Supplementary Information

Soil microorganisms in a temperate agroforestry system are shaped by soil depth and fertilization rate

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Contents:

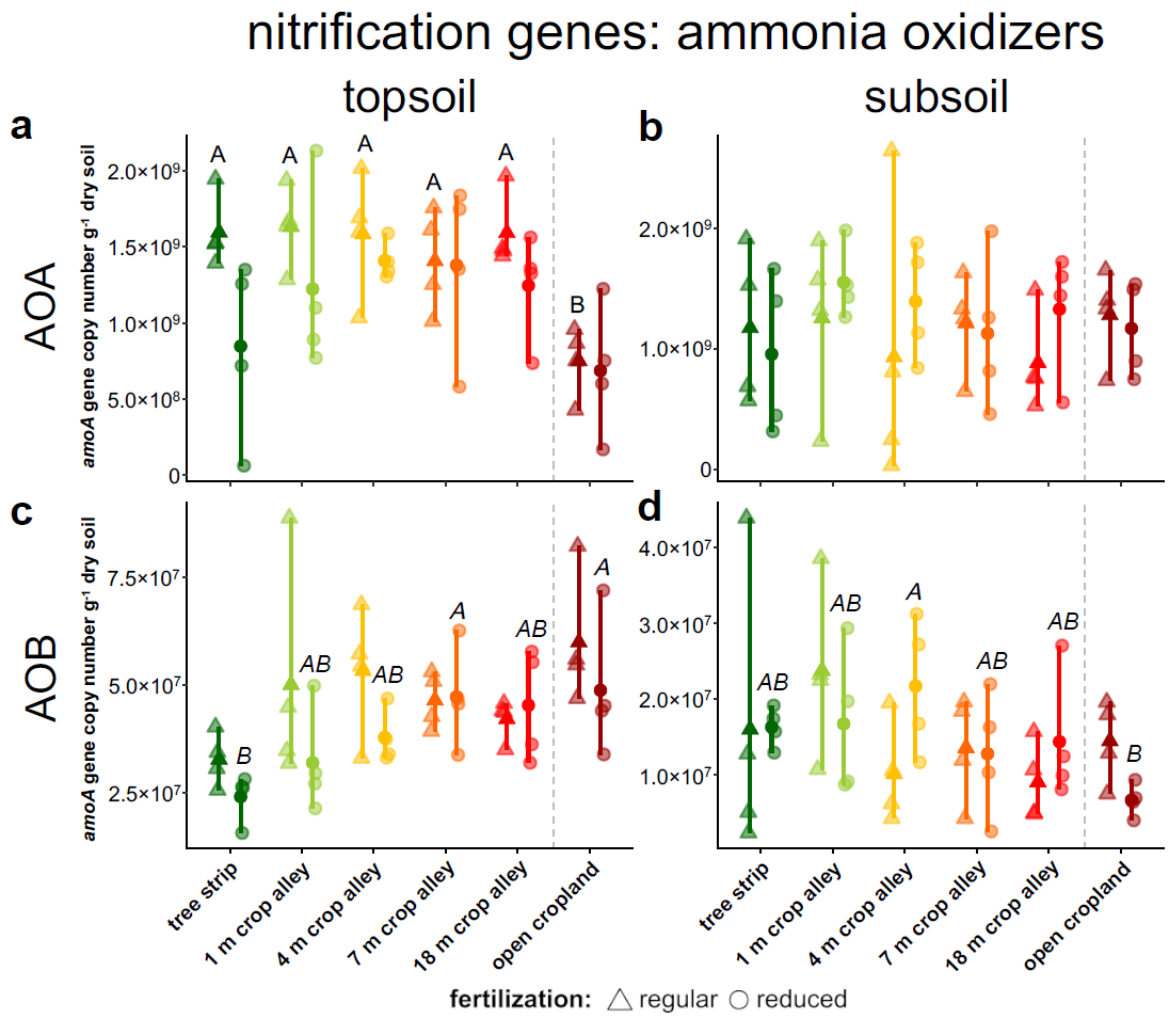

Fig. S1. Nitrification gene abundance of ammonia oxidizers in the topsoil and subsoil of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Panels **a** and **b** show the gene abundance of AOA in the topsoil and subsoil, respectively. Panels **c** and **d** show the gene abundance of AOB in the topsoil and subsoil, respectively. Transparent triangles and circles represent individual data points (n = 4). Non-transparent triangles and circles represent the means for regular and reduced fertilization, respectively. Vertical bars range from the minimum to the maximum. Asterisks indicate statistically significant differences between reduced and regular fertilization rates at each sampling position (Student's t-test or Wilcoxon Rank Sum test at $p < 0.05$). Different letters of the same font indicate statistically significant differences among sampling positions within each fertilization rate (generalized linear models (GLM) at $p < 0.05$, p-values were adjusted using the Tukey method). The absence of asterisks or letters indicates the absence of statistically significant differences. AOA = ammonia-oxidizing archaea, AOB = ammonia-oxidizing bacteria.

