

Supplementary Information

Table S1. Primers and standard sequences used for Fluidigm qPCR.

Target Gene	Sequence (5' → 3')	Amplicon Length	GenBank Accession*
16S rRNA	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	160	KR190116
<i>aacA</i>	GTGTAACACGCAAGCACGAT AGCCTCCGCGATTTCATAC	158	KM589496.1
<i>aadA5</i>	ATCTTGCGATTTTGCTGACC TGTACCAAATGCGAGCAAGA	158	KR108291.1
<i>aadD</i>	ATGGGGATGATGTTAAGGCT TCACTTCCACCTTCCACTCA	113	JQ320084.1
<i>acrD</i>	GGCAATCCTGTTGTGTCTGA ACATGAGATTATCGAGGCCG	145	CP013029.1
<i>amp</i>	CCTCTTGCTCCACATTTGCT ACAACGTTTGCTGTGTGACG	149	KR010387.1
<i>arr2</i>	TTACAAGCAGGTGCAAGGAC GCTCCATCAAGGCTGAAAAG	100	KR868994.1
<i>bla_{KPC}</i>	GATACCACGTTCCGCTCTGG GCAGGTTCGGTTTTGTCTC	213	KU216748.1
<i>bla_{NDM-1}</i>	ATTAGCCGCTGCATTGAT CATGTGCGAGATAGGAAGTG	117	KP765738.1
<i>bla_{NPS}</i>	GGACCATCGTCATCGAGTCT ATTCGCAATCGAATACTGGG	148	NG_035412.1
<i>bla_{OXA}</i>	TGATGATTGTGAAGCCAAA GCCTGTAGGCCACTCTACCC	61	AY007784.1
<i>bla_{SHV}</i>	AACGGAAGTGAATGAGGCGCT TCCACCATCCACTGCAGCAGCT	98	KT218681.1
<i>bla_{VIM}</i>	CGCAGCTTTCTGGTTGGTAT CGTGTCAACCGAGTTTCTGAG	140	AJ536835.1
<i>cadA</i>	GTGAGCAGGCCAGCACTGAA TCTCGATGCGGTAGGTGGTC	215	AP014839.2
<i>catB8</i>	GGGGAAGTTCCTTTCTGAGCA CCCCGCTTCCTATAGAACAA	135	KM382180.1
<i>chrA</i>	TCACGCCGGAATATAACTAC CGTACCCTGATCAATCACTT	229	CP011343.2
<i>cmlB</i>	TAATTGGCGGTATCCCTTG AGCGTAAGCTGAATTGTGCC	107	NG_036654.1
<i>copA</i>	GTGTACGGTCCGCTGGTTAT CTTGAACACTCCGGTCCAG	297	CP003041.1
<i>ctx-m-32</i>	CGTCACGCTGTTGTTAGGAA CGCTCATCAGCACGATAAAG	116	KT265731.1
<i>dfp13</i>	AATCGGTCCGCATTTATCTG TTGGTAAGGGCTTGCCTATG	134	KF914295.1
<i>ereB</i>	TCTGCATTATGCCAACGGTA TCTGCTCACTTTGTGGGTTTT	117	AF466411.1
<i>ermB</i>	GATACCGTTTACGAAATTGG GAATCGAGACTTGAGTGTGC	324	KF864551.1

