

Supplementary Material

1 Supplementary Tables

Table S1. Sampling scheme showing the samples used for each type of analysis.

Analysis Date	HPLC-MS	qPCR	16S rRNA gene amplicon seq.	<i>intI1</i> gene cassette
7 th Dec 20	✓	✓	✓	✓
4 th Jan 21	✓	✓		✓
11 th Jan 21	✓	✓	✓	✓
18 th Jan 21	✓	✓		✓
25 th Jan 21	✓	✓		✓
1 st Feb 21	✓	✓	✓	✓
24 th Feb 21		✓		
25 th Feb 21		✓		
26 th Feb 21		✓	✓	✓
27 th Feb 21		✓		
28 th Feb 21		✓		✓
1 st Mar 21		✓		
2 nd Mar 21		✓		
3 rd Mar 21		✓		
4 th Mar 21	✓	✓	✓	✓

Table S2. Multiple reaction monitoring (MRM) conditions for the analysis of SMX, SDZ and SMZ.

Analyte	Q1 (m/z)	Q3 (m/z)	Declustering Potential DP (V)	Entrance Potential EP (V)	Collision Energy CE (V)	Cell Exit Potential CXP (V)
SMX-d4	258.2	160.0	55	10	22	12
SDZ	251.1	156.1	45	10	22	14
		92.0			37	10
SMZ	279.0	185.9	60	10	25	8
		124.1			32	8
SMX	254.0	156.0	55	10	22	12
	254.0	108.0	55	10	32	10

Table S3. Overview of primers used in this study.

Gene	Primers*	Sequence (5'-3')	NCBI Ref.Seq.	Amplicon size (bp)	Reference	
16S	27F	AGAGTTTGATCMTGGCTCAG	-	1465	Lane D. J., 1991	
	1492R	CGGTTACCTTGTTACGACTT			Turner et al., 1999	
	519qF	CCAGCAGCCGCGGTAATAC		-	410	
	909qR	CCGTCAATTCCTTTRAGTTT				
	341F-TS	ACACTCTTTCCCTACACGACGCT CTTCCGATCT CCTACGGGNGGC WGCAG	-	194		
	518R-TS	GACTGGAGTTCAGACCTGTGCTC TTCCGATCT WTTACCGCGGCTG CTGG				
<i>sul1</i>	sul1qF	TGTCGAACCTTCAAAAGCTG	WP_00025 9031.1	113	Wang et al., 2014	
	sul1qR	TGGACCCAGATCCTTTACAG				
<i>sul2</i>	sul2qF	ATCTGCCAAACTCGTCGTTA	WP_00104 3260.1	89	Wang et al., 2014	
	sul2qR	CAATGTGATCCATGATGTCG				
<i>intI1</i>	5'CS	AAACGGATGAAGGCACGAAC	WP_00084 5048.1	variable	Frank et al., 2007	
	3'CS	ATTGCGATAACAAGAAAAAGCC				
	intI1qF	CGAACGAGTGGCGGAGGGTG		312	Gillings et al., 2015	
	intI1qR	TACCCGAGAGCTTGGCACCCA				

*q in primer name indicates the use in qPCR

Table S4. Site-specific phylotypes and phylotypes introduced by wastewater discharge on a family level (bold face). Only phylotypes with a frequency over 1 % were considered for the analysis.

Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
<i>Caulobacteraceae</i>	<i>Flavobacteriaceae</i>	<i>Rhodobacteraceae</i>			
<i>Nocardiaceae</i>		<i>Diplorickettsiaceae</i>			
<i>Uncl. Rhizobiales</i>		<i>Saprospiraceae</i>			
		<i>Peptostreptococcaceae</i>			

Table S5. List of gene cassette inserts found for each site.