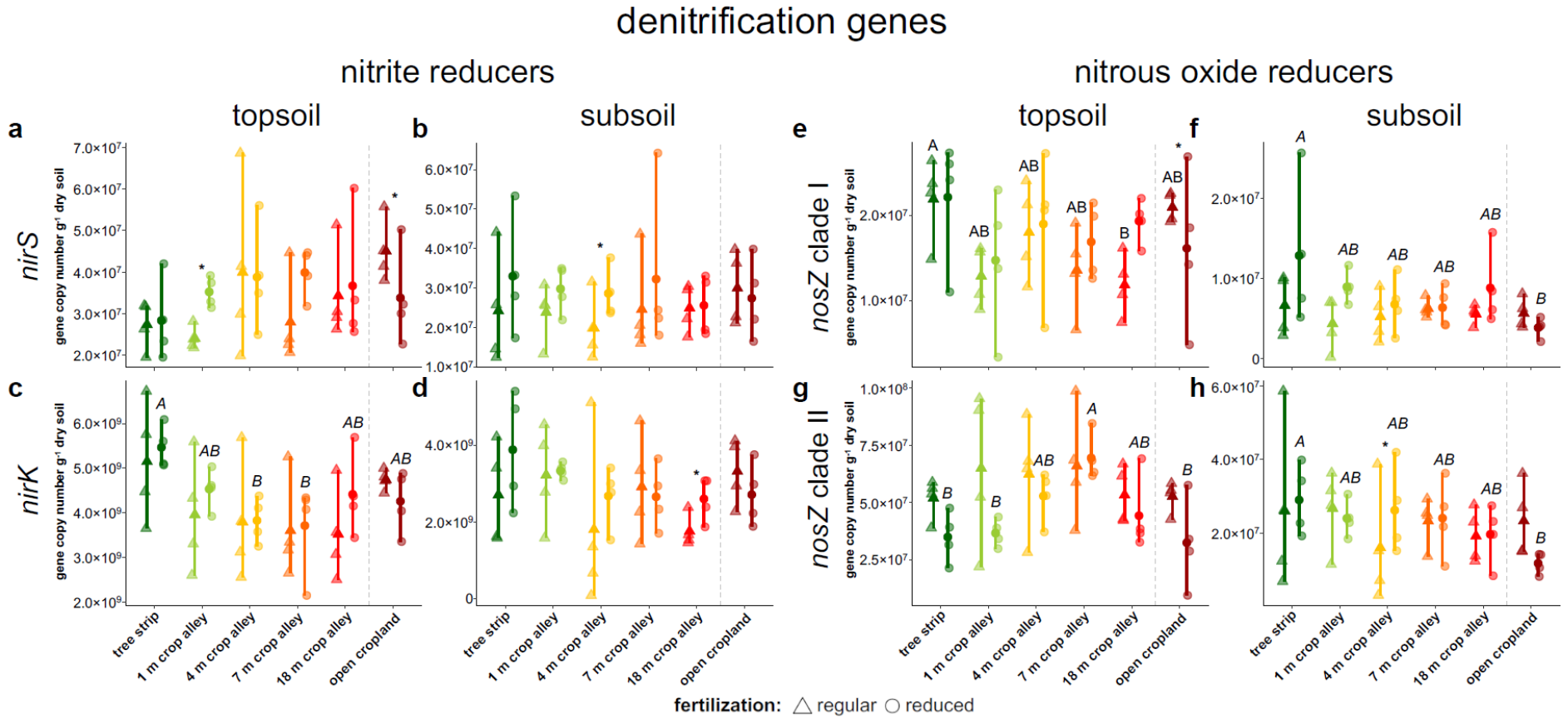

Fig. S2. Denitrification gene abundance of nitrite reducers and nitrous oxide reducers in the topsoil and subsoil of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Panels **a** and **b** show the gene abundance of *nirS* in the topsoil and subsoil, respectively. Panels **c** and **d** show the gene abundance of *nirK* in the topsoil and subsoil, respectively. Panels **e** and **f** show the gene abundance of *nosZ* clade I in the topsoil and subsoil, respectively. Panels **g** and **h** show the gene abundance of *nosZ* clade II in the topsoil and subsoil, respectively. Transparent triangles and circles represent individual data points (n = 4). Non-transparent triangles and circles represent the means for regular and reduced fertilization rates, respectively. Vertical bars range from the minimum to the maximum. Asterisks indicate statistically significant differences between reduced and regular fertilization rates at each sampling position (Student's t-test or Wilcoxon Rank Sum test at $p < 0.05$). Different letters of the same font indicate statistically significant differences among sampling positions within each fertilization rate (generalized linear models (GLM) at $p < 0.05$, p-values were adjusted using the Tukey method). The absence of asterisks or letters indicates the absence of statistically significant differences.

