soil systems

MDPI

Article

Microbial Community Structure and Enzyme Activity in Soils under Plastic and Straw Mulching System in Strawberry Cultivation

Katherine Muñoz [1,*], Sören Thiele-Bruhn [2], Kilian G.J. Kenngott [3], Maximilian Meyer [3], Dörte Diehl [3], Zacharias Steinmetz [3] and Gabriele E. Schaumann [3]

[1] Group of Organic and Ecological Chemistry, Institute for Environmental Sciences, ES Landau, University of Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany
[2] Soil Science, Faculty of Spatial & Environmental Sciences, Trier University, 54286 Trier, Germany; thiele@uni-trier.de
[3] Group of Environmental and Soil Chemistry, Institute for Environmental Sciences, ES Landau, University of Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany; kenngott@uni-landau.de (K.G.J.K.); meyer-maximilian@uni-landau.de (M.M.); diehl@uni-landau.de (D.D.); steinmetz-z@uni-landau.de (Z.S.); schaumann@uni-landau.de (G.E.S.)
* Correspondence: munoz@uni-landau.de

Supplementary Materials

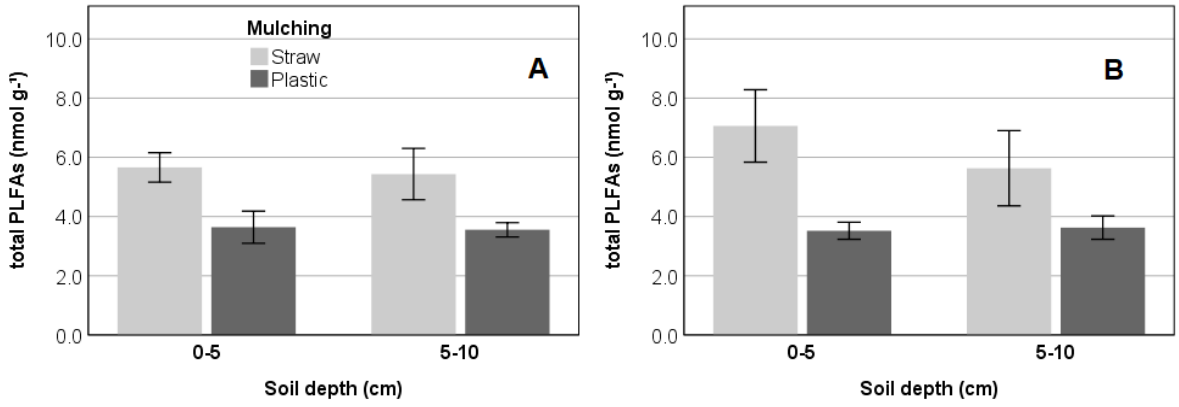

Figure S1. Total PLFAs differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

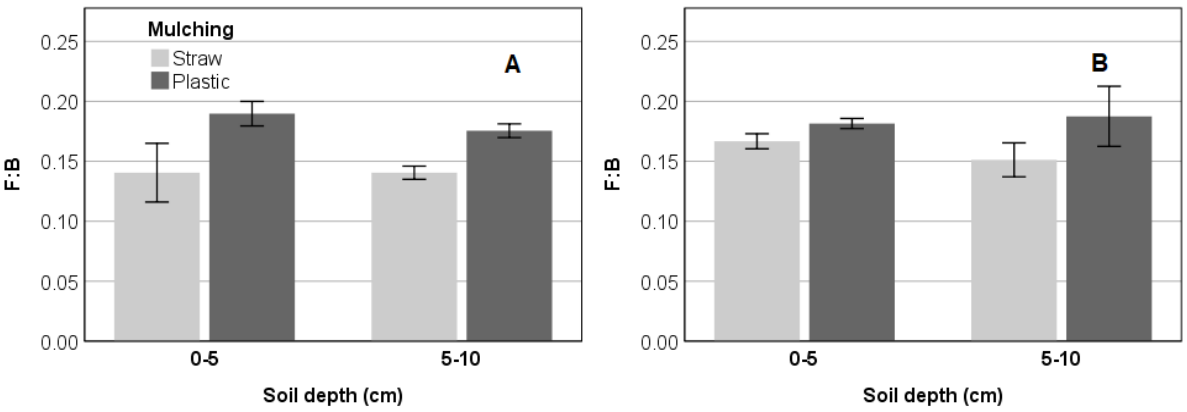

Figure S2. Fungi:bacteria ratio (F:B) ratios differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

