Fate and Transport of Determinants of Antimicrobial Resistance in Variably Saturated Terrestrial Environments

by

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TABLE OF CONTENTS

LIST OF TABLES ..... **vi**
LIST OF FIGURES ..... **viii**
ABSTRACT ..... **xi**
LIST OF ABBREVIATIONS USED ..... **xii**
ACKNOWLEDGMENTS ..... **xviii**
Chapter 1 Introduction **1**
1.1 Introduction **1**
1.1.1 Antimicrobial Resistance 1
1.1.2 Determinants of Antimicrobial Resistance 2
1.1.3 Surveillance of Antimicrobial Resistance 3
1.1.4 Technological Applications for Mitigation 5
1.2 Research Questions **6**
1.3 Research Objectives **7**
1.4 Thesis Organization **8**
Chapter 2 Fate of Antibiotic Resistance Genes In Two Arctic Tundra Wetlands Impacted By Municipal Wastewater **9**
2.1 Introduction **10**
2.2 Methodology **13**
2.2.1 Site Descriptions 13
2.2.2 Reference Wetlands 17
2.2.3 Hydraulic and Hydrology Characterization 18
2.2.4 Water Quality Sampling and Analysis 19
2.2.5 Sampling and Analysis of Soil and Water for Gene Targets 20
2.2.6 Data Analysis 22
2.2.7 First-Order Rate Constant Determination 22
2.2.8 Statistical Analysis 23

2.3 Results and Discussion 23

2.3.1 Hydraulics and Hydrology 23

2.3.2 General Water Quality 27

2.3.3 Distributions of Gene Target Concentrations 30

2.3.4 Absolute Abundance in Raw Wastewater 31

2.3.5 Absolute Abundance in Wetlands 32

2.3.6 Absolute Abundance in Soil 33

2.3.7 Environmental Resistome 37

2.3.8 Correlations Between ARGs and Water Quality Indicators (WQI) 37

2.3.9 First-Order Rate Constants of ARGs 40

2.4 Conclusions 42

Chapter 3 Lateral Flow Sand Filters Are Effective For Removal Of Determinants Of Antimicrobial Resistance From Domestic Wastewater 43

3.1 Introduction 44

3.2 Material and Methods 46

3.2.1 Sand Filters Description 46

3.2.2 Water Sampling 48

3.2.3 Conventional Analysis 49

3.2.4 Antibiotic Analysis 50

3.2.5 Genetic Analysis 51

3.2.6 Cell-Associated and Cell-Free DNA Analysis 52

3.2.7 Antibiotic Resistant Bacteria Enumeration 53

3.2.8 Sodium Bromide Tracer Tests 53

3.2.9 Statistical Analysis 54

3.3 Results and Discussion 55

3.3.1 Conventional Parameters 55
3.3.2 Hydraulic Characterization of Filters 57
3.3.3 Raw Wastewater and Septic Tank Effluent 57
3.3.4 Filter Gene Marker Removal Performance 62
3.3.5 Antibiotic Resistant Bacteria 63
3.3.6 Correlations Between Gene Markers and Water Quality Parameters 65
3.3.7 Apportionment of Cell-Associated and Cell-Free Gene Markers 67
3.3.8 Antibiotics 70
3.4 Conclusions 70
Chapter 4 Preliminary Modeling of Attenuation Of Antimicrobial Resistance Determinants In Lateral Flow Sand Filter Wastewater Treatment Systems 72
4.1 Introduction 73
4.2 Materials and Methods 75
4.2.1 Lateral Flow Sand Filter Description 75
4.2.2 Field Observed Hydraulic Tracer Tests 76
4.2.3 Microbial Techniques 77
4.2.4 Sample Collection 78
4.2.5 Sorption Experiment 79
4.2.6 Degradation Experiment 80
4.2.7 HYDRUS Modeling 81
4.2.8 Statistical Analysis 87
4.3 Results and Discussion 89
4.3.1 Gene and Bacteria Attenuation Characteristics 89
4.3.2 Model Calibration 94
4.3.3 Modeling Results 96

4.4 Discussion of the Model Approach 98

4.5 Conclusions 101

Chapter 5 Conclusions and Recommendations 102

5.1 Conclusions 102

Research Question 1: Persistence of Determinants of Antimicrobial Resistance Downgradient of Rural and Remote Sources 102

Research Question 2: Characterization of the Environmental Resistome 103

Research Question 3: Performance of Alternative Wastewater Treatment Technologies for Removal of Antimicrobial Determinants 103

Research Question 4: Mechanisms and Rates of Attenuation of Determinants of Antimicrobial Resistance in Porous Media 104

Research Question 5: Simulation of Attenuation of Determinants of Antimicrobial Resistance with Conventional Reactive Transport Models 105

5.2 Major Contributions 106

5.3 Recommendations 107

5.3.1 Improvements to Tundra Wetland Treatment Areas 107

5.3.2 Documentation of Baseline Environmental Resistome with Standardized Methods 107

5.3.3 Lateral Flow Sand Filtration 108

5.3.4 Mobilization and Attenuation of Determinants of Antimicrobial Resistance in Groundwater 108

5.3.5 Investigation of the Role of Biological Mats 109

References 110

APPENDIX A — SUPPLEMENTAL FIGURES 135

APPENDIX B — SUPPLEMENTAL TABLES 157

APPENDIX C — COPYRIGHT PERMISSIONS 176

APPENDIX D — ELECTRONIC SUPPLEMENTAL INFORMATION 177

LIST OF TABLES

Table 2.1 Minimum, maximum, and mean concentrations of water quality parameters for raw wastewater, wetland influent and effluent, and from reference wetland sites obtained during the spring freshet (Spring), and end of the summer treatment season (Summer). Mean concentrations are displayed in parentheses and when no range is reported the samples had the same value. 28

Table 2.2 Comparison of ARG absolute abundances in the wetland effluent from Cambridge Bay from Chaves-Barquero et al. (2016) and Sanikiluaq and Naujaat, Nunavut. 33

Table 2.3 First-order rate constants (k_{20}) for the absolute abundances of the gene targets and *E. coli* in the wetlands..... 41

Table 3.1 Summary of sampling schedule and details for Raw, STE and sand filters 1 -6. A double check mark indicates two samples collected per day..... 49

Table 3.2 Summary of conventional wastewater parameter results presented as mean values ± standard deviation (n = 10). 56

Table 3.3 Summary of hydraulic characteristics of the sand filters determined from the bromide tracer tests. 57

Table 3.4 Summary of average gene marker concentrations in the total, cell associated, and cell-free DNA fractions for raw wastewater, septic tank effluent (STE) and sand filters (SF) 1 – 3 samples collected on July 16 and July 23, 2018. Bolded numbers indicate absolute abundances above the LOQ, italicized numbers are below the LOQ, <DL means are below the detection limit. 68

Table 4.1 Assumed and calibrated hydraulic and advection dispersion parameterization values for the three lateral flow sand filters (SFs) (SF4, SF5, and SF6) (values adapted from Sinclair et al., 2014). Values in the square brackets were calibrated to enable matching of the simulated data to the field observed data. 84

Table 4.2 Field observed gene and bacteria influent and effluent concentrations used to parameterize and validate the SFs models from Hayward et al. (2019).