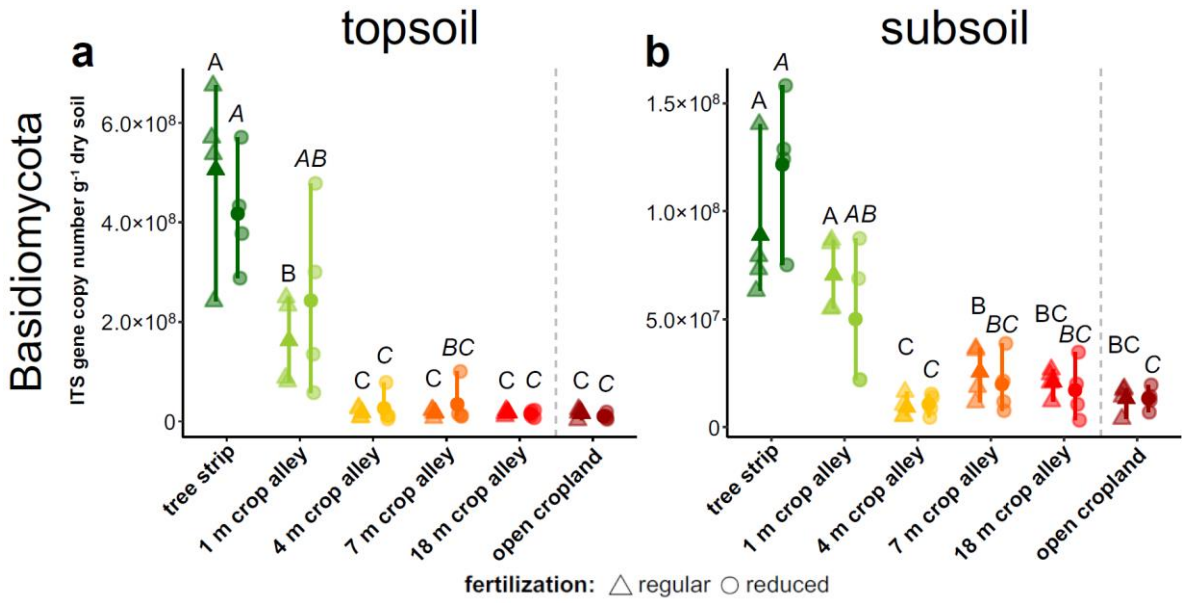

Fig. S3. Gene abundance of Basidiomycota in the topsoil (a) and subsoil (b) of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Transparent triangles and circles represent individual data points (n = 4). Non-transparent triangles and circles represent the means for regular and reduced fertilization rates, respectively. Vertical bars range from the minimum to the maximum. Asterisks indicate statistically significant differences between reduced and regular fertilization rates at each sampling position (Student's t-test or Wilcoxon Rank Sum test at $p < 0.05$). Different letters of the same font indicate statistically significant differences among sampling positions within each fertilization rate (generalized linear models (GLM) at $p < 0.05$, p-values were adjusted using the Tukey method). The absence of asterisks or letters indicates the absence of statistically significant differences.

