Table S1: Sample information on the 18 soils used in this study. A cross (†) indicates no 16S rRNA sequence data was retrieved for the specified sample while an asterisk (*) means functional libraries were too small to be considered in cross-soil comparisons of ARG content. The soil identifier refers to the soil names used in ref. 8.

Site	**Sample Name**	**Soil Identifier**	**Soil Type**	**Added N (kg ha^{-1} y^{-1})**	**pH**	**C:N ratio**	**Total Organic C (mg / g soil^{-1})**	**Total N (mg / g soil^{-1})**	**Extractable N (mg kg soil^{-1})**
Cedar Creek	S04	CC A22	Grassland	0	7.3	14.2	16.8	1.2	0.8
Cedar Creek	S05	CC A45	Grassland	0	7.2	13.3	12.1	0.9	0.6
Cedar Creek	S06	CC A54	Grassland	0	7.2	13.0	18.5	1.4	0.6
Cedar Creek	S07	CC D08	Grassland	34	6.8	13.4	17.2	1.3	1.3
Cedar Creek	S08†	CC D23	Grassland	34	6.9	13.2	15.5	1.2	1.6
Cedar Creek	S09	CC D38	Grassland	34	7.0	13.2	21.6	1.6	1.0
Cedar Creek	S10	CC H17	Grassland	272	6.1	3.3	15.0	1.5	10.3
Cedar Creek	S11	CC H40	Grassland	272	6.1	11.5	22.5	2.0	12.4
Cedar Creek	S12	CC H52	Grassland	272	6.0	11.0	16.6	1.4	12.0
Kellogg Biological Station	S13	KBS 201	Agricultural	0	6.1	10.3	13.1	1.3	0.4
Kellogg Biological Station	S14	KBS 204	Agricultural	101	6.5	10.8	13.9	1.3	0.7
Kellogg Biological Station	S15	KBS 209	Agricultural	291	5.5	9.4	12.0	1.3	15.5
Kellogg Biological Station	S16	KBS 301	Agricultural	0	6.6	11.4	14.8	1.3	0.7
Kellogg Biological Station	S17	KBS 304	Agricultural	101	6.1	10.7	16.9	1.6	0.7
Kellogg Biological Station	S18*	KBS 309	Agricultural	291	5.0	9.5	17.0	1.8	18.7
Kellogg Biological Station	S19	KBS 401	Agricultural	0	6.2	11.4	14.0	1.2	0.9
Kellogg Biological Station	S20	KBS 404	Agricultural	101	6.0	11.4	16.8	1.5	1.3
Kellogg Biological Station	S21*	KBS 409	Agricultural	291	5.1	9.9	13.0	1.3	16.0

Table S2: Catalog of the 18 antibiotics used in functional selections for drug resistance from 18 U.S. soils.

Antibiotic	**Code**	**Selection Concentration (µg/ml)**	**Antibiotic Class**	**Antibiotic Type**
Aztreonam	AZ	8	B-lactam	Synthetic
Chloramphenicol	CH	8	Amphenicol	Natural
Ciprofloxacin	CI	0.5	Fluoroquinolone	Synthetic
Colistin	CL	8	Polymyxin	Natural
Cefepime	CP	8	β-lactam	Semi-Synthetic
Cefotaxime	CT	8	β-lactam	Semi-Synthetic
Cefoxitin	CX	64	β-lactam	Semi-Synthetic
D-Cycloserine	CY	32	Amino Acid Derivative	Natural
Ceftazidime	CZ	16	β-lactam	Semi-Synthetic
Gentamicin	GE	16	Aminoglycoside	Natural
Meropenem	ME	16	β-lactam	Semi-Synthetic
Penicillin	PE	128	β-lactam	Natural
Piperacillin	PI	16	β-lactam	Semi-Synthetic
Piperacillin-Tazobactam	PI-TZ	16-PI/4-TZ	β-lactam	Semi-Synthetic
Tetracycline	TE	8	Tetracycline	Natural
Tigecycline	TG	2	Tetracycline	Semi-Synthetic
Trimethoprim	TR	8	Folate Synthesis Inhibitor	Synthetic
Trimethoprim-Sulfamethoxazole	TR-SX	2-TR/38-SX	Folate Synthesis Inhibitor	Synthetic

Table S3: Tests for association of ARG mechanism with bacterial phyla for all comparisons with at least one ARG (those depicted in figure 2B). Significant (α<0.05, Fisher's exact test) relationships after Bonferroni correction for 45 hypotheses are indicated in bold and sorted by ARG function and two-tailed p-value. The third column denotes whether an ARG mechanism is enriched or depleted in the indicated phylum. N.S. Not significant.