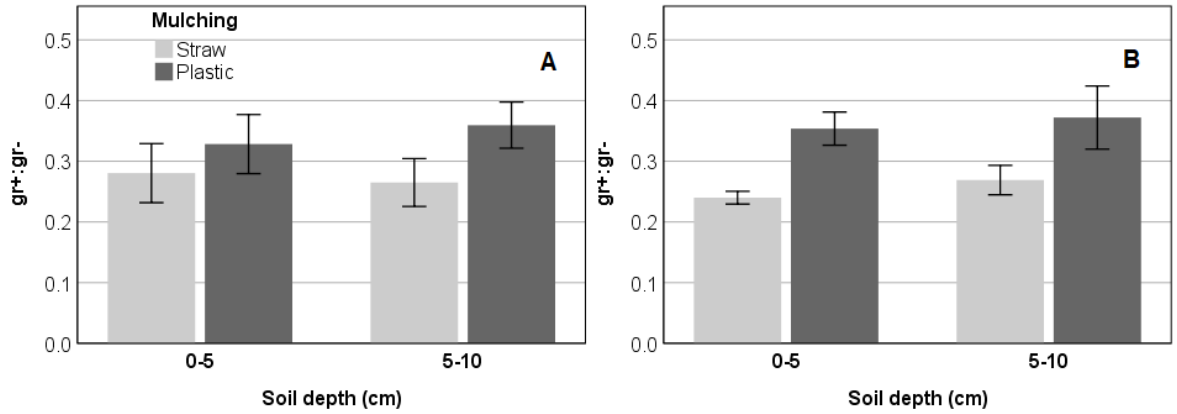

Figure S3. Ratio gram+:gram− bacteria (gr+:gr−) differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

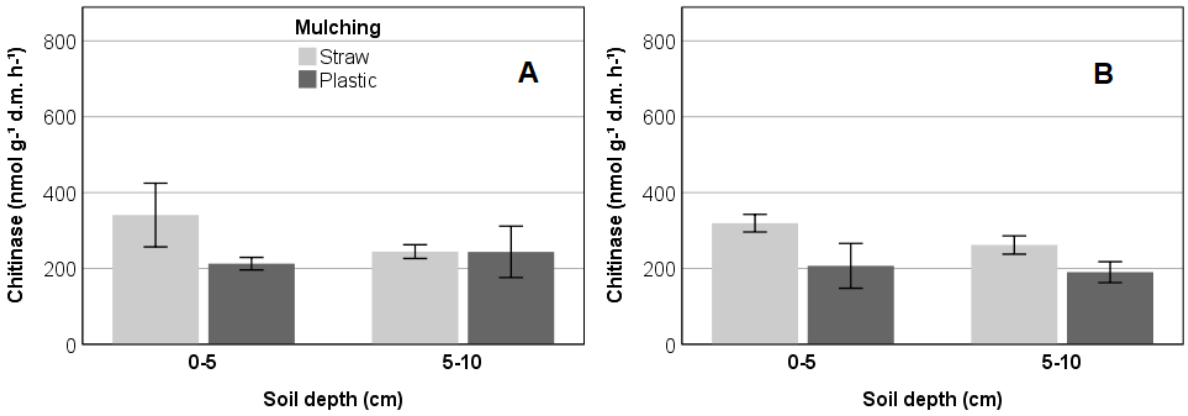

Figure S4. Chitinase activity differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