The 16S rRNA and ARGs were based on the arithmetic mean of n = 10 samples and the total heterotrophic bacteria and ARB were based on the geometric mean of n = 4 samples..... 88
Table 4.3 First order degradation rates (k) for 16S rRNA, *sul1*, *tetO*, and *ermB* with fine, medium, and coarse sand and without sand (STE) at 4 and 15°C..... 91
Table 4.4 First order degradation rates (k) for total heterotrophic bacteria (on TSA) and ARB with resistance to sulfamethoxazole (50 mg/L), tetracycline (10 mg/L), or erythromycin (50 mg/L) in STE (no sand) and fine, medium, and coarse sand stored at 4 and 15°C for 21 d..... 92
Table 4.5 Partition coefficients (K_d) for the genes and bacteria in the three sand medias. 93

LIST OF FIGURES

Figure 2.1 Map of study sites in Nunavut, Canada. 14
Figure 2.2 a) Satellite image overview of Sanikiluaq and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points. 16
Figure 2.3 a) Satellite image overview of Naujaat and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points. 17
Figure 2.4 Hydrographs from a) the Sanikiluaq wetland; and b) the Naujaat wetland over the treatment season in 2016. The black dots represent the discrete influent measurements collected during the site visits..... 26
Figure 2.5 Distribution of absolute gene target abundances and 16S rRNA gene target absolute abundances at the sample locations in a) Sanikiluaq and, b) Naujaat. The stars on top x-axis are indicative of significant difference of distributions with paired student t-tests at p <0.05. The middle lines represent the mean values, the bottom and top of the boxes represent the 25^{th} and 75^{th} percentiles, and the whiskers represent the 10^{th} and 90^{th} percentile of the distribution of gene targets. The raw data distributions were combined over the entire treatment season per site..... 31
Figure 2.6 Absolute abundance of ARGs in the raw wastewater (raw), influent (In), mid-point (Mid) and effluent (Out) water and soil samples from the wetlands during spring and summer sampling periods. 35
Figure 2.7 Relative abundance of ARGs in the raw wastewater, and soil and water, in the wetlands during spring and summer sampling periods. Note: as the dropdown bars become increasingly negative in the graphs, the gene target in question represents less of the overall proportion of genes in the bacterial population (i.e., is less enriched) 36
Figure 2.8 a) Loadings plot of the PCA results, use of red text is for contrast; and (b) scores plot of the gene targets and WQI PCA results. Ellipses denote scores groupings of spring samples (green), summer samples (red), raw truck samples (blue), and reference samples (orange). Abbreviations

indicate influent (I), mid-point (M), effluent (E), reference (R), spring (Sp), and summer (S). 39

Figure 3.1 Schematic of the sand filter experimental layout (not to scale). Sand filter (SF)1 and SF4 filter media consist of fine-grained sand, SF2 and SF5 are medium grained sand, and SF3 and SF6 are coarse grained sand. SF1 – SF3 are on a 5% slope and SF4 – SF6 are on a 30% slope..... 48

Figure 3.2 Absolute abundances of gene markers in the raw wastewater, septic tank effluent (STE), and sand filter (SF) 1 – 6 for the duration of the study (n = 10). The middle lines represent the median values, the dotted lines represent the means, the bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the gene concentrations. The dashed line represents the limit of quantification for the sand filter effluent. Difference in letters denotes significant difference of the gene absolute abundances at $p < 0.05$ for the Tukey test. The raw wastewater and septic tank effluent samples were not analyzed statistically as the differences in sand filter performance were of primary interest. 59

Figure 3.3 Relative abundances of gene markers in the raw wastewater, septic tank effluent (STE), and sand filters (SF) 1 – 6 for the duration of the study (n =10). The middle lines represent the median values, the dotted lines represent the means, the bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the gene concentrations. Difference in letters denotes significant difference between the gene absolute abundances at $p < 0.05$ for the Tukey test. 61

Figure 3.4 Geometric mean of total and antibiotic resistant bacteria in samples of raw wastewater, septic tank effluent (STE), and sand filter (SF) effluents (sampled on July 16 and 23, 2018, n = 4). The error bars represent one standard deviation. The *E. coli* is presented as the geometric mean of two samples collected on July 16 and 23, 2018. The *E. coli* concentrations for the SF effluent were all below 1 CFU/mL. 64

Figure 3.5 Principal component analysis (PCA) of the gene marker concentrations and water quality indicators along the treatment train. This illustrates the: a) loadings plot of the gene markers and other parameters (n = 10 sample events), use of red text is for contrast; and b) scores plot of the PCA results of the wastewater sampling. Ellipses denote groupings of scores of sand filters 2 – 6 (blue); sand filter 1 (green); and the raw wastewater and septic tank effluent samples (red). The numbers in brackets represent the percentage of variance described in the dataset by the first and second components. 66
Figure 4.1 3D view of the three lateral flow sand filters SF4 (fine grained), SF5 (medium grained), and SF6 (coarse grained), on a 30% slope, which receive septic tank effluent (STE) into gravel distribution trenches. 77
Figure 4.2 The boundary conditions of each of the lateral flow sand filters modeled in Hydrus 2D. The dimensions are shown in Figure 4.1. 82
Figure 4.3 The effluent tracer response curves of observed versus truncated simulated bromide (Br^-) concentrations for 7.5 g Br^- mass injections for the sand filters (SF). The peak Br^- concentration of the curve is denoted by C_p. 95
Figure 4.4 Results of the field observed ARG effluent concentrations and the modeling simulations for each sand filter (SF) with a decay rate at 4 and 15°C. The observed data bar represents the mean value of field observed data and the error bar represents one standard deviation of n = 10 samples.. 97
Figure 4.5 Results of the simulations of sand filter (SF) effluent concentrations of: a) total heterotrophic bacteria (TSA); and bacteria resistant to b) 50 mg/L sulfamethoxazole; c) 10 mg/L of tetracycline; and d) 50 mg/L of erythromycin. The model results represent bacterial decay rates at 4 and 15°C compared to field observed data for each sand filter (SF). The bar graphs represent the mean of the field observed data with the error bars representative of one standard deviation (n = 4 samples). 98
Figure 4.6 Observed versus simulated gene target concentrations. 100
Figure 4.7 Observed versus simulated total heterotrophic bacteria and ARB concentrations. 100

ABSTRACT

Antimicrobial resistance (AMR) is a major global public health threat which contributes to reduced effectiveness of antibiotics for treatment of bacterial infections in humans. Residuals of antimicrobial products from anthropogenic uses creates a selective environment and shifts the microbial populations in our municipal wastewater to become resistant. This leads to high concentrations of bacterial resistance or "hot spots." The link between clinical incidence of AMR infections and the environmental dimension impacted by anthropogenic activities has been demonstrated to be important. Surveillance of AMR hot spots in the environment is conducted by monitoring environmental compartments for determinants of AMR including: antibiotic resistance genes (ARGs), mobile genetic elements, and antibiotic resistant bacteria (ARB).

This thesis explores the fate of AMR determinants in variably saturated terrestrial environments that are used for municipal wastewater treatment and disposal. Specific focus was on rural, developing and remote regions, due to the challenges in the provision of adequate wastewater treatment in jurisdictions which are reliant on decentralized wastewater treatment. Often residents in these communities are particularly vulnerable to AMR infections.

AMR contamination from municipal wastewater was studied in communities in the Canadian Arctic. Hydrology of wetland receiving environments played an important role in the dissemination of AMR contaminants in the environment. Reference wetlands representative of background conditions with limited anthropogenic impacts had relatively low levels of determinants of AMR indicative of the environmental resistome.

Technologies for mitigation of the spread of AMR from sources in rural regions were studied. Removal of contaminants of AMR via physical filtration in lateral flow sand filters—used as a type of domestic on-site wastewater treatment system—was studied. This type of filtration technology was effective in attenuation of AMR contaminants with 2.9 to 5.4 log reductions for ARGs observed.

A commercially available computer model (HYDRUS 2D/3D) was used to simulate the attenuation processes within the sand filters. Prediction of ARB was well represented in the modeling but prediction of ARGs and other genetic elements could be improved. This thesis represents one of the first studies to observe and model the fate and transport of determinants of AMR in subsurface environments.

