Silver and hematite nanoparticles had a limited effect on the bacterial community structure in soil cultivated with *Phaseolus vulgaris* L.

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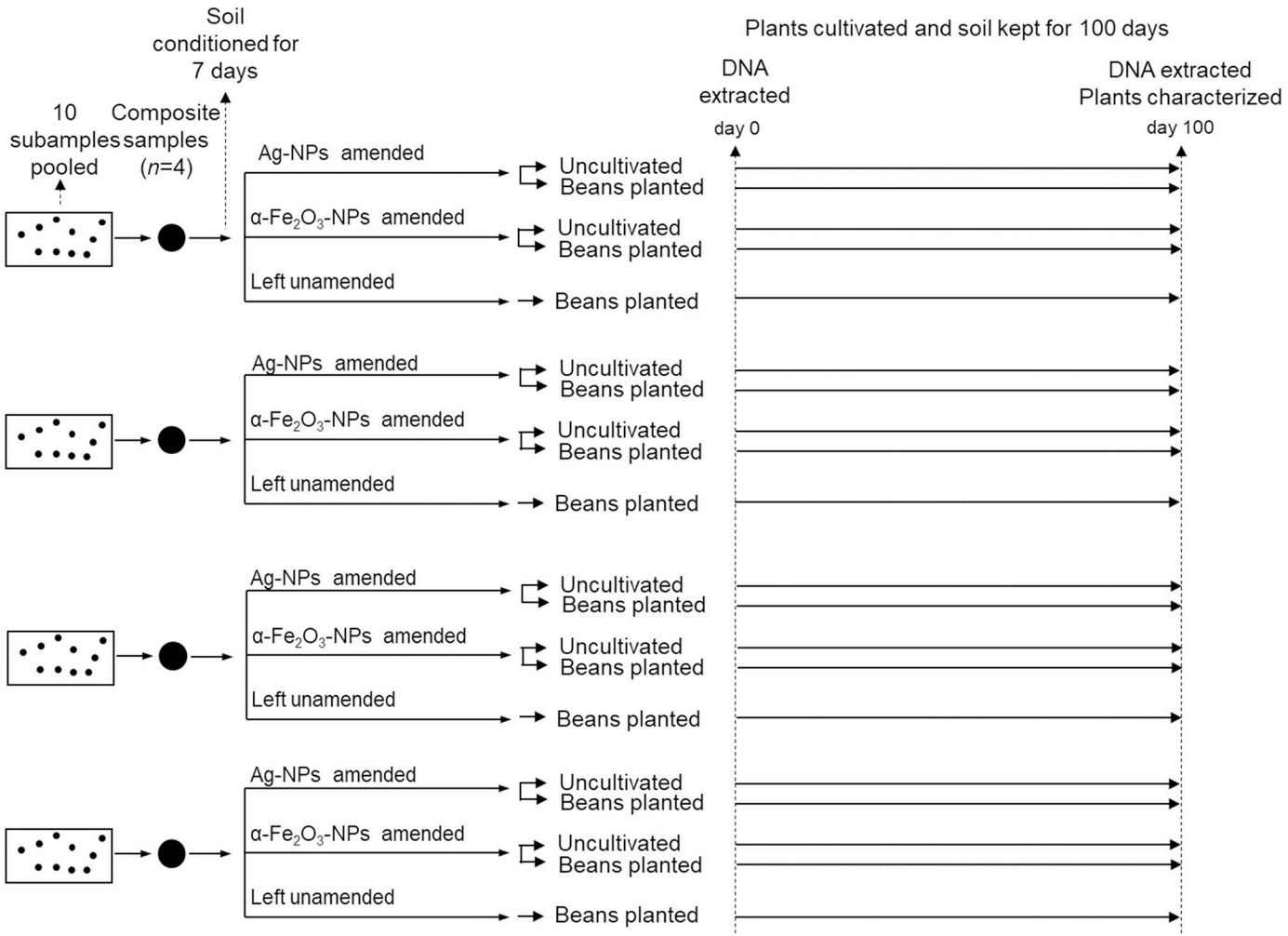
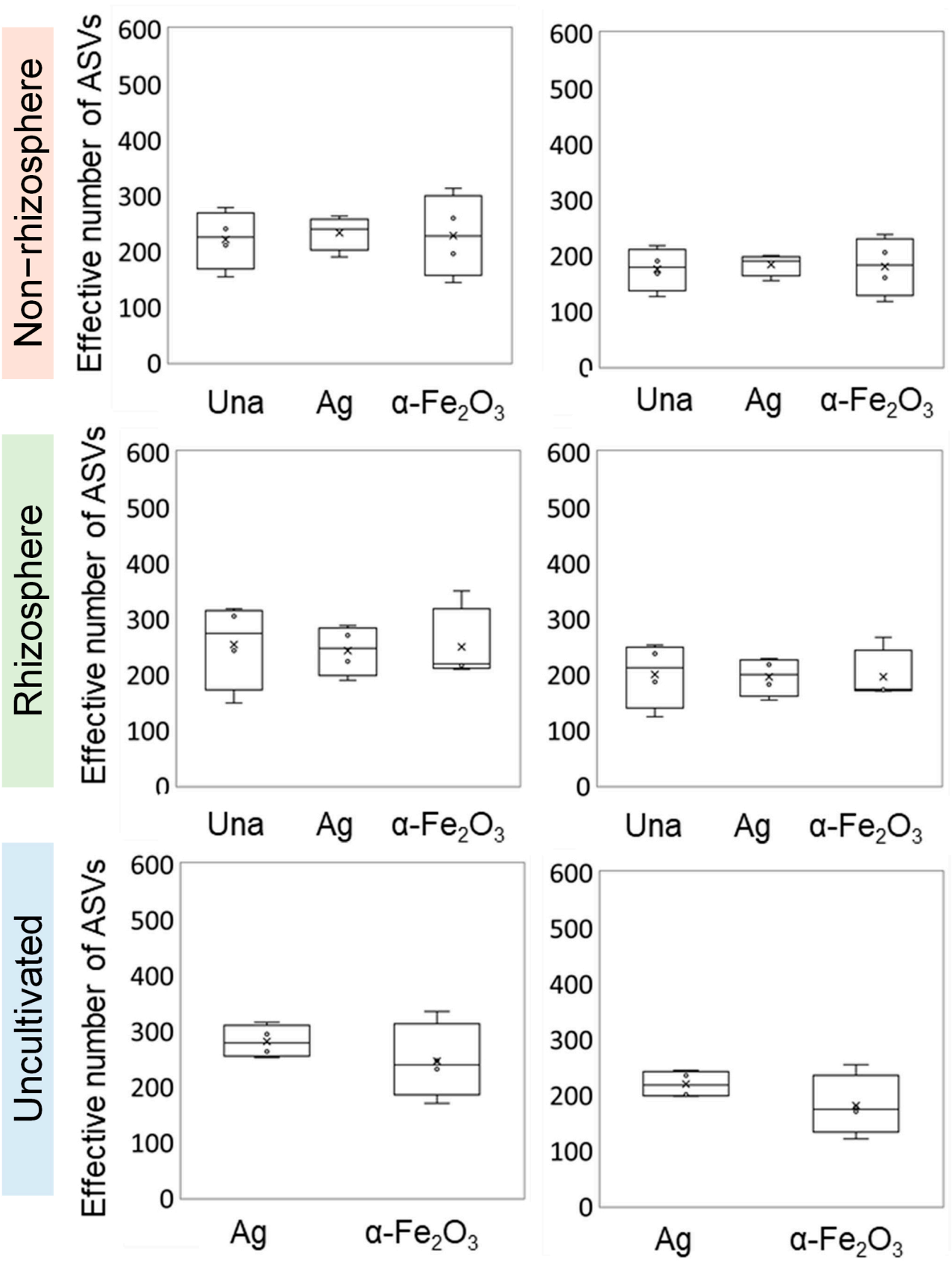


