Supplemental information

Temporal dynamics of the soil bacterial

community following *Bacillus* invasion

Panji Cahya Mawarda, Sarah L. Lakke, Jan Dirk van Elsas, and Joana Falcao Salles

Supplementary Information

Temporal Dynamics of Soil Bacterial Community Following *Bacillus* Invasion

Panji Cahya Mawarda[1,2], Sarah L Lakke[1], Jan Dirk van Elsas[1], Joana Falcao Salles[1]

[1]Department of Microbial Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborg 7, 9747 AG, Groningen, The Netherlands
[2]Research Unit for Clean Technology, National Research and Innovation Agency Republic of Indonesia (BRIN), Komplek LIPI, Jalan Sangkuriang No 21, Bandung 40135, Indonesia

Table S1. [Initial Abundance of Culturable Bacteria Before inoculation (ANOVA, p > 0.05)]. Related to the STAR method "Determination of initial bacterial abundance, *Bacillus* spp invasion, and survival monitoring"

Treatment	CFU/gram soil	log CFU/gram soil
10^-1	970000000	8.986
10^-1	1050000000	9.021
10^-1	1040000000	9.017
10^-3	1040000000	9.017
10^-3	1190000000	9.075
10^-3	1160000000	9.064
10^-6	980000000	8.991
10^-6	1150000000	9.061
10^-6	400000000	8.602

Table S2. [Successional Groups of Invaded Bacterial Community by *B. mycoides* M2E15]. Related to the STAR method "Bacterial community dynamics" and Figure S8.

a) 10-1 treatment

Taxon	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Genus	1 Rhodanobacter	1 Unidentified genus	1 Kaistobacter	2 Rhodoplanes	1 Terracoccus	2 Unidentified genus	7 Bacillus	1 Phenylobacterium
	1 Unidentified Genus		4 Unidentified genus	1 Unidentified genus	6 Unidentified genus		1 Kaistobacter	4 Unidentified genus
				1 Lysobacter	1 Gemmata		1 Sphingomonas	1 Gemmata
				1 Rummelibacillus	1 Plancomyces		1 Unidentified genus	1 Rhodoplanes
				1 Nocardiodes				
				5 Massilia				

Taxon	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Family	1 Xanthomonodaceae	1 Planococcaceae	1 Sphingomonadaceae	1 Hypomicrobaceae	2 Pirellulaceae	1 Unidentified family	7 Bacillaceae	1 Caulobacteraceae
	1 Acetobacteraceae		1 Erythrobacteraceae	1 Chitinopagaceae	2 Chitinophagaceae	1 Chitinopagaceae	3 Sphingomonadaceae	1 Gemmataceae
			2 Pirellulaceae	1 Xanthomonadaceae	1 Intrasporangiaceae			1 Pirellulaceae
			1 Xanthomonadaceae	1 Planococcaceae	1 Gemmataceae			1 Acetobacteraceae
				1 Nocardiodaceae	1 Unidentified family			1 Erthrobacteraceae
				5 Oxalobacteraceae	1 Planctomycetaceae			1 Hypomicrobiaceae

b) 10-3 treatment

Taxon	Group 1	Group 2	Group 3	Group 4
Genus	1 Burkholderia	6 Bacillus	1 Cryocola	2 Unidentified genus
	1 Unidentified genus	1 Streptomyces	2 Unidentified genus	1 Rhodanobacter

Taxon	Group 1	Group 2	Group 3	Group 4
	1 Kienococcus	1 Kaistobacter	1 Mesorhizobium	
			1 Alicyclobacillus	
Taxon	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Family	1 Burkholderiaceae	6 Bacillaceae	1 Microbacteriaceae	1 Bradyrhizobiaceae
	1 Isophaeraceae	1 Streptomycetaceae	1 Haliangiaceae	1 Geodermatophilaceae
	1 Kienosporiaceae	1 Sphingomonadaceae	1 Phyllobacteriaceae	1 Xanthomonadaceae
			1 Alicyclobacillaceae	
			1 Bradyrhizobiaceae	

a) 10-6 treatment

Taxon	Group 1	Group 2	Group 3
Genus	3 Rummelibacillys	5 Unidentified genus	8 Bacillus
	1 Arthrobacter	3 Flavisolibacter	1 Oryzihumus
	2 Unidentified genus		1Unidentified genus
			1 Sporosarcina
Taxon	**Group 1**	**Group 2**	**Group 3**
Family	3 Planococcaceae	5 unidentified family	8 Bacillaceae
	1 Micrococcaceae	3 Chitinophagaceae	1 Intrasporangiaceae
	2 Oxalobacteraceae		1 Geodermatophilaceae
	1 Solibacteraceae		1 Planococcaceae