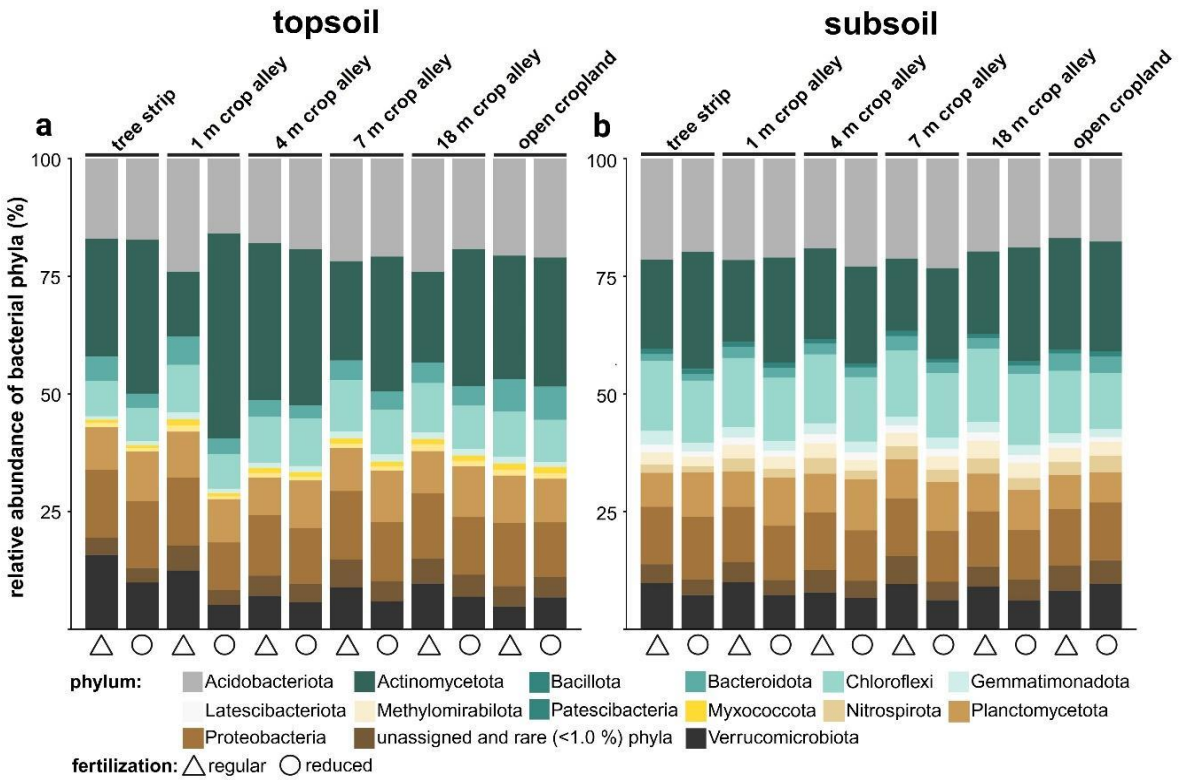

Fig. S4. Community composition of bacteria in the topsoil (a) and subsoil (b) of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Panels a and b show the mean relative abundance of bacterial classes in the topsoil and subsoil, respectively. Triangles and circles represent regular and reduced fertilization rates, respectively.