Figure S1. Soil sampling, experimental design and characterization

A)

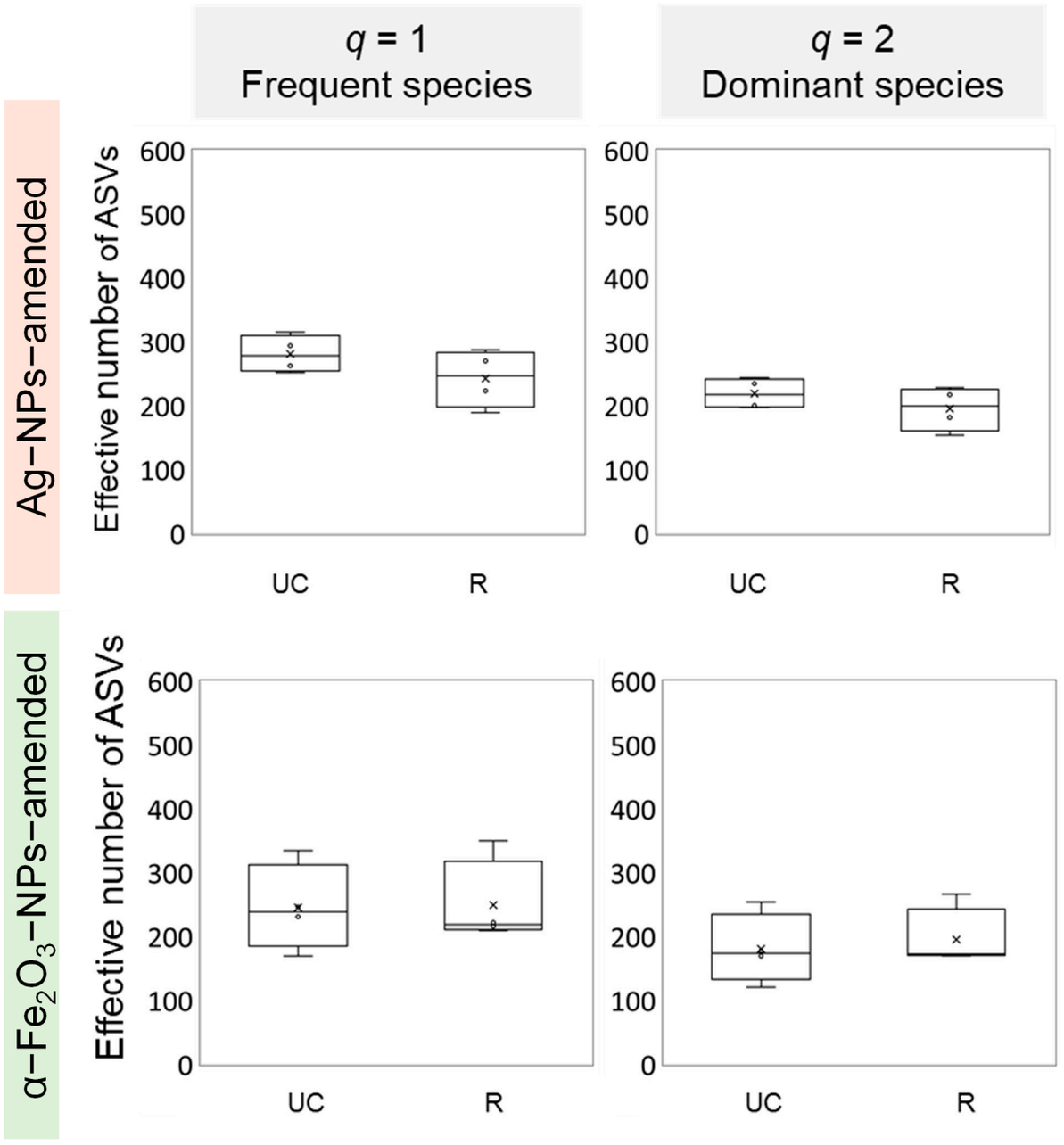


B)

	p values	
	$q = 1$	$q = 2$
Non-rhizosphere	0.981	0.981
Rhizosphere	0.874	0.874
Uncultivated	0.248	0.248

Figure S2. Alpha diversity of the soil bacterial amplicon sequence variants (ASVs). A) Boxplots of effective number of ASVs at $q = 1$ and $q = 2$ in the non-rhizosphere, rhizosphere and uncultivated soil left unamended (Una) or amended with Ag-NPs (Ag) or α-Fe_2O_3-NPs (α-Fe_2O_3) and B) a Kruskall-Wallis test applied to determine the effect of nanoparticles on $q = 1$ and $q = 2$ in the non-rhizosphere, rhizosphere and uncultivated soil.

A)



B)

	p values	
	$q = 1$	$q = 2$
Ag–NPs–amended	0.248	0.248
α–Fe_2O_3–NPs–amended	0.773	0.564

Figure S3. Effect of cultivation of beans (*Phaseolus vulgaris* L.) (rhizosphere (R) versus uncultivated (UC) soil) on the alpha diversity of the soil bacterial amplicon sequence variants (ASVs). A) Boxplots of effective number of ASVs at $q = 1$ and $q = 2$ in soil amended with Ag-NPs or α-Fe_2O_3-NPs and B) a Kruskall-Wallis test applied to determine the effect of cultivation of bean plants (rhizosphere versus uncultivated soil) on $q = 1$ and $q = 2$ in the Ag-NPs or α-Fe_2O_3-NPs amended soil.