<i>ermF</i>	CGACACAGCTTTGGTTGAAC GGACCTACCTCATAGACAAG	269	KP265720.1
<i>floR</i>	TCGTCATCTACGGCCTTTTC CTTGACTTGATCCAGAGGGC	148	KR186206.1
<i>imp13</i>	AGGAGCGGCTTTACCTGATT CGCTCCACAAACCAATTGAC	158	JN091097.1
<i>intI1</i>	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	246	KR262557.1
<i>intI2</i>	GACGGCTACCCTCTGTTATCTC TGCTTTTCCCACCCTTACC	154	KF534916.1
<i>intI3</i>	GGATGTCTGTGCCTGCTTG GCCACCACTTGTTTGAGGA	100	HE616889.1
<i>mefE</i>	CCTGCAAATGGCGATTATTT AATAGCAAGCACTGCACCAG	159	EU908679.1
<i>merA</i>	GTGCCGTCCAAGATCATG GGTGGAAGTCCAGTAGGGTGA	321	KF856624.1
<i>mexB</i>	GTGTTCGGCTCGCAGTACTC AACCGTCGGGATTGACCTTG	204	L11616.1
<i>nikA</i>	AATATCAGGCAGACGGTTCG AGGGTGAAGGTCCAGGTTTT	45	CP007037.1
<i>qacF</i>	TGGCTGTTTCAATCTTTGGC GCCCATACAGCGTAAGCAAT	132	KC441951.1
<i>qnrA</i>	AGGATTTCTCACGCCAGGATT CCGCTTTCAATGAAACTGCA	83	JN103331.1
<i>qnrB</i>	AAATATGGCTCTGGCACTCG CTTTCAGCATCGCACGACTA	151	JN166690.1
<i>rcnA</i>	GGGCACTCAAAAACGATGAT GCGAAATAGTTGCTGCCAGT	54	CP013029.1
<i>strB</i>	GCCTGTTTTTCCTGCTCATT CGCAGTTCATCAGCAATGTC	110	NG_041759.1
<i>sul1</i>	CCGTTGGCCTTCCTGTAAAG TTGCCGATCGCGTGAAGT	29	KR338352.1
<i>sul2</i>	GACAGTTATCAACCCGCGAC GTCTTGACCCGAATGCATAA	107	KM194586.1
<i>sul3</i>	TCCGTTACAGCAATTGGTGCAG TTCGTTACAGCCTTACACCAGC	83	AY617070.1
<i>tetA</i>	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	170	KF483599.2
<i>tetL</i>	TCGTTAGCGTGCTGTCATTC GTATCCCACCAATGTAGCCG	227	KP658723.1
<i>tetM</i>	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	366	KJ545575.2
<i>tetS</i>	CAAGGATTGTACGGTTGGA TTTCGAAGCTAAGATATGGCTC	129	JX865374.1
<i>tetW</i>	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	126	KC790464.1
<i>tetX</i>	AGCCTTACCAATGGGTGTA TTCTTACCTTGACATCCCG	237	JQ990987.1
<i>vanA</i>	GTAGGCTGCGATATTCAAAGC	189	LC088032.1

	CGATTCAATTGCGTAGTCCAA		
<i>vanB</i>	TTGCATGGACAAATCACTGG	319	KF823968.1
	GCTCGTTTTCTGATGGATG		

*Standard sequences used for Fluidigm microfluidic qPCR assays

Reference: Sandberg, Kyle. (2016). Quantification and Characterization of Antibiotic Resistance Gene Profiles In Freshwater Sediment. Retrieved from the University of Minnesota Digital Conservancy, <http://hdl.handle.net/11299/202144>.

Table S2. Yields and purity of DNA extracts of the sediments collected in October 2016. Values were used to calculate gene abundance in sediment g⁻¹ dry weight.

Sample	Replicate	DNA concentration (ng/μl)	260:280	260:230
CHLP	sed1	19.3	1.88	1.08
	sed2	19.7	1.91	1.25
	sed3	18.8	1.67	1.19
OO	sed1	20.6	1.86	1.69
	sed2	18.9	1.9	1.77
	sed3	20.2	1.88	1.67
PP	sed1	9.5	1.87	1.05
	sed2	11.4	1.79	1.82
	sed3	13.7	1.82	1.39

Table S3. List of all metal homeostasis and antibiotic resistance genes detected by the GeoChip 5.0.