ARG Mechanism	Bacterial Phylum	Enriched/Depleted?	2-tailed p-value	Adj. p-value
MFS_Transporter	**Actinobacteria**	**Enriched**	**5.39E-08**	**2.42595E-06**
MFS_Transporter	**Acidobacteria**	**Depleted**	**1.57E-05**	**0.0007**
MFS_Transporter	**unknown phylum**	**Depleted**	**3.10E-05**	**0.0014**
MFS_Transporter	**Proteobacteria**	**Enriched**	**0.0004**	**0.018**
MFS_Transporter	Bacteroidetes	Neither	0.0016	0.072
MFS_Transporter	Firmicutes	Neither	0.4692	N.S.
β-lactamase	**Verrucomicrobia**	**Enriched**	**1.08E-07**	**4.878E-06**
β-lactamase	**Actinobacteria**	**Depleted**	**6.01E-06**	**0.0003**
β-lactamase	**Acidobacteria**	**Enriched**	**1.89E-05**	**0.0008**
β-lactamase	**Cyanobacteria**	**Enriched**	**2.76E-05**	**0.0012**
β-lactamase	Firmicutes	Neither	0.0112	N.S.
β-lactamase	unknown phylum	Neither	0.0316	N.S.
β-lactamase	Proteobacteria	Neither	0.0612	N.S.
β-lactamase	Bacteroidetes	Neither	0.0954	N.S.
ABC_Transporter	Firmicutes	Neither	0.0298	N.S.
ABC_Transporter	unknown phylum	Neither	0.1052	N.S.
ABC_Transporter	Proteobacteria	Neither	0.2622	N.S.
ABC_Transporter	Bacteroidetes	Neither	0.4794	N.S.
ABC_Transporter	Actinobacteria	Neither	0.5046	N.S.
ABC_Transporter	Verrucomicrobia	Neither	0.5170	N.S.
ABC_Transporter	Acidobacteria	Neither	0.9570	N.S.
Acetyltransferase	Proteobacteria	Neither	0.0808	N.S.
Acetyltransferase	Firmicutes	Neither	0.1094	N.S.
Acetyltransferase	Verrucomicrobia	Neither	0.2190	N.S.
Acetyltransferase	unknown phylum	Neither	0.2866	N.S.
Acetyltransferase	Acidobacteria	Neither	0.3048	N.S.
Acetyltransferase	Actinobacteria	Neither	0.8146	N.S.
Acetyltransferase	Bacteroidetes	Neither	0.9990	N.S.
Methyltransferase	Proteobacteria	Neither	0.0202	N.S.
Methyltransferase	Firmicutes	Neither	0.7906	N.S.
Methyltransferase	Bacteroidetes	Neither	0.8176	N.S.
Methyltransferase	Actinobacteria	Neither	0.9356	N.S.
Other_ARG	Firmicutes	Neither	0.0034	N.S.
Other_ARG	Actinobacteria	Neither	0.0196	N.S.
Other_ARG	unknown phylum	Neither	0.1646	N.S.
Other_ARG	Acidobacteria	Neither	0.4192	N.S.
Other_ARG	Verrucomicrobia	Neither	0.5694	N.S.
Other_ARG	Bacteroidetes	Neither	0.7994	N.S.
Other_ARG	Proteobacteria	Neither	0.8158	N.S.
Other_ARG	Cyanobacteria	Neither	0.9482	N.S.
Phosphotransferase	Cyanobacteria	Neither	0.0720	N.S.
Phosphotransferase	Actinobacteria	Neither	0.1198	N.S.
Phosphotransferase	Acidobacteria	Neither	0.3768	N.S.
Phosphotransferase	unknown phylum	Neither	0.5836	N.S.
Phosphotransferase	Proteobacteria	Neither	0.8018	N.S.

Table S4: Resistance Gene families that most significantly differ between CC and KBS soils. Categories listed are those with unadjusted p-values (Wilcoxon rank sum test) below 0.05; all were elevated in CC soils. Two-tailed p-values are provided in the penultimate column and adjusted for 51 hypotheses via the Bonferroni method (final column). No individual resistance category retains statistical significance after adjustment (although multiple summed categories are robust to the correction). Rather, individual ARG categories are listed to highlight the specific ARGs that most strongly discriminate between CC and KBS soils.

Resistance Category ID	Annotation	p-value	Adj. p-value
All ARGs	Summed Counts for all ARG Categories	0.0002	0.0089
β-lactamase	Summed Counts for all β-lactamase families	0.0002	0.0089
MFS Transporters	Summed Counts for all MFS families	0.0012	0.0624
TIGR00710	Bcr Efflux Pump	0.0021	N.S.
ClassD	Class D β-lactamase	0.0033	N.S.
ABC Transporters	Summed Counts for all ABC families	0.0042	N.S.
SubclassB3	Subclass B3 β-lactamase	0.0052	N.S.
PF07690.11	Major Facilitator Superfamily	0.0052	N.S.
PF01061.19	ABC-2 type transporter	0.0065	N.S.
ClassA	Class A β-lactamase	0.0115	N.S.
TIGR01188	drrA, ABC resistance transporter	0.0122	N.S.
L1	L1 (subclass B3) β-lactamase	0.0225	N.S.
PF00905.17	Penicillin binding protein transpeptidase domain	0.0225	N.S.
VEB-PER	VEB/PER (class A) β-lactamase	0.0376	N.S.
TIGR00711	EmrB: drug resistance MFS transporter	0.0412	N.S.

