Supplementary Information:

Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940

Charles W. Knapp[1,3], Jan Dolfing[1], Phillip A. I. Ehlert[2], David W. Graham[1]

[1] School of Civil Engineering & Geosciences, Newcastle University, Newcastle upon Tyne, United Kingdom NE17RU

[2] Alterra, Wageningen University and Research Center, Soil Science Centre 6700 AA Wageningen, The Netherlands

[3] Current address: Department of Civil Engineering, University of Strathclyde, Glasgow, Scotland G1 1XN

Includes: Eight pages, 3 tables and 3 figures

Table S1. Regression analysis of log-transformed 16S-rRNA gene abundances versus time for each archived sample site.

Sample Site	Correlation coefficient (r^2)	Coefficient	Standard Error of Coefficient	Significance (*p*)
A	< .001	.000	.009	.964
B	.227	-.010	.007	.163
C	.478	-.022	.012	.128
D	.054	-.005	.012	.707
E	.065	-.005	.009	.580

Table S2. Abundance of bacterial 16S-rRNA genes (log (gene number per gram of dry soil)) and proportion of resistance genes per 16S-rRNA. Standard errors of the mean are presented in brackets.

Site	Year	Bacterial 16S-rRNA gene [a]	*bla*SHV / 16S	*bla*TEM / 16S	*bla*CTX / 16S	*tet*(B) / 16S	*tet*(L) / 16S	*tet*(M) / 16S	*tet*(O) / 16S	*tet*(Q) / 16S	*tet*(W) / 16S	*erm*B / 16S	*erm*C / 16S	*erm*E / 16S	*erm*F / 16S	*van*B / 16S
A	1960	9.36	-4.29	-3.21	-3.28	-5.56	-5.77	-4.46	-6.83	-6.11	-5.43	-3.88	-4.35	-3.16	-4.82	-4.94
		(.01)	(.06)	(.28)	(.25)	(.15)	(.41)	(.14)	(.58)	(.10)	(.10)	(.01)	(.31)	(.46)	(.25)	(.11)
	1962	9.03	-4.22	-2.59	-3.09	-4.89	-5.31	-4.12		-5.14	-4.90	-4.09	-3.92	-2.93	-4.91	-4.61
		(.05)	(.00)	(.03)	(.07)	(.14)	(.40)	(.25)		(.21)	(.03)	(.46)	(.03)	(.25)	(.18)	(.29)
	1968	9.07	-4.03	-3.01	-3.42	-5.12	-5.42	-3.58	-6.61	-4.93	-4.71	-4.30	-4.00	-3.09	-5.10	-4.22
		(.15)	(.19)	(.11)	(.35)	(.14)	(.10)	(.12)	(.50)	(.22)	(.01)	(.42)	(.03)	(.02)	(.02)	(.19)
	1971	8.56	-3.10	-1.77	-2.26	-4.90	-5.59	-3.52	-5.02	-5.09	-4.73	-3.28	-3.60	-2.86	-4.46	-3.90
		(.23)	(.07)	(.60)	(.27)	(.36)	(.29)	(.04)	(.32)	(.55)	(.42)	(.07)	(.33)	(.10)	(.66)	(.20)
	1973	8.84	-3.56	-2.22	-2.83	-5.73	-4.93	-4.21	-7.06	-5.14	-5.09	-3.91	-3.99	-3.10	-5.36	-3.50
		(.48)	(.85)	(.57)	(.50)	(.63)	(.16)	(.52)	(.34)	(.43)	(.58)	(.04)	(.41)	(.21)	(.10)	(.63)
	1975	8.83	-3.30	-2.56	-2.50	-4.63	-4.60	-3.68	-6.35	-4.62	-4.72	-3.69	-3.64	-2.75	-4.14	-4.49
		(.17)	(.10)	(.28)	(.47)	(.05)	(.23)	(.05)	(.24)	(.35)	(.04)	(.11)	(.14)	(.18)	(.24)	(.17)
	1981	9.18	-4.17	-2.47	-2.95	-5.03	-5.15	-3.69	-6.48	-5.01	-4.41	-4.61	-3.95	-2.95	-4.88	-4.07
		(.22)	(.10)	(.14)	(.23)	(.19)	(.10)	(.28)	(.46)	(.12)	(.48)	(.10)	(.28)	(.28)	(.24)	(.23)
	1984	9.30	-3.99	-2.72	-3.29	-4.67	-6.24	-3.41		-4.59	-4.29	-3.69	-3.78	-2.22	-3.61	-4.07
		(.01)	(.19)	(.06)	(.34)	(.17)	(.19)	(.11)		(.55)	(.22)	(.42)	(.45)	(.05)	(.07)	(.21)
	1986	9.01	-3.96	-2.30	-2.66	-4.13	-6.09	-3.05	-5.10	-3.81	-3.51	-3.00	-3.10	-2.25	-3.35	-3.80
		(.08)	(.33)	(.32)	(.03)	(.09)	(.83)	(.35)	(.07)	(.00)	(.17)	(.09)	(.09)	(.16)	(.14)	(.28)
B	1950	9.58	-3.74	-1.70	-2.62	-4.09	-4.11	-3.25	-7.29	-4.98	-4.64	-4.37	-3.23	-2.54	-5.14	-4.05
	1956	9.67	-3.77	-2.06	-2.43	-4.87	-4.50	-3.72	-6.48	-4.24	-4.48	-2.90	-3.31	-2.79	-4.03	-3.40
		(.30)	(.30)	(.46)	(.26)	(.71)	(.12)	(.07)	(.04)	(.64)	(.78)	(.63)	(.82)	(.33)	(.45)	(1.24)
	1962	9.62	-3.65	-1.74	-2.17	-4.29	-4.29	-3.01	-5.06	-3.69	-4.26	-2.45	-3.00	-2.83	-3.94	-3.50
		(.05)	(.33)	(.20)	(.46)	(.31)	(.34)	(.38)	(.19)	(.06)	(.58)	(.08)	(.38)	(.16)	(.41)	(.59)
	1968	9.48	-3.18	-2.00	-2.29	-4.52	-4.13	-3.07	-5.68	-3.74	-4.23	-3.22	-3.11	-2.53	-4.37	-3.25