Metal Homeostasis			
Gene Name	Metal	Role	Gene Annotation
Al	aluminum	transport	aluminum resistance protein
arsc	arsenic	detoxification	arsenate reductase
arsB	arsenic	transport	arsenical pump membrane protein, putative
arsA_fungi	arsenic	transport	arsenical pump-driving ATPase
arxa	arsenic	detoxification	molybdopterin oxidoreductase
arra	arsenic	detoxification	molybdopterin oxidoreductase
aoxb	arsenic	detoxification	arsenite oxidase
arasm	arsenic	detoxification	arsenite S-adenosylmethyltransferase
atr1	boron	transport	Pc12g00240
bor1	boron	transport	boron efflux transporter, putative
CadA	cadmium	transport	hypothetical protein ATEG_02507
cadBD	cadmium	transport	conserved hypothetical protein
cd_metallothionein_ciliophora	cadmium	sequester	subfamily 7a metallothionein 1
chaA	calcium	transport	sodium/calcium exchanger membrane region
Ca_vacuolar_exchanger	calcium	transport	vacuolar calcium ion transporter
ChrA	chromium	transport	hypothetical protein BC1002_5263
chrr	chromium	detoxification	putative NADPH-dependent FMN reductase
CorC	cobalt	transport	hypothetical 33.3 kDa protein
cusF	copper	transport	hypothetical protein CFU_2969
pcoA	copper	detoxification	copper resistance protein A
copA	copper	transport	copper-exporting ATPase
CusA	copper	transport	outer membrane copper (silver) and drug transport protein (RND family)
CutA	copper	transport	cutA gene product
cueo	copper	detoxification	multicopper oxidase family protein
ycnJ	copper	transport	possible copper (Cu) export protein
pcoC	copper	transport	copper resistance protein C
cusC	copper	transport	outer membrane lipoprotein
pcoE	copper	transport	putative copper-binding protein PcoE
dps	iron	storage	putative DNA-binding stress protein
cirA	iron	transport	TonB-dependent receptor
feoB	iron	transport	ferrous iron transport protein B
fhuE	iron	transport	TonB-dependent siderophore receptor
fepA_iron	iron	transport	TonB-dependent outer membrane siderophore receptor
bfr	iron	storage	bacterioferritin
fecA	iron	transport	TonB-dependent receptor
fhuA	iron	transport	putative ferrichrome-iron receptor FhuA
siderophore_transporter	iron	transport	siderochrome-iron transporter MirC
sidC	iron	transport	ferrichrome synthetase Sib1
fiu	iron	transport	Putative TonB-dependent receptor
ferroxidase_high_affinity	iron	transport	conidial pigment biosynthesis oxidase Abr1/brown 1
sidA	iron	transport	L-ornithine 5-monooxygenase
vacuolar_iron_transport	iron	transport	hypothetical protein SS1G_06910

iron_permease_high_affinity	iron	transport	plasma membrane iron permease
entB	iron	transport	2,3-dihydroxybenzoate-AMP ligase
ferric_reductase_transporter	iron	transport	ferric reductase transmembrane component (Ferric-chelate reductase)
Pyoverdin_pvcC	iron	transport	pyoverdin chromophore biosynthetic protein pvcC
pbrA	lead	transport	heavy metal translocating P-type ATPase
pbrT	lead	transport	lead uptake protein
pbrD	lead	transport	PbrD protein
mgtE	magnesium	transport	magnesium (Mg ²⁺) transporter-like protein
mgtA	magnesium	transport	P-type HAD superfamily ATPase
mntH_Nramp	manganese	transport	NRAMP family Mn ²⁺ /Fe ²⁺ transporter
psaA_5f0_Mn	manganese	transport	periplasmic solute binding protein
merT	mercury	transport	Mercuric transport protein MerT
merP	mercury	transport	heavy metal translocating P-type ATPase
mer	mercury	detoxification	pyridine mercuric reductase
merb	mercury	detoxification	Alkylmercury lyase
metc	mercury	detoxification	putative mercury transport protein MerC
merF	mercury	transport	hypothetical protein OA307_1783
merE	mercury	transport	transporter
merg	mercury	transport	drug efflux transporter
merH	mercury	transport	mercuric transporter MerH
nikA	nickel	transport	extracellular solute-binding protein family 5
NiCoT	nickel	transport	hypothetical protein LDG_7536
nreB	nickel	transport	probable NreB protein
nikC	nickel	transport	nickel transport system permease protein NikC
trkGH	potassium	transport	H(+)-transporting two-sector ATPase
trkA	potassium	transport	potassium transporter peripheral membrane component
kefBC	potassium	transport	sodium/hydrogen exchanger
kdpA	potassium	transport	unnamed protein product
kup	potassium	transport	low affinity potassium transport system protein Kup
ktrBD	potassium	transport	Trk-type potassium uptake protein KtrB
trk_fungi	potassium	transport	hypothetical protein PTT_14075
Se	selenium	detoxification	selenate reductase subunit alpha (Selenatereductase molybdenum subunit)
silaffin	silicon	biosynthesis	silaffin precursor
silP	silver	transport	copper/silver-translocating P-type ATPase
silC	silver	transport	OprM
silA	silver	transport	Resistance-Nodulation-Cell Division Superfamily transporter
nhaA	sodium	transport	pH-dependent sodium/proton antiporter
nhaB	sodium	transport	sodium/proton antiporter
nhaP	sodium	transport	putative sodium/proton antiporter