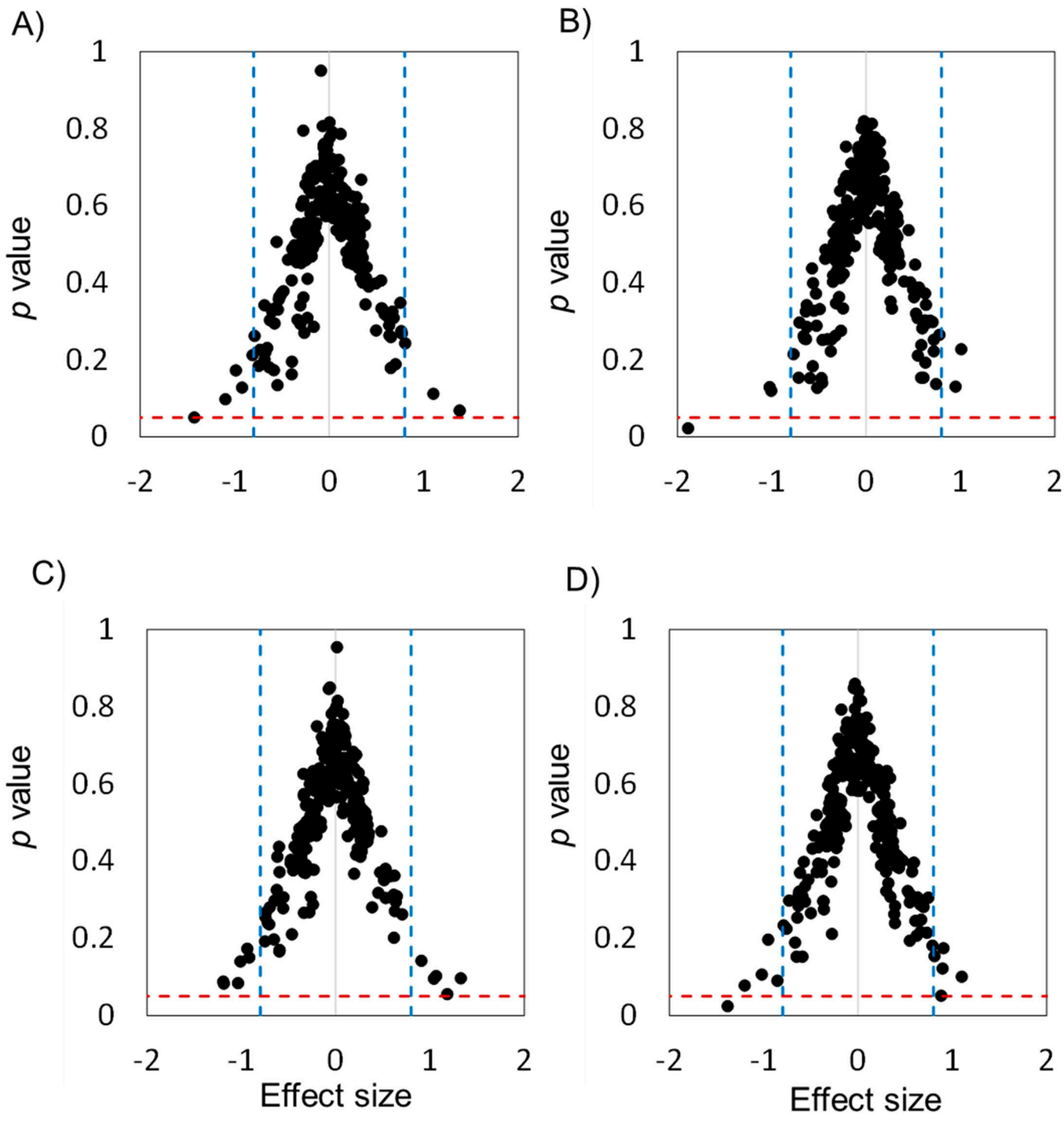
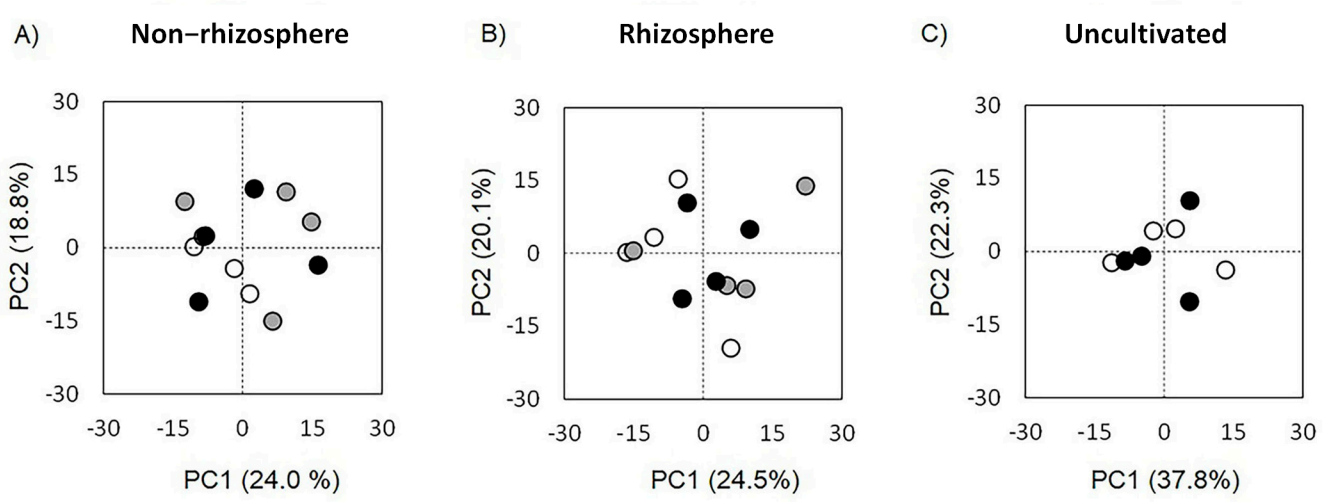


