Supplemental Materials: Elucidating the impact of common stormwater pollutants on antibiotic resistance: the role of heavy metals, nutrients, and salts

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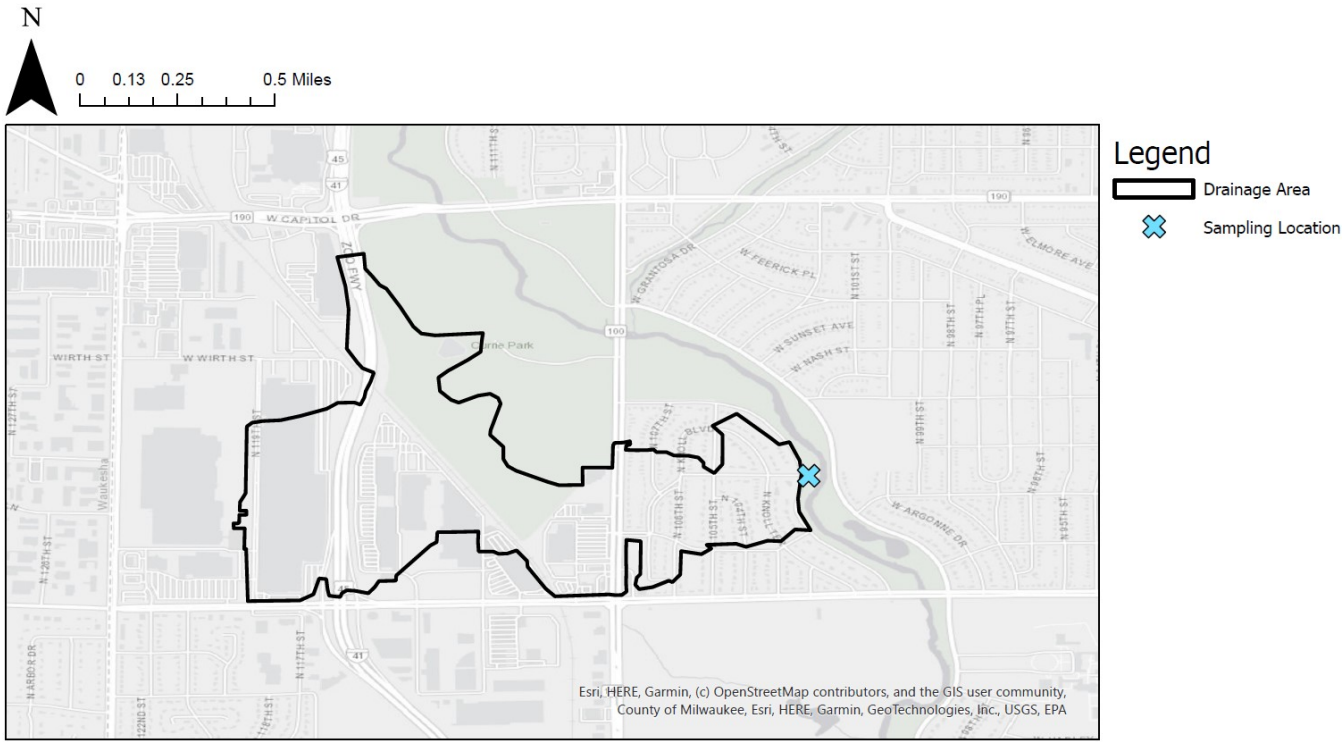

Fig. A1. This figure depicts the stormwater outfall sampling point, marked with a blue cross, along with the corresponding drainage area. The land cover within the drainage area was characterized as 100% development, with 62.4% of the area being impervious based on the 2019 National Land Cover Database.

Stream Gage Data

Stream gage data is not available for the nutrient microcosm due to ice conditions at the stream gage.

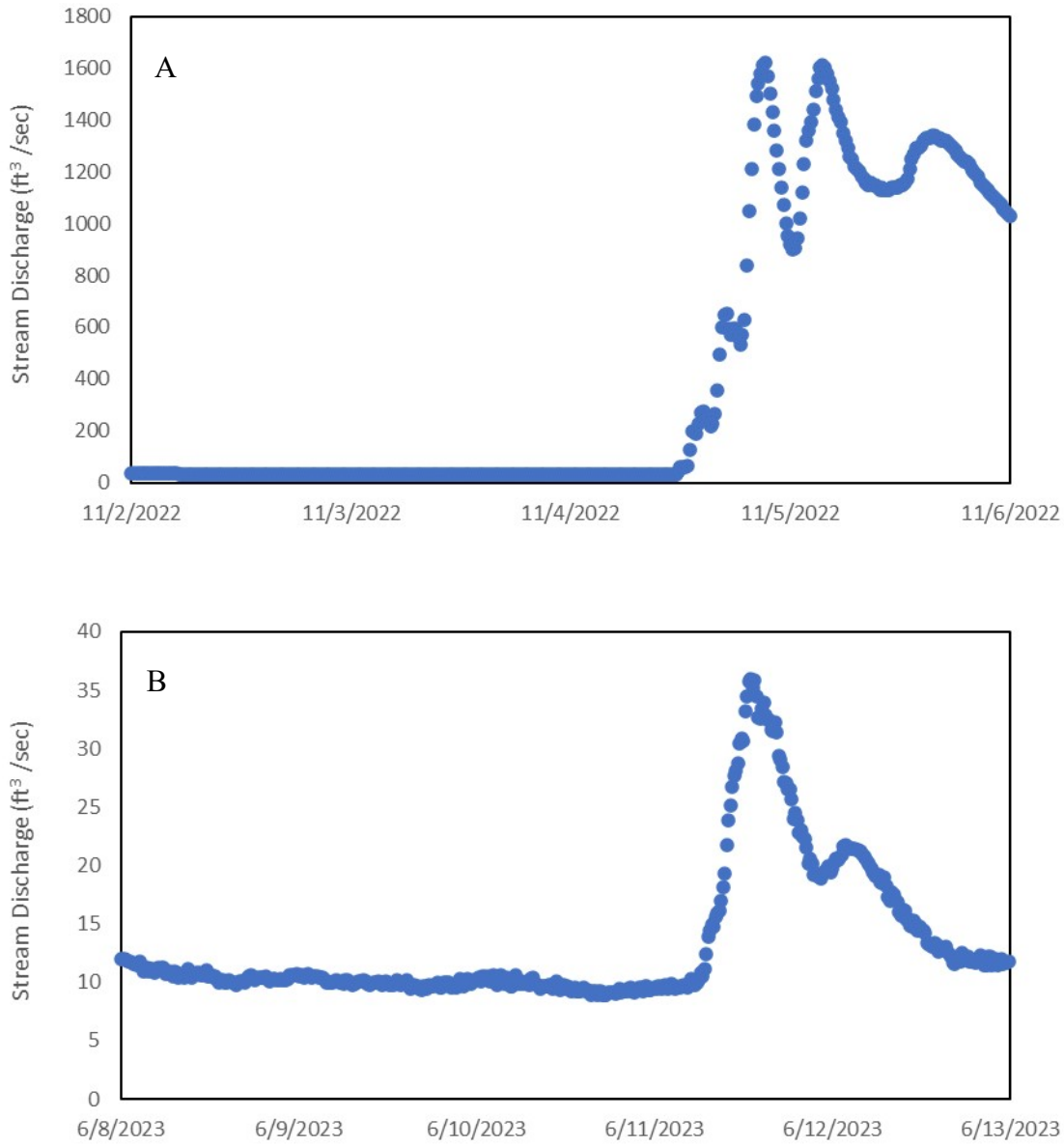

Fig. A2. Stream gage data for (A) heavy metal microcosm and (B) road salt microcosm