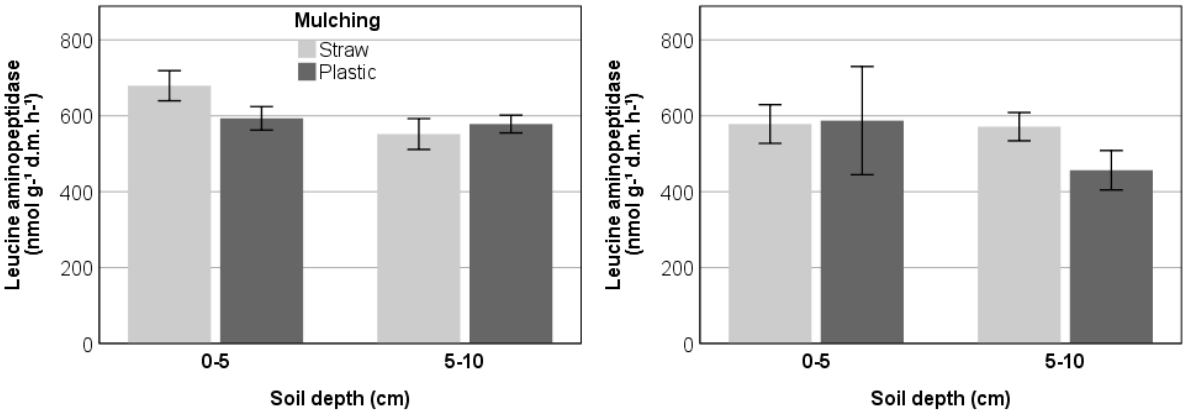

Figure S5. Leucine aminopeptidase activity differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

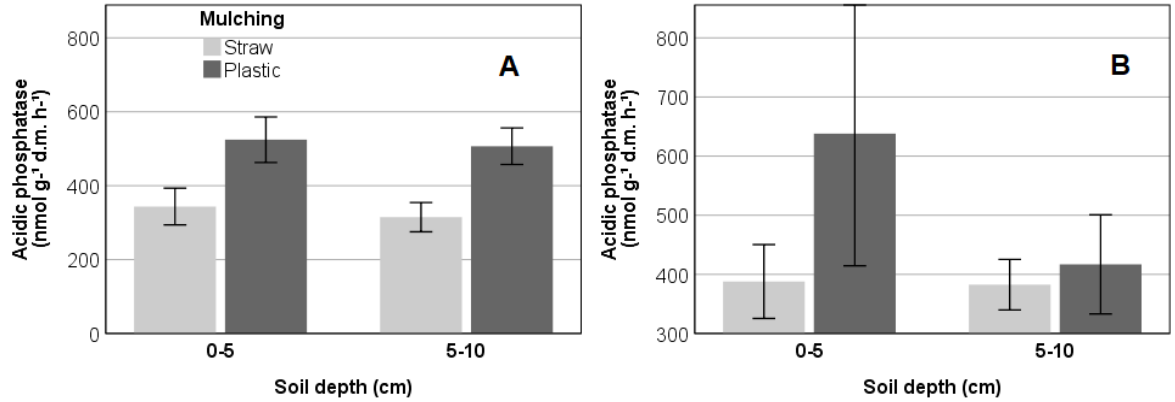

Figure S6. Acidic phosphatase activity differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

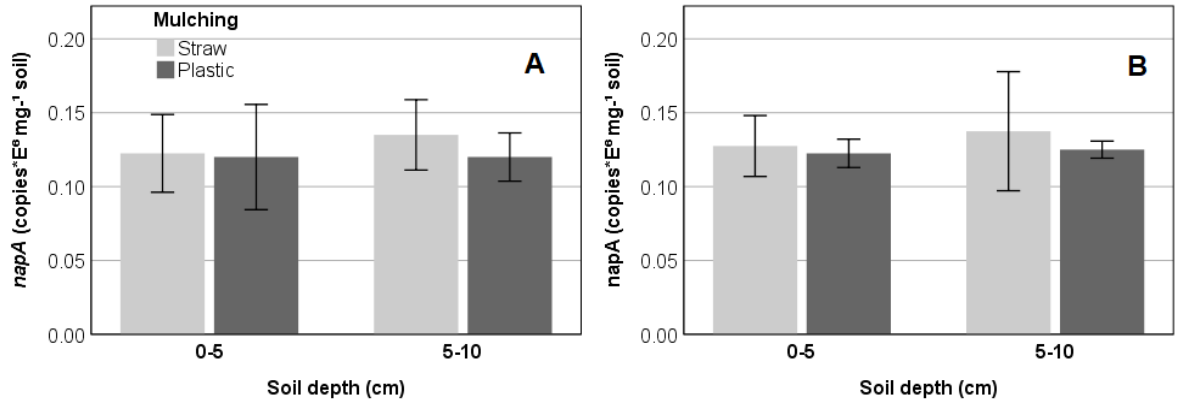

Figure S7. *napA* genes differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