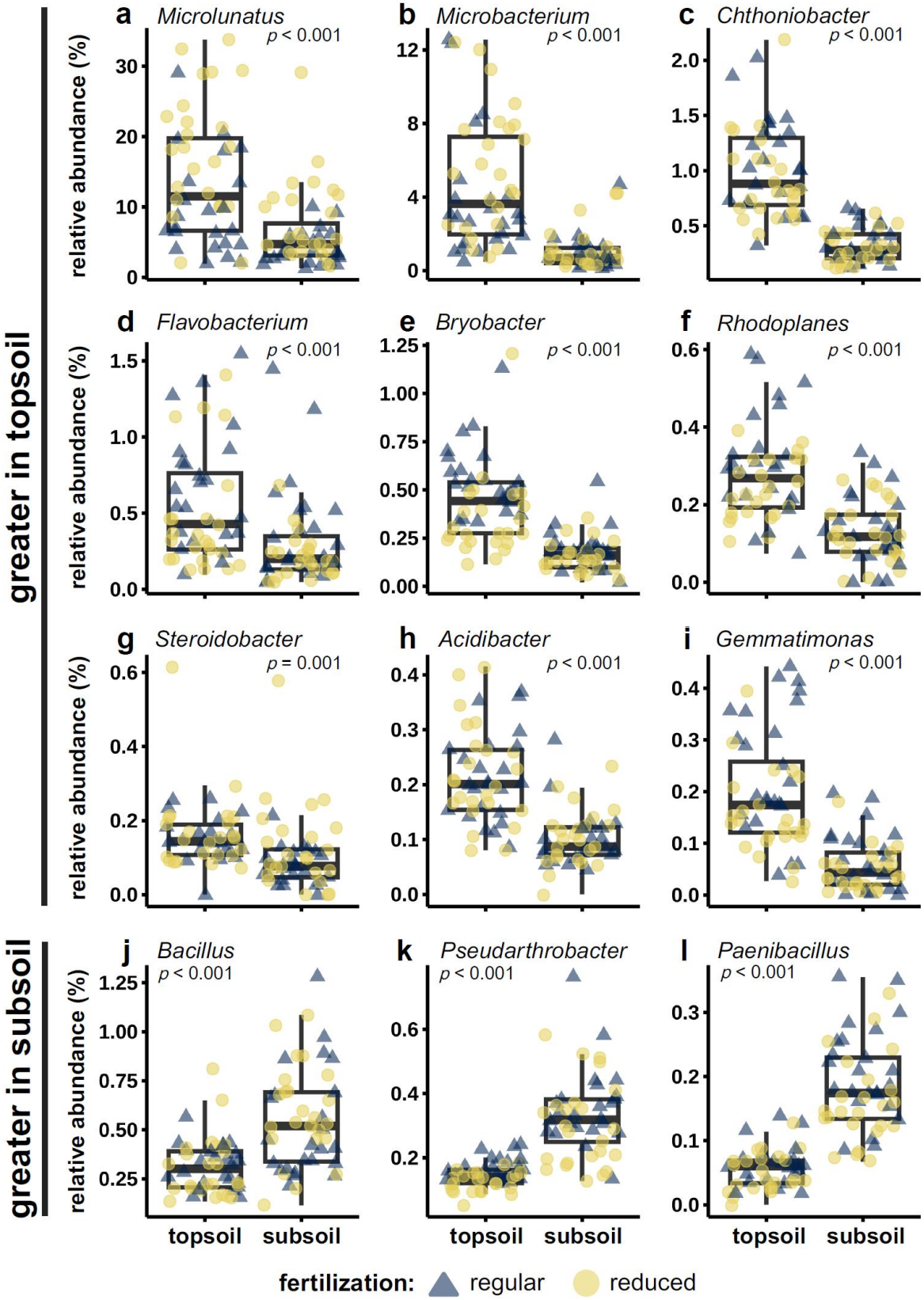

Fig. S5. Relative abundance of bacterial genera altered by soil depth in paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Triangles and circles represent individual data points (n = 48). p-values were obtained using linear models for differential abundance analysis (LinDA).