Table S3. [Successional Groups of Invaded Bacterial Community by *B. pumilus* ECO-B-02]. Related to the STAR method "Bacterial community dynamics" and Figure S9.

a) 10-1 treatment

Taxon	Group 1	Group 2	Group 3
Genus	2 Bacillus	2 Rhodanobacter	1 Sphingomonas
	1 Brevibacillus	1 Massilia	
	1 Arthrobacter	7 Unidentified genus	
	1 Unidentified genus		

Taxon	Group 1	Group 2	Group 3
Family	2 Bacillaceae	4 Chitinopagaceae	1 Sphingomonadaceae
	1 Paenibacillaceae	2 Xanthomonadaceae	
	1 Chitinopagaceae	1 Patulibacteraceae	
	1 Micrococcaceae	1 Caulobacteraceae	
		1 Oxalobacteraceae	
		1 Pirellulaceae	

b) 10-3 treatment

Taxon	Group 1	Group 2	Group 3	Group 4	Group 5
Genus	6 Bacillus	1 Kaistobacter	1 Candidatus Solibacter	1 Unidentified genus	1 Rreyranella
	1 Alicyclobacillus	1 Mucilagnibacter	4 Lysobacter		1 Devosia
	1 Conexibacter	1 Devoisa	1 Nocardiodes		
	1 Rgodoplanes		1 Phenylobacterium		
	1 Terracoccus		1 Massilia		
	1 Unidentified genus				

Taxon	Group 1	Group 2	Group 3	Group 4	Group 5
Family	6 Bacillaceae	1 Sphingomonadaceae	1 Solibacteraceae	1 Unidentified family	1 Rhodospirilaceae
	1 Alicyclobacillaceae	1 Sphingobacteriaceae	4 Xanthomonadaceae		1 Hypomicrobiaceae
	1 Conexibacteraceae	1 Hypomicrobiaceae	1 Nocardiiodaceae		
	1 Hypomicrobiaceae		1 Caulobacteraceae		
	1 Intrasporagiaceae		1 Xanthomonadaceae		
	1 Bradyrhizobiaceae				

c) 10-6 treatment

Taxon	Group 1	Group 2	Group 3
Genus	3 Rummelibacillus	5 Unidentified genus	8 Bacillus

Taxon	Group 1	Group 2	Group 3
	2 Arthrobacter	3 Flavisolibacter	1 Oryzihumus
	2 Unidentified genus		1 unidentified genus
	1 Unidentified genus		1 Sporosarcina
Family	3 Planococcaceae	5 Unidentified family	8 Bacillaceae
	1 Micrococcaceae	3 Chitinopagaceae	1 Intrasporangiaceae
	2 Oxalobacteraceae		1 Geodermatophilaceae
	1 Solibacteraceae		1 Planococcaceae

