Supplementary Materials

Soil bacterial diversity is positively correlated with decomposition rates during early phases of maize litter decomposition

Authors

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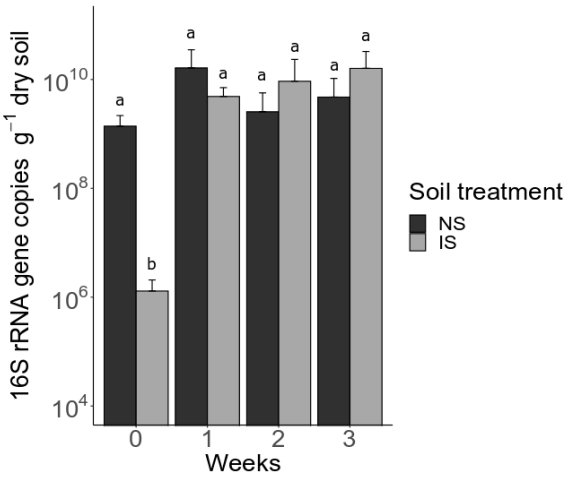
(a)



(b)

		Week 0	Week 1	Week 2	Week 3
Shannon-Weaver Diversity Index	NS	3.27 ± 0.15 *	3.23 ± 0.09 *	2.90 ± 0.09 *	2.05 ± 0.68 *
	IS	2.68 ± 0.10	0.95 ± 0.04	1.75 ± 0.52	2.28 ± 0.10
Pielou's evenness	NS	0.87 ± 0.01 *	0.95 ± 0.03 *	0.97 ± 0.01 *	0.97 ± 0.02 *
	IS	0.67 ± 0.01	0.35 ± 0.02	0.48 ± 0.13	0.70 ± 0.03

Figure S1. Results of 16S rRNA gene copy number quantification and alpha-diversity index (Shannon-Weaver Diversity Index and Pielou's evenness) calculation based on qPCR and T-RFLP profiles during the preliminary test. (a) Copy numbers (mean + standard deviation of three replicates) of the 16S rRNA gene representing bacterial abundance in the natural soil (NS) and the inoculated soil (IS) during 3 weeks of the preliminary test. Samples were taken every week (week 0, 1, 2, and 3). Black and gray colors represent NS and IS, respectively. Bacterial abundance was log-transformed before statistical tests to improve the normality of the data distribution. We performed a two-way ANOVA to investigate the interaction between the time point and soil treatment on bacterial abundance, followed by Tukey's HSD test for pairwise comparisons. Different letters indicate significant differences ($p<0.05$) according to Tukey's HSD test. (b) Alpha-diversity indices (Shannon-Weaver Diversity Index and Pielou's evenness) of the soil bacterial communities based on T-RFLP profiles in NS and IS during the preliminary test. Values represent the mean ± the standard deviation of three replicates. We performed a two-way ANOVA to investigate the interaction between the time point and soil treatment on each diversity index, followed by Tukey's HSD test for pairwise comparisons. Asterisks indicate significant differences ($p<0.05$) according to Tukey's HSD test.