mrpA	sodium	transport	NADH:ubiquinone oxidoreductase subunit 5 (chain L)/multisubunit Na ⁺ /H ⁺ antiporter, MnhA subunit
natB	sodium	transport	conserved hypothetical protein
nhaD	sodium	transport	NhaD family sodium/proton antiporter
nqrB	sodium	transport	Na ⁺ (+)-translocating NADH-quinone reductase subunit B
nhaC	sodium	transport	antiporter (Na /H antiporter)
terZD	tellurium	transport	tellurite resistance protein TerA
terc	tellurium	detoxification	Integral membrane protein TerC
tehb	tellurium	detoxification	Hemagglutinin protein
TerD	tellurium	transport	stress protein
TerZ	tellurium	transport	tellurium resistance protein
zntA	zinc	transport	Heavy metal translocating P-type ATPase
znuA	zinc	transport	high-affinity zinc transporter periplasmic component
znuC	zinc	transport	ABC transporter-related protein
troA	zinc	transport	zinc ABC transporter, periplasmic zinc-binding protein
zitB	zinc	transport	cation diffusion facilitator family transporter
adcA	zinc	transport	zinc ABC transporter, zinc-binding lipoprotein
zrt2	zinc	transport	hypothetical protein CPAR2_210740
zupT_ygiE	zinc	transport	zinc transporter ZupT
yiip_fieF	zinc	transport	cation-efflux pump FieF
zrt1	zinc	transport	DEHA2B16170p
msc2	zinc	transport	Msc2p
cot1	zinc	transport	DEHA2G03278p
zrt3	zinc	transport	hypothetical protein NDAI_0A00880
zrc1	zinc	transport	Pc12g02060
czcD	cadmium,cobalt,zinc	transport	cation efflux family protein
czcA	cadmium,cobalt,zinc	transport	heavy metal efflux pump, CzcA family
czcC	cadmium,cobalt,zinc	transport	HelC protein
rcnA	cobalt,nickel	transport	nickel/cobalt efflux protein RcnA
cnrA	cobalt,nickel	transport	heavy metal efflux pump, CzcA family
cnrC	cobalt,nickel	transport	putative nickel and cobalt resistance protein
corA	cobalt/magnesium	transport	putative metal-ion transporter
rndA	multiple metals	transport	cation efflux system protein CzcA
smtA	multiple metals	sequester	metallothionein
metallothionein	multiple metals	sequestration	putative metallothionein
Antibiotic Resistance			
Gene Name	Antibiotics	Role	Gene Annotation
B_lactamase_A	β-lactams	degradation	beta-lactamase
b_lactamase_b	β-lactams	degradation	THIN-B proetin
B_lactamase_C	β-lactams	degradation	beta-lactamase
b_lactamase	β-lactams	degradation	Beta-lactamase
fosb	fosfomycin	degradation	fosfomycin resistance protein FosB
fosa	fosfomycin	degradation	ORF1 protein
fosx	fosfomycin	degradation	fosfomycin resistance family protein
qnr	quinolone	subunit modification	QnrB31

Tet	tetracycline	transporter	tetracycline resistance protein
tetx_resistance	tetracyclines	degradation	TetX
Van	vancomycin	subunit modification	putative lactate dehydrogenase VanHE
vgb	virginiamycin	degradation	Virginiamycin B lyase
Mex	multiple antibiotics	transporter	Acr family drug resistance transporter
MFS_antibiotic	multiple antibiotics	transporter	major facilitator superfamily MFS_1
SMR_antibiotics	multiple antibiotics	transporter	putative multidrug transporter, SMR family, DMT Superfamily protein
MATE_antibiotic	multiple antibiotics	transporter	multidrug transport protein (MATE family)
ABC_antibiotic_transporter	multiple antibiotics	transporter	ABC transporter
ABC_multidrug_fungi	multiple antibiotics	transporter	TPA: ABC multidrug transporter (Eurofung)