Fig. S1 [Overview of the experimental set up. (a and d) After the first inoculation, survival monitoring and destructive sampling were initially planned for Day 0, Day 3, Day 10, Day 15, Day 30, and Day 90 post inoculation (p.i). However, 3 days p.i, both invaders were below the detection limit of 1 log CFU/g soil and the pH of microcosms dropped from 7 to 4.9, except for the sterile soil controls, which remained at pH 7. Therefore, a week after the first inoculation, we adjusted the experimental set up and design (b and e - Transitions). At this stage, the unused jars (the jars containing soil microcosms that have not been destructively sampled) of the 10-1, 10-3 and 10-6 treatments were divided equally. The control jars were discarded and the new ones were made. We adjusted the pH to 7.0 by adding $Ca(OH)_2$ to these 10-1, 10-3 and 10-6 microcosms, while sterile distilled water was added to the new control. Those jars were incubated for 2 weeks to allow the establishment of the resident bacterial community. The second inoculation was done 3 weeks after the first inoculation. The survival monitoring and destructive sampling were now scheduled at Day 0, Day 1, Day 3, Day 7, Day 15, Day 28, and Day 57 after the second inoculation for *B. mycoides* M2E15 (c), while for *B. pumilus* ECO B 02 (f), it was scheduled at Day 0, Day 1, Day 3, Day 32, and Day 60 after the second inoculation]. Related to the STAR method "Diversity gradient experiment: set up and design".
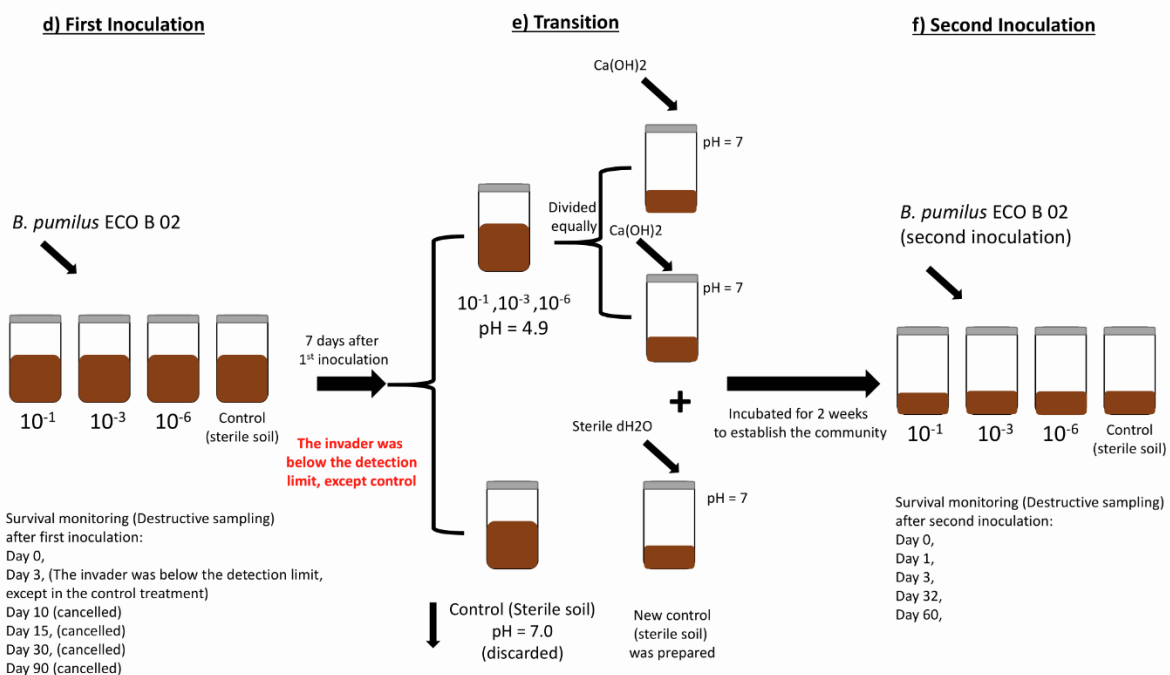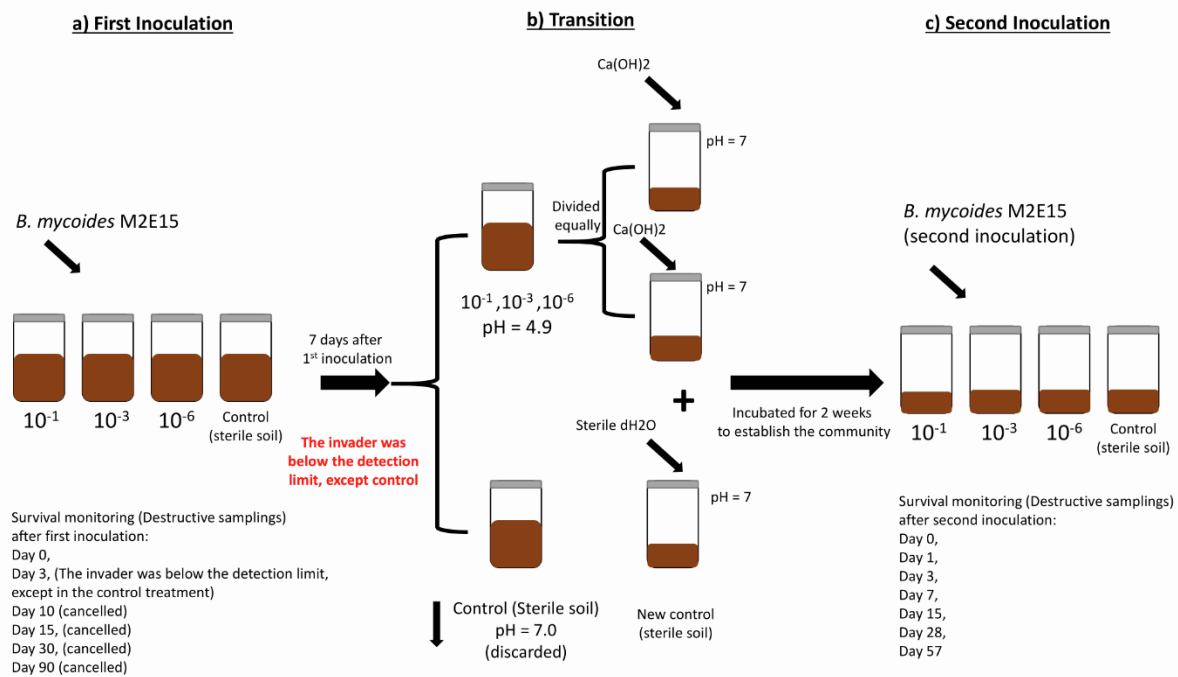
Fig. S2 [Survival of total spore of a) *B. mycoides* M2E15 and b) *B. pumilus* ECO-B-02 in all treatments]. Related to Figure 1.
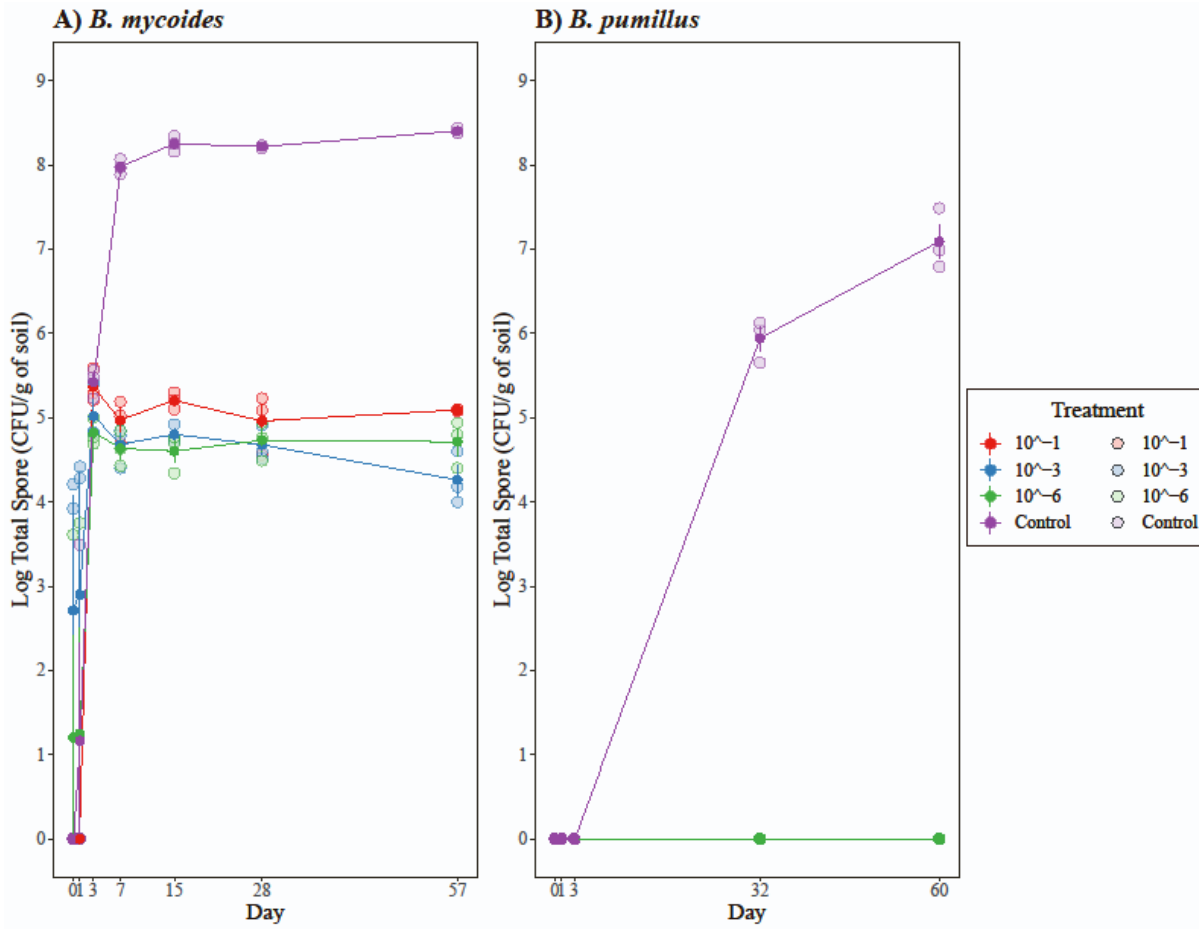
Fig. S3 [Correlation between species evenness over time in bacterial community invaded by a) *B. mycoides* M2E15 and b) *B. pumilus* ECO-B-02]. Related to Figure 2.
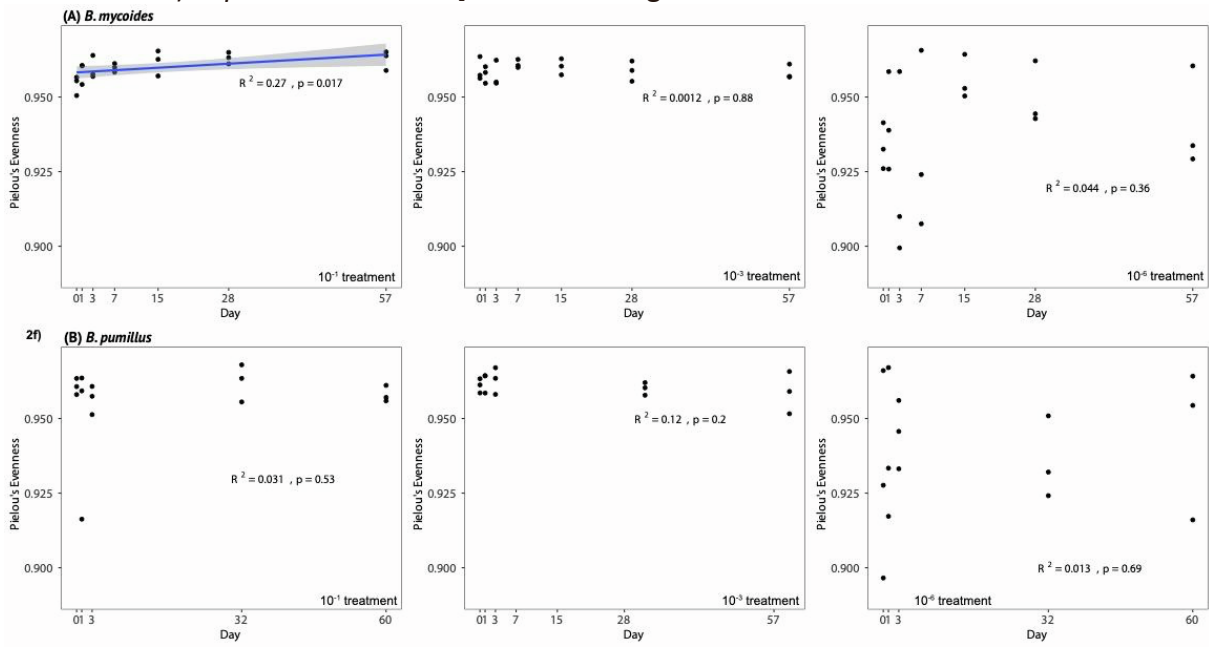