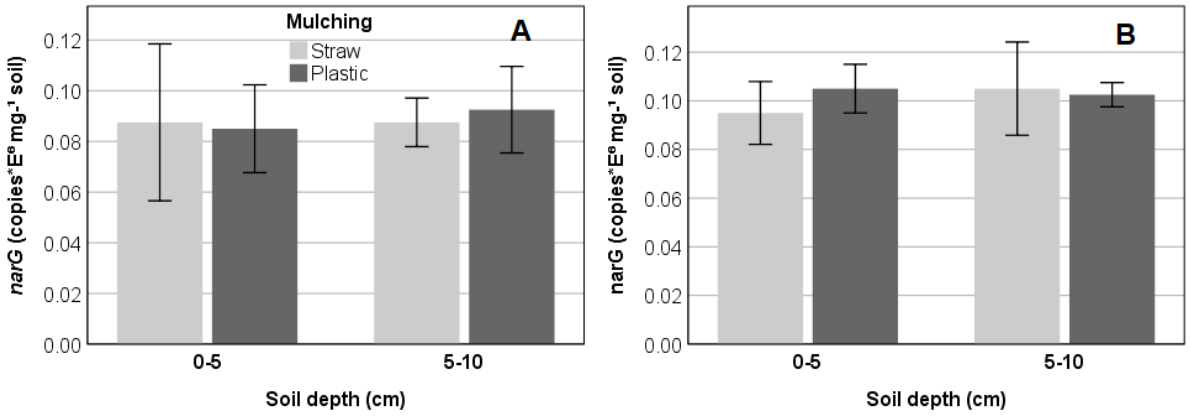

Figure S8. *narG* genes differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

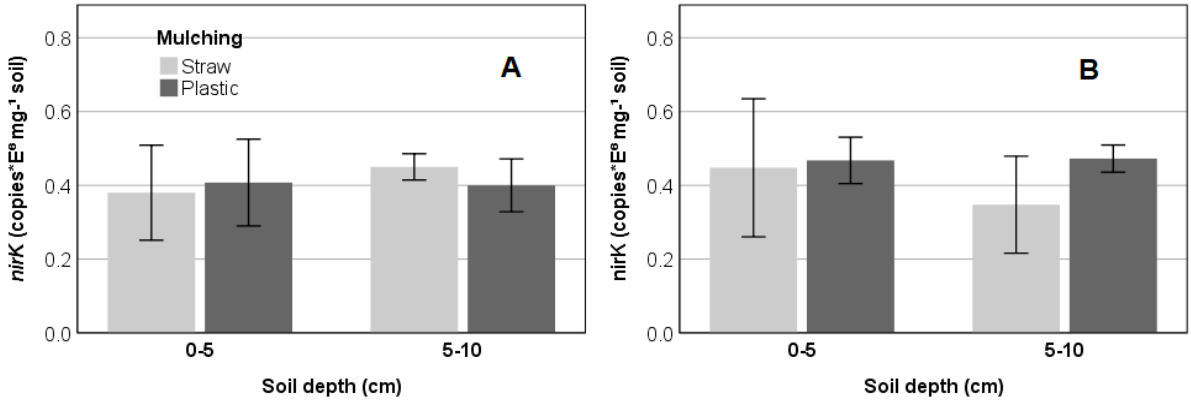

Figure S9. *nirK* genes differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

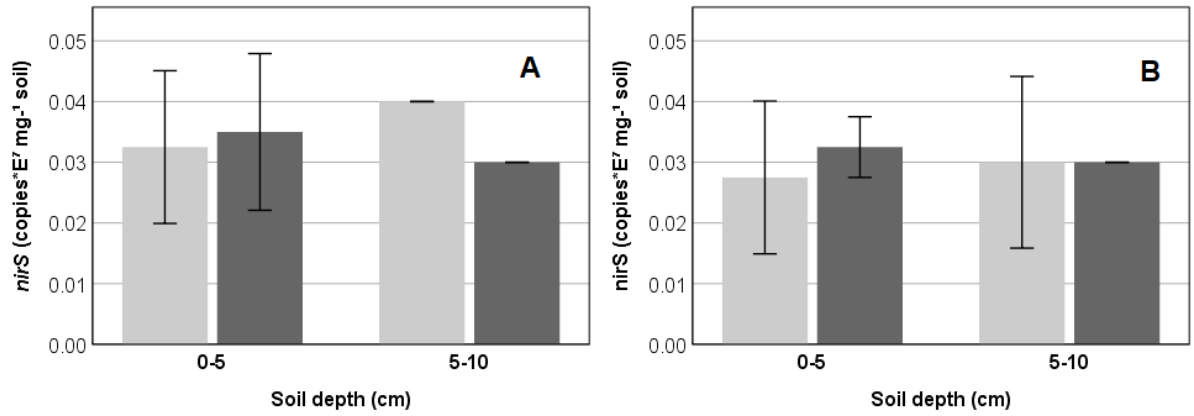

Figure S10. *nirS* genes differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