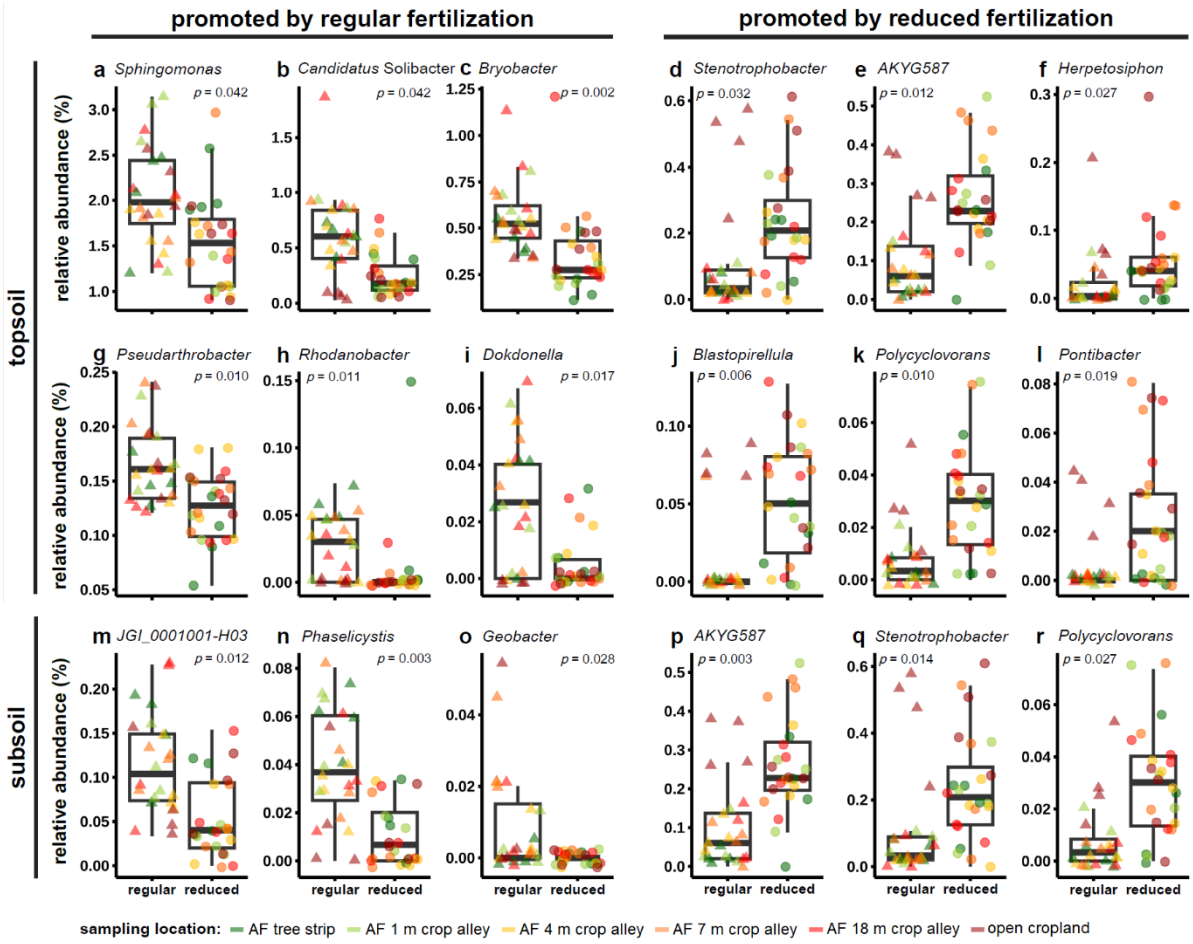

Fig. S6. Relative abundance of bacterial genera promoted by regular (a – c, g – i, m – o) and reduced fertilization rates (d – f, j – l, p – r) in the topsoil (a – l) and subsoil (m – r) of paired temperate alley-cropping agroforestry and reference open-cropland systems in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Triangles and circles represent individual data points (n = 24). p-values were obtained using linear models for differential abundance analysis (LinDA).

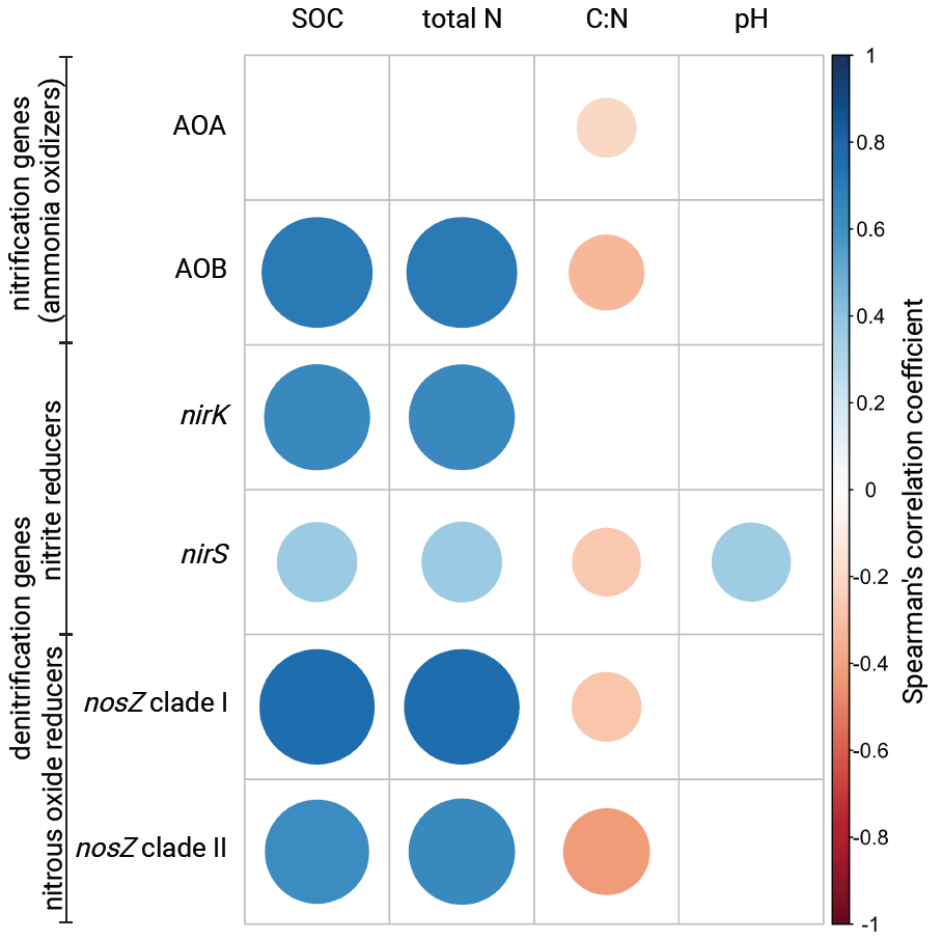

Fig. S7. Relationships between abundance of soil-N-cycling genes and soil properties in the topsoil and subsoil of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Relationships were analyzed using Spearman's rank correlation test across soil depth (topsoil: 0 – 30 cm soil depth; and subsoil: 30 – 60 cm soil depth), fertilization rate (regular and reduced), and sampling position (agroforestry tree strip; 1 m, 4 m, 7 m and 18 m distances within the agroforestry crop alley; and reference open cropland) (n = 96). The diameter of the circles is proportional to the absolute magnitude of the correlation coefficient. Only statistically significant correlations at $p < 0.05$ are shown. SOC = soil organic carbon, AOA = ammonia-oxidizing archaea, AOB = ammonia-oxidizing bacteria.