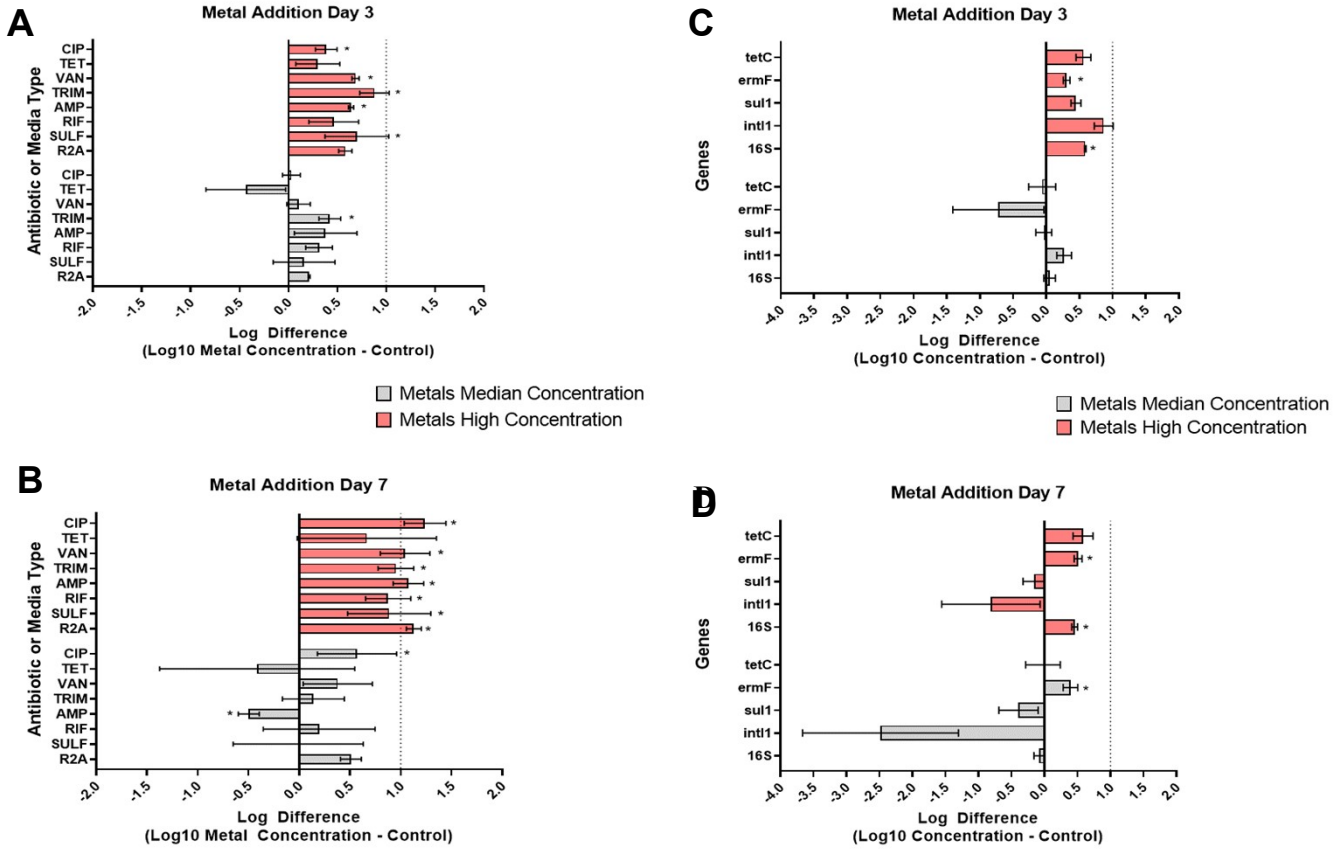

Fig. A3. Results of the ARB and ARG analysis of the heavy metal microcosm. Log difference in ARB concentration between the control samples and the microcosm samples at a high or median concentration on day 3 (A) and day 7 (B). Log difference in ARG concentration between the control samples and the microcosm at a high or median concentration on day 3 (C) and day 7 (D). Stars indicate a statistical difference ($p<0.05$) in absolute concentration of ARB and ARG when exposed to metals in comparison to the control. Error bars represent standard deviation from the mean ($n\geq6$).

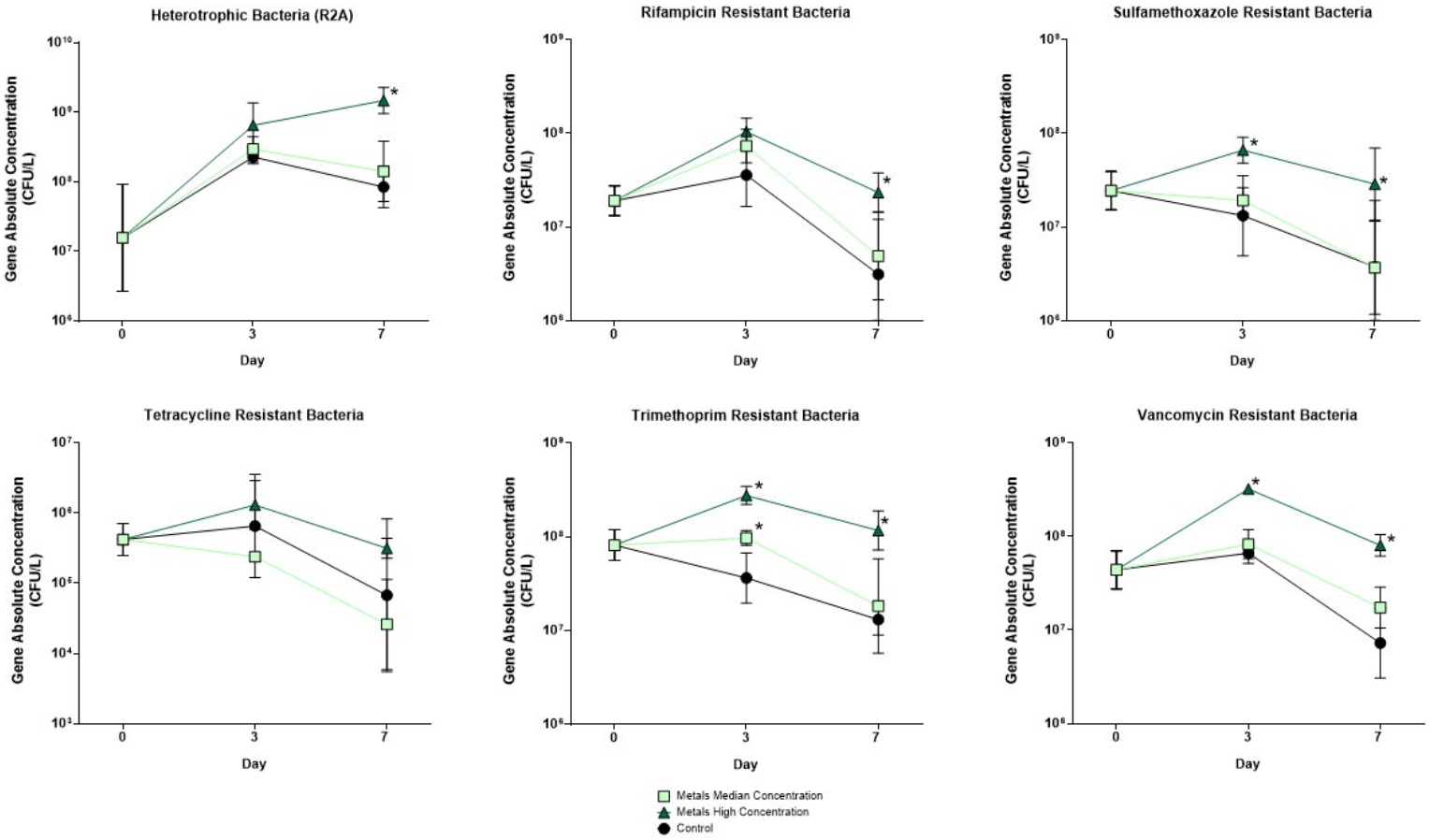

Fig. A4. Concentration of ARB over the time of the heavy metal microcosm experiment. Error bars represent standard deviation from the mean ($n \geq 6$). * indicates a statistical difference ($p < 0.05$) of the microcosm dosed condition from the control at the respective timepoint.