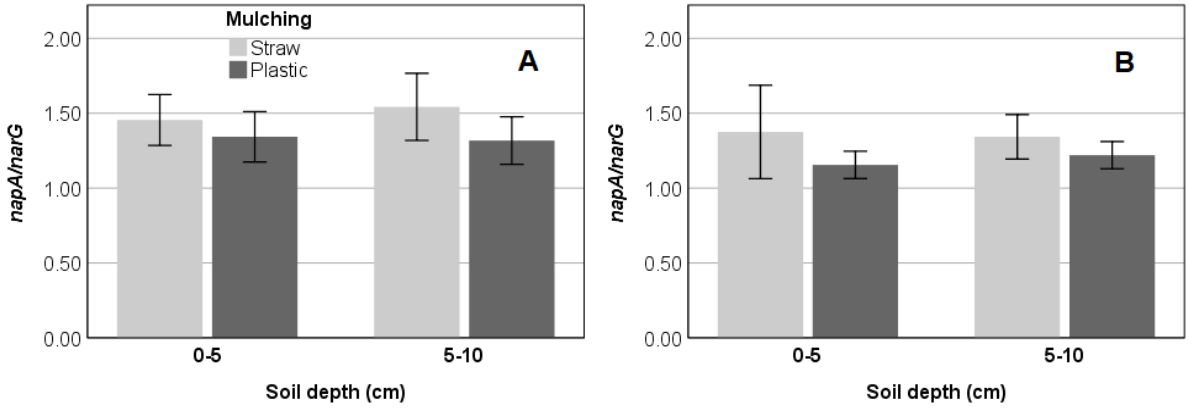

Figure S11. *napA/narG* ratios differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

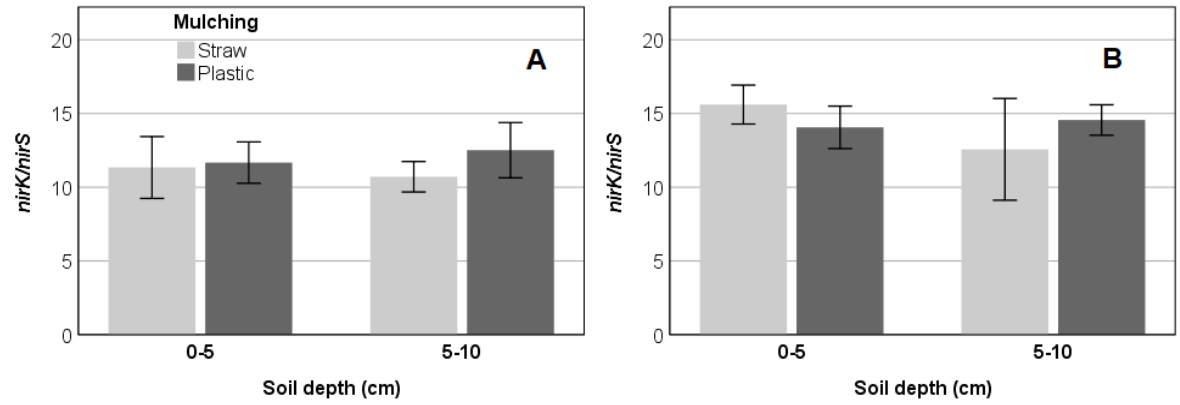

Figure S12. *nirK/nirS* ratios differentiated by mulching system (Straw vs. Plastic) and by soil depth (0–5 and 5–10 cm). Graphic A: Corresponds to samples collected in April (S1) and graphic B, to samples collected in May (S2).

Table S1. Primers and standard species used for real time PCR of 16S rRNA and functional genes [1–5].