LIST OF ABBREVIATIONS USED

Al	Aluminum
ADE	Advection Dispersion Equation
AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
APHA	American Public Health Association
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Gene
Ba	Barium
BEEC	Bio-Environmental Engineering Centre
bla_{CTX-M}	Class A β-lactamase Gene
bla_{TEM}	Class A β-Lactamase Gene
Br^-	Bromide Ion
C	Concentration
C_0	Initial Concentration
Ca	Calcium
$CaCO_3$	Calcium Carbonate
$CBOD_5$	Five-Day Carbonaceous Biochemical Oxygen Demand
Cd	Cadmium
Ce	Cesium

CFU	Colony Forming Units
Cr	Chromium
Cu	Copper
D_{10}	Effective Size at 10% Passing
DEM	Digital Elevation Model
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
E. coli	*Escherichia coli*
EPA	Environmental Protection Agency
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, *Enterobacter* spp.
ermB	Macrolide-Lincosamide-Streptogramin Type B Resistance Gene
Fe	Iron
FWS	Free Water Surface
h	Matric Potential (Pressure head)
ha	Hectare
HDPE	High Density Polyethylene
HF183	Human Specific Bacteroides Marker
HGT	Horizontal Gene Transfer
HHRA	Human Health Risk Assessment

HLR	Hydraulic Loading Rate
Hmt	Human Mitochondrial Gene
HRT	Hydraulic Retention Time
ICP-ms	Inductively Coupled Mass Spectrometry
int1	Class I Integrase Gene
k	First Order Rate Constant
K	Potassium
K	Unsaturated Hydraulic Conductivity
k_{20}	First Order Rate Constant Adjusted to 20°C
K_d	Partition Coefficient
K_s	Saturated Hydraulic Conductivity
LOD	Limit of Detection
LOQ	Limit of Quantification
mecA	Methicillin Resistance Gene
Mg	Magnesium
Mn	Manganese
Na	Sodium
NaBr	Sodium Bromide
NaCl	Sodium Chloride
NH_3-N	Un-Ionized Ammonia Nitrogen

NH_4+	Ammonium
OWTS	On-site Wastewater Treatment System
PCA	Principal Components Analysis
PLC	Programmable Logic Controller
PS	Peptone Saline
QMRA	Quantitative Microbial Risk Assessment
qnrS	Fluoroquinolone Resistance Gene
qPCR	Quantitative Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
RTD	Residence Time Distribution
RTK	Real-Time Kinematic
RWT	Rhodamine WT Dye
S	Sink Term
SAO	Senior Administrative Officer
Sb	Antimony
Se	Selenium
SF	Sand Filter
SPE	Solid Phase Extraction
Sr	Strontium
STE	Septic Tank Effluent

sul1	Class I Sulfonamide Resistance Gene
sul2	Class II Sulfonamide Resistance Gene
TAN	Total Ammonia Nitrogen
TC	Total Coliform
tetO	Tetracycline Resistance Gene
TIS	Tanks-In-Series
TN	Total Nitrogen
TP	Total Phosphorus
TSA	Tryptone Soy Agar
TSS	Total Suspended Solids
U	Uranium
vanA	Vancomycin Type A Resistance Gene
VSS	Volatile Suspended Solids
WHO	World Health Organization
WQI	Water Quality Indicator
WSP	Wastewater Stabilization Pond
WTA	Wetland Treatment Area
WWTP	Wastewater Treatment Plant
WWTS	Wastewater Treatment System
Zn	Zinc

Θ	Volumetric Water Content

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CHAPTER 1 INTRODUCTION

1.1 Introduction

1.1.1 Antimicrobial Resistance

The progressively reduced effectiveness of antibiotics for the treatment of infections threatens to undermine the advances that have been made in the 21st century of modern medicine, with the reality of a post-antibiotic era within the realm of near future possibilities (WHO, 2014). Outbreaks of multi-drug resistant infections are becoming increasingly common from bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp. (ESKAPE) (Medina & Pieper, 2016; Pendleton et al., 2013). If left unchecked, antimicrobial resistance will have dire impacts on global public health, food security, medicine, and the economy (Laxminarayan et al., 2016; Ahmed et al., 2017). This problem that modern society grapples with is comparable in scale to the threat posed to humanity by anthropogenic induced climate change (Woolhouse & Farrar, 2014). In fact, antimicrobial resistance is projected to increase with the predicted progression of climate change (MacFadden et al., 2018).

Antimicrobial resistance (AMR) develops in bacteria typically as a result of selective pressure placed by naturally derived antimicrobials, which contributes to a Darwinian narrative of survival of the fittest for bacteria subjected to exposure to antimicrobials (Holmes et al., 2016). AMR is in fact an ancient survival strategy of bacteria, where specific ancestral genetic elements associated with modern day AMR, such as β-lactamases enzymes, have been observed through phylogenetic analysis to have originated over 2 billion years ago (Aminov, 2009). Simply put, AMR is how bacteria have originally evolved to withstand the selective pressures placed by naturally occurring chemical exposure in their environments. In modern times, antimicrobials are used extensively for various applications including but not limited to: clinical use of antibiotics for treatment of human infections, industrial processes, veterinary use, growth promotion in livestock and poultry, and aquaculture (Bacquero et al., 2008). The use of these antimicrobials leads to

the development of hot spots of AMR in the environment receiving by-products and waste streams associated with these reservoirs (Berendock et al., 2015).

1.1.2 Determinants of Antimicrobial Resistance

Quantification of determinants of AMR is not straightforward as there are numerous mechanisms for acquisition of resistance and hundreds to thousands of potential genetic elements which express a myriad of resistance functions. Bacteria that are able to survive exposure to antimicrobials have acquired antimicrobial resistance genes (ARGs), which encode for a plethora of resistance functions. The acronym ARG will be synonymous with antibiotic resistance genes throughout the thesis. There is an ARG which confers resistance to virtually every type of antimicrobial commercially available today. Mechanistically, there are a few ways that bacteria can acquire antimicrobial resistance, which includes: through vertical transmission via genetic mutation, horizontal transmission via conjugation (e.g., cell-to-cell exchange), transformation (e.g., bacterial acquisition from 'free genetic elements'), and transduction (e.g., gene transfer mediated by bacteriophages) (Founou et al., 2016; Dantas & Sommer, 2014). ARGs are transferred between antibiotic resistant bacteria (ARB) facilitated by mobile genetic elements (MGE) including plasmids, integrons, and transposons, all of these elements may be considered determinants of AMR. The problem with the mechanistic way in which the genetic elements are exchanged by microbes is that horizontal gene transfer (HGT) is not necessarily species specific. In fact, due to this ease of trans-species acquisition, the link between environmental ARB and clinically diagnosed pathogens has become increasingly likely (Wright, 2010).

The cellular acquisition of elements of AMR occurs via various mechanisms, with the most important mechanism being cell-to-cell transfer mediated by HGT conjugation which involves exchange of plasmids. Plasmids are relatively small, self-replicating, extrachromosomal molecules of DNA (Amábile-Cuevas & Chicurel, 1993). There is potential for determinants of AMR, including ARGs which may be housed within plasmids or chromosomal DNA from lysed cell materials, to exist extracellularly. This potential for extracellular ARGs to exist in the environment could be problematic due to the small molecular size of plasmids, allowing them to be readily mobilizable in environmental settings. This dilemma is similar to the severe problem posed by bacteriophages

downgradient of wastewater sources, whereby the molecular size of bacteriophages (20-40 nm in diameter) contributes to their potential long-range transport in saturated subsurface environments (Kauppinen et al., 2018; Gotkowitz et al., 2016). Theoretically, bacteria are much larger (*ca.* 0.5-2 µm) than viruses and more easily immobilized in porous media due to physical filtration (Jin & Flury, 2002). Ascertainment of intracellularly housed and extracellular DNA with conventional quantitative polymerase chain reactions (qPCR) methods is not standardized and is difficult to validate (Vikesland et al., 2017). Testing and validation of methods to distinguish between intracellular and extracellular determinants of AMR is currently lacking. Contrary to chemical contaminants, which are typically subject to sorption, dilution, and degradation processes exclusively, ARGs can be present in genomes and as extracellular DNA (i.e., cell-free DNA), and persist in their hosts and propagate through cell division cycles which complicates typical attenuation considerations (Berendonk et al., 2015). In order to properly represent the attenuation of determinants of AMR within soil environments, the sorption and degradation properties of the biological contaminants to soil media should be quantified.