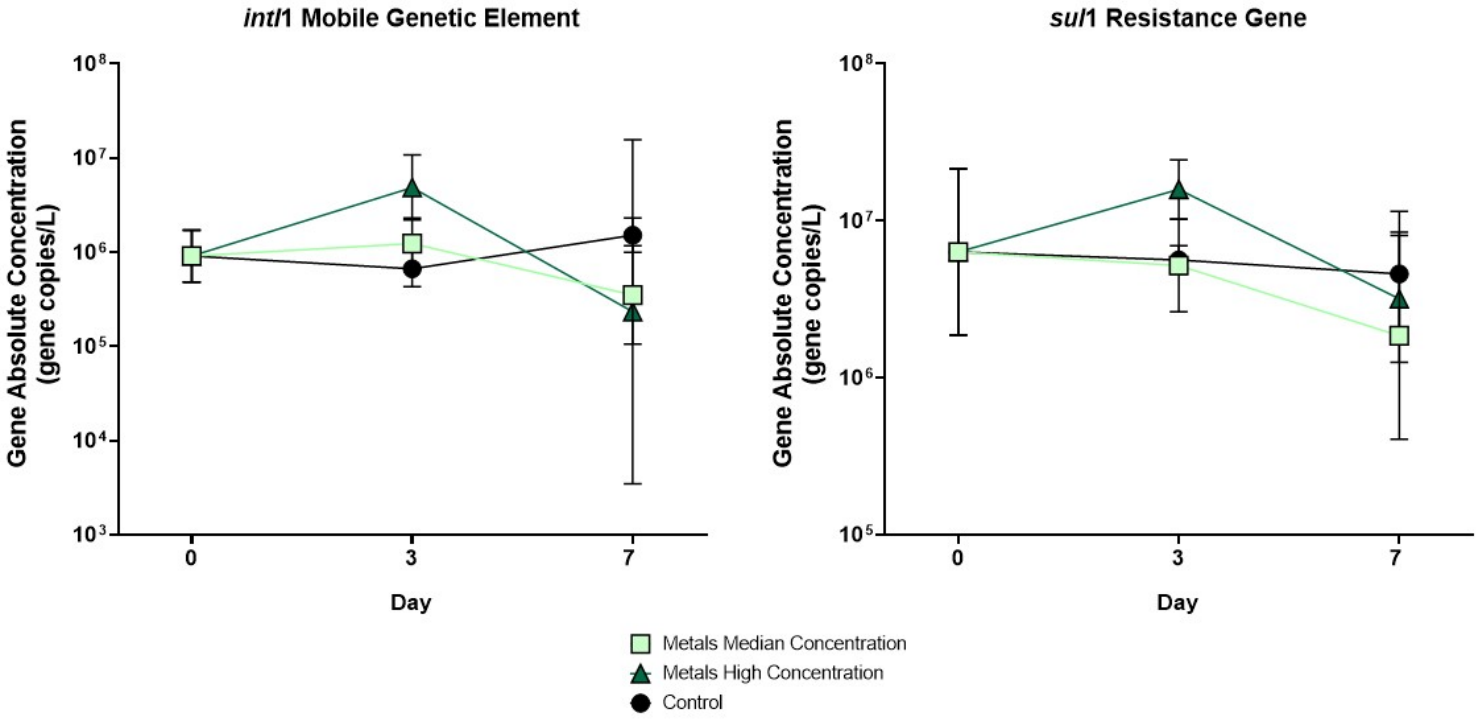

Fig. A5. Concentration of ARG over the time of the heavy metal microcosm experiment. Error bars represent standard deviation from the mean (n≥6).

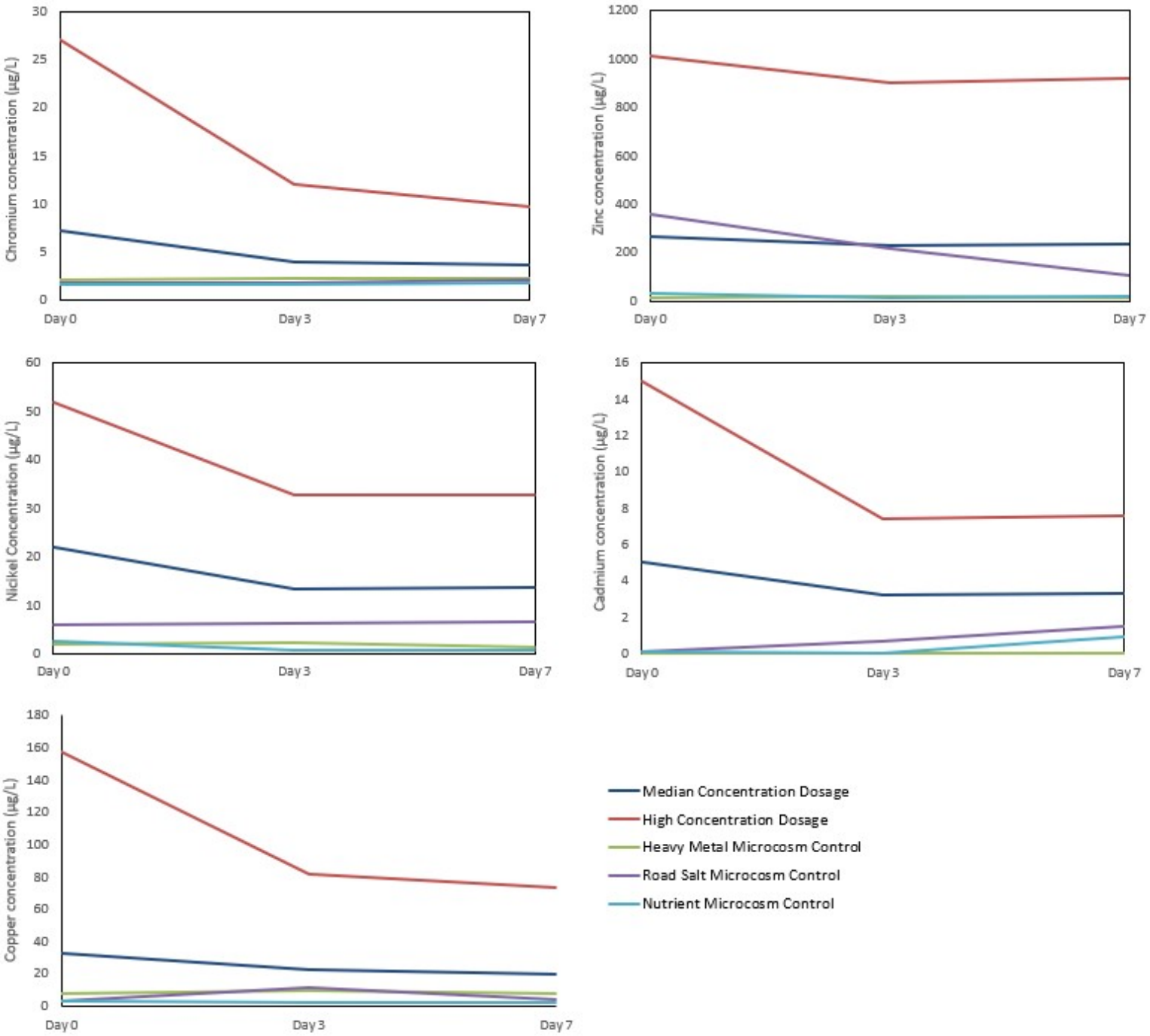

Fig. A6. Concentrations of chromium, zinc, nickel, cadmium, and copper in the different microcosm experiments. The red line indicates the concentration under the high concentration dosage. The dark blue line indicates the concentration under the median concentration dosage. The control during the metal microcosm experiment is indicated in the green line, while the controls during the nutrient microcosm and the road salt microcosm are also displayed as the purple and light blue lines, respectively.