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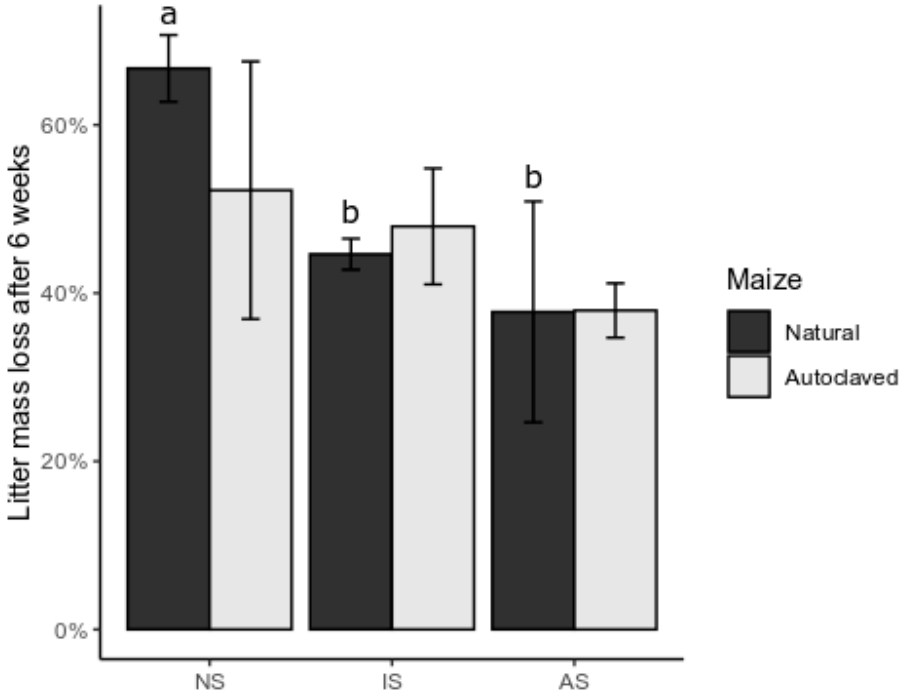

Figure S2. Percentage of litter mass loss of natural and autoclaved maize litter in soils after 6 weeks of incubation (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil). Shown are mean ± standard deviation of three replicates. We performed a one-way ANOVA to investigate the effect of maize treatments and soil treatments on the mass loss of natural and autoclaved maize litter after 6 weeks, followed by the pairwise t-test and the Benjamini-Hochberg adjustment of p-values. No significant effect of maize treatments was determined. Different letters indicate significant differences ($p<0.05$) according to the pairwise t-test.

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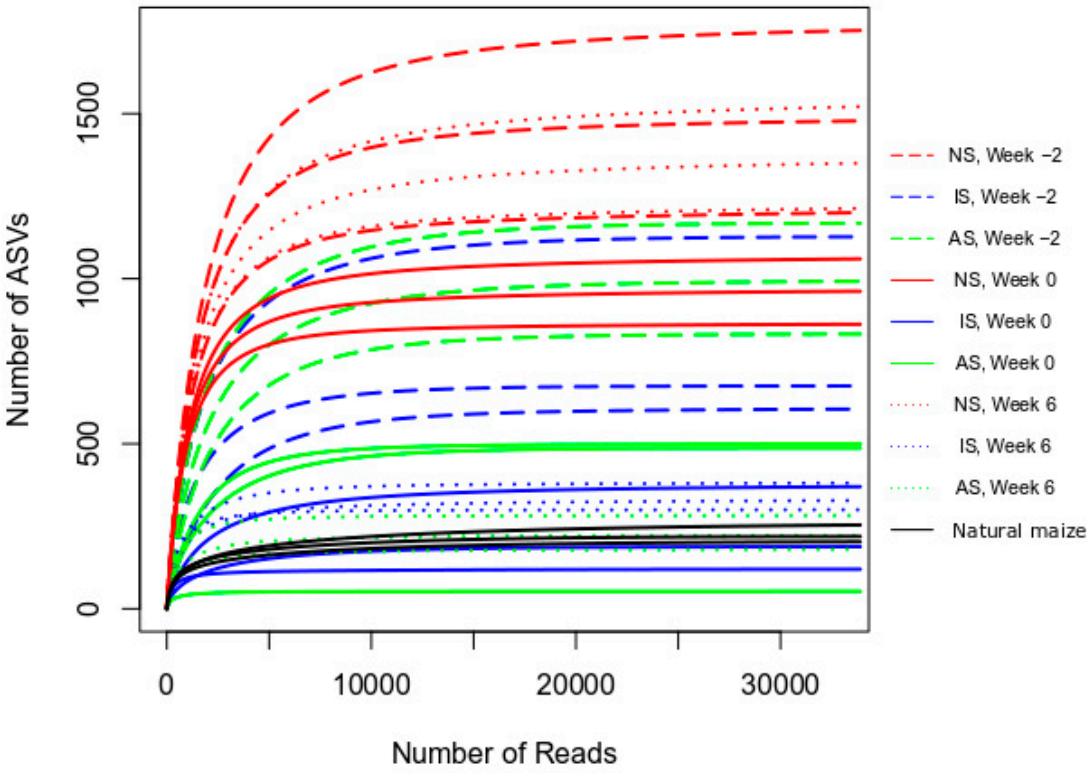