1.1.3 Surveillance of Antimicrobial Resistance

Surveillance of AMR prevalence in the environment can be conducted by monitoring prevalence and abundances of determinants of AMR. This involves detecting, tracking, and quantification of ARGs using a specific gene target panel detected with qPCR. This targeted method has been broadly applied to characterize AMR hot spots and dissemination, with an advantage being the sensitive low-level detection afforded by this method and quantitative capabilities. Despite the extensive use of this targeted gene strategy to monitor AMR prevalence, the gene target panels are not standardized and there remains uncertainty on which gene elements to target. Technological advances in sequencing now present the opportunity for metagenomic analyses to provide a broader and more comprehensive, yet less sensitive, characterization of AMR in environmental compartments (Vikesland et al., 2017).

Often times, surveillance of AMR hot spots in environmental compartments uses qualitative comparison metrics between anthropogenic ‘affected’ areas and relatively non-impacted areas. Work has been carried out to try to characterize the environmental

resistome, which is the innate resistance of environmental microbes that reside naturally in the environment (D'Costa et al., 2006). Relatively geographically isolated regions such as the high arctic may provide a benchmark closer to natural levels of AMR in the soil and water environment (McCann et al., 2019). Benchmark quantification of determinants of AMR is important to be able to track the spread of AMR over time. Even geographically isolated polar regions are prone to dissemination of determinants of AMR as suggested by Hernández and González-Acuña (2016). A recent study by Li et al. (2018) demonstrated that contaminants of AMR such as ARGs can be disseminated by atmospheric deposition in urban areas. Furthermore, long-range global transport of microbial contaminants have been suggested by Mayol et al. (2017). Therefore, the potential for atmospheric deposition of ARGs in arctic regions may also be possible.

The 'One Health' approach to tackling the problem of increasing clinical incidence of AMR involves gaining a better understanding of the rates of antimicrobial use across health care, food production, other relevant industries, and within the environmental dimension (WHO, 2018). The environmental dimension of antimicrobial resistance is defined as the impact on the environment resulting from antibiotic use in agriculture, aquaculture, anthropogenic waste processing, and industrial manufacturing (Topp et al., 2018). Understanding links between clinical incidence of infection and the environment have become increasingly important to fully understand the development of AMR (Woolhouse et al., 2015). Good progress has been made to characterize hot spots of AMR from anthropogenic sources in the environment. Studies have shown the development of hot spots of AMR in the environment around a variety of sources such as: municipal wastewater effluent from treatment plants (Xu et al., 2015; Marti et al., 2013), hospital effluent (Rodriguez-Mozaz et al., 2015), aquaculture (Jang et al., 2018; Muziasari et al., 2016), and agriculture (Murray et al., 2019; Cheng et al., 2016).

Despite, the expanding body of knowledge on environmental sources of AMR, there still remains many unanswered questions on particular environmental reservoirs. For instance, populations that reside in rural and developing regions often times do not have access to advanced centralized wastewater treatment facilities. Up to 25% of the population in North America are reliant on decentralized on-site wastewater treatment systems (OWTS) for

provision of domestic wastewater treatment (Garcia et al., 2013). The potential for regionally distributed hot spots of AMR from decentralized wastewater facilities in rural and developing regions has been identified by authors such as McConnell et al. (2018a). More research is needed to verify the risk of AMR hot spot development potential in other rural environments, which have anthropogenic impacts. Specifically, northern Canadian environments, which are largely reliant on passive municipal wastewater treatment are not well studied in terms of potential hot spot sources and extent of AMR contamination. Making this particularly poignant, Daley et al. (2019) demonstrated through quantitative microbial risk assessment (QMRA) modeling, that there is a high likelihood of incidence of enteric gastrointestinal illnesses in arctic communities in Nunavut, Canada. This was predicted as likely resulting from exposure to pathogens from wastewater effluent within the communities.

1.1.4 Technological Applications for Mitigation

A review on the critical knowledge gaps for tackling the environmental dimensions of AMR recognized that there is a need to assess technological interventions to mitigate the spread of AMR in the environment (Larsson et al., 2018). Specifically, the review highlighted that low- and middle-income countries are most in need of assessment of low cost and low-tech solutions to provide treatment of determinants of AMR (Larsson et al., 2018). Low technology options such as OWTS are cost effective and practical for implementation in rural and developing countries. Many of these types of de-centralized wastewater treatment technologies are largely untested for their efficacy of AMR mitigation. Assessment of the potential human exposure routes to AMR in the various environmental compartments would be useful to inform QMRA modeling, which should take place in conjunction with advancement of existing treatment technologies that are ideally low cost and implementable (Ashbolt et al., 2013; Ashbolt et al., 2018). Many types of decentralized wastewater treatment technologies may pose a risk of introduction of contaminants of AMR into the shallow subsurface environment. Currently, this risk is largely unknown and there are no tools that have been demonstrated to be effective for prediction of attenuation of contaminants of AMR in the vadose zone. Furthermore, research should be done to: determine how to optimize wastewater treatment technologies,

and develop predictive mathematical models for representing ARG attenuation (Waseem et al., 2017).

1.2 Research Questions

Based on the literature, the fate of determinants of AMR within variably saturated terrestrial environments, in particular, is not well understood. Numerous studies have detected these contaminants in various subsurface and wetland environments, but the information required to quantitatively predict their fate is still lacking. To address this research gap, the following research questions have been identified:

i) What is the persistence and rates of attenuation of determinants of AMR in variably saturated environments receiving rural and remote domestic wastewater sources?

ii) What are the background levels of determinants of AMR in variably saturated environments that are relatively un-impacted by anthropogenic activities that could be characteristic of ARG abundance in the environmental resistome?

iii) What is the performance of on-site wastewater treatment technologies for removal of determinants of AMR from domestic wastewater?

iv) What are the dominant mechanisms and rates of attenuation of determinants of AMR in porous filtration media?

v) Can fate of determinants of AMR in variably-saturated porous media systems be predicted using conventional reactive transport models?

1.3 Research Objectives

The research questions were addressed with the following research objectives:

1. Assess the sources and attenuation of determinants of AMR in variably saturated terrestrial environments downgradient of rural and remote sources of municipal wastewater;

2. Characterize background levels of determinants of AMR in geographically isolated terrestrial environments that have relatively low anthropogenic impacts;

3. Evaluate the treatment performance of conventional technology for on-site wastewater treatment systems for attenuation of determinants of AMR;

4. Determine whether design factors such as grain size and filter slope have an effect on treatment performance of on-site wastewater treatment systems;

5. Verify the compartmentalization of intracellular and extracellular ARGs within on-site wastewater treatment systems;

6. Characterize sorption and degradation coefficients for determinants of AMR in various types of porous media; and

7. Apply a preliminary numerical modeling to represent the contaminant hydrogeology of determinants of AMR in variably saturated terrestrial environments.

1.4 Thesis Organization

The thesis presented herein is organized with the following structure:

CHAPTER 1 provides a background for the state of the research on AMR in the environment, outlines the knowledge gaps at the time of this thesis, and identifies the research questions and objectives.

CHAPTER 2 addresses research objectives 1 and 2. This work focuses on the characterization of determinants of AMR in variably saturated wetland environments downstream of municipal wastewater treatment facilities in the Canadian Arctic. A version of this research was published in *Science of the Total Environment*.

CHAPTER 3 addresses research objectives 3, 4, and 5. This work was focused on a treatment performance assessment of pilot-scale on-site wastewater treatment systems, which were characterized by sand media filtration, for attenuation of determinants of AMR. A version of this work was published in *Water Research*.

CHAPTER 4 addresses research objectives 6 and 7. This work applied a preliminary conventional reactive transport model for variably saturated porous media environments for simulation of fate and transport of determinants of AMR in on-site wastewater treatment systems using sand filtration technology.