Fig. S4 [Variation in the community diversity following *Bacillus* invasion. PCoA plot of Bray-Curtis distances between each diversity treatments invaded by A) *B. mycoides* M2E15, and B) *B. pumilus* ECO-B-02; PCoA plot of Weighted Unifrac distances between each diversity treatments invaded by C) *B. mycoides* M2E15, and D) *B. pumilus* ECO-B-02. Centroids for each sampling time are shown along with their standard errors (error bars)]. Related to Figure 3.
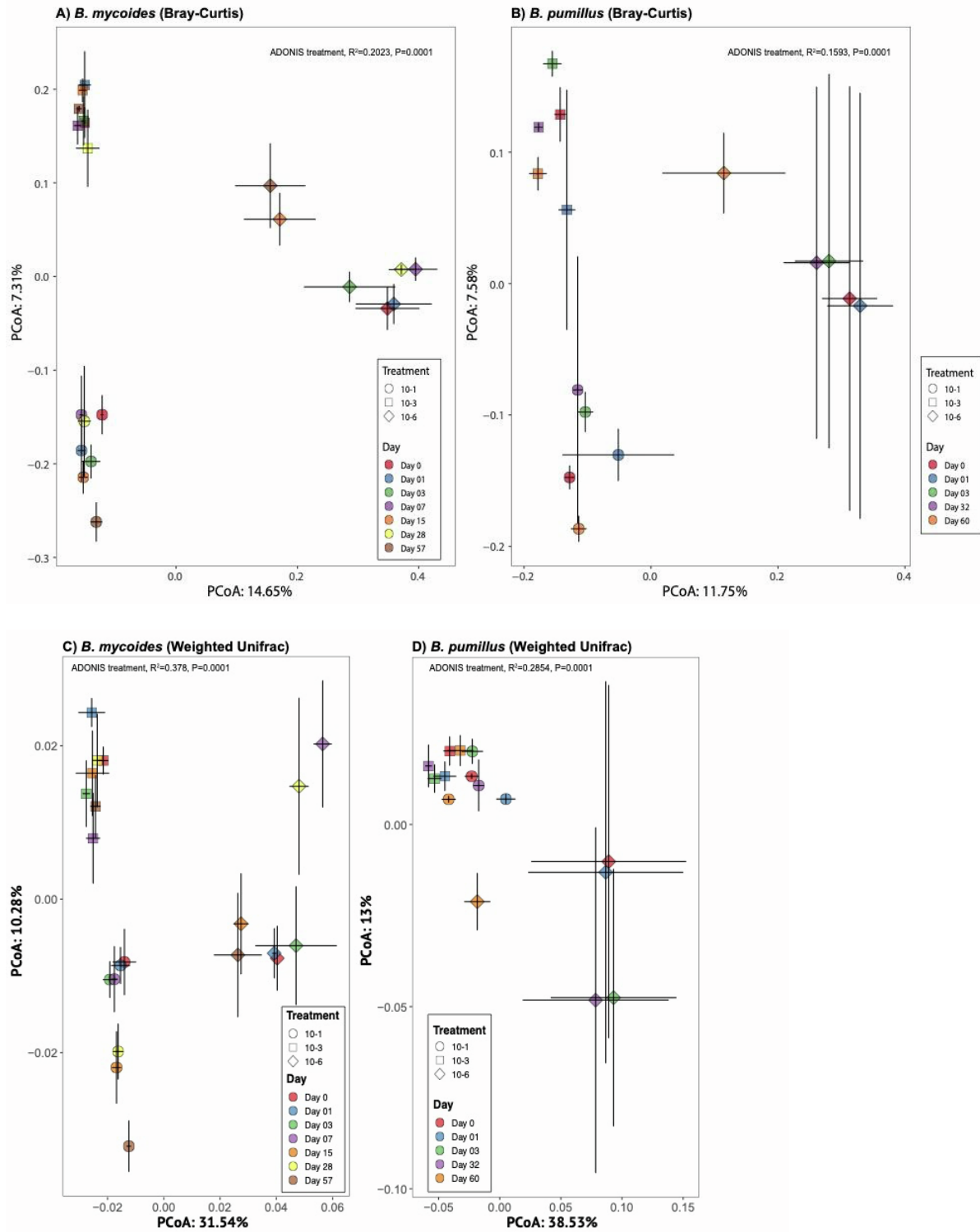
Fig. S5 [Beta diversity based on bray-curtis (A-B) in bacterial community invaded by *B. mycoides* M2E15 and *B. pumilus* ECO-B-02]. Related to Figure 3.
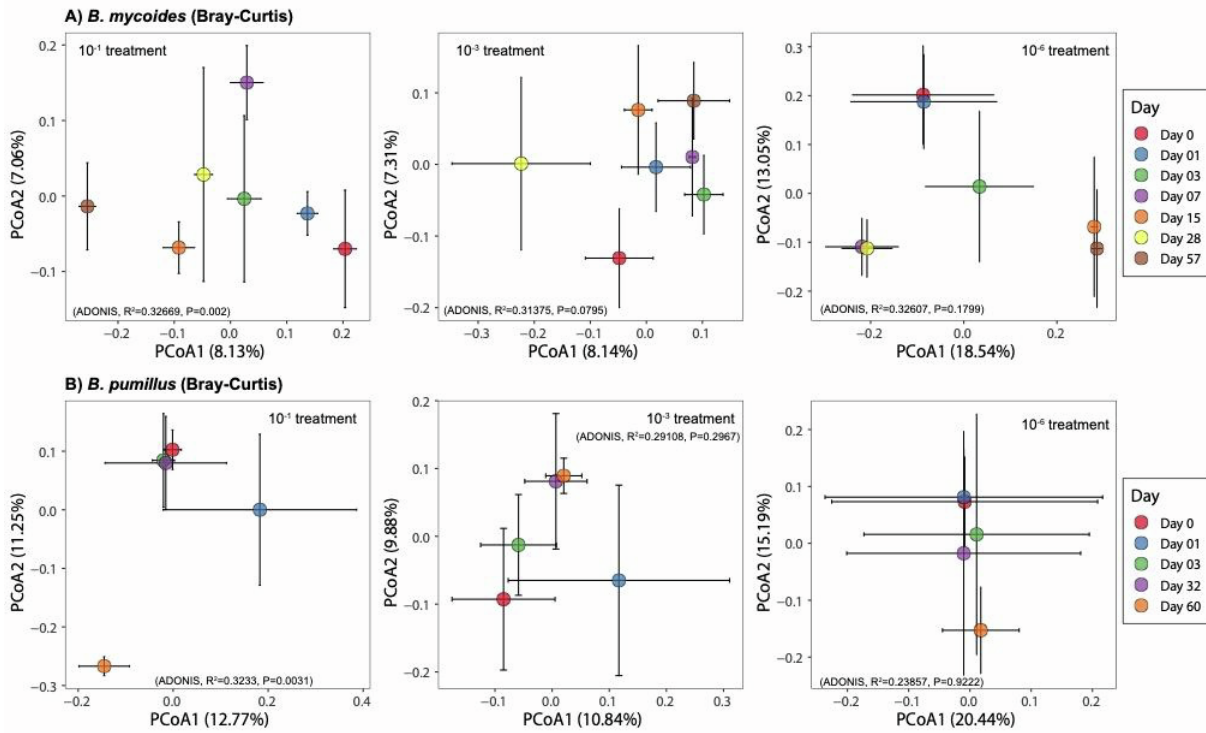
