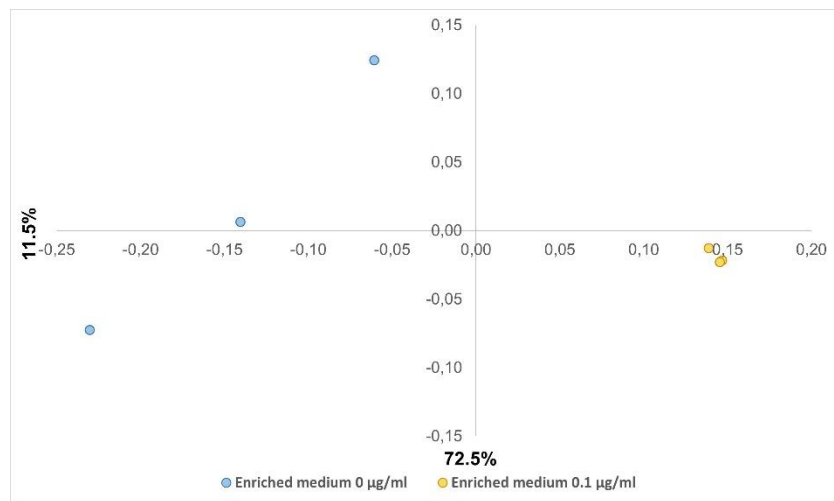
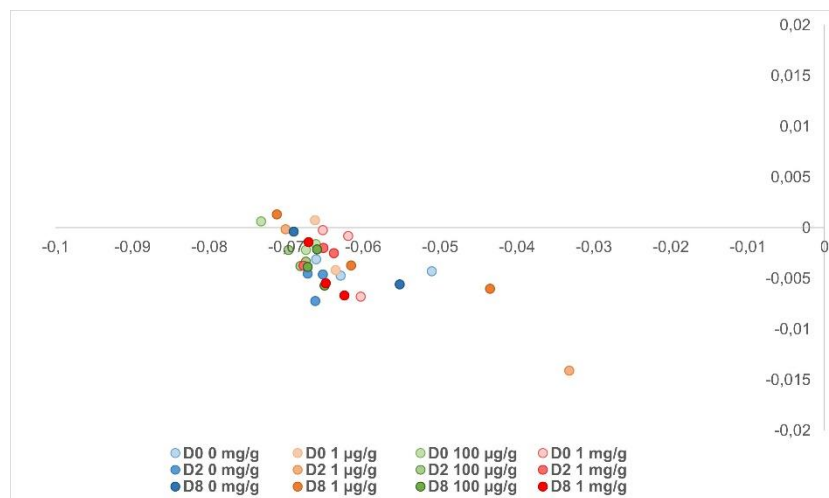


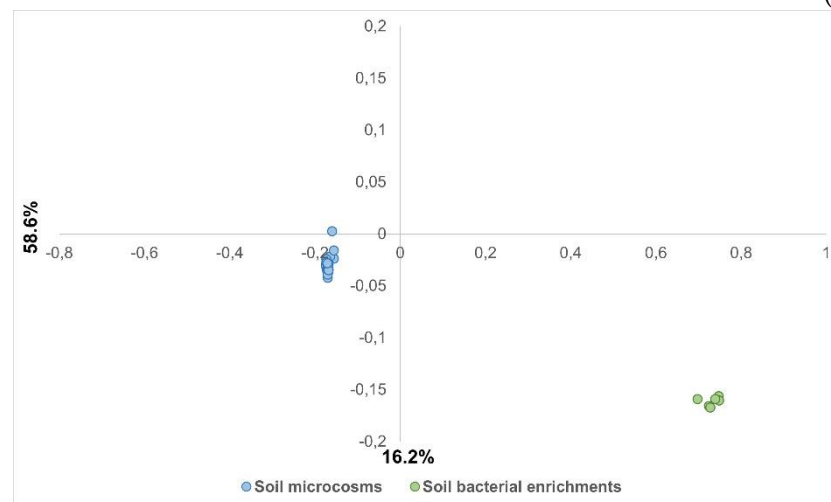
Supplementary Materials



(a)



(b)



(c)

Figure S1. (a) Bacterial community composition PCoA of: (a) soil microcosms and bacterial enrichments; (b) soil microcosms; (c) soil bacterial enrichments.