Site	Year	Bacterial 16S-rRNA gene [a]	*bla*SHV 16S	*bla*TEM 16S	*bla*CTX 16S	*tet*(B) 16S	*tet*(L) 16S	*tet*(M) 16S	*tet*(O) 16S	*tet*(Q) 16S	*tet*(W) 16S	*erm*B 16S	*erm*C 16S	*erm*E 16S	*erm*F 16S	*van*B 16S
		(.16)	(.09)	(.50)	(.07)	(.30)	(.23)	(.25)	(.30)	(.15)	(.09)	(.61)	(.18)	(.15)	(.01)	(.57)
	1971	9.51	-3.52	-2.25	-2.21	-4.57		-3.68	-5.20	-5.73	-4.48	-2.89	-3.60	-3.03	-4.08	-3.53
		(.02)	(.18)	(.41)	(.23)	(.10)		(.45)	(.31)		(.17)	(.66)	(.48)	(.23)	(.30)	(.55)
	1974	9.27	-3.89	-2.58	-2.20	-4.71	-4.59	-3.86	-6.63	-4.40	-4.24	-2.35	-3.04	-2.64	-5.23	-4.13
C	1942	8.87	-3.87	-1.85	-2.39		-4.05		-5.47	-3.49		-2.47		-2.63	-3.61	
	1950	9.25	-3.03	-1.78	-2.18	-4.02	-4.08	-2.37	-5.03	-3.21	-3.86	-2.18	-3.38	-2.52	-5.08	-3.94
	1951	9.07	-2.44	-1.24	-.81	-3.80		-2.76	-4.99	-3.27		-1.94	-2.60		-3.34	-2.76
	1966	8.22	-1.77	-.63	-.56	-3.28		-2.04		-2.60	-3.18	-1.69	-2.35	-1.41	-2.29	-2.43
	1973	8.76	-2.47	-.81	-1.65	-3.75	-3.24	-1.89	-3.78	-3.21	-2.78	-1.87	-2.17	-1.06	-3.47	-3.70
	1975	8.10	-2.18	-.50	-.56	-2.96	-2.48	-1.63	-2.65	-2.62	-1.95	-2.08	-1.23	-1.60		-2.33
D	1976	9.89	-2.68	-2.86		-2.46		-3.01	-1.89	-5.75	-3.32	-2.53	-2.83	-5.65	-5.76	
	1980	8.83	-1.50	-2.53		-1.45		-3.14		-5.21	-3.36	-2.53	-2.27			
	1990	9.46	-2.45	-1.06		-1.04		-1.42	-1.73	-3.35	-2.81	-2.52	-3.66	-5.65		
	2002	9.45	-2.63	-1.69		-1.64		-2.84	-1.44	-4.27	-3.72	-3.05	-2.73	-6.57		
	2008	9.31	-2.20	-1.00		-0.98		-1.28	-1.19	-3.08	-2.83		-2.15		-4.44	
F	1978	9.74	-2.94	-2.98		-2.62		-2.42	-2.81	-5.54		-2.96	-2.50		-4.42	
	1979	10.05	-3.06	-4.12		-3.00		-3.45	-2.21	-5.59	-3.27	-3.30	-3.55	-5.84	-6.52	
	1986	9.94	-3.34	-2.53		-2.46		-3.00	-2.31	-4.63	-4.03		-3.58	-5.79	-6.24	
	1987	10.34	-3.43	-3.34		-3.06		-2.74	-3.06	-5.40	-4.42	-3.43	-3.26		-6.67	
	1994	10.07	-3.16	-3.06		-2.79		-2.24	-2.39	-4.10	-3.39		-2.62		-6.26	
	1995	9.75	-2.05	-3.81		-2.03		-2.19	-2.18	-4.26	-2.83	-3.33	-2.53	-5.29	-6.32	
	2008	9.82	-3.26	-3.24		-2.90		-1.58	-1.74	-3.09	-3.09		-3.18	-5.62	-5.34	

