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Supplementary information

Effects of dry- and wet-sieving of soil on identification and interpretation of microbial community composition

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Table S1. Description of the primers used to target each community and the annealing temperature of each Q-PCR assays.

Target gene	Primer	Sequence 5'-3'	Annealing temp. (°C) and time (s)	References
Bacterial	519F	GCCAGCAGCCGCGGTAAT	58 (30 s)	Lane, 1991
16SrRNA	907R	CCGTCAATTCCTTTGAGTTT		Stubner and Meuser, 2000
Archaeal	Arch 0025F	CTGGTTGATCCTGCCAG	58 (30 s)	Vetriani et al., 1999
16SrRNA	Arch 364R	ACGGGGCGCACGAGGCGCGA		Vetriani et al., 1999
Fungal	ITS1f	TCCGTAGGTGAACCTGCGG	50 (45 s)	Gardes and Bruns, 1993
ITS	5.8s	CGCTGCGTTCTTCATCG		Vilgalys and Hester, 1990
nifH	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	62.5 (60 s)	Rösch and Bothe, 2005
	nifHRb	TGSGCYTTGTCYTCRCGGATBGGCAT		Rösch and Bothe, 2005
amoA	amoA_F	GGHGACTGGGAYTTCTGG	55.3 (30 s)	Holmes et al., 1995
Bacteria	amoA_R	CCTCKGSAAAGCCTTCTTC		Okano et al., 2004
amoA	amoAF	STAATGGTCTGGCTTAGACG	55 (35 s)	Francis et al., 2005
Archaea	amoAR	GCGGCCATCCATCTGTATGT		Francis et al., 2005
narG	NARG F	TCGCCSATYCCGGCSATGTC	63 (30 s)	López-Gutiérrez et al., 2004
	NARG R	GAGTTGTACCAGTCRGCSGAYTCSG		López-Gutiérrez et al., 2004
nirS	NIRS4Q F	GTSAACGYSAAGGARACSGG	63 (30 s)	Braker et al., 1998
	NIRS6Q R	GASTTCGGRTGSGTCTTSAYGAA		Braker et al., 1998
nosZ	nosZ1840_F	CGCRACGGCAASAAGGTSMSSGT	67 (30 s)	Henry et al., 2006
	nosZ2090_R	CAKRTGCAKSGCRTGGCAGAA		Henry et al., 2006

Table S2. Overview table of the ANOVA of the aggregate distribution with sites, soil fractions and sieving methods as factors. Significant P values ($P < 0.05$) are shown in bold.

Factors	F values	P value
Sites	0.04	0.84
Fractions	**31.93**	**9.64 10-^{10}**
Sieving	0.07	0.79
Sites:fractions	**5.45**	**0.004**
Sites:sieving	0.0043	0.95
Fractions:sieving	**26.12**	**9.83 10^{-9}**
Sites:fractions:sieving	**8.65**	**0.00024**

Table S3. Overview table of the ANOVA of the relative abundance of microbial genes, with sites, soil fractions and sieving methods as factors. Significant P values (P < 0.05) are shown in bold.

Factors		Bacteria	Fungi	nifH	AOB	AOA	narG	nirS	nosZ
Sites	F value	**296.87**	**65.35**	**277.08**	**116.70**	**6.36**	0.69	**191.4**	**147.83**
	P values	**<2 10-16**	**6.09 10-10**	**<2 10-16**	**1.99 10-13**	**0.016**	0.41	**<2 10-16**	**1.14 10-14**
Fractions	F value	**3.06**	0.55	1.01	0.94	0.88	0.49	1.06	0.73
	P values	**0.027**	0.70	0.41	0.45	0.49	0.75	0.39	0.57
Sieving	F value	0.996	1.35	3.67	**12.66**	1.36	3.34	**18.28**	**10.07**
	P values	0.324	0.25	0.06	**0.00098**	0.25	0.07	**0.0001**	**0.003**
Sites:fractions	F value	2.49	1.24	0.37	1.29	0.86	2.49	0.43	0.86
	P values	0.059	0.31	0.82	0.29	0.50	0.06	0.78	0.50
Sites:sieving	F value	0.52	1.90	0.38	0.03	0.24	0.07	0.0007	0.50
	P values	0.47	0.17	0.54	0.86	0.62	0.79	0.98	0.48
Fractions:sieving	F value	1.45	0.53	0.16	0.19	0.60	0.062	0.18	0.079
	P values	0.24	0.71	0.96	0.94	0.67	0.99	0.94	0.99
Sites:fractions:sieving	F value	0.41	1.26	1.37	0.87	1.60	0.58	0.56	0.59
	P values	0.80	0.30	0.26	0.49	0.19	0.68	0.69	0.67

Table S4. Overview table of the ANOVA of the microbial gene express as percentage of the bacterial 16S rRNA gene copies, with sites, soil fractions and sieving methods as factors. Significant P values ($P < 0.05$) are shown in bold.

