Supplementary Information

Losses in microbial functional diversity reduce the rate of key soil processes

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Gene	Primers	Sequence (5' –3')	Thermal conditions	Reference
qPCR				
amoA (AOA)	CrenamoA23f CrenamoA616r	ATGGTCTGGCTWAGACG GCCATCCATATGTATGTCCA	95°C, 10 min, 39 cycle 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, 40 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Hallin et al., 2009.
nosZ	nosZ2F nosZR	CGC RAC GGC AAS AAG GTS MSS GT CAK RTG CAK SGC RTG GCA GAA	95°C, 15 min, 1 cycle 95°C for 15 s, 65 to 60°C for 30 s (- 1°C by cycle), 72°C for 30 s, 80°C for 15 s, 6 cycles 95°C for 15 s, 60°C for 30 s, 72°C for 30 s, 80°C for 15 s, 40 cycles 95°C for 15 s, 60 to 95°C, 1 cycle	Henry et al., 2006
pmoA	pmoA-189F pmoA-650R	GGN GAC TGG GAC TTC TGG GAA SGC NGA GAA GAA SGC	95°C, 180S, 1 cycle 95°C for 15 s, 53°C for 15 s, 72°C for 30 s, 40 cycles 83°C for 15 s, 1cycle	Bourne et al., 2001
TRFLP				
amoA	FAM - CrenamoA23F CrenamoA616R	ATG GTC TGG CTW AGA CG GCCATCCATCTGTATGTCCA	95°C for 5 min, 35 cycle 95°C for 30 s, 56°C for 30 s, 72°C for 60 s, 72°C for 10 min, 1 cycle	Tourna et al., (2008)
nosZ	VIC nosZ 1211F nosZ 1917R	CGYTGTTCMTCGACAGCCA CATGTGCAGNGCRTGGCAGAA	95°C for 5 min, 30 cycle 95°C for 60 s, 55°C for 30 s, 72°C for 60 s, 72°C for 7 min, 1 cycle	Scala & Kerkof, 1998.
pmoA	VIC- pmoA-189F pmoA-650R	GGN GAC TGG GAC TTC TGG ACGTCCTTACCGAAGGT	96°C for 5 min, 30 cycles 94°C for 60 s, 56°C for 60 s, 72°C for 60 s, 72°C for 5 min, 1 cycle	Bourne et al., 2001

Table S1. Details of QPCR primers and PCR cycling conditions used in this study.

Table S2. Spearman correlation between specialized functions and the functional diversity of microbial groups responsible for performing associated functions in two soil types. Original soil (DC) are not included in statistical analyses. P-values as follows: $^{a}P = 0.06$; $^{*}P<0.01$ and $^{**}P < 0.001$, respectively.

Diversity of	Function	Site A	Site B
amoA	NO ₃ production	0.94**	0.86**
nosZ	N ₂ O flux	-0.81**	-0.83**
pmoA	CH ₄ flux	-0.42 ^a	-0.77**

Table S3. Partial correlation (Spearman) between diversity of different microbial groups and their associated functions controlling for biomass (determined by qPCR using similar genes used for diversity analysis for different microbial groups) and dissolved organic C (Fig S2), simultaneously. P-values as follows: **P <0.001. Original soil (DC) are not included in statistical analyses. Only significant values are shown.

		Soil A	Soil B
Microbial groups	Functions	ρ	ρ
Diversity of nitrifiers	NO ₃ production	0.85**	0.91**
Diversity of denitrifiers	N ₂ O flux	-0.55*	-0.74**
Diversity of methanotrophs	CH ₄ flux		-0.75**

Table S4. Partial correlation (Spearman) between diversity of denitrifiers and their associatedfunctions N2O production controlling for nitrate production. P-values as follows: **P <0.001.</td>Original soil (DC) are not included in statistical analyses.

		Soil A	Soil B
Microbial groups	Functions	ρ	ρ
Diversity of denitrifiers	N ₂ O flux	-0.77**	-0.58**



Figure S1. Mean (\pm SE) values for specialized functions across different dilutions from two sites. DC represents the original soil (not included in statistical analyses). DX to D10 represent diluations from 10⁰ to 10⁻¹⁰.



Figure S2. Mean (\pm SE) values for dissolved organic C (DOC) across different dilutions from two sites. DC represents the original soil (not included in statistical analyses). DX to D10 represent dilutions from 10⁰ to 10⁻¹⁰.

Reference

- Bourne, D.G., McDonald, I. RMurrell, J.C., 2001. Comparison of pmoA PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. Applied and environmental microbiology 67, 3802-3809.
- Hallin, S., Jones, C. M., Schloter, M., Philippot, L., 2009. Relationship between N cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. The ISME Journal 3, 597-605.
- 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.
- Scala, D.J., Kerkhof, L.J., 1998. Nitrous oxide reductase (nosZ) gene-specific PCR primers for detection of denitrifiers and three nosZ genes from marine sediments. FEMS Microbiology Letters 162, 61-68.
- 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.