Figure S4. Volcano plot comparing the relative abundance of bacterial groups assigned up to the taxonomic level of genus in A) the Ag-NPs amended non-rhizosphere versus the unamended non-rhizosphere soil, B) the Ag-NPs amended rhizosphere versus the unamended rhizosphere soil, C) the unamended non-rhizosphere soil versus the α-Fe_2O_3-NPs amended non-rhizosphere and D) the unamended rhizosphere soil versus the α-Fe_2O_3-NPs amended rhizosphere. The expected *p* value of the Kruskal-Wallis test is given in the y-axis and the effect size is given in the x-axis [38]. The effect size, which is defined as the difference between groups divided by the maximum dispersion within group A or B, was calculated with the ALDEx2 package using the aldex.ttest argument. A negative value indicates that the relative abundance of the microbial group was higher in the first mentioned soil than in the second mentioned soil and a positive value the opposite. Blue dotted line indicates a large effect (≤ -0.8 and ≥ 0.8, [37]) and a red dotted line $p = 0.05$.



D)

	F	R^2	*p*
Non−rhizosphere	0.918	0.169	0.591
Rhizosphere	0.781	0.148	0.821
Uncultivated	0.983	0.141	0.478

Figure S5. Principal component analysis (PCA) with all the putative metabolic functions in the A) non-rhizosphere, B) rhizosphere and C) uncultivated soil left unamended (●), or amended with Ag-NPs (○) or α-Fe_2O_3-NPs (●) and D) a permutational analysis of variance (perMANOVA) test to determine the effect of application of the different nanoparticles on the putative metabolic functions at day 100.

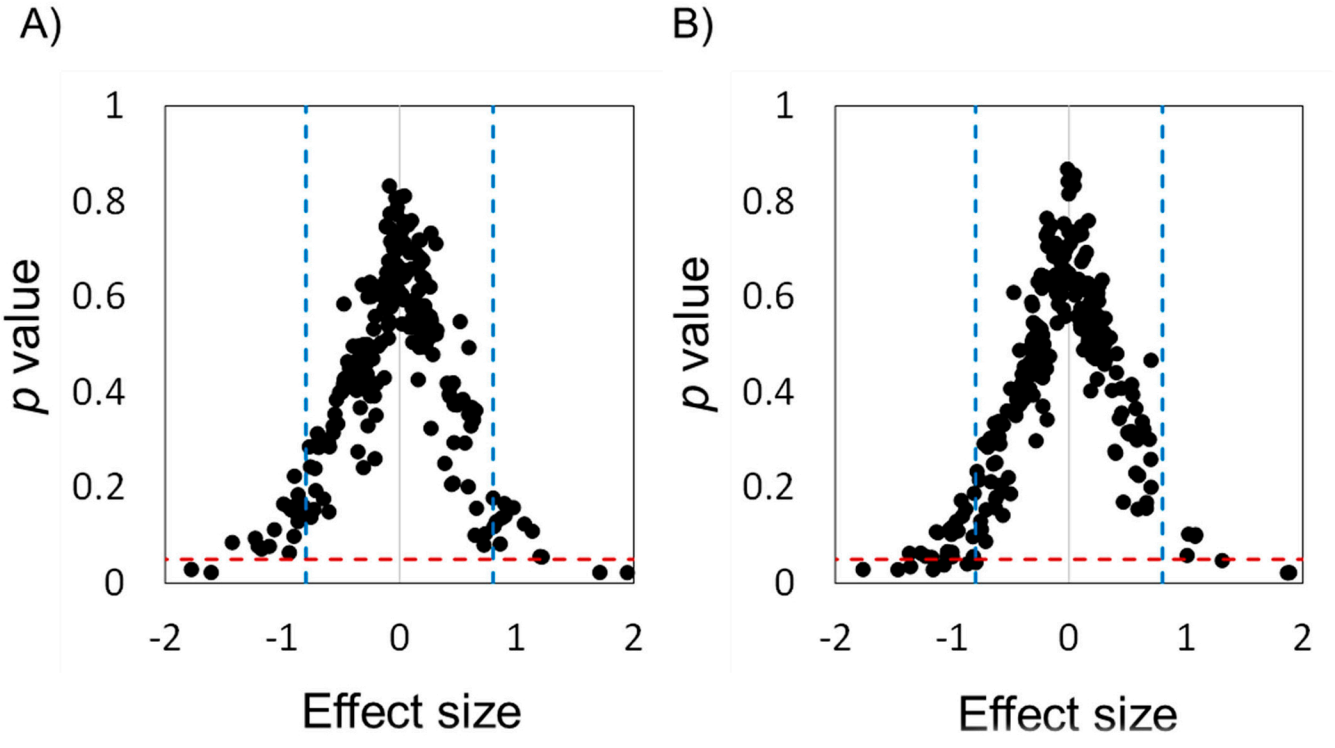
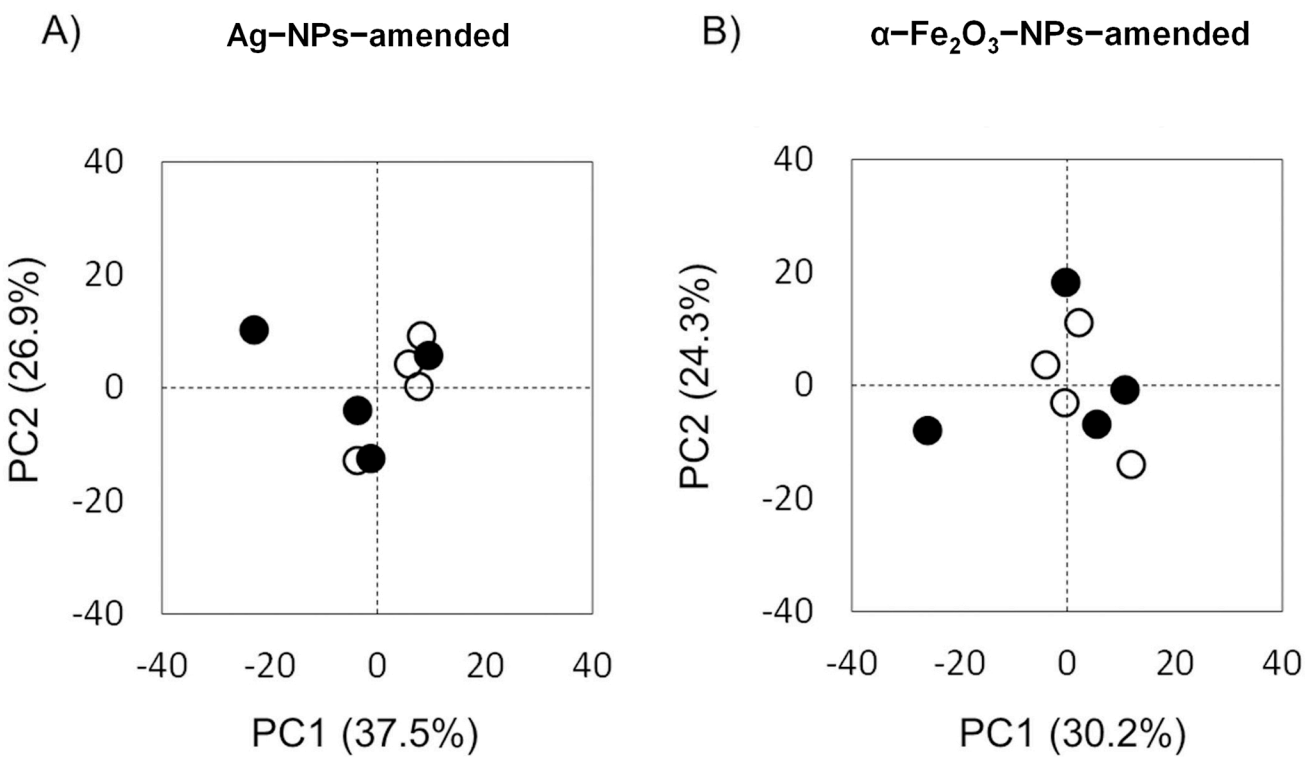