Figure S3. Rarefaction curves of the number of observed amplicon sequence variants (ASVs) in soil (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil) and natural maize samples (n=3). Soil samples were analyzed at different time points (week –2, 0, and 6), while natural maize litter prior to litter application was analyzed. The vertical and horizontal axes represent the number of ASVs and sequence reads, respectively. Red, blue, green, and black colors stand for NS, IS, AS, and natural maize litter, respectively. Long dash, solid, and dotted lines represent week –2, 0, and 6, respectively, for the soil samples. .

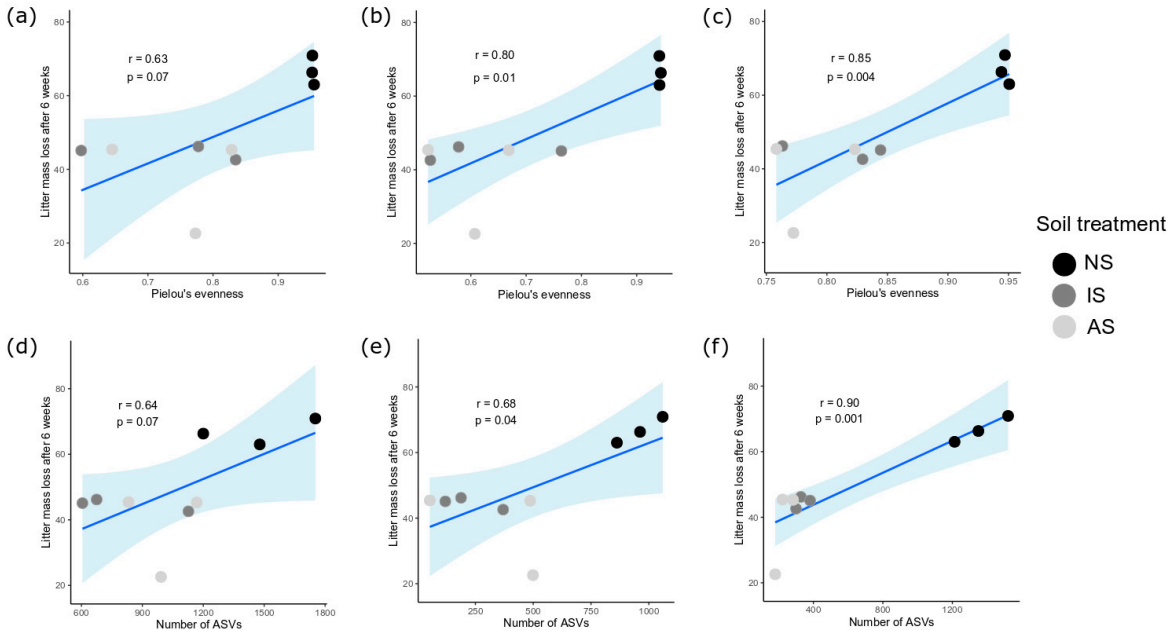

Figure S4. Scatter plots and regression lines showing the correlation between litter mass loss after 6 weeks and the alpha-diversity indices (Pielou's evenness and the number of ASVs on week -2 (a and d), week 0 (b and e), and week 6 (c and f)). Additionally, the 95% confidence interval, the Pearson's correlation coefficient (r), and p-value (p) are displayed. Black, gray and light gray dots indicate different soil treatments (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil, n=3).