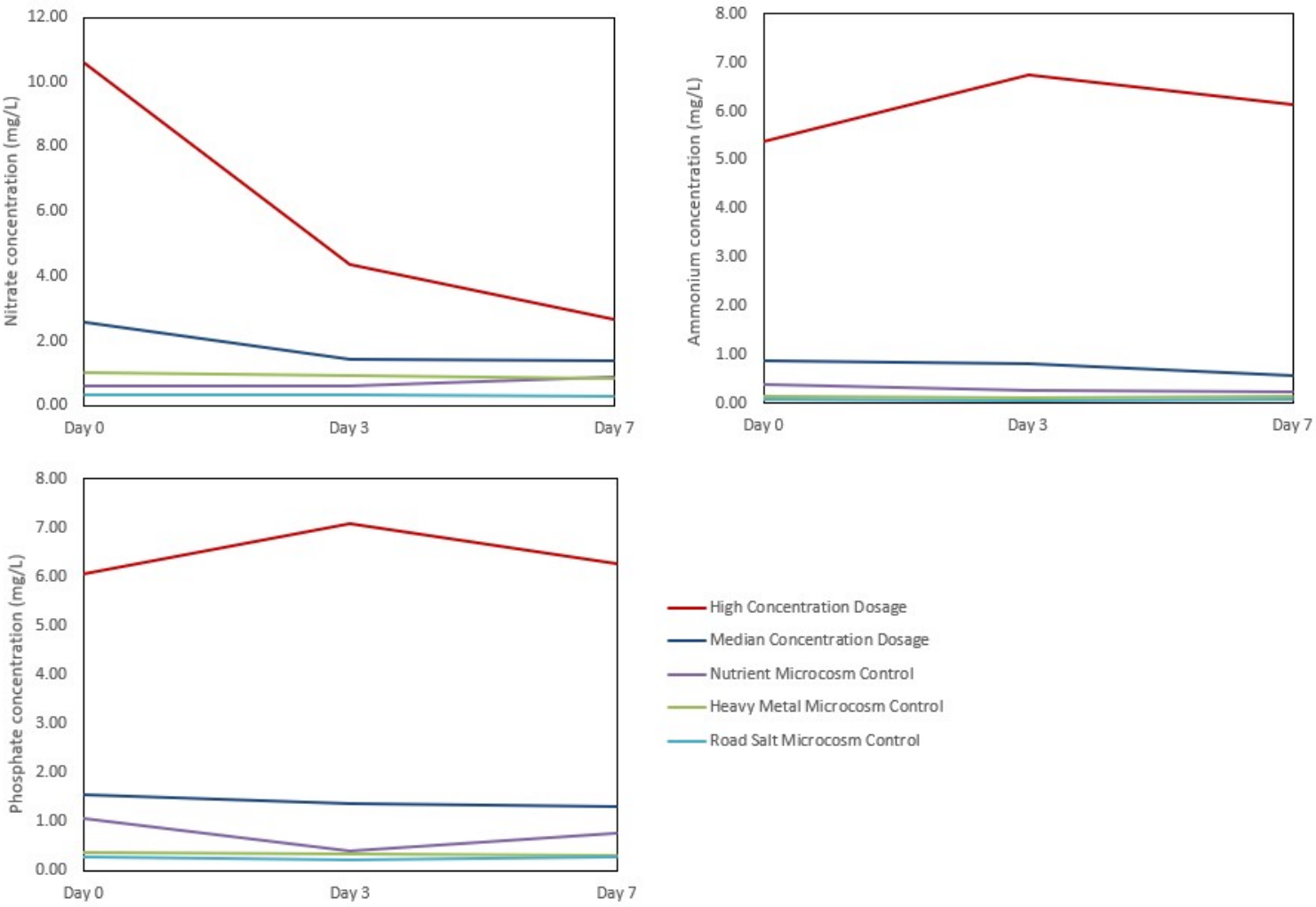

Fig. A7. Concentrations of phosphate, ammonium, and nitrate in the different microcosm experiments. The red line indicates the concentration under the high concentration dosage. The dark blue line indicates the concentration under the median concentration dosage. The control during the nutrient microcosm experiment is indicated in the purple line, while the controls during the heavy metal microcosm and the road salt microcosm are also displayed as the green and light blue lines, respectively.

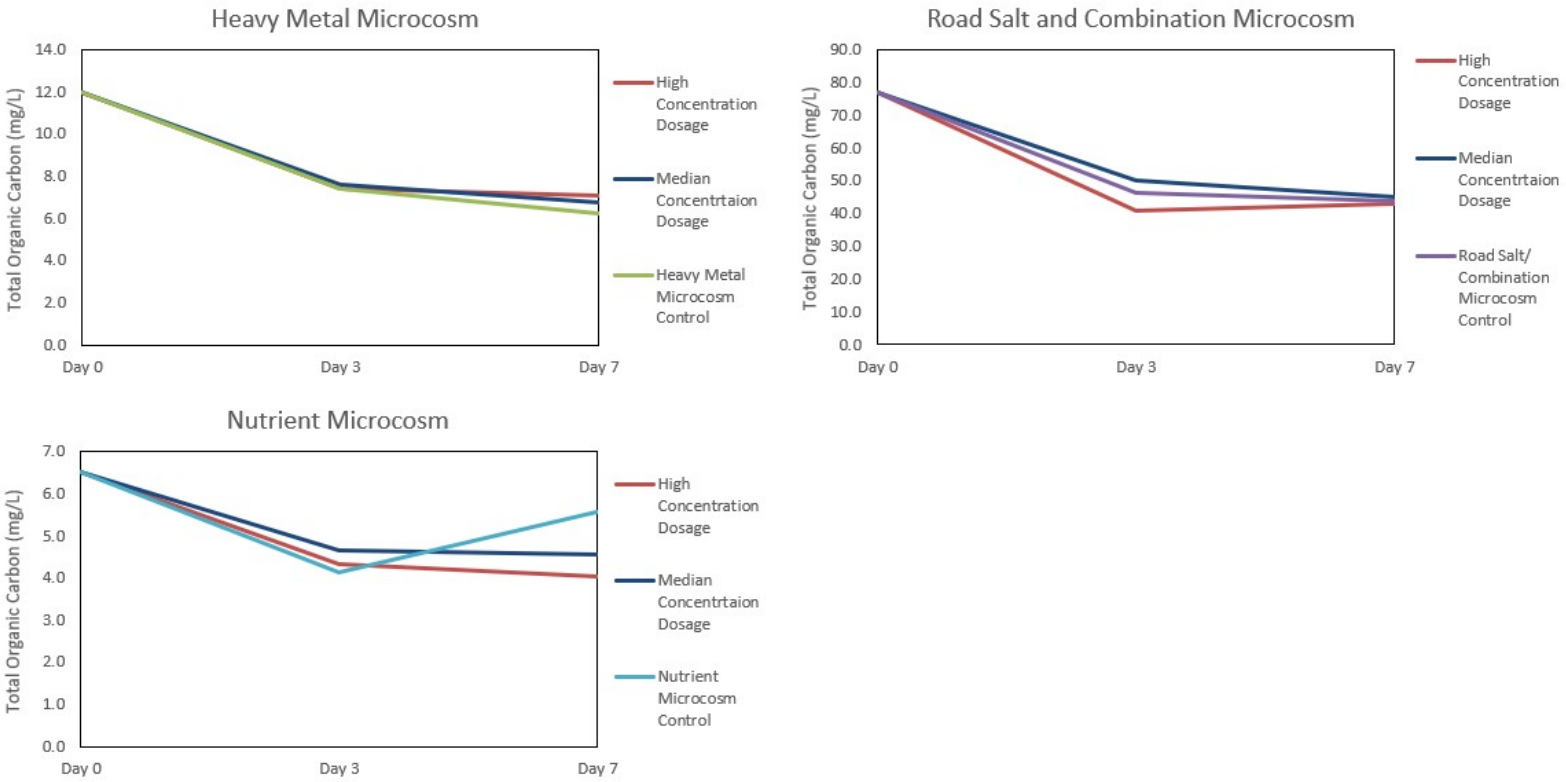

Fig. A8. Concentrations dissolved organic carbon in the different microcosm experiments.