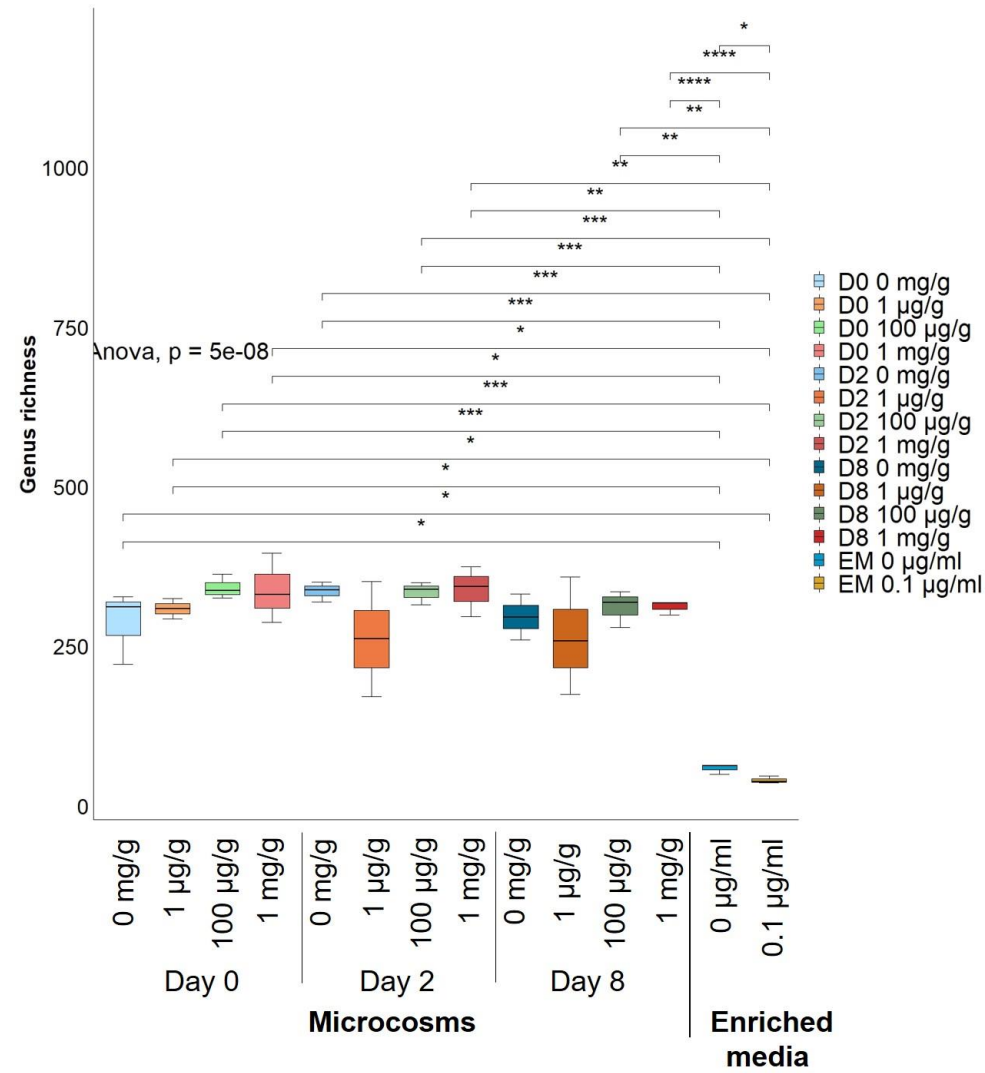


Figure S2. Bacterial richness measured in soil microcosms and enriched media polluted at different gentamicin concentrations. * p -value ≤ 0.05 ; ** p -value ≤ 0.01 ; *** p -value ≤ 0.001 ; **** p -value ≤ 0.0001 . $n=3$.

Table S1a. Genes related to aminoglycoside resistance that increased their average relative abundance over time in soil microcosms at different gentamicin (Pearson coefficient between time and gentamicin concentration ≥ 0.9). No significant increase over time in gentamicin-contaminated soils was detected for any of these genes. *ceoB* and *smeB*: multidrug efflux pumps that include aminoglycoside efflux. *acrD* and *amrB*: aminoglycoside efflux pumps. *baeR*: promoter of *acrD* expression. n=3.