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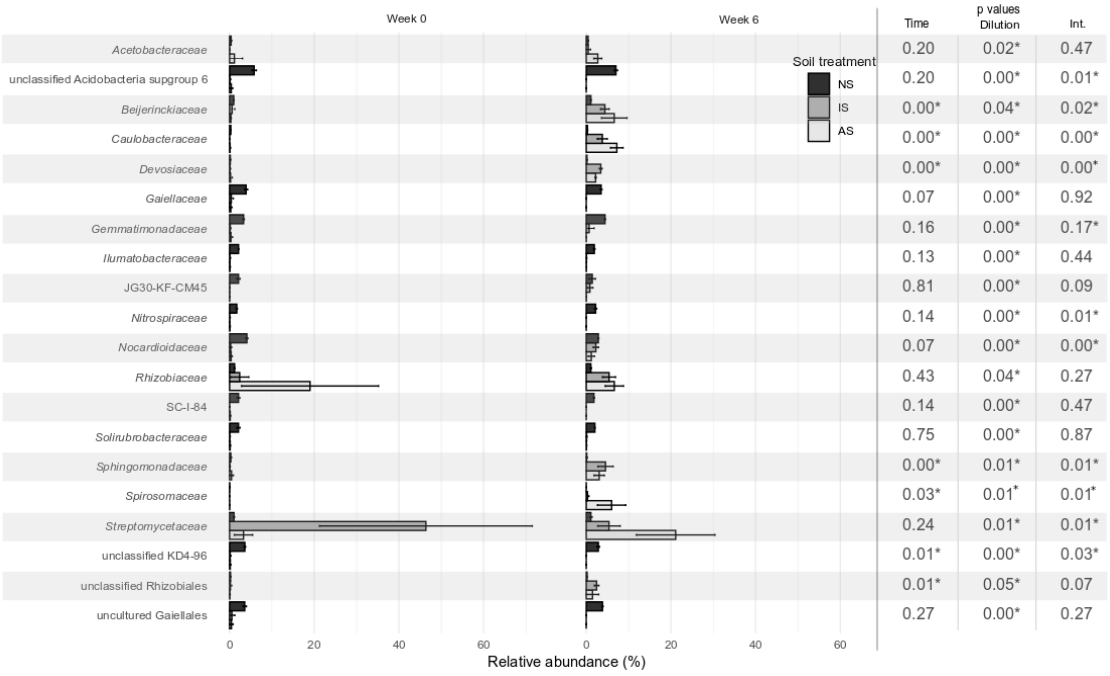

Figure S5. Relative abundance of dominant bacterial families (average relative abundance >2%) at different time points (week 0 and 6) and among soil treatments (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil). Shown are mean values ± standard deviation of three replicates. The vertical and horizontal axes represent dominant bacterial families and their relative abundance, respectively. Black, gray, and white colors indicate NS, IS, and AS, respectively. Values on the right-hand side show p-values of the two-way ANOVA, followed by Benjamini-Hochberg adjustment. Asterisks indicate significant differences ($p<0.05$) according to Tukey's HSD test.