CHAPTER 5 provides the overall conclusions and recommendations from the thesis research.

CHAPTER 2 FATE OF ANTIBIOTIC RESISTANCE GENES IN TWO ARCTIC TUNDRA WETLANDS IMPACTED BY MUNICIPAL WASTEWATER

Abstract[1]

In the Canadian Arctic, it is common practice to discharge municipal wastewater into tundra wetlands. Antibiotic resistant bacteria and the antibiotic resistance genes they contain can be present in municipal wastewater and there is a scarcity of knowledge on ARGs in wastewater in Arctic environments. This study was initiated on the fate of ARGs in tundra wetland ecosystems impacted by anthropogenic wastewater sources in Arctic communities. In the summer season of 2016, two wetlands were studied in the Inuit communities of Sanikiluaq and Naujaat in Nunavut, Canada. Genomic DNA was extracted from both soil and water during the spring freshet and late summer in the wetlands, and a suite of nine clinically relevant ARGs (*sul1*, *sul2*, *mecA*, *vanA*, *qnrS, ermB*, *tetO,* bla_{TEM}, $bla_{CTX\text{-}M}$), and an integron gene (*int1*) were analyzed using qPCR. Hydrological and water quality measurements were conducted in conjunction with the microbiological sampling. Gene targets were consistently present in the wastewater, and throughout both wetlands, except for *vanA* and *mecA*. Concentrations of ARGs were greater during the spring freshet, due to short hydraulic retention times (< 2 days), which coincided with decreased treatment performance. The natural resistome in un-impacted wetlands had above limit of quantification concentrations of *int1, sul1*, *sul2,* $bla_{CTX\text{-}M}$ in water in Naujaat, and *sul1*, *qnrS* and *tetO* in soil in Sanikiluaq. First-order rate constants were widely variable and

[1] Note: A version of this chapter is published in Science of the Total Environment.

Hayward, J. L., Jackson, A. J., Yost, C. K., Hansen, L. T., & Jamieson, R. C. (2018). Fate of antibiotic resistance genes in two Arctic tundra wetlands impacted by municipal wastewater. *Science of the Total Environment, 642*, 1415-1428.

specific to the gene target. ARGs were present in concentrations elevated above baseline reference sites in tundra wetlands influenced by municipal wastewater, and hydrological conditions had a large impact on their spatial distribution and levels.

2.1 Introduction

The development of AMR has become a prevalent global public health issue (Davies & Davies, 2010). Antibiotics are present in municipal wastewater, originating from partially metabolized medications used by humans and disposal of un-used antibiotics (Nagulapally et al., 2009). Antibiotic resistant bacteria, and associated ARGs, can accumulate and persist in human and agricultural wastes and then be applied or released into terrestrial and aquatic environments (Ashbolt, 2013; Czekalski et al., 2012). These AMR contaminants pose human health risks as previously curable infections are now becoming resistant to conventional antibiotics (Ashbolt, 2013; Laxminarayan et al., 2013). Research has begun to demonstrate that some environments can behave as reactors for ARB proliferation, such as wastewater treatment plants (Czekalski et al., 2012; Rizzo et al., 2013). Soil and water environments can function as environmental reservoirs for ARGs (Martinez, 2008; Taylor et al., 2011), and it has been demonstrated that some ARGs are part of the ancient soil microbiome (D'Costa et al., 2011). Potential for conference of ARGs between environmental soil bacteria and human pathogens has been suggested by Forsberg et al. (2012). In some regions the baseline environmental levels of ARGs have been observed to be increasing under anthropogenic pressures (Knapp et al., 2009). There is concern that increasing anthropogenic use of antibiotics is contributing to selective pressure and increased risk of horizontal gene transfer to human pathogens (Qiu et al., 2012). Characterization of the persistence of ARGs is crucial for human health risk assessment (HHRA) (Bouki et al., 2013). Specifically, quantification of the clinically relevant AMR bacteria within the potential exposure sites to humans and animal vectors within the environment is required to inform QMRA, and this type of data has been reported to be limited (Huijbers et al., 2015). One knowledge gap in the HHRA process includes quantification of ARGs and AMR bacteria hot spots in the environmental dimension, specifically soil and aquatic systems (Ashbolt et al., 2013).

The extreme climate and remoteness of the expansive region of the Canadian Arctic restricts wastewater management options, increases costs and often leads to difficulties with operation and maintenance of wastewater infrastructure (Johnson et al., 2014; Yates et al., 2012). The majority of communities in Canada's Far North use centralized methods of wastewater management with passive treatment systems comprised of lagoons (Johnson, 2008); also referred to as wastewater stabilization ponds (WSPs). In many communities, these systems are not engineered, and consist of a natural depression in the landscape. Due to permafrost, most communities have trucked water distribution systems for drinking and wastewater with storage on an individual household level (Daley et al., 2014). Engineered lagoons operate as one-year detention-controlled discharge storage ponds which remain frozen most of the year. Typically, discharge occurs at the end of a three-month "treatment season" spanning from late-June to early-September (Ragush et al., 2015). Conversely, non-engineered systems release wastewater in an uncontrolled manner at the start of the spring thaw or freshet, continuing throughout the summer. The lagoons often discharge to tundra wetlands, which are natural features of the landscape where polishing of effluent has been observed prior to discharge into primarily marine receiving water environments (Yates et al., 2012; Hayward et al., 2014; Balch et al., 2018). These wetland areas have been used for wastewater disposal for decades (Balch et al., 2018), and have been termed Wetland Treatment Areas (WTAs). The treatment performance and attenuation of conventional wastewater contaminants within tundra WTAs has been observed to have high inter- and intra-system variability governed largely by the natural hydrology of the landscape and temperature with increased treatment observed when hydraulic retention times (HRTs) are sufficient to allow treatment (Hayward et al., 2014; Balch et al., 2018). For example, Yates et al. (2012) reported WTAs were effective for wastewater treatment with contaminant removal rates in six WTAs over an Arctic summer which ranged from 47 – 94% for five-day biochemical oxygen demand ($CBOD_5$), 39 – 98% for total suspended solids (TSS), >99% for *Escherichia coli* (*E. coli)*, 84 – 99% for un-ionized ammonia (NH_3-N), and 80 – 99% for total phosphorus (TP).

It has been observed that the removal of human pathogens from some lagoons in the Canadian Arctic may be inadequate (Huang et al., 2017). Due to lack of disinfection, there may be an elevated risk of development of AMR in the microbial communities associated

with Arctic wastewater treatment systems (WWTSs), which is exacerbated by low ambient temperatures and low biological diversity in the receiving waters (Gunnarsdóttir et al., 2013). A potential contributing problem in Arctic environments includes the demonstrated potential for longer survival time of some bacteria in cold temperatures (Howell et al., 1996). Although population sizes in the Canadian Arctic are relatively small—with few commercial agriculture and aquaculture industries—there is still cases of higher reported incidence of enteric infection in comparison to southern Canada (Harper et al., 2015) and AMR could be problematic (Gunnarsdóttir et al., 2013). Since WWTSs are located within or near many northern and remote communities, human-environment interactions could pose a risk of exposure to pathogens associated with wastewater via direct contact with the landscape or aquatic environment, or through contact with wildlife vectors (Daley et al., 2015; Pardhan-Ali et al., 2013; Founou et al., 2016; Harper et al, 2011). The possibility for wildlife to act as vectors for spreading of AMR from urban to rural areas in Arctic environments has been demonstrated in Alaskan seagulls carrying antibiotic resistant strains of *E. coli* (Atterby et al., 2016; Ramey et al., 2017).

