07x + 3.586	R ² = 0.90	P<0.05
			NO ₃ -N	y = 6E-07x + 1.18	R ² = 0.52	P<0.05
			NH ₄ -N	y = -1E-07x + 8.49	R ² = 0.81	P<0.05
			PNR	y = -8E-08x + 3.04	R ² = 0.68	P<0.05
		DMPP	N ₂ O	y = 2E-07x + 6.69	R ² = 0.77	P<0.05
			NO ₃ -N	y = 9E-07x - 0.84	R ² = 0.88	P<0.05
			NH ₄ -N	y = -1E-07x + 8.02	R ² = 0.63	P<0.05
			PNR	y = -6E-08x + 1.8	R ² = 0.77	P<0.05
		DMPP + Cd	N ₂ O	y = 4E-07x + 4.0625	R ² = 0.93	P<0.05
			NO ₃ -N	y = 8E-07x + 0.63	R ² = 0.78	P<0.05
			NH ₄ -N	y = -9E-08x + 6.84	R ² = 0.78	P<0.05
			PNR	y = -5E-08x + 1.739	R ² = 0.63	P<0.05

Table. S3c. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Red soil	<i>NosZ</i>	CK	N ₂ O	y = 3E-11x + 13.663	R ² = 0.005	P<0.05
			NO ₃ -N	y = 1E-10x + 4.7042	R ² = 0.114	P<0.05
			NH ₄ -N	y = -7E-11x + 7.5401	R ² = 0.36	P<0.05
			PNR	y = -6E-11x + 2.8035	R ² = 0.61	P<0.05
		Cd	N ₂ O	y = 1E-10x + 10.78	R ² = 0.13	P<0.05
			NO ₃ -N	y = 4E-10x + 2.93	R ² = 0.44	P<0.05
			NH ₄ -N	y = -9E-11x + 8.0	R ² = 0.67	P<0.05
			PNR	y = -6E-11x + 3.04	R ² = 0.78	P<0.05
		DMPP	N ₂ O	y = 3E-11x + 8.49	R ² = 0.27	P<0.05
			NO ₃ -N	y = 2E-10x + 2.2	R ² = 0.96	P<0.05
			NH ₄ -N	y = -4E-11x + 7.9	R ² = 0.97	P<0.05
			PNR	y = -2E-11x + 1.7	R ² = 0.91	P<0.05
		DMPP + Cd	N ₂ O	y = 4E-11x + 9.31	R ² = 0.104	P<0.05
			NO ₃ -N	y = 3E-10x + 4.4	R ² = 0.75	P<0.05
			NH ₄ -N	y = -3E-11x + 6.36	R ² = 0.66	P<0.05
			PNR	y = -2E-11x + 1.60	R ² = 0.79	P<0.05

Table. S3d. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Red soil	AOA	CK	N ₂ O	y = 5E-07x + 7.8	R ² = 0.90	P<0.05
			NO ₃ -N	y = 2E-07x + 5.28	R ² = 0.21	P<0.05
			NH ₄ -N	y = -1E-07x + 7.08	R ² = 0.48	P<0.05
			PNR	y = -6E-08x + 2.13	R ² = 0.46	P<0.05
		Cd	N ₂ O	y = 3E-07x + 6.9	R ² = 0.68	P<0.05
			NO ₃ -N	y = 5E-07x + 2.3	R ² = 0.66	P<0.05
			NH ₄ -N	y = -1E-07x + 7.9	R ² = 0.86	P<0.05
			PNR	y = -6E-08x + 2.8	R ² = 0.79	P<0.05
		DMPP	N ₂ O	y = 4E-08x + 7.68	R ² = 0.75	P<0.05
			NO ₃ -N	y = 2E-07x + 3.6	R ² = 0.85	P<0.05
			NH ₄ -N	y = -2E-08x + 7.4	R ² = 0.65	P<0.05
			PNR	y = -1E-08x + 1.5	R ² = 0.74	P<0.05
		DMPP + Cd	N ₂ O	y = 9E-08x + 7.9	R ² = 0.33	P<0.05
			NO ₃ -N	y = 3E-07x + 3.4	R ² = 0.93	P<0.05
			NH ₄ -N	y = -4E-08x + 6.49	R ² = 0.85	P<0.05
			PNR	y = -2E-08x + 1.6	R ² = 0.92	P<0.05

Table. S3e. Simple linear regression analysis of selected genes with selected parameters

Soil	Gene	Treatments	Selected parameters	Equation	R ²	P value
Red soil	AOB	CK	N ₂ O	y = 2E-07x + 6.74	R ² = 0.59	P<0.05
			NO ₃ -N	y = 2E-07x + 1.71	R ² = 0.56	P<0.05
			NH ₄ -N	y = -8E-08x + 8.3	R ² = 0.92	P<0.05
			PNR	y = -5E-08x + 2.95	R ² = 0.88	P<0.05
		Cd	N ₂ O	y = 3E-07x + 6.2	R ² = 0.80	P<0.05
			NO ₃ -N	y = 3E-07x + 3.1	R ² = 0.56	P<0.05
			NH ₄ -N	y = -8E-08x + 7.9	R ² = 0.88	P<0.05
			PNR	y = -4E-08x + 2.7	R ² = 0.73	P<0.05
		DMPP	N ₂ O	y = 1E-07x + 8.2	R ² = 0.93	P<0.05
			NO ₃ -N	y = 3E-07x + 10.13	R ² = 0.32	P<0.05
			NH ₄ -N	y = -2E-08x + 6.4	R ² = 0.06	P<0.05
			PNR	y = -2E-08x + 1.09	R ² = 0.25	P<0.05
		DMPP + Cd	N ₂ O	y = 2E-07x + 7.83	R ² = 0.89	P<0.05
			NO ₃ -N	y = 2E-07x + 11.2	R ² = 0.34	P<0.05
			NH ₄ -N	y = -3E-08x + 5.7	R ² = 0.36	P<0.05
			PNR	y = -1E-08x + 1.08	R ² = 0.26	P<0.05

References

- Cui, P., Fan, F., Yin, C., Song, A., Huang, P., Tang, Y., Zhu, P., Peng, C., Li, T., Wakelin, S.A., 2016. Long-term organic and inorganic fertilization alters temperature sensitivity of potential N₂O emissions and associated microbes. *Soil Biology and Biochemistry* 93, 131-141.
- Hallin, S., & Lindgren, P. (1999). PCR Detection of Genes Encoding Nitrite Reductase in Denitrifying Bacteria. *Applied and Environmental Microbiology*, 65, 1652 - 1657.
- 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. *Appl Environ Microbiol* 72: 5181-5189.
- Michotey, Valerie & Méjean, Vincent & Bonin, Patricia. (2000). Comparison of Methods for Quantification of Cytochrome cd1-Denitrifying Bacteria in Environmental Marine Samples. *Applied and Environmental Microbiology - AEM*. 66. 1564-1571.
10.1128/AEM.66.4.1564-1571.2000.
- 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. *Appl Environ Microbiol* 63: 4704-4712.
- Throbäck N., Enwall K., Jarvis A., Hallin, S. (2004) Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol Ecol* 49: 401-417.
- Tourna, M., Freitag, T.E., Nicol, G.W., Prosser, J.I., 2008. Growth, activity and temperature responses of ammonia- oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology* 10, 1357-1364.

Zhang, L., Hu, H., Shen, J., He, J., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. The ISME journal 6, 1032-1045.