Fig. S6 [Taxa bar plot bacterial community invaded by a) B. mycoides M2E15 and b) B. pumilus ECO-B-02 in 10-1, 10-3, and 10-6 treatments]. Related to the STAR method "Bacterial community dynamics".
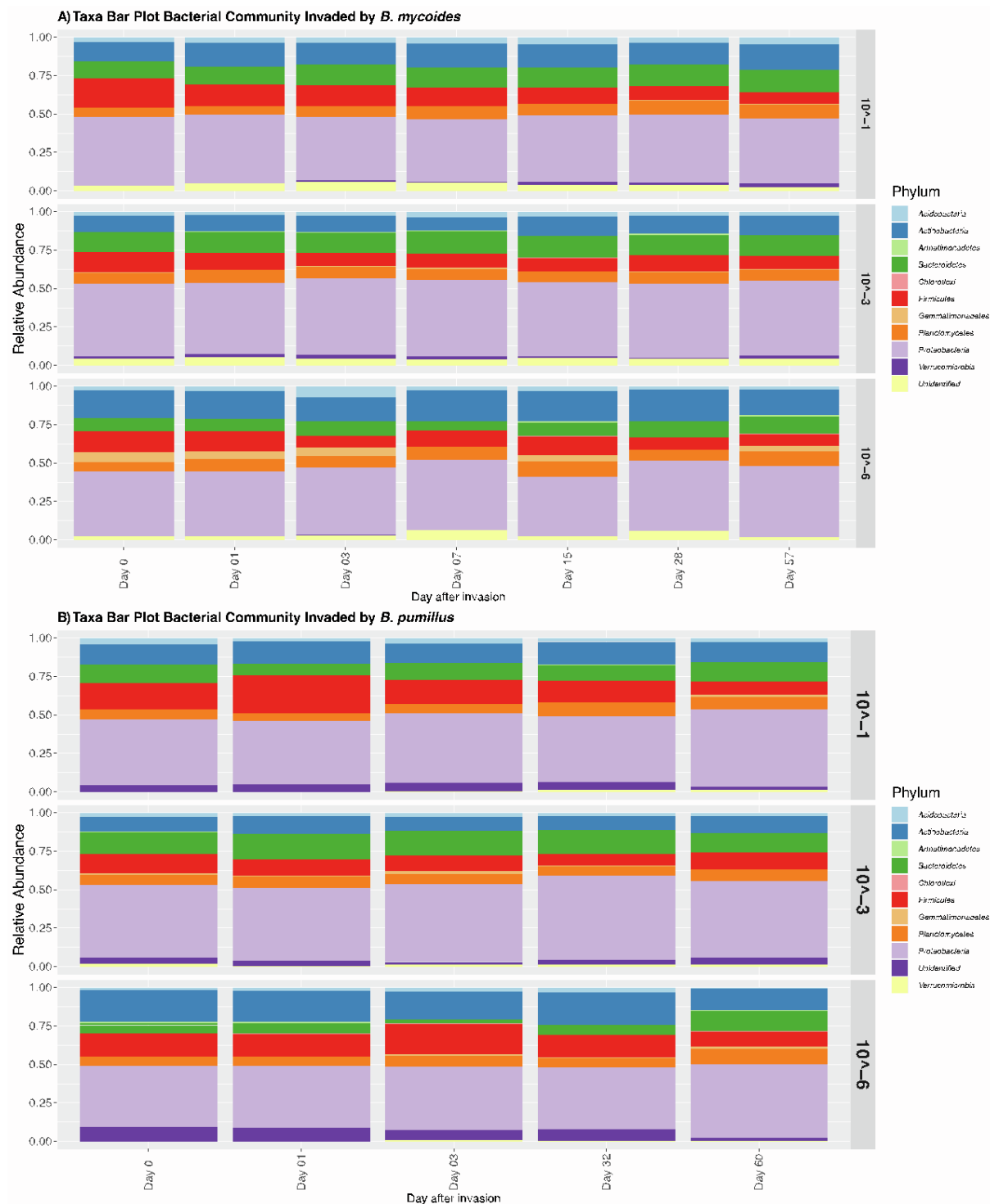
Fig. S7 [Temporal dynamics of bacterial ASVs clusters along secondary succession after *B. mycoides M2E15* invasion in a) 10^{-1} treatment, b) 10^{-3} treatment, and c) 10^{-6} treatment. The intensity of the red color indicates the relative abundance of each ASVs. Family and Genus membership within each group are shown in supplementary table 2]. Related to the STAR method "Bacterial community dynamics".