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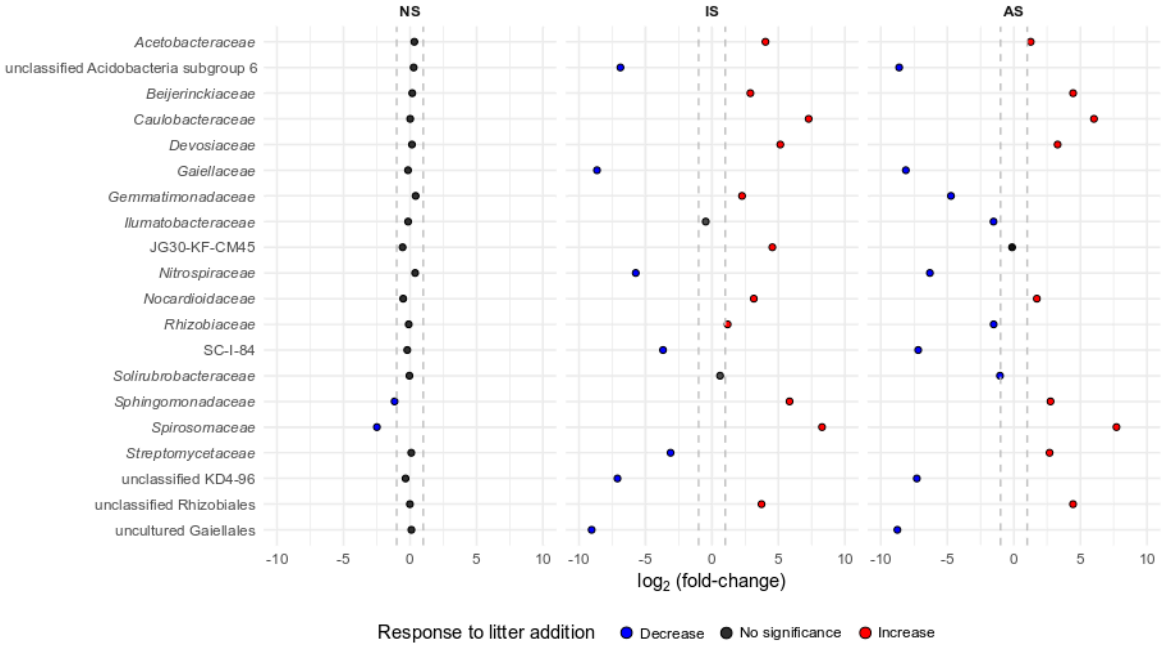

Figure S6. Fold changes in the relative abundance of dominant bacterial families (average relative abundance >2%) in response to litter addition. The fold changes (log2) were calculated by dividing the mean relative abundance of three replicates on week 6 by that on week 0. The vertical and horizontal axes represent dominant bacterial families and their fold-changes in soils (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil), respectively. Two vertical dashed lines show a threshold of fold changes (fold changes of –2 and +2). Red and blue dots indicate the bacterial families with significantly higher and lower relative abundance on week 6 than week 0.

Table S1. RNA concentration in the natural soil (NS), inoculated soil (IS), and autoclaved soil (AS) sampled on week –2 (n=3).

Soil	Replicate	RNA concentration (ng g^{-1} dry soil)
NS	1	59.88
NS	2	72.71
NS	3	94.14
IS	1	0.00
IS	2	0.28
IS	3	0.10
AS	1	0.10
AS	2	0.20
AS	3	0.29

Table S2. Primer sequences used for qPCR analysis and sequencing.

Target gene	Primer	Sequence	Reference
16S rRNA gene for qPCR	FP 16S	5′-GGTAGTCYAYGCMSTAAACG-3′	Bach *et al*. (2002)[48]
	RP 16S	5′-GACARCCATGCASCACCTG-3′	
ITS for qPCR	ITS 1	5′-TCCGTAGGTGAACCTGCGG-3′	White *et al*. (1990)[49]
	ITS 4	5′-TCCTCCGCTTATTGATATGC-3′	
16S rRNA gene for sequencing	S-D-Bact-0008-a-S-16	5′-AGAGTTTGATCMTGGC-3′	Klindworth *et al*. (2013)[51]
	S-D-Bact-0343-a-A-15	5′-CTGCTGCCTYCCGTA-3′	

Table S3. Reads obtained from the 16S rRNA gene sequencing. Values in the table show the number of raw reads and reads after quality filtering, denoising, merging, removing chimeric sequences, sequences assigned to chloroplasts at the order level, and singletons for the natural soil (NS), inoculated soil (IS), and autoclaved soil (AS) sampled on week –2, 0 and 6, and natural maize litter prior to being applied to the soils (n=3).