Table S4. Measured concentrations of elements in the sediments. Analytical details were provided by OSU OARDC STAR Laboratory: Samples were digested with quality control materials (Soil CK 13 [STAR lab] and NIST Standard 2709a) to provide detection limits for the three soils (not reported). Additionally, blanks and standard check solutions were measured every 15-20 samples.

	λ (nm)	Instrument Detection Limit (ppm)	CHLP		OO		PP	
g kg ⁻¹ dry sediment								
Ca	422.673	0.004	60.6	81.4	22.9	22.5	31.3	29.4
Al*	396.152	0.0006	18.2	17.3	26.0	24.9	32.6	34.2
Fe*	238.204	0.0006	18.6	16.6	22.9	22.4	23.2	23.7
Mg*	285.213	0.003	22.7	30.5	11.4	11.3	12.2	12.0
K*	766.491	0.072	6.5	6.6	5.6	5.4	12.3	13.0
Si*	288.158	0.001	1.6	1.5	1.6	1.4	1.7	1.6
Mn*	257.610	0.00008	0.6	0.6	1.8	1.6	0.7	0.7
P	213.618	0.003	0.5	0.6	1.5	1.4	0.7	0.7
S	180.669	0.012	0.5	0.6	1.4	1.4	0.4	0.4
Mg kg ⁻¹ dry sediment								
Na*	588.995	0.0318	249.4	295.3	228.0	238.1	382.1	405.0
Ba	455.403	0.004	115.1	130.9	251.6	241.4	171.3	181.1
Zn*	206.200	0.001	67.6	93.0	131.2	125.2	69.9	72.2
Sr	421.552	0.0001	112.8	129.6	55.9	54.6	65.1	65.0
V	292.401	0.0005	45.7	42.2	57.7	56.2	70.0	72.6
Cr*	267.716	0.004	20.9	19.9	33.1	32.1	37.0	38.6
Ni*	216.555	0.0005	24.5	23.9	27.3	26.2	34.9	35.9
Cu*	324.754	0.0003	20.4	43.6	27.8	26.9	26.2	22.8
Li	610.365	0.003	19.5	19.7	25.7	24.6	36.3	38.2
B*	208.956	0.004	17.0	25.6	14.1	13.2	44.0	47.4
Pb*	220.353	0.002	15.2	13.6	20.5	20.7	13.9	14.0
Co*	228.615	0.004	10.9	9.0	11.4	10.9	14.7	15.5
As*	193.696	0.004	10.6	7.9	9.1	8.9	7.0	7.4
Mo	202.032	0.001	7.0	6.0	2.1	2.4	5.1	4.6
Se*	196.026	0.004	2.0	1.8	3.0	3.5	<0.9	<0.9
Tl*	276.789	0.008	1.0	2.3	4.6	1.8	<0.05	1.2
Cd*	226.502	0.008	1.7	1.5	2.5	2.4	1.9	1.9
Sb	206.834	0.005	0.7	0.8	0.7	<0.4	1.0	1.5
Be	234.861	0.004	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9

* Metals for which metal homeostasis genes were detected by either the Fluidigm or GeoChip platforms.