However, there have been limited studies conducted specifically on AMR in the Arctic due to the logistical and financial constraints associated with travel to these remote communities. Chaves-Barquero et al. (2016) conducted a study in Cambridge Bay, Nunavut, Canada to assess the concentrations of pharmaceuticals and ARGs (conferring resistance to tetracycline and sulfonamide) in the effluent from a WSP and downstream tundra wetland. They concluded that ARGs were found in the WSP and wetland system and were largely diluted in the marine receiving waters. Neudorf et al. (2017) studied three WWTSs in Nunavut, Canada including two WSP systems (Pond Inlet and Clyde River), and one mechanical treatment plant (Iqaluit). Their study demonstrated that ARGs (2 log gene copies per mL) were present in the effluent discharged into the receiving environments, which could pose a risk for horizontal gene transfer to pathogens. The actual risk posed to human health by ARGs in Arctic receiving environments is unknown at this point. To address this knowledge gap, Neudorf et al. (2017) stated that further research into ARG prevalence and behavior in Arctic environments is warranted.

It is hypothesized that the ARG concentrations in the WTAs will follow trends observed in previous conventional wastewater quality studies on tundra wetlands which showed improvements in water quality as the treatment season progressed (Yates et al., 2012; Hayward et al., 2014; Balch et al., 2018). In addition, it is hypothesized that the geographic location of the wetlands may contribute to the observed treatment, with higher latitudes having potentially less precipitation leading to decreased dilution, and lower temperatures, which can slow biological treatment. The overall objective of this study was to quantify the levels of nine ARG targets in soil and water within two tundra wetlands impacted by municipal wastewater in the northern territory of Nunavut, Canada. This study specifically examined the: (i) seasonal hydrologic variability effects on the spatial distribution and levels of ARGs with the two treatment wetlands, (ii) natural resistome of un-impacted reference wetlands, and (iii) kinetics of ARG removal.

2.2 Methodology

2.2.1 Site Descriptions

The two study sites were located in Sanikiluaq (56°32’34” N, 079°13’30” W) and Naujaat (66°31’19”N, 086°14’16”W) in Nunavut, Canada (Figure 2.1). Sanikiluaq is in the subarctic region of Canada which was selected as a low latitude site with slightly higher precipitation and average summer air temperatures. Naujaat is located close to the boundary of the Arctic Circle and was selected as representative of a higher latitude site with less precipitation and lower average summer air temperatures. The populations are 882 and 1,082 for Sanikiluaq and Naujaat, respectively (Statistics Canada, 2017a & b). Average air temperatures in Sanikiluaq range from –28°C to –19°C in January, and from 6°C to 16°C in July. Total precipitation averages 671 mm, with 422 mm as rainfall, and 2,488 mm as snow (249 mm Snow Water Equivalent), (Government of Canada, 2016a). In Naujaat, average air temperatures range from –34°C to –28°C in January, and from 4°C to 13°C in July. Total precipitation averages 339 mm, with 124 mm as rainfall, and 2,154 mm as snow (215 mm Snow Water Equivalent), (Government of Canada, 2016b).

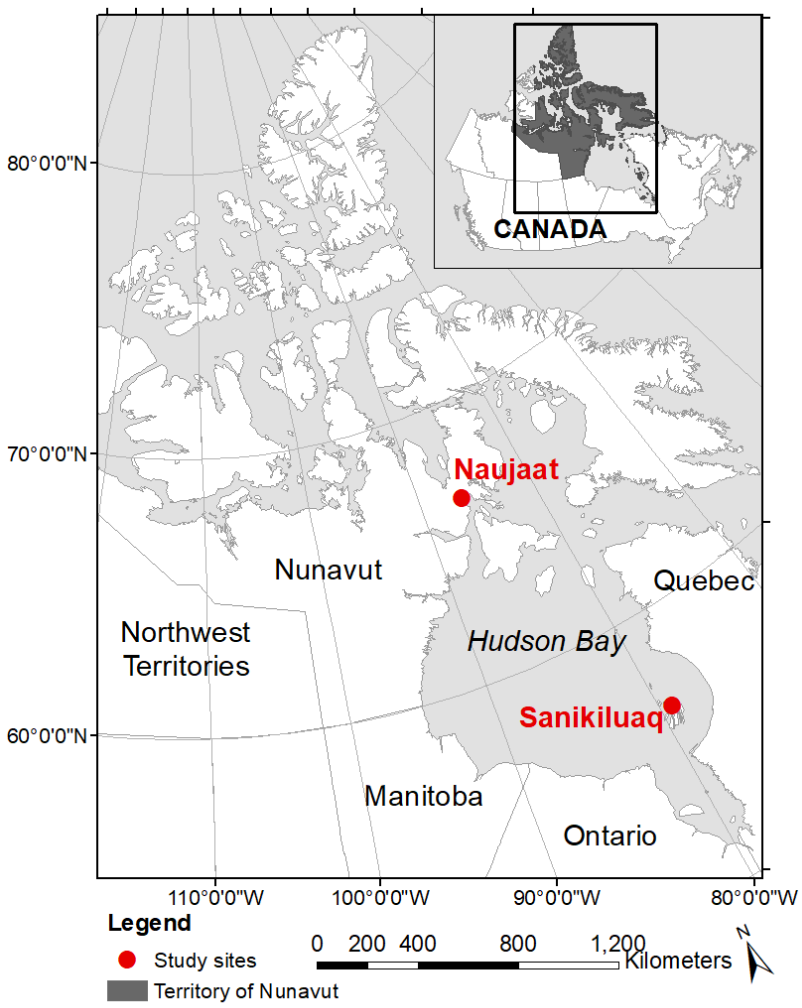

Figure 2.1 Map of study sites in Nunavut, Canada.

Both communities use trucked drinking water distribution and waste collection services. Approximately 88 to 100 m^3/day of wastewater is collected in each of the hamlets (Hamlet of Sanikiluaq, 2015; Hamlet of Repulse Bay, 2015). In each of the communities, wastewater is deposited into lagoons, which consist of natural depressions in the landscape. In Sanikiluaq one side of the lagoon has an engineered berm to retain the wastewater. Both WTAs remain frozen throughout the winter months with freeze-up occurring in early to late-September and thaw occurring in late-May in Sanikiluaq and mid-June in Naujaat. Permafrost was not encountered at depths of up to 1.5 m in Sanikiluaq, and the depth of the active layer in Naujaat was generally greater than 0.3 m in the wetland. Effluent from the lagoons flows by gravity throughout the treatment season into the downgradient WTAs

as shown in Figures 2.2 and 2.3. The areas and lengths of the study wetlands are approximately: 3.4 ha and 1 km (Sanikiluaq); and 3.7 ha and 1.2 km (Naujaat). The vegetation in the study wetlands are characterized by various willows, sedges, fireweed, mosses, and grasses; the upland areas were characterized by berries, white heather, lichens, grasses, and mountain avens (the latter only in Naujaat). The watershed areas contributing external hydrologic inputs into the wetlands were 170 ha and 96 ha, for Sanikiluaq and Naujaat, respectively (Figures 2.2 and 2.3). Ultimately, the wetlands discharge effluent into marine receiving environments. Data collection was conducted from May 20th – 31st and September 1st – 9th, 2016 in Sanikiluaq, and from June 10th – 21st and August 24th – 31st, 2016 in Naujaat. The May and June trips were representative of the spring period, when snow and ice melt is occurring; while the August and September trips were representative of the summer period, which is characteristically more arid in many tundra wetlands (Hayward et al., 2014).

