Supplementary Information

Title: Metagenomic profiling of historic Colorado Front Range flood impact on distribution of riverine antibiotic resistance genes

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Figure S1: Relative abundance of ARGs as determined by metagenomic analysis of site 1 and site 5 Poudre River bulk water samples. (A) Mechanisms of antibiotic resistance and (B) classes of ARGs determined by metagenomic analysis and annotation against the Comprehensive Antibiotic Resistance Database.

Figure S2: Relative abundance (ARG copies / 16S rRNA gene copies) of ARGs as determined by metagenomic analysis of site 1 and site 5 Poudre River bulk water samples.
Figure S3: Quantification of select ARGs by quantitative polymerase chain reaction (qPCR) in Poudre River sediment (bars) and bulk water (points), normalized to 16S rRNA genes at 12 months (-12) before the flooding occurred and at five time points following the flooding. X-axis indicates sites and months relative to the flooding event. (*) indicates gene detected below quantification limit in sediment and (+) in water. Error bars represent standard deviation of triplicate qPCR measurements in water and standard deviation of triplicate samples in sediment.
Figure S4: Phyla accounting for greater than 1% of the total OTUs in bulk water, determined by 16S rRNA gene sequencing.
Figure S5: Phyla accounting for greater than 1% of the total OTUs in bulk water, determined by 16S rRNA gene sequencing. Triplicate sediment samples were sequenced separately and results averaged.

Figure S6: Spearman’s Rank Correlation Coefficient between abundance of ARGs normalized to 16S rRNA genes, as determined by qPCR, and water quality parameters. Statistically significant (p<0.05) correlations are indicated in bold.
Figure S7: Rarefaction curves for metagenomic samples.

Figure S8: ARG copies determined by qPCR in WWTP effluent, normalized to 16S rRNA gene copies.
Table S1: Characteristics of metagenomic data. All sequences have been deposited in MG-RAST under project name “Fate and Transport of Antibiotics and Antibiotic Resistance Genes during Historic Colorado Flood.”

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Table S2: Antibiotic concentrations in Poudre River bulk water (ng/L). Standard deviation of replicate samples denoted in parentheses and months indicate months post-flood. “W” denotes Wastewater Treatment Plant samples. Antibiotics abbreviations are denoted as follows:

- anhydrotetracycline (ATC), azithromycin (AZI), clarithromycin (CLA), chlorotetracycline (CTC), doxycycline (DOX), erythromycin (ERY), 4-epitetracycline (ETC), oxytetracycline (OTC), sulfamerazine (SMR), sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SPD), tetracycline (TC), and tylosin (TYL). Sulfameter, sulfamethiazole, sulfamerazine, sulfachloropyridazine, sulfathiazole, roxithromycin, spiramycin, 4-epichlorotetracycline, anhydrochlorotetracycline, demeclocycline (surrogate), minocycline (internal standard), phenyl-13C6-sulfamethazine (13C6-SMZ), d4-sulfamethoxazole (d4-SMX), N-methyl 13C-erythromycin, and d10-carbamazepine (internal standard) were not detected in any samples.
Table S3: p-values for Kruskal-Wallis rank sum tests for correlations between ARGs and antibiotics of metals. Significant (p<0.05) values indicated in bold.

Table S4: Correlation matrix using Spearman's rank sum correlation coefficients. Statistically significant (p<0.05) correlations are indicated in bold.

Table S5: Metal concentrations in Poudre River bulk water (µg/L). Standard deviation of replicate samples denoted in parentheses.