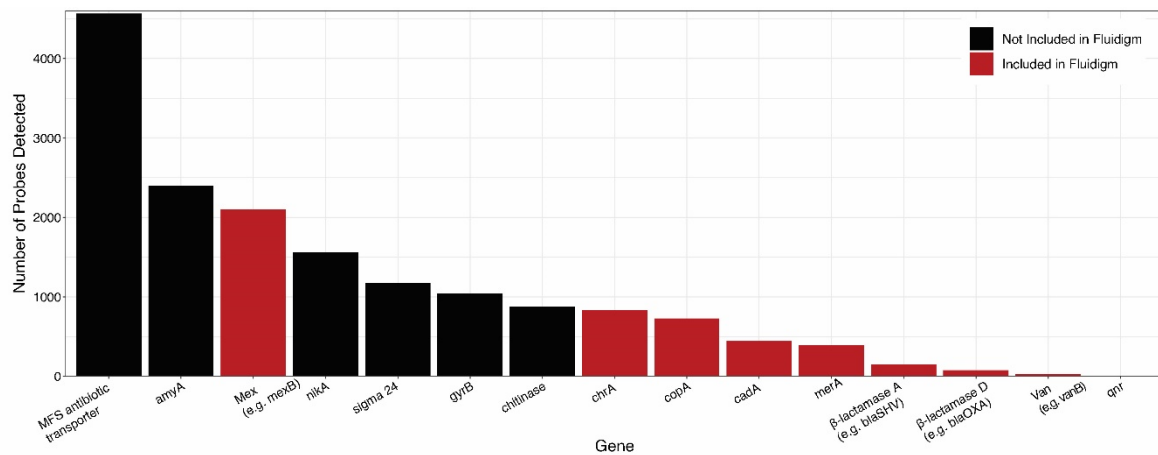


Figure S1. The number of gene probes detected in the GeoChip. This includes the 8 genes represented on the Fluidigm, as well as the seven other most commonly detected genes.

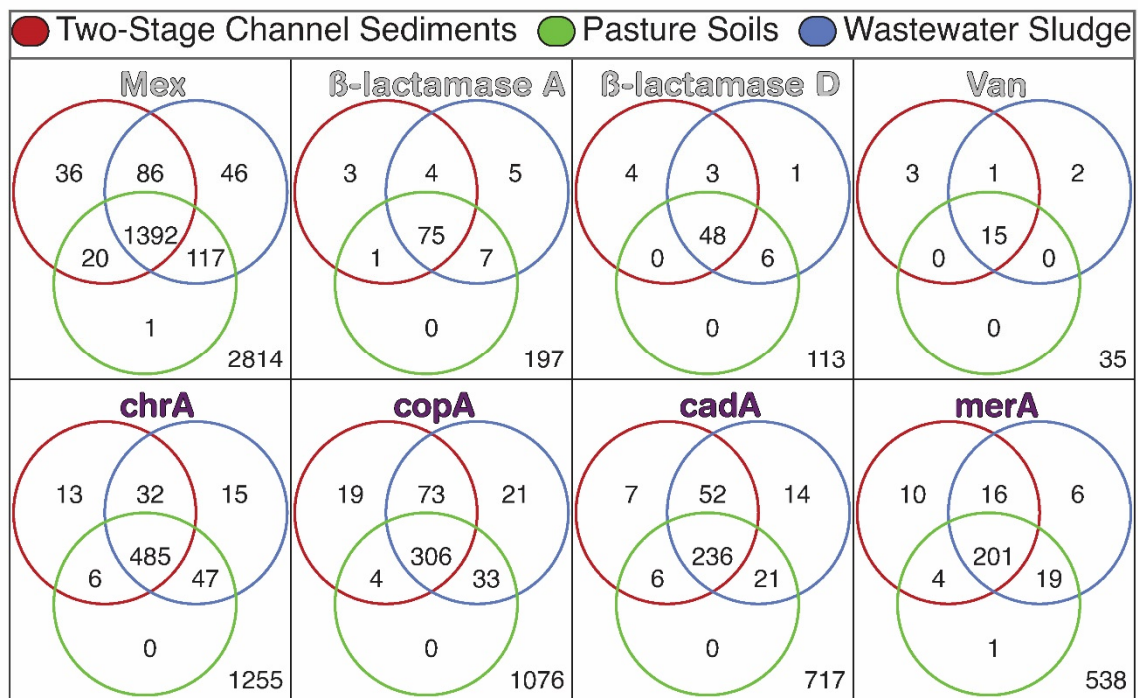


Figure S2. The number of indicator species identified for each of the GeoChip studies. The number in the bottom right indicate the total number of probes belonging to each gene. Indicator species analysis also included the full Tibetan restored grassland data set (not shown) allowing for indicators to be identified for the three sites.