Site 3	Site 4	Site 5	Site 6
<i>aadA1</i> ^{*1} <i>aadA1</i> ^{*1} <i>aadA1</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA23</i> ^{*1} <i>aadA24</i> ^{*1} <i>aadA25</i> ^{*1} <i>aadA4</i> ^{*1} <i>aadA6/aadA10</i> ^{*1} <i>aadA6/aadA10</i> ^{*1} <i>aadA6/aadA10</i> ^{*1} <i>bla</i> _{BEL-1} ^{*2} <i>bla</i> _{BEL-1} ^{*2} <i>bla</i> _{GES-11} ^{*4} <i>bla</i> _{OXA-33} ^{*3} <i>bla</i> _{OXA-36} ^{*3} <i>bla</i> _{OXA-392} ^{*3} <i>bla</i> _{OXA-4} ^{*3} <i>bla</i> _{OXA-824} ^{*3} <i>cmlA5</i> ^{*5} <i>dfrA7</i> ^{*6} <i>ereA2</i> ^{*7} <i>ereA2</i> ^{*7} <i>ereA2</i> ^{*7} <i>ereA2</i> ^{*7} <i>qacL</i> ^{*8}	<i>aadA1</i> ^{*1} <i>aadA1</i> ^{*1} <i>aadA1</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA11</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA23</i> ^{*1} <i>aadA24</i> ^{*1} <i>aadA6/aadA10</i> ^{*1} <i>bla</i> _{BEL-1} ^{*2} <i>bla</i> _{OXA-33} ^{*3} <i>bla</i> _{OXA-36} ^{*3} <i>ereA2</i> ^{*7} <i>qacL</i>	<i>aadA2</i> ^{*1} <i>aadA6/aadA10</i> ^{*1}	<i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>aadA2</i> ^{*1} <i>bla</i> _{OXA-36} ^{*3}

Gene family | Drug class

*1 ANT(3'') aminoglycoside nucleotidyltransferase | aminoglycosides

*2 BEL beta-lactamase | cephalosporins, penams, monobactams

*3 OXA beta-lactamase | carbapenems, cephalosporins, penams

*4 GES beta-lactamase | carbapenems, cephalosporins, penams

*5 Major facilitator superfamily (MFS) antibiotic efflux pump | phenicol

*6 Trimethoprim resistant dihydrofolate reductase dfr | diaminopyrimidines

*7 Macrolide esterase | Macrolides

*8 Small multidrug resistance (SMR) antibiotic efflux pump | disinfecting agents and antiseptics

2 Supplementary Figures

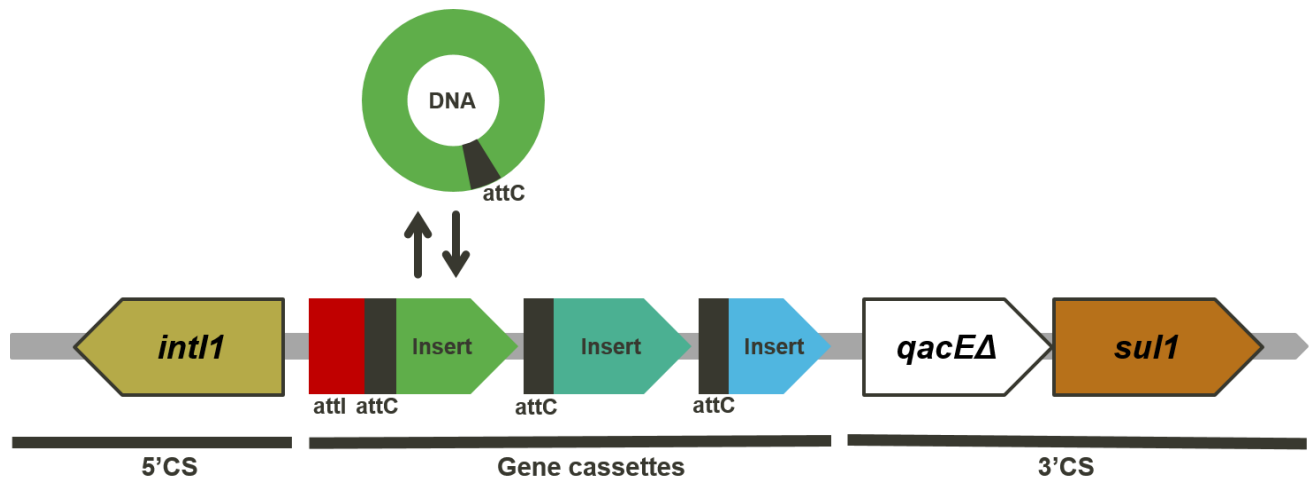


Figure S1. Structure of the Class 1 integron.