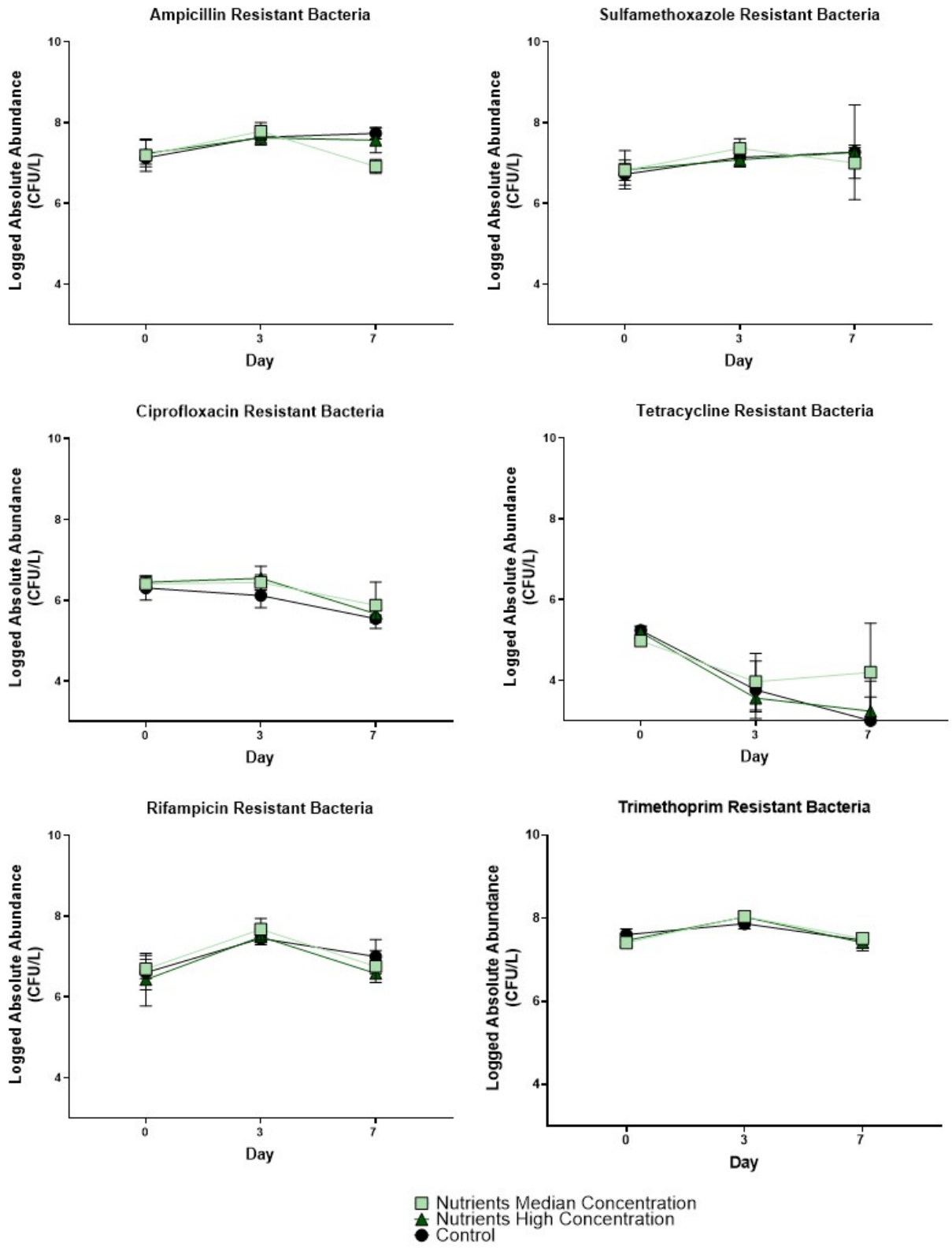

Fig. A9. Concentration of ARB over the time of the nutrient microcosm experiment. Error bars represent standard deviation from the mean (n≥6).

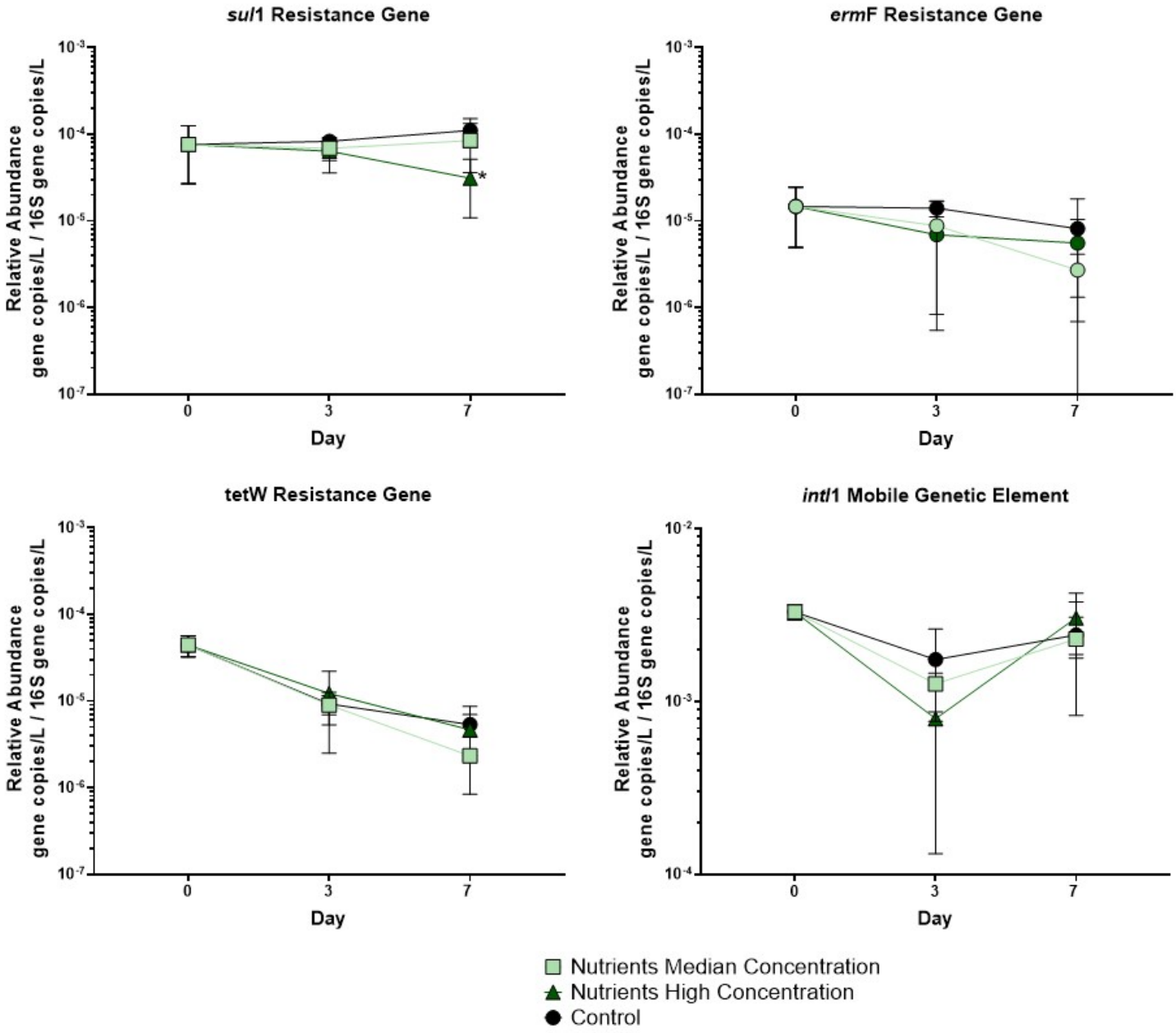

Fig. A10. Concentration of ARG over the time of the nutrient microcosm experiment. Error bars represent standard deviation from the mean (n≥6).