Factors		nifH/16S rRNA	AOB/ 16S rRNA	narG/16S rRNA	nirS/16S rRNA	nosZ/16S rRNA
Sites	F value	**33.47**	**1391.1**	**34.31**	2.56	0.01
	P values	**9.50 10-7**	**<2.2 10-16**	**7.49 10-7**	0.12	0.91
Fractions	F value	**8.13**	**6.92**	0.85	**11.56**	**8.57**
	P values	**6.80 10-5**	**0.00025**	0.5	**2.48 10-6**	**4.94 10-5**
Sieving	F value	**4.44**	**19.63**	0.14	**47.6**	**26.84**
	P values	**0.041**	**7.13 10-5**	0.71	**2.58 10-8**	**7.54 10-6**
Sites:fractions	F value	2.33	4.86	1.61	2.59	**8.36**
	P values	0.07	0.0027	0.19	0.051	**6.12 10-5**
Sites:sieving	F value	**4.95**	0.32	2.8	1.026	0.27
	P values	**0.03**	0.58	0.1	0.32	0.61
Fractions:sieving	F value	**3.06**	3.2	0.44	**5.17**	**3.88**
	P values	**0.027**	0.02	0.78	**0.0019**	**0.01**
Sites:fractions:sieving	F value	**3.71**	1.4	0.15	2.51	**3.64**
	P values	**0.01**	0.24	0.96	0.57	**0.01**

Table S5: Overview table of the ANOVA of the relative abundance of bacterial phylum, with sites, soil fractions and sieving methods as factors. Significant P values ($P < 0.05$) are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Factors	Sites	Fractions	Sieving	Sites:fractions	Sites:sieving	Fractions:sieving	Sites:fractions:sieving
Acidobacteria		*	***	**	*		***
Actinobacteria		***	***	*	**		**
Armatimonadetes			***		*		***
Bacteriodetes		*	***				*
Chlorobi		*					
Chloroflexi	*	***	***	*			
Cyanobacteria			***		**		*
Firmicutes		*	***				***
Gemmatimonadetes			***				*
Nitrospirae	***	***		**			
Planctomycetes	***	**					
Proteobacteria	***	***					
Verrucomicrobia			***				**
WS3				*			