[a] 16S-rRNA genes per g-dry soil.

Table S3. Exponential rate coefficients associated with detected ARG genes and heavy metals in archived soils based on unitized data from 1940-2008.

	Rate Coefficient (yr^{-1})	Standard Error of Rate	Correlation Coefficient (r^2)	Significance (*p*)
Tetracyclines				
tet(B)	.044	.018	.144	.017
tet(L)	-.005	.027	.002	.841
tet(M)	.057	.012	.318	< .001
tet(O)	.077	.017	.360	< .001
tet(Q)	.081	.018	.297	< .001
tet(W)	.035	.016	.098	.032
Erythromycins				
erm(B)	.017	.015	.033	.245
erm(C)	.029	.013	.089	.038
erm(E)	.029	.012	.117	.018
erm(F)	.022	.019	.030	.241
Beta-lactamases				
bla_{TEM-1}	.052	.015	.189	.001
bla_{SHV-1}	.026	.013	.077	.051
$bla_{CTX-M-1}$	.018	.020	.020	.379
ampC	.021	.032	.040	.531
Other				
*van*B	.044	.021	.108	.044
Metals				
Arsenic	.007	.003	.081	.038
Cadmium	-.005	.004	.031	.210
Copper	-.001	.004	.001	.860
Nickel	.002	.002	.009	.505
Zinc	.001	.006	.001	.861
Lead	.004	.009	.004	.657

Fig. S1. Antibiotic production in the United States (kton per year) from 1950 to 1986. (Source: Committee on Human Health Risk Assessment of using Subtherapeutic Antibiotics in Animal Feeds, *Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feed.* 1989, Institute of Medicine, Division of Health Promotion and Disease Prevention: National Academy Press, Washington, D.C.)