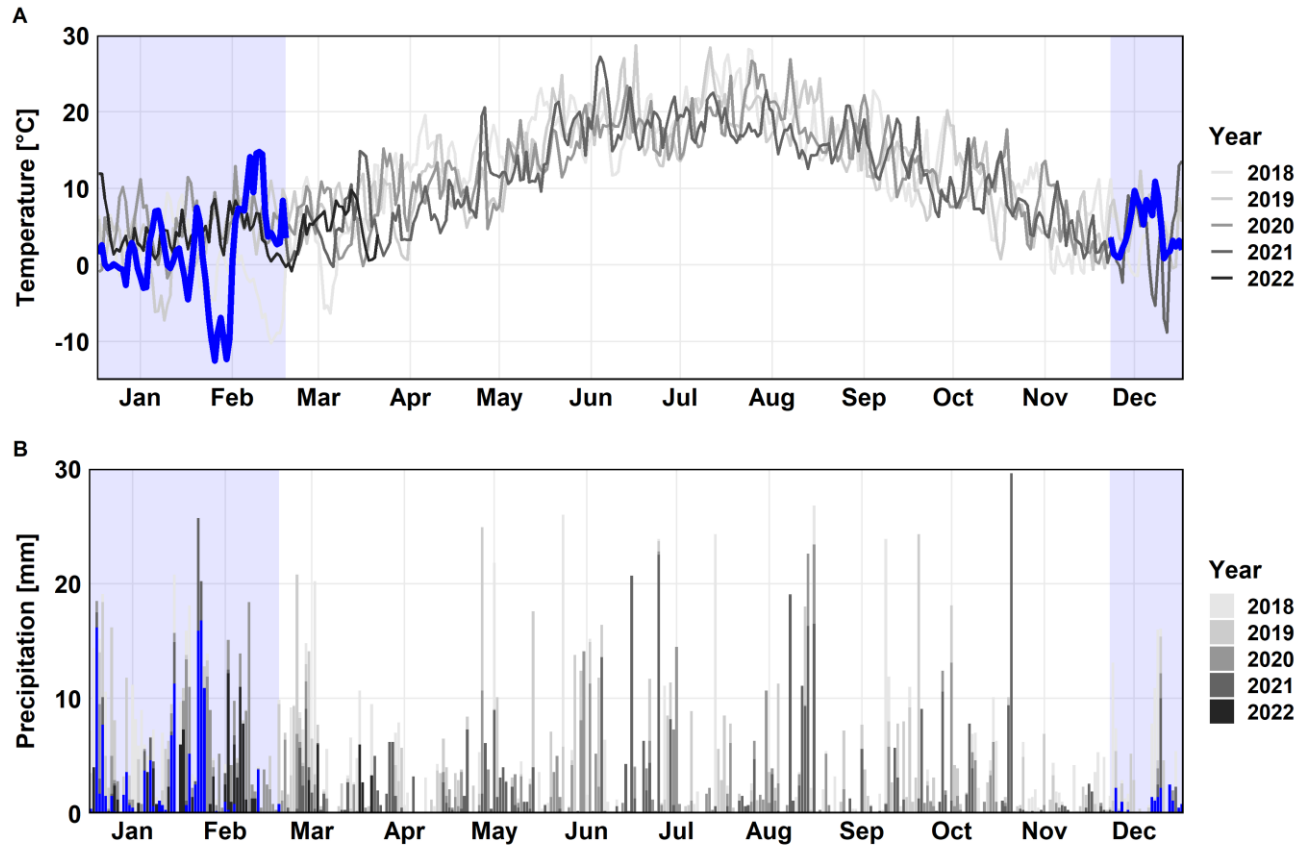