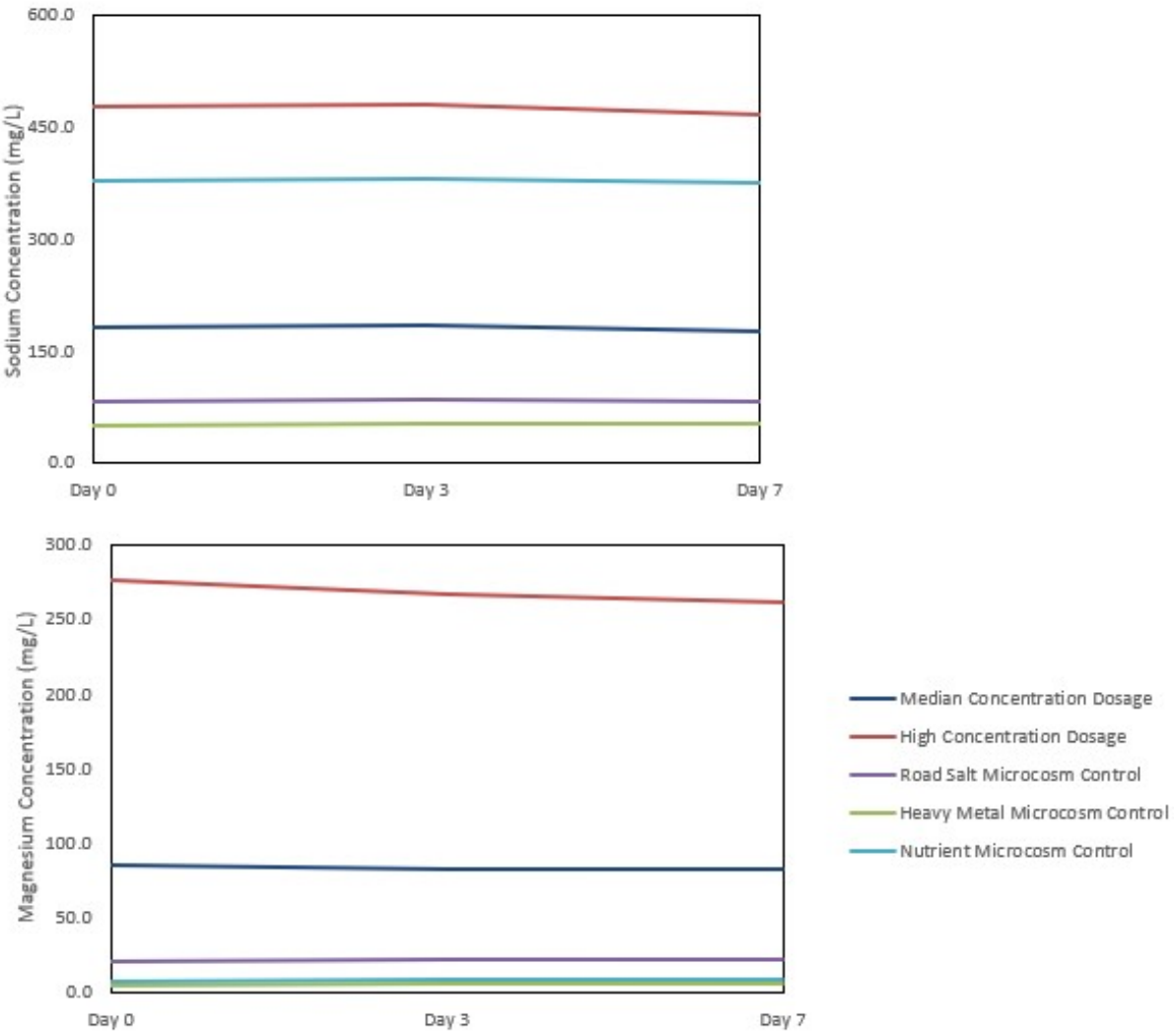

Fig. A11. Concentrations of road salts – sodium and magnesium – in the different microcosm experiments. The red line indicates the concentration under the high concentration dosage. The dark blue line indicates the concentration under the median concentration dosage. The control during the road salt microcosm experiment is indicated in the purple line, while the controls during the heavy metal microcosm and the nutrient microcosm are also displayed as the green and light blue lines, respectively.

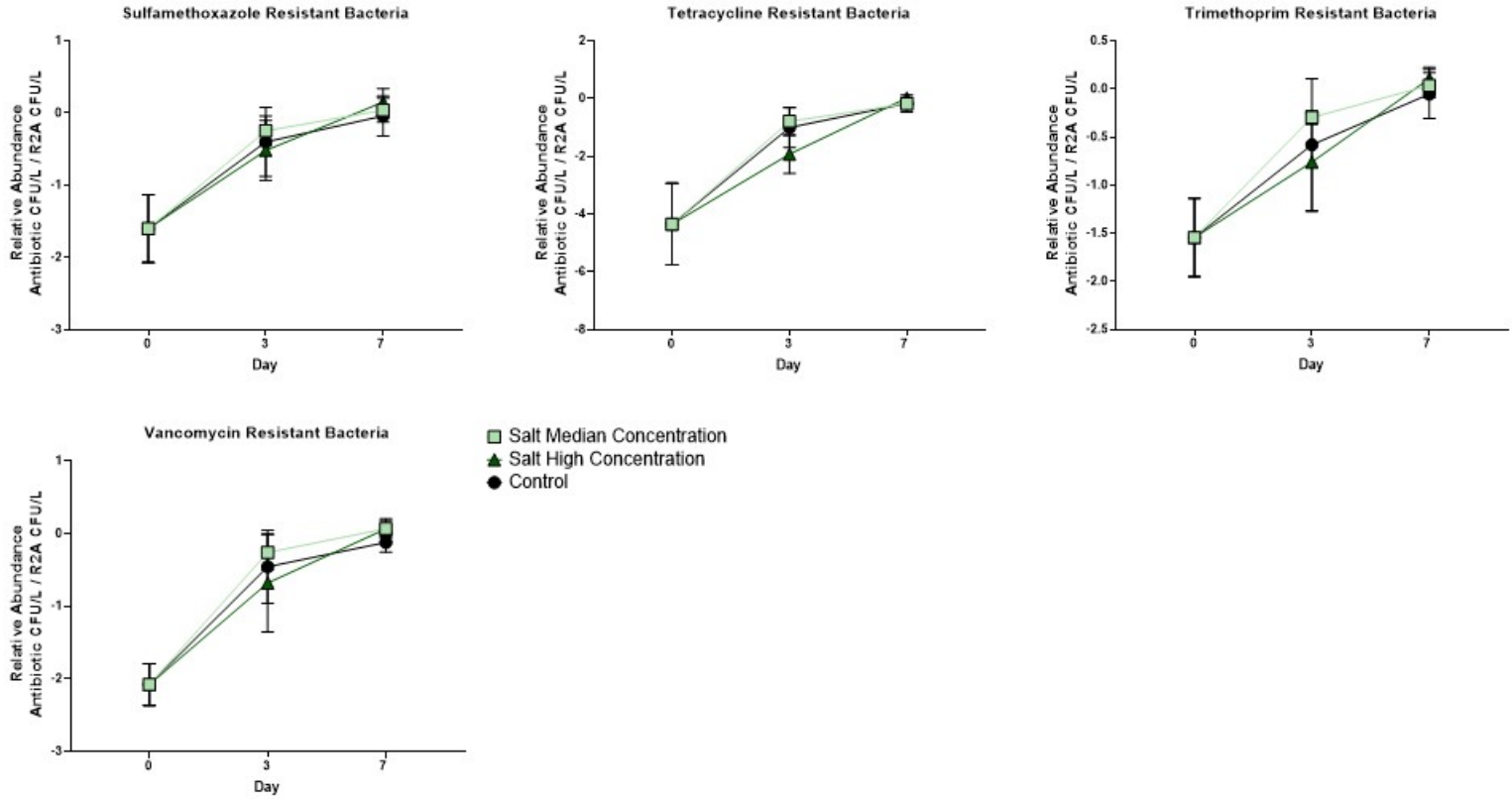

Fig. A12. Change in ARB concentrations across the microcosm experiment when exposed to a median and high salt concentration. Error bars represent standard deviation from the mean (n≥6).