Table S1. Effects of sampling depth, fertilization rate, sampling distance, and their interactions on soil microbial abundance and the abundance of soil N-cycling genes in paired temperate alley-cropping agroforestry and reference open-cropland systems in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). The effects were analyzed using Type II likelihood ratio tests from the generalized linear models (GLM). Bold fond indicates statistical significance ($p < 0.05$). AOA = ammonia-oxidizing archaea, AOB = ammonia-oxidizing bacteria.

		archaea		**bacteria**		**fungi**		**Basidiomycota**		**AOA**		**AOB**		***nirK***		***nirS***		***nosZ* clade I**		***nosZ* clade II**	
source of variance	***df***	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**	χ^2	***p*-value**
soil depth[a]	1	52.340	< **0.001**	174.844	< **0.001**	191.486	< **0.001**	16.634	< **0.001**	0.238	0.626	122.991	< **0.001**	29.884	< **0.001**	9.514	**0.002**	105.770	< **0.001**	83.657	< **0.001**
fertilization rate[b]	1	14.439	< **0.001**	6.489	**0.011**	0.018	0.894	0.059	0.808	0.384	0.536	1.027	0.311	1.724	0.189	3.231	0.072	3.488	0.062	3.055	0.080
sampling position[c]	5	2.967	0.705	13.570	**0.019**	188.792	< **0.001**	306.132	< **0.001**	5.429	0.366	3.811	0.577	11.634	**0.040**	2.834	0.726	12.954	**0.024**	8.017	0.155
soil depth[a] × fertilization rate[b]	1	2.326	0.127	0.464	0.496	0.080	0.778	0.489	0.484	2.929	0.088	1.199	0.274	0.320	0.572	0.416	0.519	0.160	0.689	2.286	0.131
soil depth[a] × sampling position[c]	5	9.588	0.088	11.438	**0.043**	4.350	0.500	24.285	< **0.001**	7.521	0.185	16.234	**0.006**	2.908	0.714	5.133	0.400	5.752	0.331	5.962	0.310
fertilization rate[b] × sampling position[c]	5	5.890	0.317	15.788	**0.007**	5.658	0.341	1.969	0.853	3.226	0.665	8.530	0.129	4.042	0.543	5.121	0.401	6.891	0.229	7.499	**0.186**
soil depth[a] × fertilization rate[b] × sampling position[c]	5	4.090	0.537	5.970	0.309	4.982	0.418	4.606	0.466	1.845	0.870	6.997	0.221	1.737	0.884	1.828	0.872	3.066	0.690	2.889	0.717

df = degrees of freedom; χ^2 = likelihood-ratio chi-square

[a] two soil depths: topsoil (0 – 30 cm) and subsoil (30 – 60 cm)

[b] two fertilization rates (regular and reduced fertilization) as presented in Table 1

[c] six sampling positions, of which five were located in the alley-cropping agroforestry system (tree strip, 1 m, 4 m, 7 m, and 18 m distances from the trees within the crop alley) and one in the adjacent open cropland

Table S2. Relationships between distance gradient, soil microbial abundance, and the abundance of soil N-cycling genes in the topsoil and subsoil in a temperate alley-cropping agroforestry system under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m distances within the agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Spearman's rank correlation analysis was utilized to explore the relationships between distance gradient (i.e. 1 m, 4 m, 7 m, and 18 m into the crop alley of the agroforestry system), soil microbial abundance, and the abundance of soil N-cycling genes. Bold fond indicates statistical significance ($p < 0.05$). AOA = ammonia-oxidizing archaea, AOB = ammonia-oxidizing bacteria.

	topsoil				**subsoil**			
	regular		**reduced**		**regular**		**reduced**	
	ρ	***p*-value**	**ρ**	***p*-value**	**ρ**	***p*-value**	**ρ**	***p*-value**
archaea	-0.005	0.971	-0.02	0.892	-0.132	0.372	-0.105	0.478
bacteria	-0.16	0.277	-0.118	0.423	-0.235	0.107	-0.087	0.555
fungi	-0.394	**0.006**	-0.382	**0.007**	-0.424	**0.003**	-0.358	**0.013**
Basidiomycota	-0.467	**0.001**	-0.471	**0.001**	-0.401	**0.005**	-0.389	**0.006**
AOA	-0.109	0.461	-0.078	0.598	-0.175	0.235	-0.121	0.412
AOB	0.027	0.856	0.09	0.542	-0.163	0.269	-0.1	0.501
nirK	-0.168	0.253	-0.183	0.213	-0.27	0.063	-0.21	0.152
nirS	0.081	0.586	0.001	0.993	0.02	0.892	-0.071	0.63
nosZ clade I	0.042	0.778	0.024	0.87	-0.071	0.63	0.05	0.737
nosZ clade II	0.019	0.899	0.03	0.842	-0.071	0.63	-0.066	0.656