Gene	Primer	Sequence	Standard species	Reference
16S rRNA	16S-f	5′-GGT AGT CYA YGC MST AAA CG-3′	*Pseudomonas putida*	[1]
	16S-r	5′-GAC ARC CAT GCA SCA CCT G-3′		
nirS	nirS cd3af	5′-GTS AAC GTS AAG GAR ACS GG-3′	*Pseudomonas stutzeri*	[2,3]
	nirS R3cd	5′-GAS TTC GGR TGS GTC TTG A-3′		
nirK	nirK 876	5′-ATY GGC GGV CAY GGC GA-3′	*Azospirillium irakense*	[2,4]
	nirK 5R	5′-GCC TCG ATC AGR TTR TGG-3′		
napA	napA 3F	5′-TGG ACV ATG GGY TTY AAY C-3′	*Pseudomonas aeruginosa* PA 01	[5]
	napA 4R	5′-ACY TCR CGH GCV GTR CCR CA-3′		
narG	narG-f	5′-TCG CCS ATY CCG GCS ATG TC-3′	*Pseudomonas aeruginosa* PA 01	[5]
	narG-r	5′-GAG TTG TAC CAG TCR GCS GAY TCS G-3′		

Table S2. Composition of the mastermix for real time PCR analysis of functional genes; all data in µL.

	16S rRNA	***nirS***	***nirK***	***napA***	***narG***
3% BSA [a]	–	0.4	0.4	0.4	0.4
DMSO	–	0.5	0.5	0.4	0.4
Primer F [b]	0.8	0.4	0.4	0.4	0.4
Primer R [b]	0.8	0.4	0.4	0.4	0.4
SYBR green mix [c]	10.0	10.0	10.0	10.0	10.0
DEPC H_2O [d]	6.8	6.7	6.7	6.8	6.8
DNA [e]	1.6	1.6	1.6	1.6	1.6
Total volume	20.0	20.0	20.0	20.0	20.0

[a] 30% bovine albumin serum solution (Sigma-Aldrich. Taufkirchen. Germany).
[b] (10 pmol/µl).
[c] InnuMix SYBR® green qPCR master mix (Analytik Jena. Jena. Germany).
[d] Diethyl pyrocarbonate (DEPC)-treated distilled H_2O (Karl-Roth. Karlsruhe. Germany).
[e] Dilution of DNA extract: 1:50.

Table S3. Thermocycler temperature programs for real time PCR analysis of functional genes**.**

Temperature	Duration	Cycles
16S rRNA according to Bach et al. [1]**.**		
95 °C	10 min.	1
95 °C	20 sec.	35
60 °C	1 min.	
72 °C	30 sec.	
nirS according to Braker et al. [2], Throbäck et al. [3]**.**		
95°C	10 min.	1
94°C	45 sec.	39
57°C	45 sec.	
72 °C	45 sec.	
95°C	15 sec.	1
60°C	30 sec.	
95 °C	15 sec.	
nirK according to Braker et al. [2], Henry et al. [4]**.**		
95°C	10 min.	1
95°C	15 sec.	5 (−1°C; 30s)
63°C	30 sec.	
72 °C	30 sec.	
95°C	15 sec.	40
58°C	30 sec.	
72 °C	30 sec.	
napA according to Bru et al. [5]**.**		
95°C	10 min.	1
95°C	15 sec.	5 (−1°C; 30s)
61°C	30 sec.	
72 °C	30 sec.	
95°C	30 sec.	40
56°C	30 sec.	
72 °C	30 sec.	
80 °C	30 sec.	
narG according to Bru et al. [5].		
95°C	10 min.	1
95°C	15 sec.	5 (−1°C; 30s)
63°C	30 sec.	
72 °C	30 sec.	
95°C	30 sec.	40
58°C	30 sec.	
72 °C	30 sec.	
80 °C	30 sec.	

melting curve 60–95°C.

References

1. Bach, H.J.; Tomanova, J.; Schloter, M.; Munch, J.C. Enumeration of total bacteria and bacteria with genes for proteolytic activity in pure cultures and in environmental samples by quantitative PCR mediated amplification. *J. Microbiol. Methods* **2002**, *49*, 235–245.
2. Braker, G.; Fesefeldt, A.; Witzel, K.P. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Appl. Environ. Microbiol.* **1998**, *64*, 3769–3775.
3. Throbäck, I.N.; Enwall, K.; Jarvis, A.; Hallin, S. Reassessing PCR primers targeting *nirS, nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.* **2004**, *49*, 401–417.
4. Henry, S.; Baudoin, E.; López-Gutiérrez, J.C.; Martin-Laurent, F.; Brauman, A.; Philippot, L. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *J. Microbiol. Methods* **2004**, *59*, 327–335.
5. Bru, D.; Sarr, A.; Philippot, L. Relative abundances of proteobacterial membrane-bound and periplasmic nitrate reductases in selected environments. *Appl. Environ. Microbiol.* **2007**, *73*, 5971–5974.