ARG	Day 0				Day 2				Day 8				Increased RA over time		
	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	1 µg/g	100 µg/g	1 mg/g
<i>ceoB</i>	1,04E-05	0,00E+00	1,28E-05	7,53E-06	8,16E-07	6,48E-06	3,68E-06	7,84E-06	1,78E-06	1,83E-05	1,38E-05	1,30E-05	X		
<i>acrD</i>	0,00E+00	5,01E-06	0,00E+00	0,00E+00	2,75E-06	1,14E-06	2,16E-06	0,00E+00	0,00E+00	2,86E-06	8,79E-06	3,76E-06		X	
<i>smeB</i>	0,00E+00	2,96E-06	0,00E+00	2,80E-06	1,63E-06	0,00E+00	1,08E-06	0,00E+00	0,00E+00	2,86E-06	7,86E-06	1,44E-06		X	
<i>amrB</i>	3,91E-06	0,00E+00	0,00E+00	2,80E-06	0,00E+00	0,00E+00	1,52E-06	8,21E-07	0,00E+00	2,86E-06	4,19E-06	2,32E-06		X	
<i>baeR</i>	0,00E+00	0,00E+00	1,78E-06	1,40E-06	0,00E+00	1,14E-06	1,08E-06	0,00E+00	0,00E+00	2,86E-06	9,31E-07	2,32E-06	X		

Table S1b. Genes related to multidrug efflux that increased their average relative abundance over time in soil at different gentamicin (Pearson coefficient between time and gentamicin concentration ≥ 0.9). No significant increase over time in gentamicin-polluted soils was detected for any of these genes. n=3)

ARG	Day 0				Day 2				Day 8				Increased RA over time		
	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	1 µg/g	100 µg/g	1 mg/g
<i>muxB</i>	3,25E-05	4,20E-05	2,50E-05	4,85E-05	2,07E-05	3,50E-05	3,27E-05	3,28E-05	2,02E-05	3,34E-05	4,05E-05	2,55E-05		X	
<i>mexF</i>	2,08E-05	4,50E-06	9,46E-06	1,96E-05	1,27E-05	1,18E-05	6,27E-06	1,25E-05	3,56E-06	2,01E-05	2,21E-05	1,96E-05	X		
<i>msbA</i>	2,08E-05	9,09E-06	1,04E-05	2,40E-05	4,38E-06	9,93E-06	9,46E-06	8,74E-06	2,05E-05	1,02E-05	2,19E-05	2,28E-05	X		
<i>mexQ</i>	3,91E-06	0,00E+00	9,36E-06	8,58E-06	6,43E-06	1,91E-06	5,40E-06	1,62E-06	0,00E+00	2,11E-05	1,35E-05	9,18E-06	X		
<i>mdsB</i>	8,53E-06	5,01E-06	0,00E+00	1,23E-05	1,63E-06	8,76E-06	2,60E-06	4,75E-06	6,92E-06	1,45E-05	1,90E-05	9,18E-06	X	X	
<i>oqxB</i>	3,91E-06	3,06E-06	1,78E-06	1,26E-05	5,32E-06	9,90E-06	5,83E-06	5,36E-06	5,13E-06	1,08E-05	1,09E-05	9,81E-06	X	X	
<i>mexW</i>	0,00E+00	6,03E-06	6,15E-06	1,40E-06	0,00E+00	9,16E-06	6,86E-06	4,69E-06	1,77E-05	1,23E-05	1,12E-05	3,10E-06	X	X	
<i>acrB</i>	0,00E+00	0,00E+00	0,00E+00	7,02E-06	1,93E-06	4,20E-06	1,08E-06	4,56E-06	0,00E+00	2,86E-06	8,59E-06	2,32E-06		X	
<i>acrF</i>	0,00E+00	0,00E+00	0,00E+00	6,13E-06	1,63E-06	4,20E-06	1,08E-06	1,60E-06	0,00E+00	4,30E-06	5,12E-06	1,55E-06		X	
<i>mtrA</i>	3,91E-06	0,00E+00	0,00E+00	3,33E-06	8,16E-07	3,42E-06	0,00E+00	1,62E-06	5,13E-06	5,12E-06	2,79E-06	6,19E-06	X		
<i>rosA</i>	5,21E-06	1,53E-06	1,78E-06	0,00E+00	0,00E+00	5,71E-06	0,00E+00	1,62E-06	0,00E+00	4,51E-06	2,79E-06	2,32E-06			X

Table S1c. Genes related to tetracycline efflux that increased their average relative abundance over time in soil microcosms at different gentamicin concentrations (Pearson coefficient between time and gentamicin concentration ≥ 0.9). No significant increase over time in gentamicin-polluted soils was detected for any of these genes. N=3.