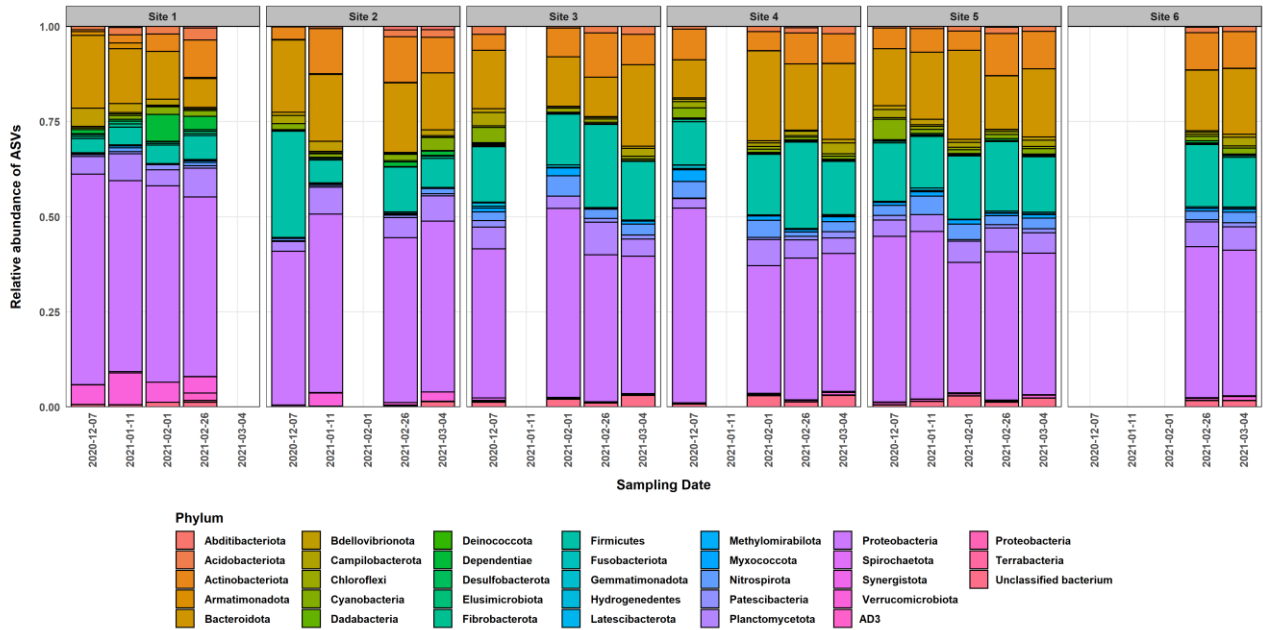