Figure S6. Volcano plot comparing the relative abundance of bacterial groups assigned up to the taxonomic level of genus in A) the Ag-NPs amended rhizosphere versus the Ag-NPs amended uncultivated soil and B) the α-Fe_2O_3-NPs amended rhizosphere versus the α-Fe_2O_3-NPs amended uncultivated soil. Details of the figure can be found in Figure S4.

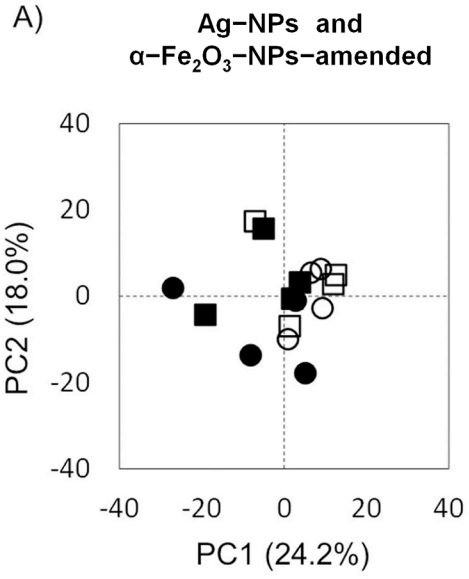


C)

	F	R^2	*p*
Ag−NPs−amended	0.968	0.139	0.566
α−Fe_2O_3−NPs−amended	1.071	0.151	0.370

Figure S7. Principal component analysis (PCA) with putative metabolic functions in A) Ag-NPs and B) α-Fe_2O_3-NPs amended uncultivated soil (○) and rhizosphere soil (●), and C) permutational analysis of variance (perMANOVA) test to determine the effect of bean (*Phaseolus vulgaris* L.) cultivation on the putative metabolic functions in each nanoparticles amended soil.