ARG	Day 0				Day 2				Day 8				Increased RA over time		
	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	1 µg/g	100 µg/g	1 mg/g
<i>tetB(60)</i>	0,00E+00	0,00E+00	1,78E-06	1,26E-05	2,75E-06	3,05E-06	1,08E-06	3,11E-06	0,00E+00	8,59E-06	4,19E-06	8,15E-06	X		
<i>tetA(58)</i>	3,91E-06	5,93E-06	1,78E-06	1,40E-06	0,00E+00	6,48E-06	3,97E-06	4,58E-06	0,00E+00	7,21E-06	6,38E-06	6,08E-06	X	X	X
<i>otrC</i>	5,21E-06	0,00E+00	0,00E+00	0,00E+00	8,16E-07	4,20E-06	1,08E-06	8,21E-07	0,00E+00	1,65E-06	1,40E-06	3,76E-06		X	X
<i>otr(A)</i>	0,00E+00	0,00E+00	2,59E-06	5,61E-06	0,00E+00	1,91E-06	3,97E-06	8,21E-07	0,00E+00	3,69E-06	1,77E-06	2,18E-06	X		
<i>adeB</i>	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	1,91E-06	0,00E+00	0,00E+00	0,00E+00	2,86E-06	6,63E-06	0,00E+00	X		

Table S1d. Genes related to other resistance mechanisms that increased their average relative abundance over time in soil microcosms under gentamicin pollution (Pearson coefficient between time and gentamicin concentration ≥ 0.9). No significant increase over time in gentamicin-polluted soils was detected for any of these genes. *Streptomyces*: mutant conferring resistance to aminocoumarin. *rphA*: rifampicin enzymatic inactivation. *Bifidobacteria*: intrinsically resistant form of *ileS* (isoleucyl-tRNA synthetase) in *Bifidobacteria* that confers resistance to mupirocin. *mupA*: alternative *ileS* in *Staphylococcus* conferring resistance to mupirocin. *bcrA*: ABC transporter conferring resistance to bacitracin. n=3.

ARG	Day 0				Day 2				Day 8				Increased RA over time		
	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	0 µg/g	1 µg/g	100 µg/g	1 mg/g	1 µg/g	100 µg/g	1 mg/g
<i>Streptomyces</i>	0,00E+00	2,28E-05	3,56E-06	7,18E-06	1,63E-06	6,48E-06	5,05E-06	7,16E-06	0,00E+00	1,11E-05	7,86E-06	7,52E-06		X	
<i>rphA</i>	3,91E-06	0,00E+00	4,27E-06	1,93E-06	2,75E-06	1,14E-06	5,05E-06	7,98E-06	5,13E-06	6,61E-06	6,38E-06	4,95E-06	X	X	
<i>Bifidobacteria</i>	0,00E+00	1,53E-06	5,33E-06	3,33E-06	8,16E-07	1,14E-06	0,00E+00	3,89E-06	0,00E+00	3,11E-06	7,31E-06	4,32E-06			X
<i>mupA</i>	0,00E+00	0,00E+00	0,00E+00	1,93E-06	1,93E-06	1,14E-06	0,00E+00	8,02E-07	0,00E+00	2,89E-06	3,00E-06	3,76E-06	X		
<i>bcrA</i>	0,00E+00	5,01E-06	0,00E+00	0,00E+00	8,16E-07	1,14E-06	1,08E-06	8,02E-07	0,00E+00	2,86E-06	1,40E-06	2,18E-06		X	X

Table S2. Gentamicin concentrations in the available fraction (in the water fraction) of soils polluted at 1 µg/g, 100 µg/g and 1 mg/g. <QL= below quantification limit.

Gentamicin concentration	Bioavailable fraction
0 ng/g	<QL
1 µg/g	<QL
100 µg/g	<QL
1 mg/g	<QL

Table S3. Assembly and ARG screening of metagenomic reads obtained from soil bacterial enrichments (at different gentamicin concentrations: 0, 0.1 µg/ml or 12 µg ml) and from soil microcosms (at different gentamicin concentrations: 0, 1 µg/g, 100 µg/g or 1 ml/g).

		Soil bacterial enrichments	Soil microcosms
Co-assembly of metagenomic reads (MEGAHIT)	Input	2908668 reads (9 samples)	9556334 reads (36 samples)
	Assembled contigs	4155	137
	Contig length	1000 – 27273 bp	1000 – 2977 bp
	Average length	2238 bp	1179 bp
	N50	2417 bp	1147 bp
Mapping (Bowtie2)	Maximum alignment rate	85.47%	0.08%
Binning	Bins with >50% completion and <10% redundancy	2 (<i>Bacillus</i> and <i>Lysinibacillus</i>)	0
ARG screening on anvi'o profiles	Hits with >60%id and >33 aa	38 hits	0 hits