Figure S2. Temperature (A) and precipitation (B) in Wernigerode city from January 2018 until March 2022. Blue lines and bars are highlighting the data from December 2020 until March 2021. Data was received from the German weather monitoring station 5490 (<https://opendata.dwd.de/>).

A



B

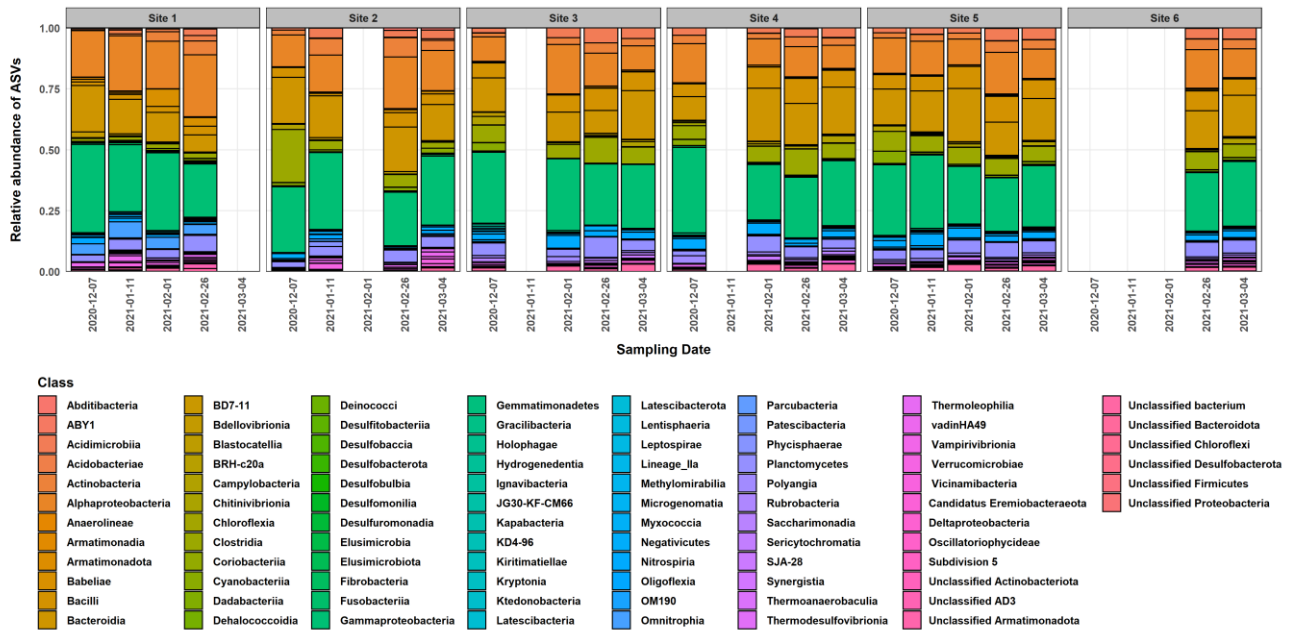


Figure S3. Microbial community structure at phylum (A) and class level (B) for each site, sorted by sampling date. For Sites 1 to 4, one outlier was removed in each data set before the analysis.

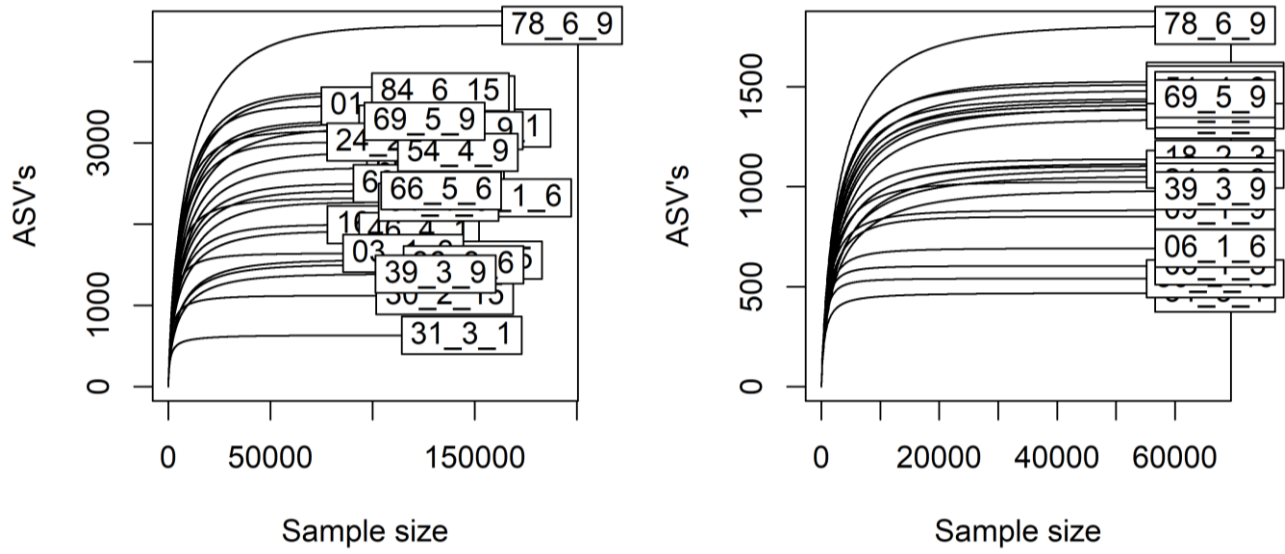


Figure S4. Rarefaction curves for 16S rRNA gene sequencing before (left) and after (right) removal of low abundance ASV's.