Sample	Replicate	Raw reads	Filtered reads	Denoised reads	Merged reads	Non-chimeric reads	Reads without chloroplast	Reads without singletons
NS, Week –2	1	232140	138656	138656	88261	76114	75951	75939
NS, Week –2	2	146807	92550	92550	56530	49764	49631	49624
NS, Week –2	3	188784	114699	114699	71817	62604	62467	62452
IS, Week –2	1	86759	52593	52593	42269	33942	33915	33899
IS, Week –2	2	122420	72327	72327	54521	46867	46653	46643
IS, Week –2	3	96466	62239	62239	56420	45506	45108	45098
AS, Week –2	1	115802	71973	71973	55666	47595	47009	46999
AS, Week –2	2	109602	66020	66020	56580	50803	48105	48099
AS, Week –2	3	128681	78522	78522	59078	49400	49142	49127
NS, Week 0	1	142445	83559	83559	48978	43324	43144	43137
NS, Week 0	2	122848	77227	77227	45193	40061	40015	40006
NS, Week 0	3	115771	68942	68942	40310	35624	35546	35530
IS, Week 0	1	108354	70021	70021	64492	48386	48356	48352
IS, Week 0	2	162905	96094	96094	84831	63763	63757	63733
IS, Week 0	3	148410	94112	94112	86103	46922	46922	46922
AS, Week 0	1	94740	62945	62945	55248	44017	43649	43584
AS, Week 0	2	87171	56144	56144	54618	40438	40438	40438
AS, Week 0	3	91505	60730	60730	54309	46882	46558	46555
NS, Week 6	1	189032	118536	118536	73905	63934	63764	63740
NS, Week 6	2	176213	104059	104059	63856	55041	54870	54851
NS, Week 6	3	153532	94737	94737	57891	50426	50274	50266
IS, Week 6	1	144396	85617	85617	78169	57042	57042	57005
IS, Week 6	2	114896	71590	71590	62358	42167	42167	42167
IS, Week 6	3	220828	139467	139467	115422	73226	73226	73226
AS, Week 6	1	170861	100926	100926	92467	59478	59478	59460
AS, Week 6	2	116606	71518	71518	65169	46576	46576	46536
AS, Week 6	3	105837	62614	62614	55070	37694	37694	37679
Fresh maize	1	190862	109485	109485	99205	69270	44126	43334
Fresh maize	2	146706	89301	89301	80606	59394	35144	34676
Fresh maize	3	192623	112238	112238	101933	71469	53644	52881

Table S4 Results of two-way PERMANOVA on the Bray-Curtis dissimilarities of soil bacterial communities in different soil treatments (NS: natural soil, IS: inoculated soil, and AS: autoclaved soil) sampled on different time points (week 0 and 6).

PERMANOVA.		
	R^2	P-value
Soil treatments	0.37	<0.01
Time points	0.09	0.01
Soil treatments*Time points	0.15	<0.01

Table S5 List of the names and the relative abundance of maize-associated ASVs that were detected in autoclaved soil (AS) on week 6

	Phylum	Family	Genus	Relative abundance %		
ASV1	Actinobacteria	*Microbacteriaceae*	*Microbacterium*	0.03	±	0.06
ASV2	Actinobacteria	*Nocardiaceae*	*Rhodococcus*	0.02	±	0.03
ASV3	Bacteroidetes	*Spirosomaceae*	*Larkinella*	0.02	±	0.03
ASV4	Proteobacteria	*Acetobacteraceae*	*Roseomonas*	0.03	±	0.06
ASV5	Proteobacteria	*Acetobacteraceae*	*Roseomonas*	0.02	±	0.03
ASV6	Proteobacteria	*Acetobacteraceae*	*Roseomonas*	0.65	±	0.41
ASV7	Proteobacteria	*Acetobacteraceae*	*Roseomonas*	0.62	±	0.45
ASV8	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.25	±	0.31
ASV9	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.27	±	0.38
ASV10	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.37	±	0.15
ASV11	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.07	±	0.12
ASV12	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.13	±	0.08
ASV13	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	0.15	±	0.26
ASV14	Proteobacteria	*Beijerinckiaceae*	*Methylobacterium*	1.65	±	2.51
ASV15	Proteobacteria	*Burkholderiaceae*	*Variovorax*	0.13	±	0.03
ASV16	Proteobacteria	*Burkholderiaceae*	*Variovorax*	0.03	±	0.06
ASV17	Proteobacteria	*Burkholderiaceae*	*Massilia*	0.30	±	0.10
ASV18	Proteobacteria	*Caulobacteraceae*	*Brevundimonas*	0.18	±	0.16
ASV19	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	0.03	±	0.06
ASV20	Proteobacteria	*Rhizobiaceae*	*Aureimonas*	0.12	±	0.16
ASV21	Proteobacteria	*Rhizobiaceae*	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	0.47	±	0.46
ASV22	Proteobacteria	*Sphingomonadaceae*	*Sphingomonas*	0.35	±	0.33
ASV23	Proteobacteria	*Sphingomonadaceae*	*Sphingomonas*	0.47	±	0.72
ASV24	Proteobacteria	*Sphingomonadaceae*	*Sphingomonas*	0.10	±	0.17
ASV25	Proteobacteria	*Sphingomonadaceae*	*Sphingomonas*	0.33	±	0.49
SUM				6.78%		