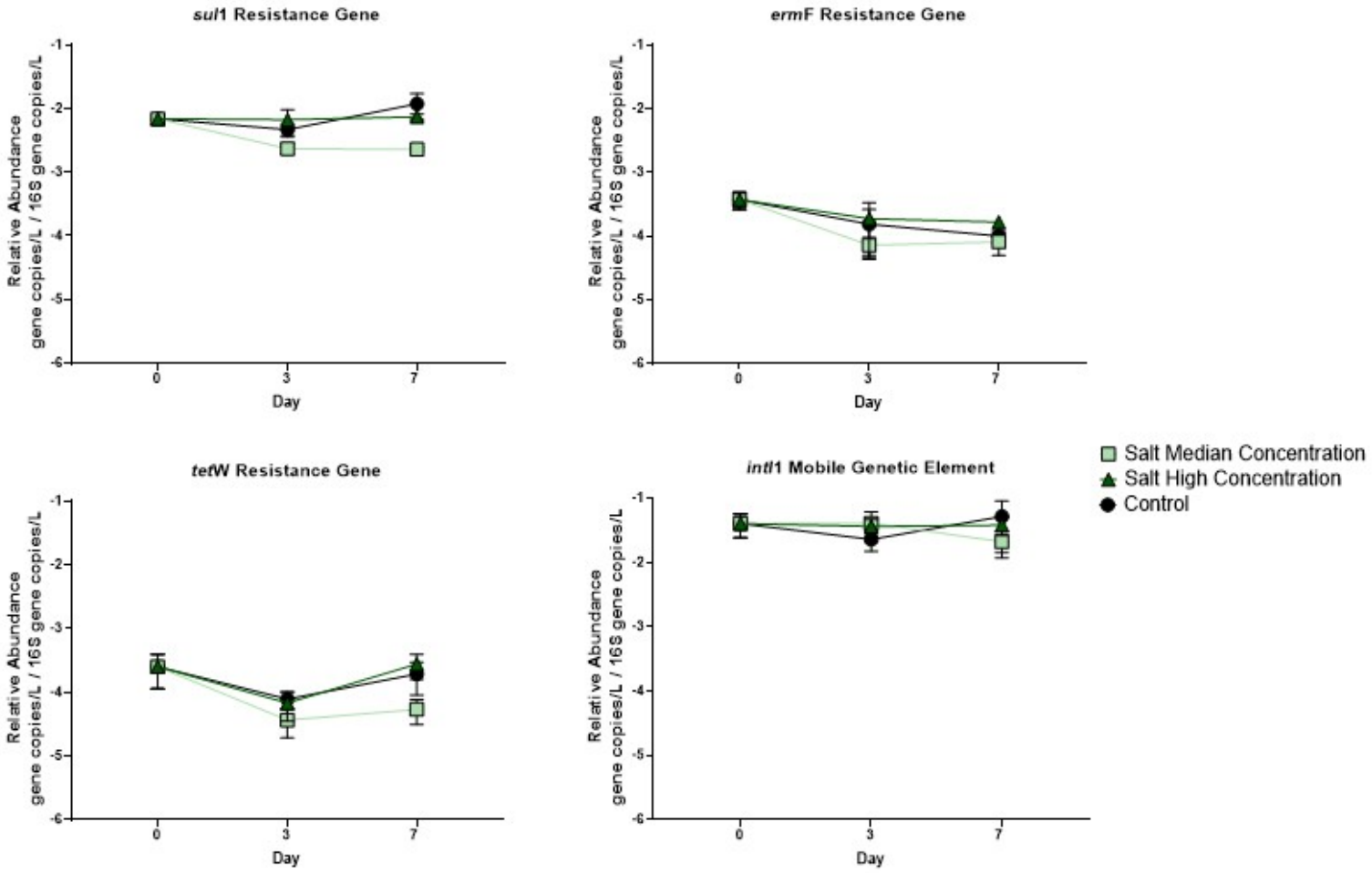

Fig. A13. Change in ARG and MGE concentrations across the microcosm experiment when exposed to a median and high salt concentration. Error bars represent standard deviation from the mean (n≥6).

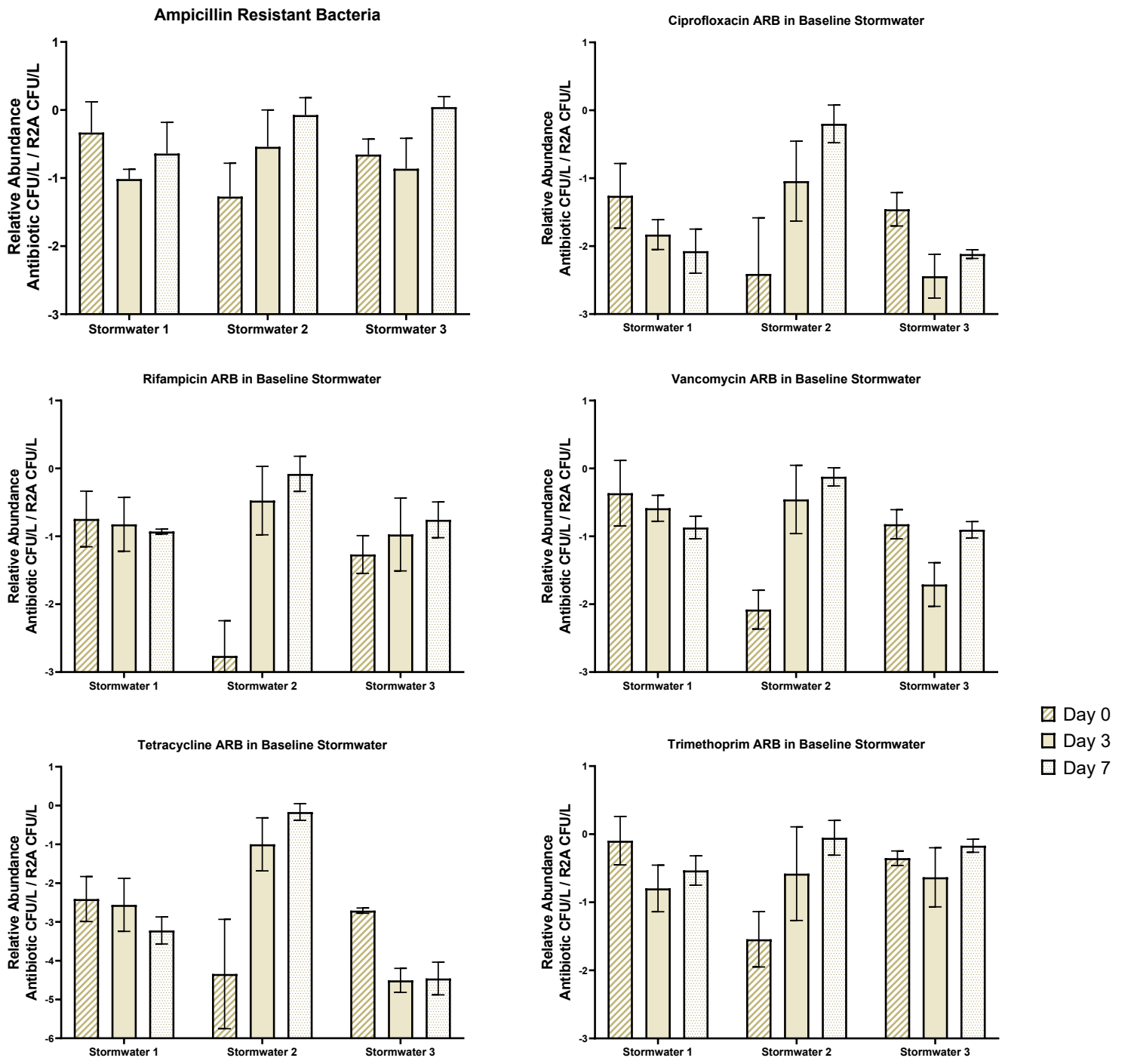

Fig. A14. ARB concentrations across control microcosm experiments, indicating the variance in ARB across time under baseline conditions, without addition of additional stormwater pollutants.