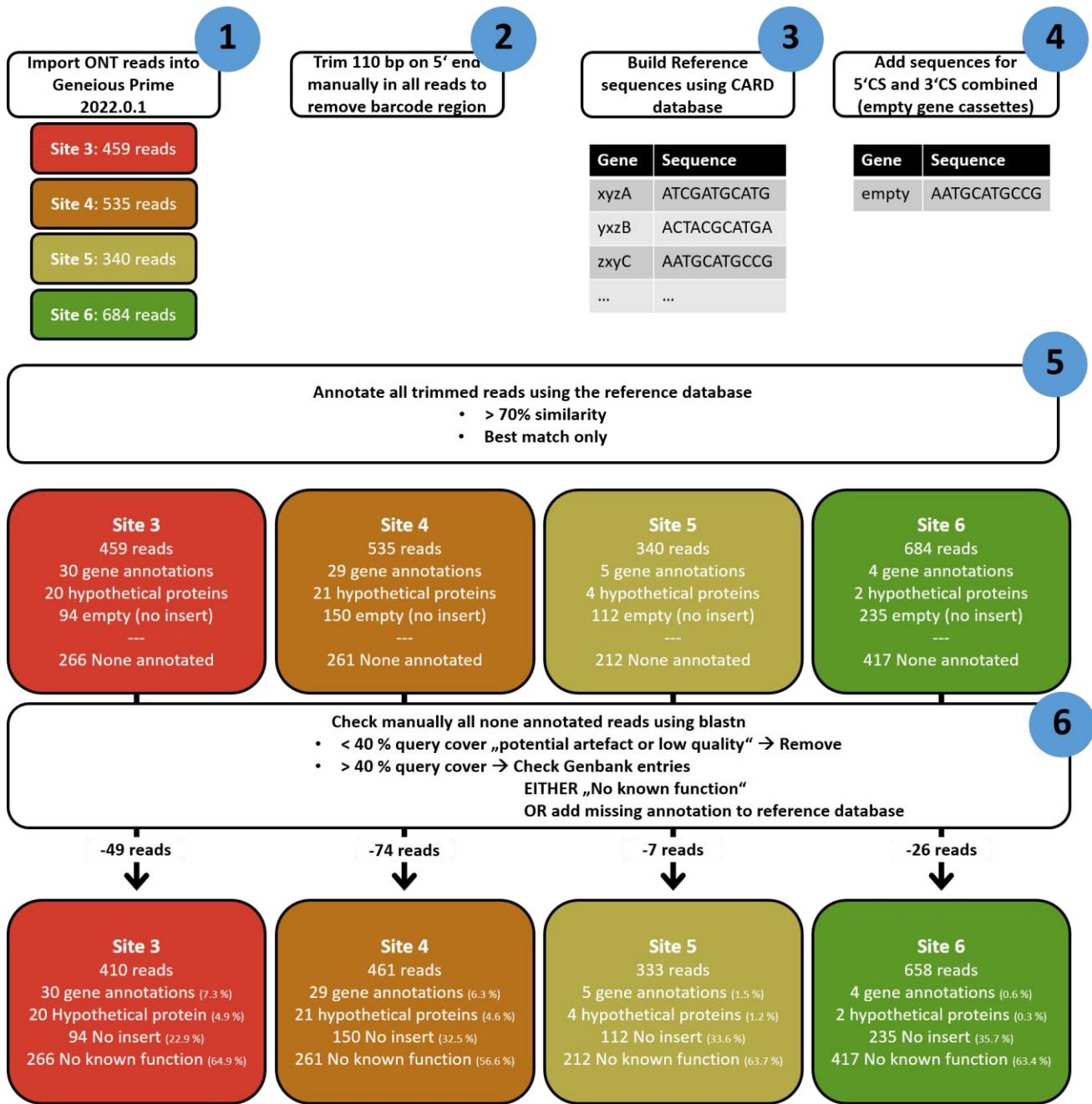


Figure S5. Workflow for the analysis of class 1 integron gene cassette inserts after ONT sequencing.