Table S5: ARGs shared across at least two soils. An ARG was considered shared if at least two sequences from two soils contained ≥99% nucleotide identity. Of the 23 ARGs shared across soils, 21 were shared within CC soils, a significant enrichment over observations from KBS ($p<0.05$, Fisher's exact test). ARG names take the convention: Soil_Antibiotic_ContigNumber_Start_Stop.

ARG 1	**ARG 2**	**ARG 3**	**ARG 4**	**ARG Mechanism**	**Soil Site**
S04_CZ_11_915_1820	S05_CH_3_188_553			β-lactamase	CC
S04_AZ_10_1449_2267	S05_AZ_3_1007_1825			β-lactamase	CC
S05_PI_43_57_872	S04_PITZ_1_55_870	S05_AZ_7_2_763		β-lactamase	CC
S05_PE_125_1138_2253	S09_PE_23_3_587			β-lactamase	CC
S06_TG_5_48_1226	S07_TG_5_227_1465	S06_TE_10_48_1226	S07_TE_13_223_1461	MFS Transporter	CC
S06_TG_4_158_1618	S08_TG_1_895_2355			MFS Transporter	CC
S07_TE_21_681_1403	S09_TE_27_78_1352			MFS Transporter	CC
S10_CX_11_1_840	S11_CT_3_97_1110			β-lactamase	CC
S10_CZ_5_324_1259	S11_CZ_4_340_1275			β-lactamase	CC
S15_CH_4_3_449	S21_CH_6_718_1983			MFS Transporter	KBS

Table S6: Resistance Gene families that most significantly differ between high-N and other CC soils. Categories listed are those with unadjusted p-values (Wilcoxon rank sum test) below 0.05. Two-tailed values are provided in the penultimate column and the group in which the ARG is elevated indicated in the final column. No resistance category retains statistical significance after multiple hypothesis correction; rather, ARG categories are listed to highlight the functions that most strongly discriminate between high-N and other CC soils.

Resistance Category ID	Annotation	p-value	Elevated In:
β-lactamase	Summed Counts for all β-lactamase families	0.0238	Other CC Soils
ClassD	Class D β-lactamase	0.0238	Other CC Soils
PF00005.22	ABC Transporter	0.0275	High-N CC Soils
SubclassB3	Subclass B3 β-lactamase	0.0476	Other CC Soils

Table S7: Significant Spearman correlations between Bray-Curtis distance matrices calculated from normalized ARG counts and phylogenetic/taxonomic distance matrices generated from 16S rRNA gene sequence. Significant correlations were observed within CC soils, and across the combined set of CC and KBS soils. No significant trend was observed for solely KBS soils.

Soil Resistomes Considered	Phylogenetic/Taxonomic 16S Metric	Spearman's rho (ρ)	p-value
All Soils	Unweighted Unifrac	0.35	0.0074
All Soils	Weighted Unifrac	0.22	0.0447
All Soils	Bray-Curtis	0.27	0.0265
CC Soils	Unweighted Unifrac	0.59	0.0052
CC Soils	Weighted Unifrac	0.42	0.0303
CC Soils	Bray-Curtis	0.44	0.032

Table S8: M^2 and p-values associated with Procrustes transformations. When all soils or only CC soils are considered, a significant association between resistome content and soil bacterial composition is observed, independent of whether phylogenetic (unweighted/weighted Unifrac) or taxonomic (Bray-Curtis) measures of 16S community structure are used. Significance is robust to transformations across either the first two or all principal coordinates.

Soils Considered	16S Metric	# Principal Coordinates Transformed	M^2	p-Value
All Soils	Bray Curtis	2	0.388	<0.0001
All Soils	Bray Curtis	15	0.179	0.0003
All Soils	Unweighted Unifrac	2	0.362	<0.0001
All Soils	Unweighted Unifrac	15	0.184	<0.0001
All Soils	Weighted Unifrac	2	0.474	0.0001
All Soils	Weighted Unifrac	15	0.273	0.0007
Cedar Creek	Bray Curtis	2	0.227	0.0028
Cedar Creek	Bray Curtis	8	0.072	0.0049
Cedar Creek	Unweighted Unifrac	2	0.236	0.0018
Cedar Creek	Unweighted Unifrac	8	0.049	0.0014
Cedar Creek	Weighted Unifrac	2	0.299	0.004
Cedar Creek	Weighted Unifrac	8	0.185	0.0078

Table S9: 16S community similarity measurements, calculated across all soils, Cedar Creek (grassland) soils, and Kellogg Biological Station (agricultural) soils. Soils considered were those used in Procrustes and Mantel analyses. By all metrics used, Kellogg Biological Station soils showed less community variability.

16S rRNA Gene Community Similarity			
Soils Considered	**Bray-Curtis Similarity**	**Unweighted Unifrac Similarity**	**Weighted Unifrac Similarity**
All	0.327	0.303	0.748
Cedar Creek	0.335	0.315	0.740
Kellogg Biological Station	0.446	0.343	0.825