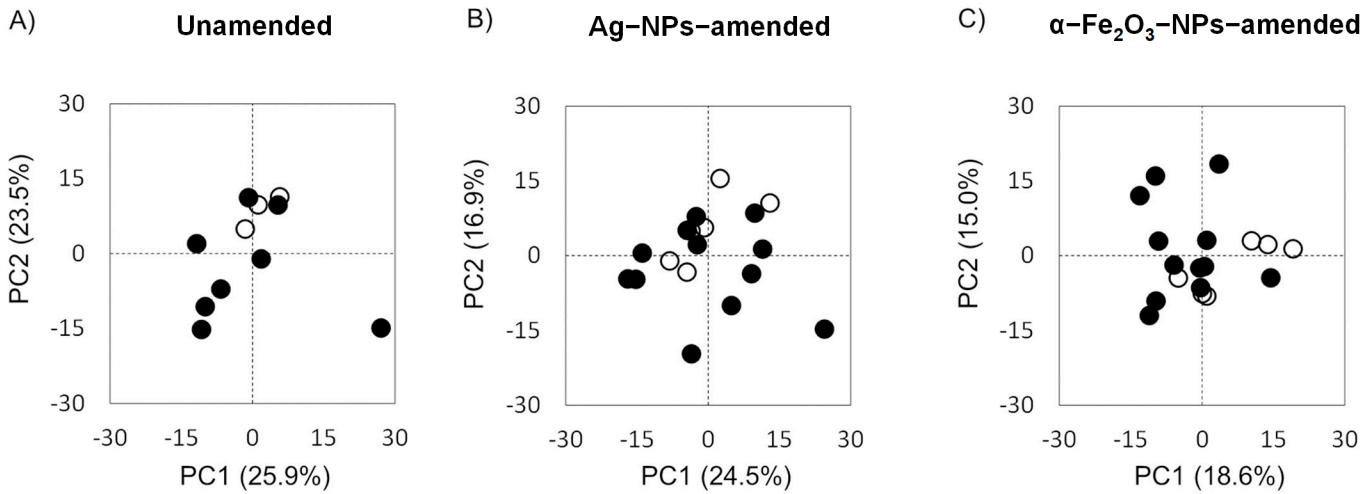
A)



B)

	F	R^2	*p*
Treatment	1.212	0.080	0.224
Plant	1.774	0.112	**0.038**
Plant × Treatment	1.161	0.225	0.223

Figure S8. A) Principal component analysis (PCA) with putative metabolic functions in the uncultivated soil amended with Ag-NPs (○), rhizosphere soil amended with Ag-NPs (●), uncultivated soil amended with α-Fe_2O_3-NPs (□), and rhizosphere soil amended with α-Fe_2O_3-NPs (■) and B) perMANOVA test to determine the effect of plant cultivation, nanoparticles application and their interaction.



D)

	F	R^2	*p*
Unamended	0.956	0.096	0.493
Ag−NPs−amended	1.174	0.068	0.292
α−Fe_2O_3−NPs−amended	1.582	0.090	**0.045**
Treatment × Day	1.193	0.077	0.167

Figure S9. Principal component analysis (PCA) with all putative metabolic functions in the A) unamended, and B) Ag-NPs and C) α-Fe_2O_3-NPs amended soil at the onset of the experiment (○) and after 100 days (●), and D) Permutational analysis of variance (perMANOVA) test used to determine the effect of time on the putative metabolic functions.

Table S1 Methods used to determine soil characteristics.

Characterization	Technique used	Reference
pH	A soil sample was mixed with distilled water in a 1:2.5 ratio and pH was determined with a potentiometer HANNA HI 110.	[18]
Electrolytic conductivity (EC)	A soil sample was mixed with distilled water in ratio 1:5 to determine EC with a conductimeter HANNA HI 2300.	[19]
Particle size distribution	Soil particle size distribution was determined by the hydrometer method.	[20]
Total carbon	Total C was determined with a Thermo Scientific™ FlashSmart™ Elemental Analyzer (Waltham, Massachusetts, USA) following the standard protocol given by the manufacturer.	
Total nitrogen	Total N was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and HgO to digest the sample.	[21]
Water holding capacity (WHC)	The WHC was measured on soil samples water-saturated in a funnel and left to stand overnight. The weight of the water retained by the soil was considered the WHC.	

Table S2 Statistical analysis applied to the data and the package in R used.