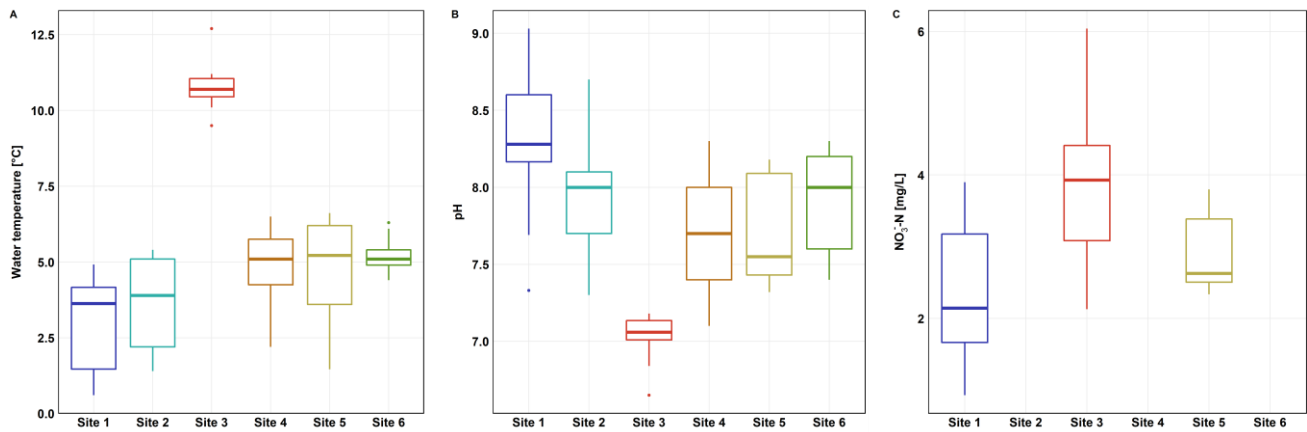


Figure S6. Physicochemical parameters measured at all Sites. Measurements were obtained from MOBICOS monitoring stations (site 1 and 5, <https://www.ufz.de/index.php?en=39611>), WWTP Silstedt (site 3, <https://www.wahb.eu/>) and manually using a SenTix41 probe (site 2, 4 and 6). Water temperature (A). pH (B) and NO₃⁻-N (C). Distinct colours were used to differentiate between individual Sites.

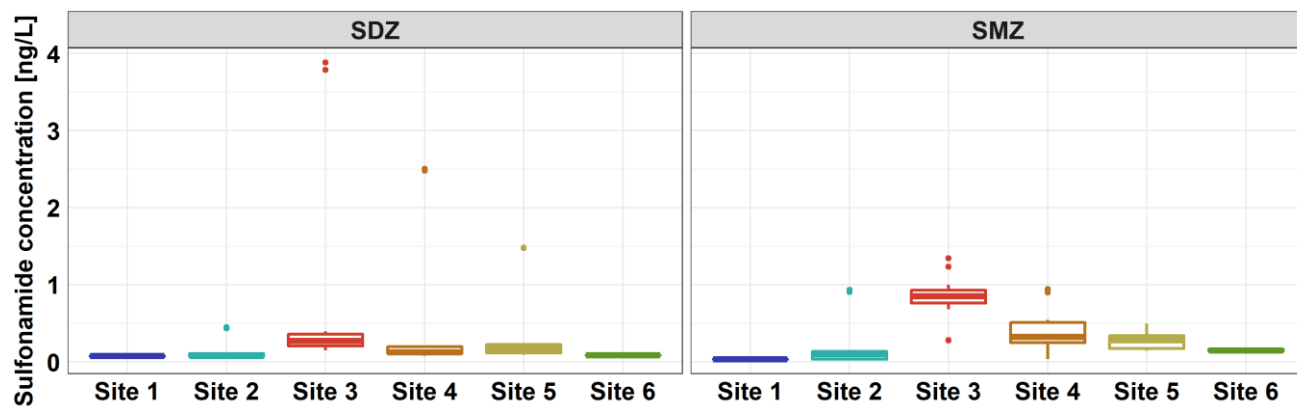


Figure S7. SDZ and SMZ concentrations for all six Sites measured by HPLC-MS/MS. The results of seven replicates are shown using box-whiskers-plots with the median represented by a horizontal line. Distinct colours were used to differentiate between individual Sites.