ρ = Spearman correlation coefficient

Table S3. General soil properties (soil organic C (SOC), total N, C:N, and soil pH; mean ± standard deviation) in the topsoil and subsoil of paired temperate alley-cropping agroforestry and reference open-cropland systems under regular and reduced fertilization rates in Germany. Soil samples within the agroforestry system were collected in the center of the tree strip as well as at 1 m, 4 m, 7 m, and 18 m distances from the trees within the 48-m-wide crop alley. At each sampling position (agroforestry tree strip; 1 m, 4 m, 7 m, and 18 m agroforestry crop alley; and reference open cropland), soil samples were collected in the topsoil (0 – 30 cm soil depth) and subsoil (30 – 60 cm soil depth). Different letters indicate statistically significant differences among sampling positions within each fertilization rate (generalized linear models (GLM) at $p < 0.05$, *p*-values were adjusted using the Tukey method). The absence of letters indicates the absence of statistically significant differences. Vertically paired (regular & reduced) bold means and standard deviations indicate statistically significant differences between fertilization rates at each sampling position (Student's *t*-test or Wilcoxon Rank Sum test at $p < 0.05$).

	fertilization rate	agroforestry system					open cropland
		tree strip	1 m	4 m	7 m	18 m	
topsoil (0 – 30 cm)							
SOC (%)	regular	1.95 ± 0.40 a	1.47 ± 0.13 ab	1.66 ± 0.38 ab	**1.41 ± 0.07** b	1.45 ± 0.16 ab	1.60 ± 0.06 ab
	reduced	2.04 ± 0.21 a	1.69 ± 0.10 b	1.63 ± 0.11 b	**1.57 ± 0.12** b	1.56 ± 0.08 b	1.71 ± 0.08 b
total N (%)	regular	0.18 ± 0.03	**0.15 ± 0.01**	0.18 ± 0.04	**0.15 ± 0.01**	0.15 ± 0.02	0.16 ± 0.01
	reduced	0.20 ± 0.02 a	**0.18 ± 0.01** ab	0.17 ± 0.01 ab	**0.17 ± 0.01** ab	0.17 ± 0.01 b	0.17 ± 0.01 ab
C:N	regular	10.66 ± 0.46 a	9.51 ± 0.30 bc	9.40 ± 0.22 bc	9.32 ± 0.17 c	9.47 ± 0.15 bc	10.06 ± 0.42 ab
	reduced	10.42 ± 0.17 a	9.51 ± 0.35 b	9.31 ± 0.25 b	9.18 ± 0.14 b	9.43 ± 0.31 b	10.21 ± 0.42 a
pH	regular	**6.33 ± 0.27** b	**6.33 ± 0.24** b	**6.38 ± 0.28** b	**6.39 ± 0.41** b	**6.20 ± 0.32** b	**7.47 ± 0.06** a
	reduced	**6.93 ± 0.42**	**7.12 ± 0.36**	**7.10 ± 0.27**	**7.07 ± 0.32**	**7.03 ± 0.43**	**7.17 ± 0.08**
subsoil (30 – 60 cm)							
SOC (%)	regular	0.89 ± 0.14	0.88 ± 0.10	**0.82 ± 0.14**	**0.75 ± 0.06**	**0.72 ± 0.06**	0.65 ± 0.17
	reduced	1.05 ± 0.18	1.06 ± 0.13	**1.03 ± 0.11**	**1.04 ± 0.10**	**1.00 ± 0.10**	0.80 ± 0.18
total N (%)	regular	0.09 ± 0.01	0.09 ± 0.01	**0.08 ± 0.02**	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.05
	reduced	0.11 ± 0.02	0.11 ± 0.02	**0.10 ± 0.02**	0.10 ± 0.02	0.09 ± 0.02	0.07 ± 0.01
C:N	regular	9.61 ± 0.08	9.69 ± 0.77	10.71 ± 2.04	9.53 ± 0.50	9.87 ± 0.67	10.86 ± 0.80
	reduced	9.79 ± 0.59	10.07 ± 1.15	10.21 ± 1.26	10.67 ± 1.21	10.92 ± 1.66	11.10 ± 0.45
pH	regular	**6.38 ± 0.15** b	6.62 ± 0.44 b	6.65 ± 0.49 b	6.71 ± 0.59 b	**6.57 ± 0.26** b	**7.70 ± 0.08** a
	reduced	**6.98 ± 0.37**	7.16 ± 0.37	7.14 ± 0.30	7.17 ± 0.32	**7.04 ± 0.40**	**7.51 ± 0.10**