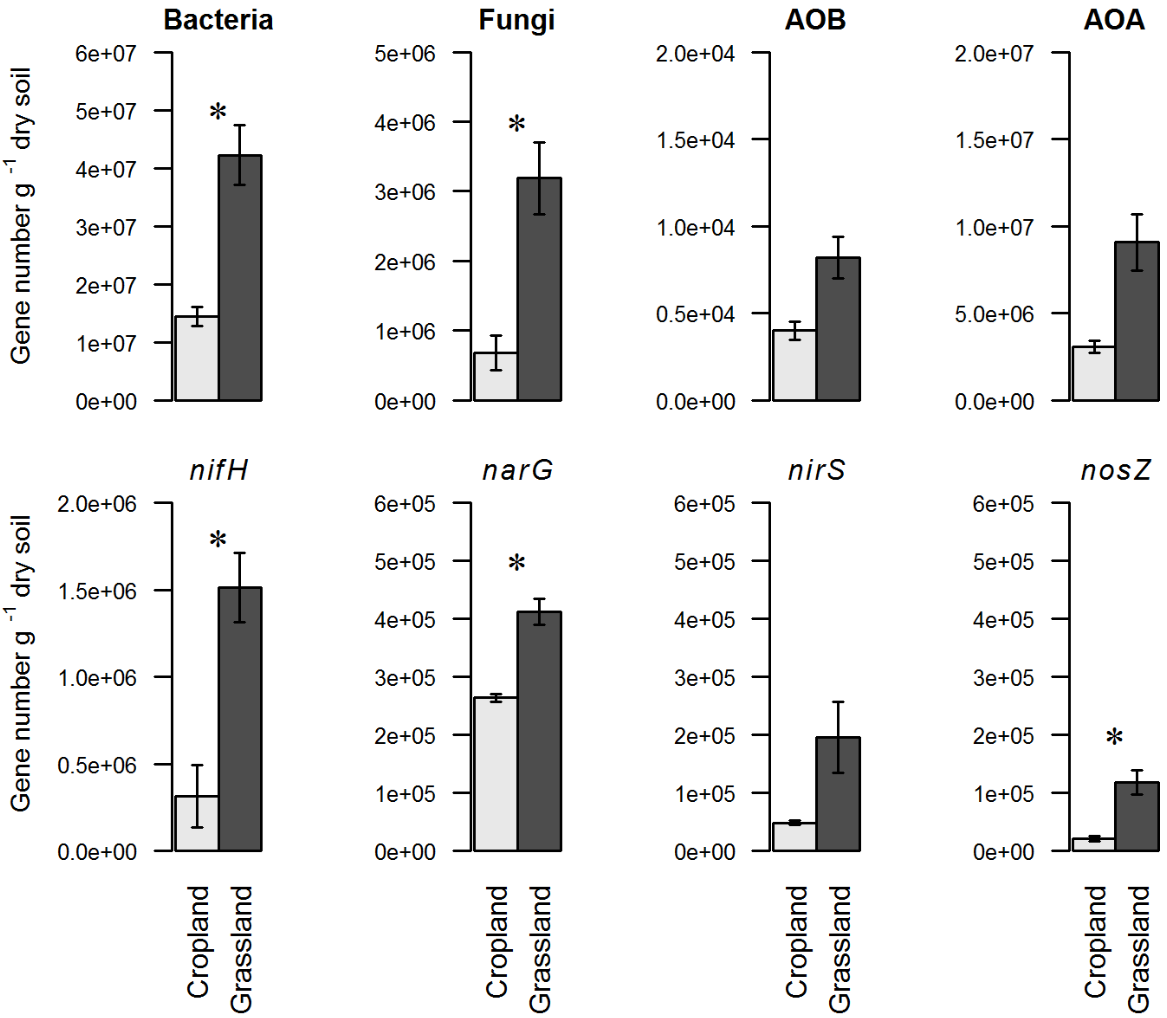

Fig. S1. Variation in microbial gene abundance of water from wet sieving method. All abundances are expressed on the basis of 1 g of dry mass of soil fractionated. Means values ± standard error (n = 3) are shown. * indicate significant ($P < 0.05$) difference between cropland and grassland for a specific gene. AOB: amoA bacteria. AOA: amoA archaea.

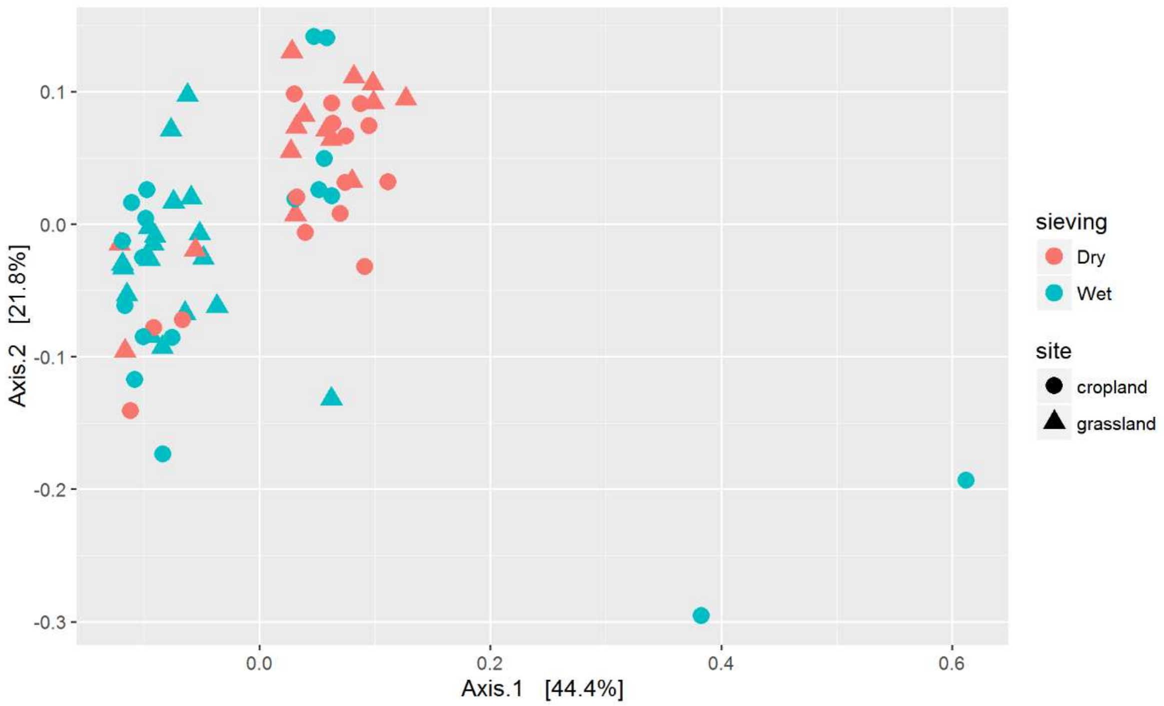

Fig. S2 PCoA of archaeal community of four soil fractions obtained by dry- or wet-sieving method and bulk soil from cropland and grassland. The PCoA was based on relative abundance of OTU and generated using Bray-Curtis distance. The samples isolated from the rest of the samples correspond to water from the wet-sieving.