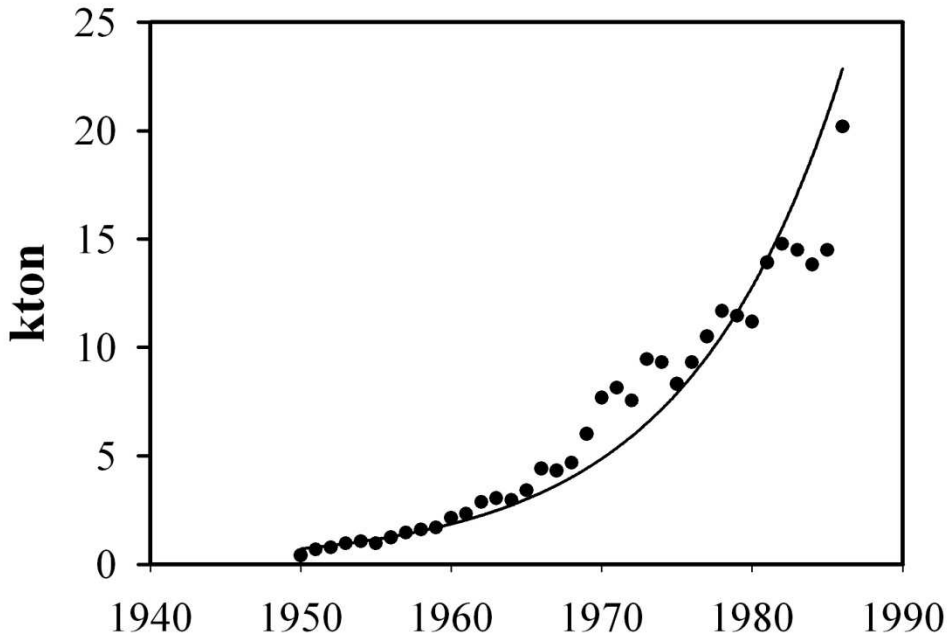

Fig. S2. Relative increase in heavy metals among soils collected at five sites in the Netherlands from 1940-2008. Relative heavy metal levels were grouped according to decade and unitized to mean 1970-1979 values for each site to account for variances in basal metal levels among sites (using the same method as for Figure 2). Each time-series represents the unbiased sum of standardized heavy metal levels from the sites. Rate coefficients for each heavy metal are presented in Table S3.