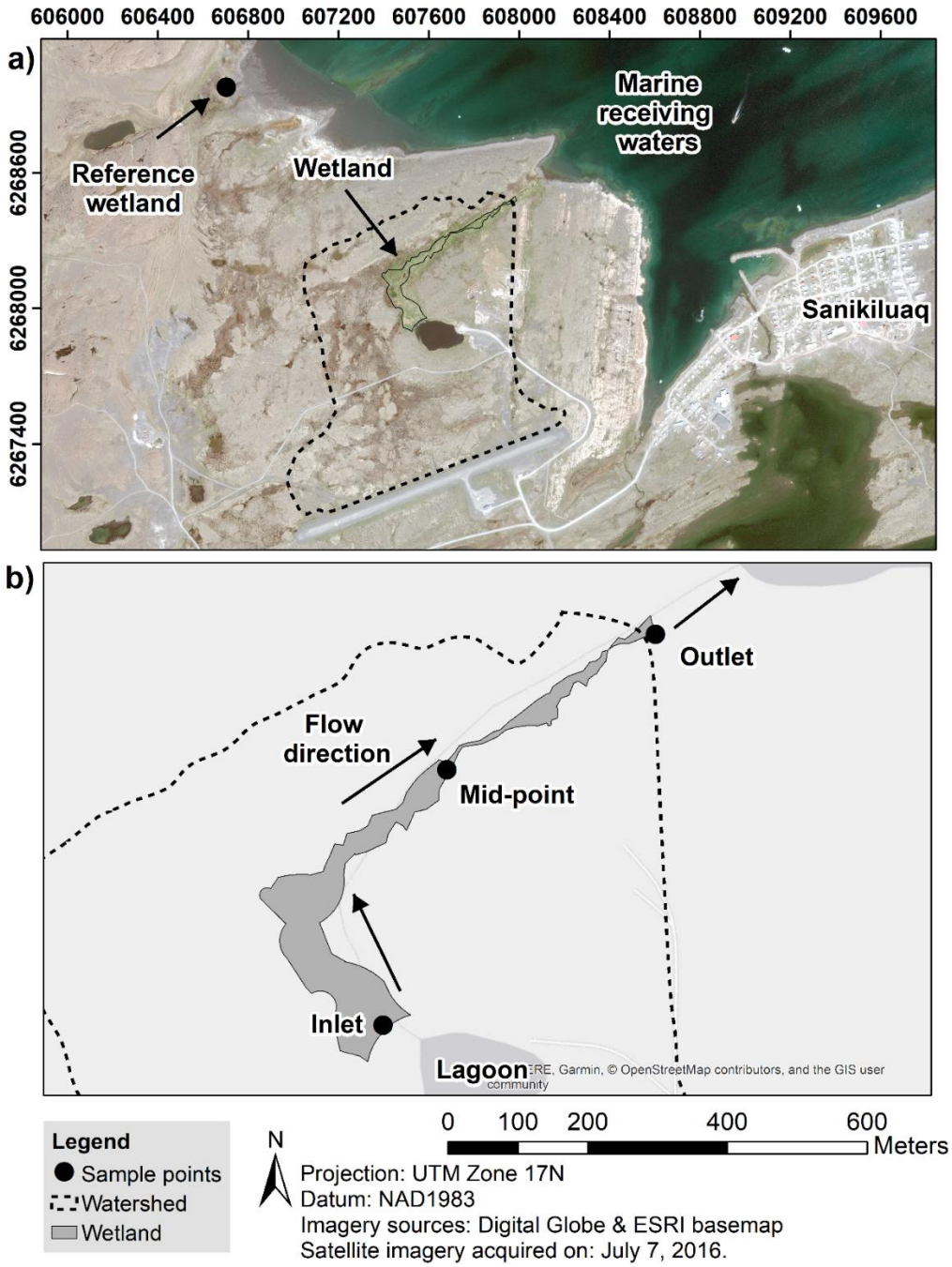

Figure 2.2 a) Satellite image overview of Sanikiluaq and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points.

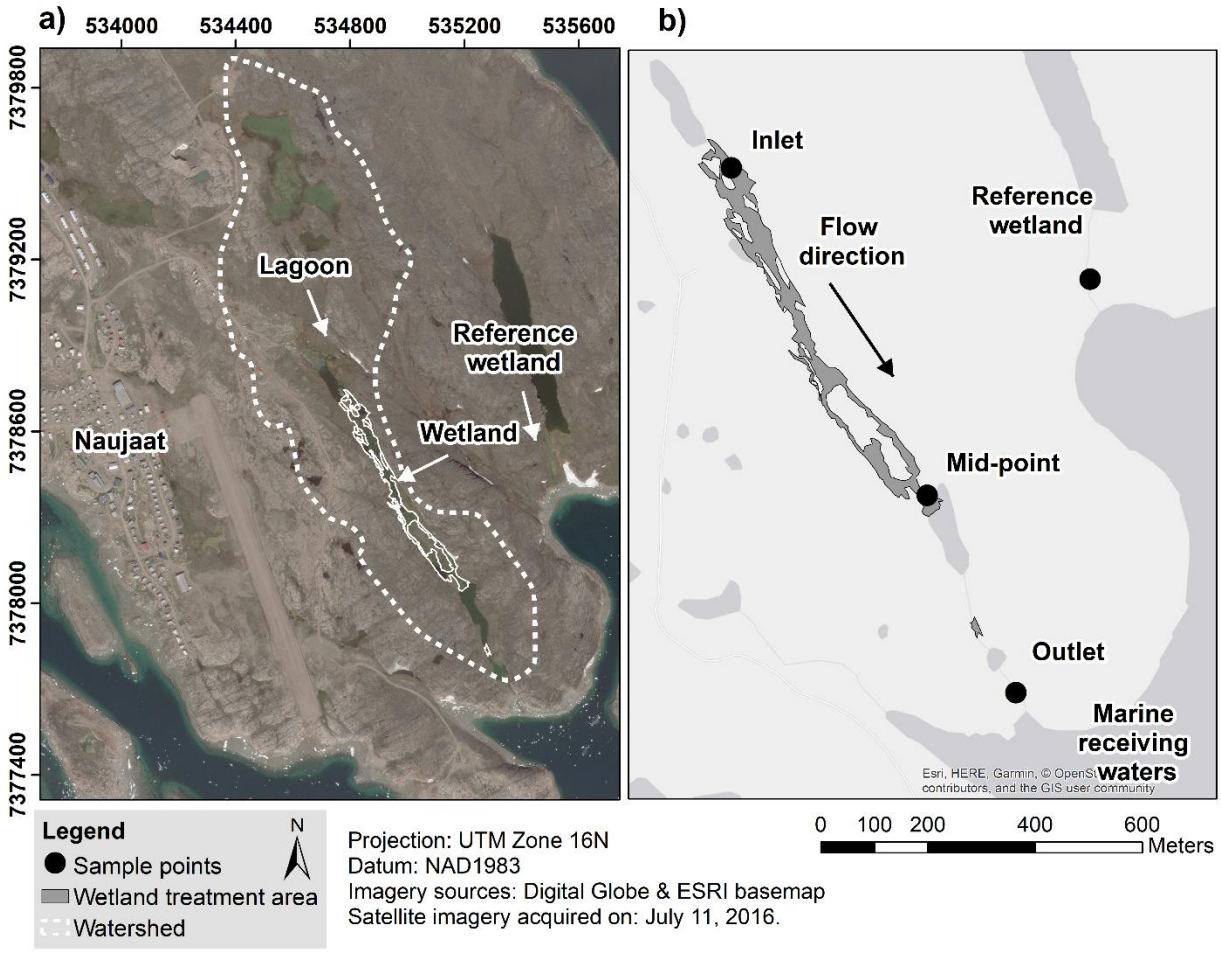

Figure 2.3 a) Satellite image overview of Naujaat and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points.

2.2.2 Reference Wetlands

A reference wetland with similar physical attributes to the WTAs was selected at each study site. This allowed for comparison of ARG levels and general water quality between the study wetlands and the natural tundra landscape. The locations of the reference wetlands are shown in Figures 2.2 and 2.3. Both reference wetlands were 70 m – 90 m upgradient of the intertidal zone and not under the influence of tidal action. Water quality and soil samples were collected at two locations in flowing water channels separated by 75 m in Sanikiluaq and 15 m in Naujaat within each of the reference wetlands. The sample sites were selected to capture running surface water flow from permanent drainage channels in the reference wetlands. The reference wetland in Sanikiluaq was sampled for

surface water twice during each site visit. Whereas, the reference wetland in Naujaat was sampled for surface water twice in June and only once in August. Therefore, six and five reference surface water samples were collected over the treatment season in Sanikiluaq and Naujaat, respectively.