Statistical analysis	Comparison	Package used and reference
clr-transformation	Centred log-ratio transformation as high-throughput data or compositional	ALDEx2 (1.18) [33]
principal component analysis (PCA)	Visualize the effect of the application of NPs, the cultivation of beans and the time on bacterial community structure or putative metabolic functions	FactoMineR (2.3.), [34]
perMANOVA	Determine the effect of the application of NPs, the cultivation of beans and the time of exposure, and their interaction on the structure and putative functionality of bacterial community	Vegan (2.5-6), [35]
permutation test of multivariate analysis of dispersions (PERMDISP)	Determine dispersion within treatments	Vegan (2.5-6), [35, 36]
non-parametric Kruskal Wallis test	Determine of the application of NPs or plant on bacterial groups	aldex.kw, ALDEx2 (1.18), [33]
effect size (the difference between the groups divided by maximum dispersion within the treatment)	Determine of the application of NPs or plant on bacterial groups. An effect size $\geq \|0.8\|$ was considered as a large effect.	aldex.effect, ALDEx2 (1.18), [33, 37]
volcano plot	The effect size was plotted versus the expected *p*-value of the Kruskal-Wallis test for each feature	aldex.ttest, ALDEx2 (1.18), [33, 38]

Table S3 Some morphological characteristics of common bean plants (*Phaseolus vulgaris* L.)

Treatment	Stem length (cm)	Root length (cm)
Unamended	173.9 ± 18 a	66.5 ± 3.6 a
Ag-NP amended	179.8 ± 16.8 a	61.8 ± 6.3 a
α-Fe_2O_3-NP amended	175.6 ± 18.1 a	60.7 ± 6.5 a

[a] Values with the same letter within a column, i.e. differences between treatments, are not significantly different at $p < 0.05$

Table S4 Heavy metal content in the roots and leaves of common bean plants (*Phaseolus vulgaris* L.)

	Roots			
Treatment	Cu	Fe	Mn	Zn
		($\mu g\ g^{-1}$)		
Unamended	22 ± 3 a	15082 ± 2888 a	402 ± 108 a	50 ± 8 a
Ag-NP amended	14 ± 2 b	7187 ± 1712 b	157 ± 39 b	32 ± 8 b
α-Fe_2O_3-NP amended	18 ± 5 ab	5229 ± 3327 b	106 ± 83 b	31 ± 6 b

	Leaves			
Unamended	13 ± 3 a	718 ± 238 a	103 ± 7 a	43 ± 6 a
Ag-NP amended	11 ± 2 a	485 ± 79 a	85 ± 3 b	39 ± 10 a
α-Fe_2O_3-NP amended	14 ± 5 a	839 ± 452 a	90 ± 9 ab	42 ± 11 a

[a] Values with the same letter within a column, i.e. differences between treatments, are not significantly different at $p < 0.05$

Table S5 Bacterial genera with a large effect size when was applied with Ag-NPs and α-Fe_2O_3-NPs

Silver nanoparticles amended vs unamended non-rhizosphere soil	**Effect size**	***p* value**
Flavitalea	**1.4**	0.068
DS-100	**1.1**	0.112
67-14	**-1.4**	0.051
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	**-1.1**	0.098
Bradyrhizobium	**-1.0**	0.172
Haliangium	**-0.9**	0.128
Silver nanoparticles amended vs unamended rhizosphere soil		
Craurococcus-Caldovatus	**0.9**	0.130
Nocardioides	**-2.3**	0.022
Ohtaekwangia	**-1.9**	0.022
Candidatus_Alysiosphaera	**-1.0**	0.129
Turicibacter	**-1.0**	0.120
Unamended non-rhizosphere soil vs α-Fe_2O_3 nanoparticles amended		
Hafnia-Obesumbacterium	**2.5**	0.021
Massilia	**1.3**	0.097
RCP2-54	**1.2**	0.056
Stenotrophomonas	**1.1**	0.102
Pseudenhygromyxa	**1.0**	0.096
Sphingomonas	**0.9**	0.142
Amycolatopsis	**-1.2**	0.083
bacteriap25	**-1.0**	0.084
Gaiella	**-1.0**	0.140
Rubrobacter	**-0.9**	0.173
KD4-96	**-0.9**	0.150
Unamended rhizosphere soil vs α-Fe_2O_3 nanoparticles amended		
Nocardioides	**2.3**	0.021
bacteriap25	**0.9**	0.175
Turicibacter	**0.9**	0.122
Flavitalea	**0.9**	0.052
Arenimonas	**0.8**	0.154
Solirubrobacter	**-1.0**	0.106
JGI_0001001-H03	**-1.0**	0.197
NB1-j	**-0.9**	0.090

Table S6 *p* values obtained from permutation test of multivariate homogeneity of groups dispersions

Nanoparticles application	***p* value**
Non-rhizosphere	0.312
Ag-NPs amended *vs* unamended	0.356
α-Fe_2O_3-NPs amended *vs* unamended	0.108
Ag-NPs *vs* α-Fe_2O_3-NPs	0.698
Rhizosphere	0.808
Ag-NPs amended *vs* unamended	0.739
α-Fe_2O_3-NPs amended *vs* unamended	0.325
Ag-NPs *vs* α-Fe_2O_3-NPs	0.831
Uncultivated	0.854
Ag-NPs *vs* α-Fe_2O_3-NPs	0.864
Plant cultivation	
Rhizosphere *vs* uncultivated	0.897
Time	
Unamended	0.212
Ag-NPs amended	0.219
α-Fe_2O_3-NPs amended	0.844