Text S1 Experimental design for the preliminary test

To determine the length of the pre-incubation period between microbial inoculation and litter addition, an incubation experiment with the natural soil (NS) and inoculated soil (IS) was carried out. First, 21 g of NS and IS were placed in Falcon™ 50 mL conical centrifuge tubes with open headspace. Under the same experimental conditions of soil moisture and room temperature as in the main text, the soils were incubated for 3 weeks. Soil samples were sampled right after the addition of the inoculum (week 0) and each of the following weeks, and stored at –20 °C for molecular analysis. For each treatment and time point, 3 parallel tubes were prepared and treated as true replicates throughout the preliminary test. Genomic DNA was extracted from 0.30 g of soil using the NucleoSpin®Soil Kit (Macherey-Nagel, Germany) following the manufacture's instruction. Soil bacterial abundance was quantified using a SYBR Green-based quantitative real-time PCR (qPCR) assay on a 7300 Real-time PCR System (Applied Biosystems, USA) as described in the main text. The optimal dilution rate of DNA extracts was 1:32 for soils. Missing values under detection limit (one sample for IS on week 2 and another sample for IS on week 3) were replaced by the values obtained with 1:64 dilution. Soil bacterial diversity was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis based on 16S rRNA gene using the primers 27f (FAM - labelled) and 1401r following the instruction by Gschwendtner et al. [100]. Briefly, PCR runs began at 94 °C for 5 min, followed by 30 cycles of amplification (94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min), and the final extension at 72 °C for 10 min. The reaction was performed in a total volume of 50 μl, containing 1×Taq buffer (Life Technologies), 2.5 mM of $MgCl_2$ (Fermentas, St. Leon Rot, Germany), 0.2 mM of dNTPs (Fermentas), 0.2 μM of each primer (Metabion), 150 μg of BSA (Sigma Aldrich), 2.5 U of Taq Polymerase (Life Technologies) and 20 ng of template DNA. The amplicons were purified using NucleoSpin®Gel and PCR Clean-up kit (Macherey Nagel, Germany) and digested with the restriction enzyme MspI (Fermentas) at 37 °C for 4 hours. The enzymatic reaction was stopped by incubating at 80 °C for 20 min. The digested amplicons were purified using NucleoSpin®Gel and PCR Clean-up kit and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA). The terminal restriction fragments (T-RFs) were separated based on their size by an ABI 3730 sequencer using MapMarker 1000 (Eurogentec, Köln, Germany) as an internal standard [100]. Size and relative abundances of the T-RFs were analyzed using GeneMapper v4.0 software (Life Technologies) and the online software T-REX (http://trex.biohpc.org/) with a binning range of 2 bp. T-RFs smaller than 50 bp and/or smaller than 1% of the total peak height were excluded. The data analysis of the T-RFLP profiles was carried out with the statistical software R (version 3.5.3) by calculating

the relative abundance of T-RFs per soil sample. Alpha diversity indices were calculated using the diversity function in the vegan package.

Reference

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