Fig. S8 [Temporal dynamics of bacterial ASVs clusters along secondary succession after *B. pumilus ECO-B-02* invasion in a) 10^{-1} treatment, b) 10^{-3} treatment, and c) 10^{-6} treatment. The intensity of the red color indicates the relative abundance of each ASVs. Family and Genus membership within each group are shown in supplementary table 3]. Related to the STAR method "Bacterial community dynamics".
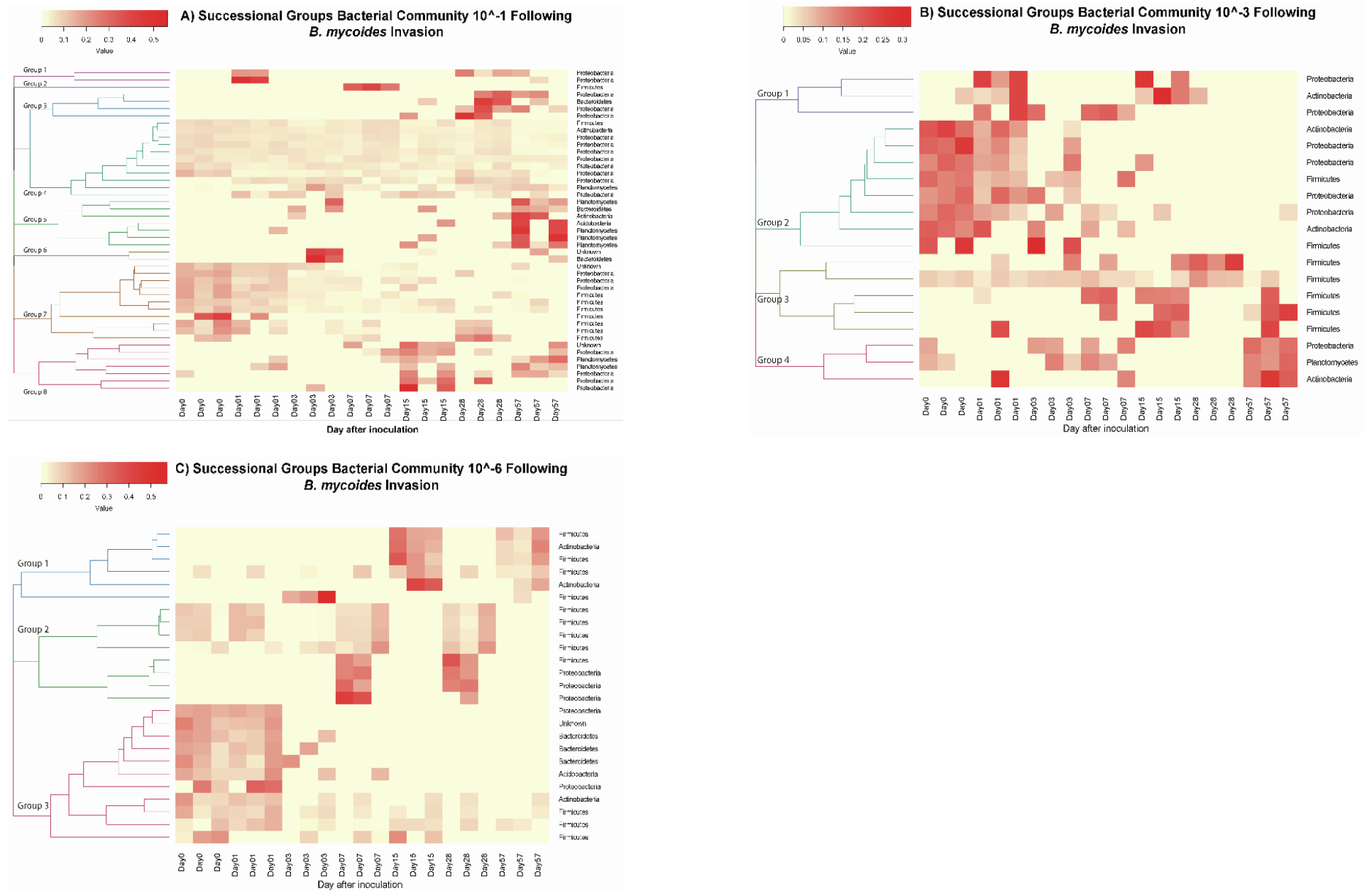
Fig. S9 [Correlation between mean βNTI and species richness in bacterial community invaded by a) *B. mycoides* M2E15 and b) *B. pumilus* ECO-B-02]. Related to Figure 4.