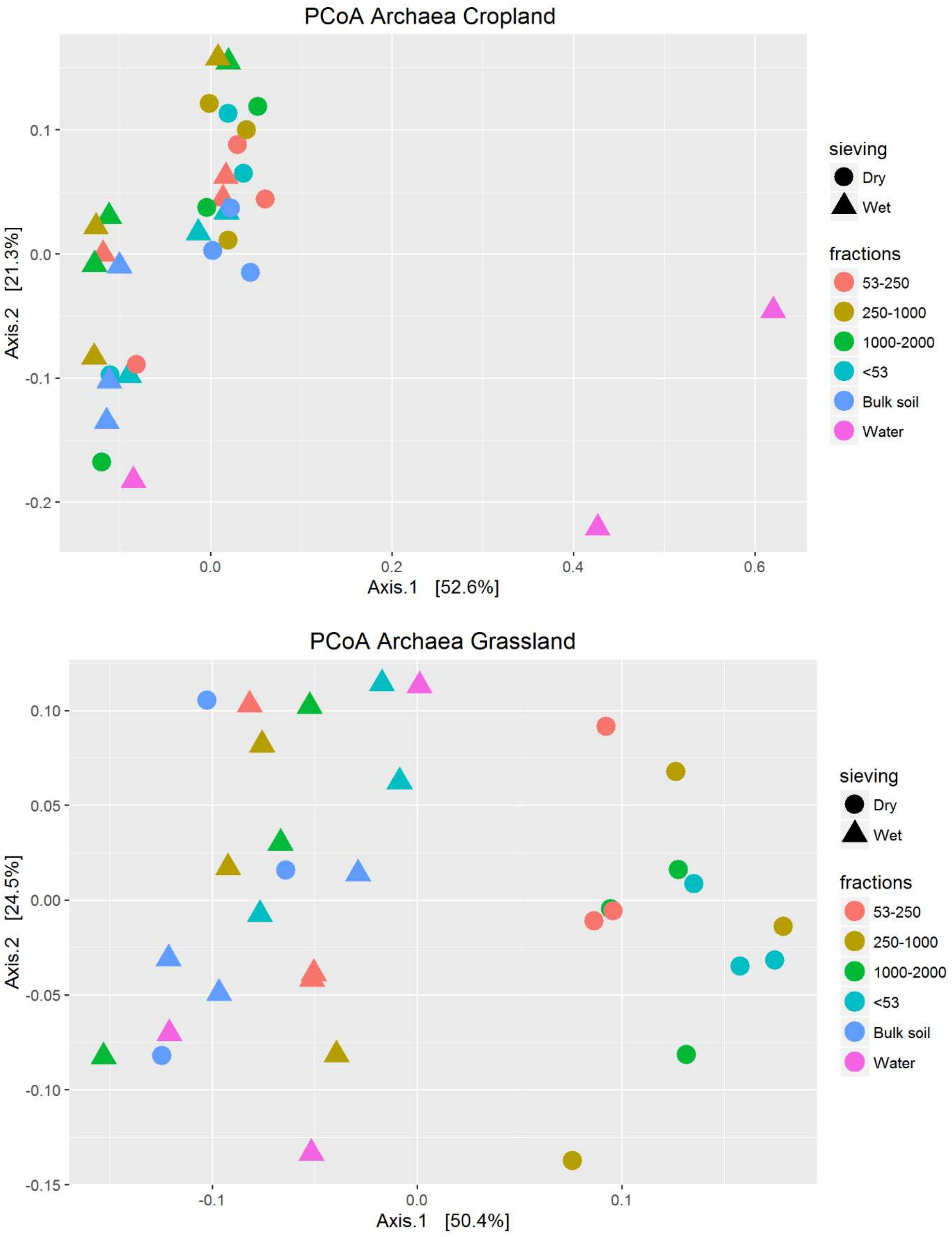

Fig. S3 PCoA of archaeal community of four soil fractions obtained by dry- or wet-sieving method and bulk soil from cropland (top) and grassland (bottom). The PCoA were based on relative abundance of OTU and generated using Bray-Curtis distance.