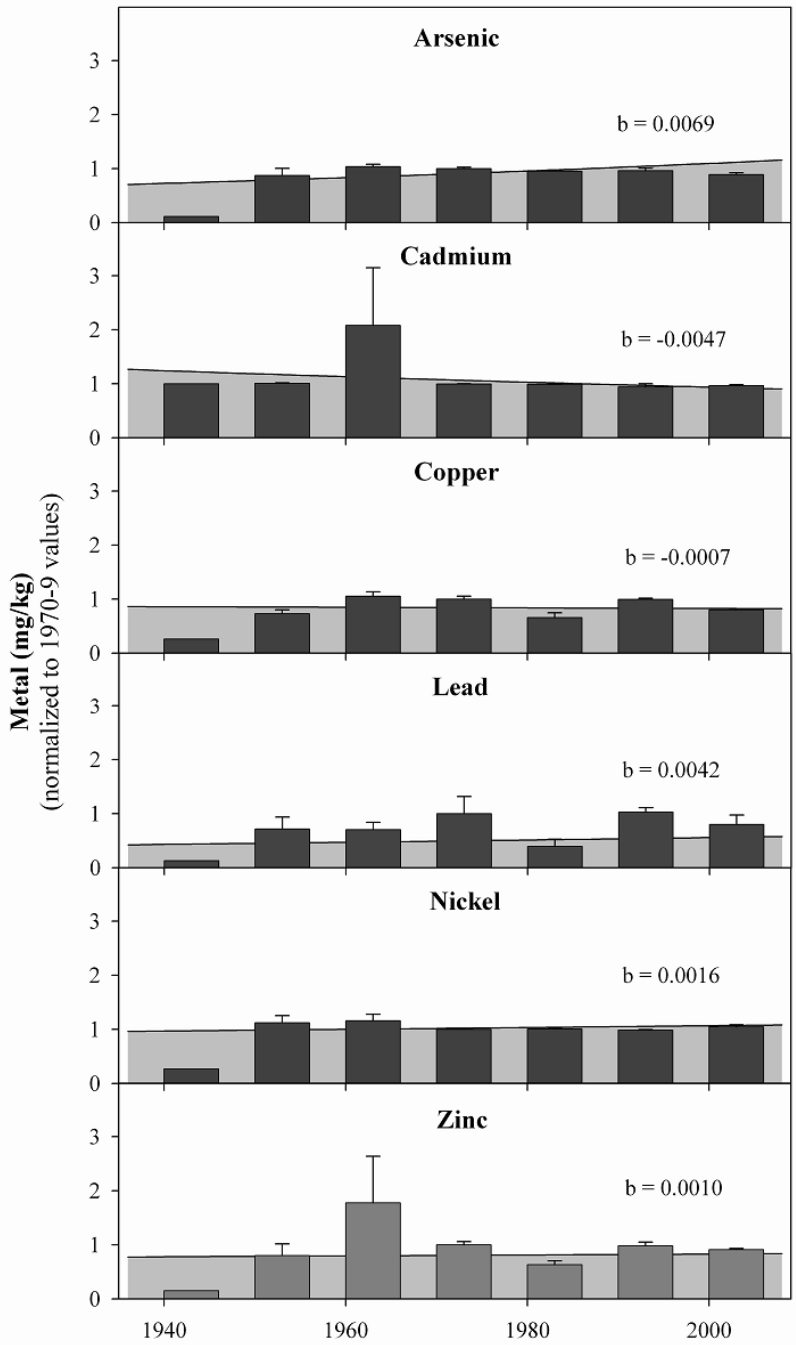
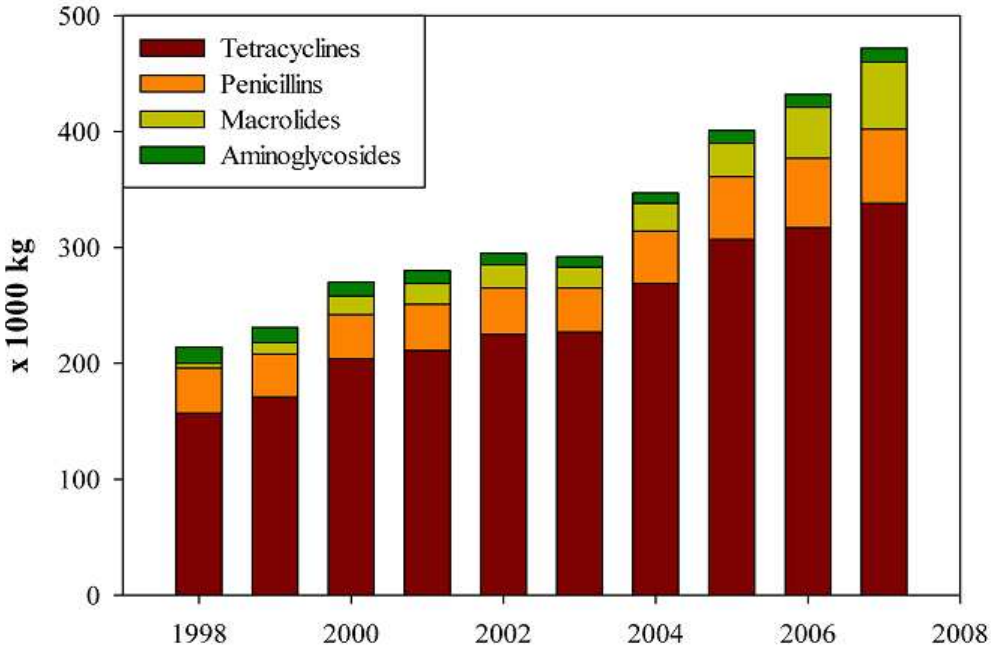
Fig. S3. Utilization of selected antibiotics in agriculture in the Netherlands between 1997 and 2007. (Source: FIDIN Werkgroep Antibioticabeleid *Antibioticarapportages*; FIDIN. Vereniging van Fabrikanten en Importeurs van Diergenessmiddelen in Nederland. The Hague).