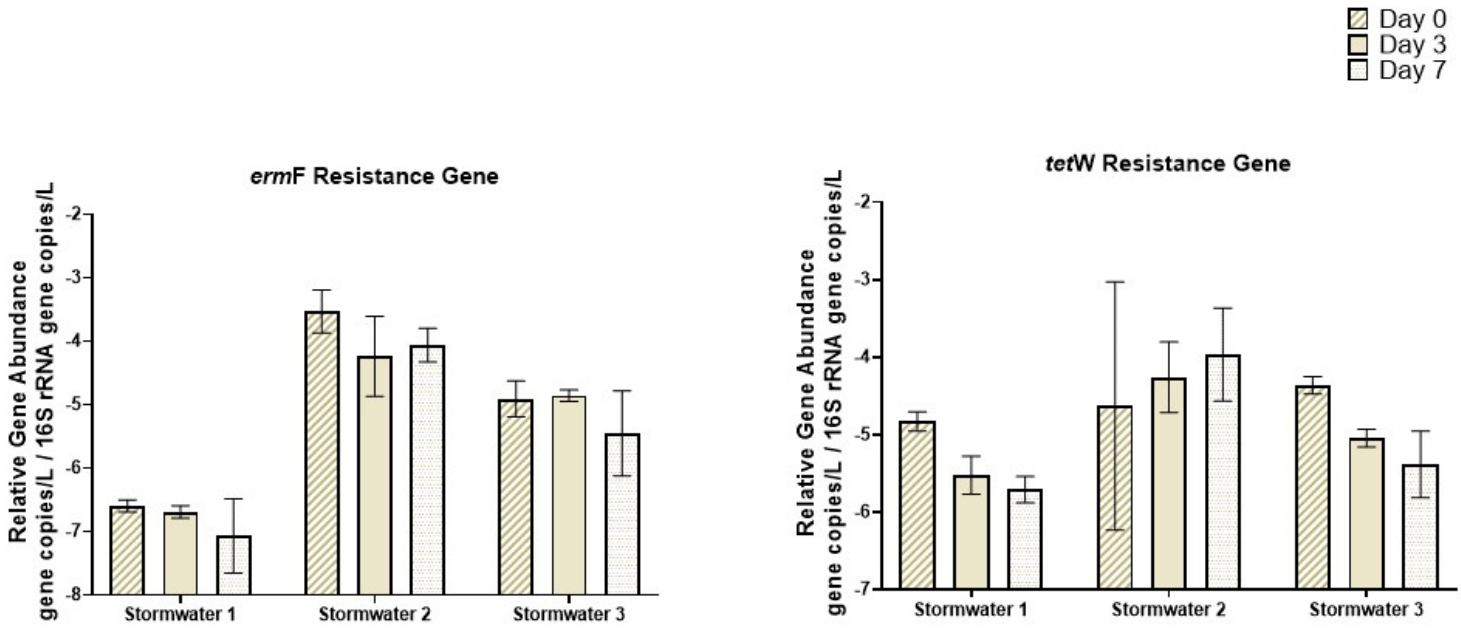

Fig. A15. ARG concentration across control microcosm experiments, indicating the variance in ARGs across time under baseline conditions, without addition of additional stormwater pollutants.

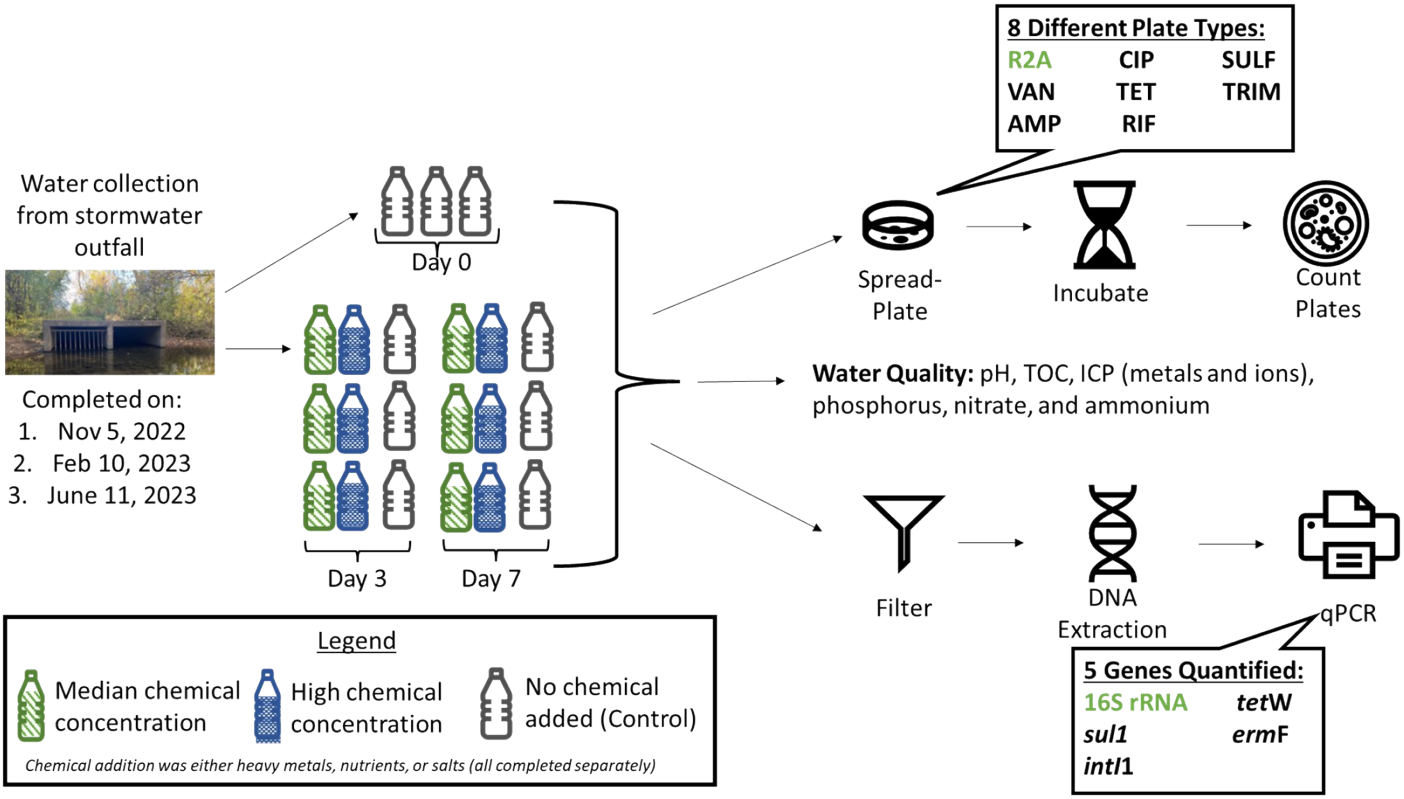

Fig. A16. Schematic of Sampling and Laboratory Methods

Table A1. qPCR conditions and primers

Gene	Annealing Temperature	Forward Primer and Reverse Primer	Reference
16S rRNA	60°C	For. (5'-CCTACGGGAGGCAGCAG-3') Rev. (5'-ATTACCGCGGCTGCTGG-3')	Muyzer et al. 1993
intI1	60°C	For. (5'-CCTCCCGCACGATGATC-3') Rev. (5'-TCCACGCATCGTCAGGC-3')	Goldstein et al. 2001
tetW	60°C	For. (5'-GCGGGATATCGTCCATTCCG -3') Rev. (5'-GCGTAGAGGATCCACAGGACG-3')	Stanton et al., 2003
ermF	60°C	For. (5'-TCGTTTTACGGGTCAGCACTT-3') Rev. (5'- CAACCAAAGCTGTGTCGTTT-3')	Graham et al., 2011
sul1	60°C	For. (5'-CCGTTGGCCTTCCTGTAAAG -3') Rev. (5'- TTGCCGATCGCGTGAAGT-3')	Wang et al., 2014