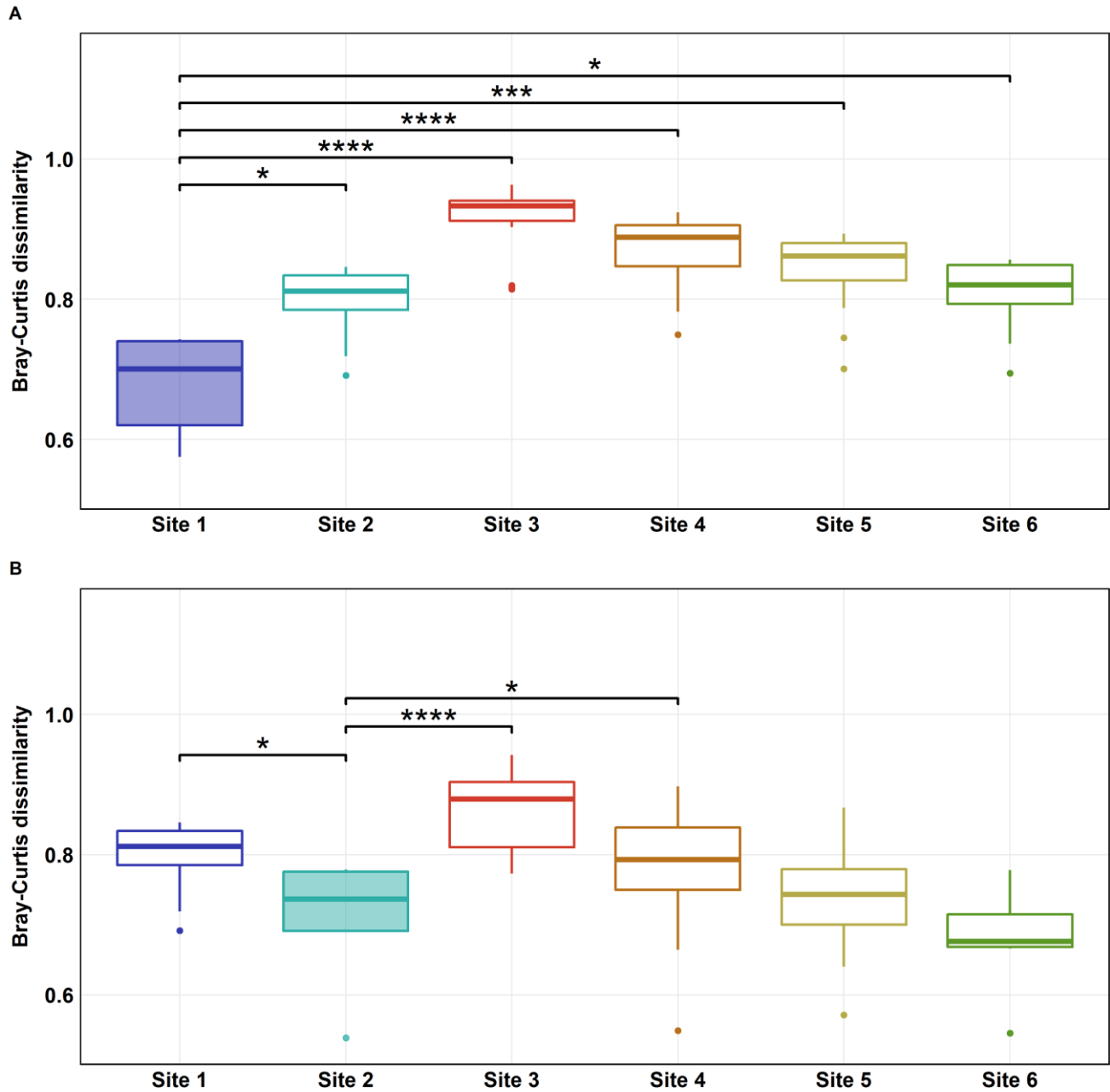


Figure S8. Bray Curtis dissimilarity in comparison to site 1 – pristine (A). Bray Curtis dissimilarity in comparison to site 2 – city (B). Significant differences between Sites are represented by asterisks (Dunn’s test, $* \leq 0.05$, $*** \leq 0.001$, $**** \leq 0.0001$). Distinct colours were used to differentiate between individual Sites.

3 References

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- Wang, N., Yang, X., Jiao, S., Zhang, J., Ye, B., and Gao, S. (2014). Sulfonamide-resistant bacteria and their resistance genes in soils fertilized with manures from Jiangsu Province, Southeastern China. *PLoS One* 9, e112626. doi: 10.1371/journal.pone.0112626