2.2.3 Hydraulic and Hydrology Characterization

Instantaneous discharge was measured within the study wetlands at the inlets, mid-points, and outlets with a 625DF2N digital pygmy meter (Gurley Precision Instruments, Troy, New York, United States). The velocity-area method was used to determine the instantaneous discharge according to Dingman (2002). Stage-discharge relationships were developed at the outlets of the wetlands and combined with continuous water level measurements collected with HOBO U20 Water Level loggers (Onset Computer Corporation, Bourne, Massachusetts, United States) to continuously measure flow. Discrete measurements of flow were conducted at the inlets.

Rhodamine WT (RWT) fluorescent dye with a standard concentration of 200 g/L RWT was used to conduct tracer tests within the wetlands during each site visit to aid with wetland delineation and to characterize the hydraulic parameters of the wetlands including hydraulic retention times HRTs and mixing and dispersion behavior. The RWT tracer tests were conducted by segmenting the wetlands and conducting multiple tracer tests during similar flow conditions. The concentrations of RWT were measured at the end of each wetland segment at discrete time intervals with an optical YSI rhodamine WT probe. HRTs of the wetland sections were determined by taking the first moment of the residence time distribution (RTD), defined by Fogler (2010). Analysis of the RTD curves, and determination of HRTs were performed with Simpson's rule quadrature formula from Fogler (2010). Additional details on the processing procedure for the tracer concentration response curves are detailed in Hayward et al. (2014). An example calculation is included in Appendix D.

The watersheds of each of the wetlands were delineated with ArcGIS ArcMap 10 software (ESRI, Redlands, California, United States). Digital Elevation Models (DEMs) with a spatial resolution of 30 m were sourced for each site from the Natural Resources Canada

online database GeoGratis (Government of Canada, 2017). A real-time kinematic (RTK) topographic survey was conducted in Sanikiluaq due to the requirement for a finer spatial resolution DEM due to the lack of topographic relief at this site. The site in Naujaat had topographic relief that did not require the spatial resolution of an RTK survey. NThe hydraulic loading rates were calculated by dividing the minimum, maximum, and average wetland discrete inflows (m^3/d) over the treatment season by the delineated wetland areas (m^2) and conversion of units to centimetres per day.

2.2.4 Water Quality Sampling and Analysis

Raw wastewater samples were collected from the pump trucks to characterize untreated wastewater quality parameters and ARG concentrations. Water samples were collected from the inlet, mid-points, and outlet of the wetlands in sterilized 1L plastic sample bottles. Water samples were also collected from two locations in each of the reference wetlands as shown in Figures 2.2 and 2.3. General water quality indicators (WQIs) of temperature, dissolved oxygen (DO), specific conductance, and pH were made *in situ* for each sample collection with a YSI600 handheld water quality sonde (YSI Inc., Yellow Springs, Ohio, United States), which has lower operational limits of –5°C. The sonde was calibrated according to manufacturer's specifications prior to each site visit for all parameters and daily for DO. Water samples for standard water quality parameters were stored chilled at 4°C and transported by aircraft to be analyzed within hold times at an accredited commercial laboratory.

Water samples were analyzed for $CBOD_5$, TSS, volatile suspended solids (VSS), total coliform (TC), *E. coli*, total nitrogen (TN), total ammonia nitrogen (TAN), NH_3-N, and TP according to standard methods (APHA, 2012). Quantification of a standard suite of 32 metals was analyzed for all water samples with inductively coupled-mass spectrometry (ICP-MS). Water samples collected in June from Naujaat were analyzed at the commercial laboratory Taiga Environmental in Yellowknife, Northwest Territories. Water samples collected from Sanikiluaq in May were analyzed at the commercial laboratory Maxxam Analytics in Montreal, Quebec. All other samples were analyzed at the commercial laboratory Maxxam Analytics in Winnipeg, Manitoba. The trace metals ICP-MS scan from Taiga Environmental Laboratory was conducted according to EPA method 200.8 (EPA,

1994). The trace metals ICP-MS scan from Maxxam Analytics Laboratory in Montreal was conducted according to method MA.200 -Mét 1.2 (Government of Québec, 2014). The trace metals ICP-MS scan from Maxxam Analytics Laboratory in Winnipeg was conducted according to Method 6020A R1 (EPA, 1998). A total of two rounds of water samples were collected from the wetlands per each site visit (e.g., spring and summer). Water samples for general water quality and gene target analysis were collected on May 25th and May 30th, 2016, and September 6th and 8th, 2016 in Sanikiluaq. Water samples for general water quality and gene target analysis were collected in Naujaat on June 16th and June 21st, 2016, and on August 29th and August 31st, 2016 (except reference samples). A total of 1L of raw wastewater sample was collected to facilitate the general water quality and gene target analysis per each pump truck sampled. In Sanikiluaq, discrete samples were collected from one truck on May 25th, two separate trucks on May 30th, and three separate trucks on September 9th, 2016. In Naujaat, discrete samples were collected from three separate trucks on June 16th, 2016 and three separate trucks on August 31st, 2016.

2.2.5 Sampling and Analysis of Soil and Water for Gene Targets

Soil samples were collected from the inlet, mid-point, and outlet of each wetland and at three locations within each of the reference wetlands. Soil samples were only collected once at each site near the end of summer due to logistical constraints. Soil samples were collected on September 8th, 2016 and August 29th, 2016, in Sanikiluaq and Naujaat, respectively. Each soil sample consisted of a composite sample of three sub-samples from each sample collection point. The soil samples were collected by cutting soil with a sterilized knife from the top 0 – 10 cm of the bottom substrate and banks of the flow paths of effluent within the study wetlands and reference wetlands. Soil samples were collected directly (either submerged or partially) in the flow paths of the effluent in the WTAs. The soil samples were kept chilled at 4°C following collection.

Approximately 30 – 500 mL of each water sample was filtered through a 0.45 µm pore size filter using a Millipore Vacuum Manifold and Microfil Filtration funnels (Millipore, Inc., Bedford, Massachusetts, United States). After filtration, the filter membrane was placed in a 15 mL falcon tube with 1mL of sterilized water to prevent dry out and immediately

frozen. The soil (stored at 4°C) and filters (stored frozen) were transported by aircraft to Dalhousie University in Halifax, Nova Scotia, Canada.

MoBio Powersoil DNA Extraction Kits (VWR International, Ville Mont-Royal, Québec, Canada) were used to extract the genomic DNA from the bacteria within the soil and water samples. Gene target copy numbers were detected using qPCR. This study was limited to only nine of the following gene targets which were quantified within the water and soil samples for this study: class I integrase gene (*int1*), sulfonamide resistance genes (*sul1* and *sul2*), methicillin resistance gene (*mecA*), vancomycin type A resistance gene (*vanA*), fluoroquinolone resistance gene (*qnrS*), macrolide-lincosamide-streptogramin type B resistance gene (*ermB),* tetracycline resistance gene (*tetO*)*,* and class A β-lactamase genes (bla_{TEM} and $bla_{CTX\text{-}M}$). The *int1* gene was analyzed because it is a genetic indicator of anthropogenic pollution and is commonly associated with genes which confer resistance to antibiotics (Gillings et al., 2015). A limitation to this study is that the antibiotic concentrations within the wastewater were not quantified. According to the Canadian AMR surveillance system report, commonly prescribed antibiotics in the northern territories include amoxicillin, azithromycin, ciprofloxacin, doxycycline, and sulfamethoxazole (Government of Canada, 2016c). Of these prescribed antibiotics, the following genes can confer resistance: bla_{TEM}, $bla_{CTX\text{-}M}$ and *mecA* (amoxicillin); *ermB* (azithromycin); *qnrS* (ciprofloxacin); *tet* (doxycycline); and *sul1* and *sul2* (sulfamethoxazole) respectively (McConnell, 2017). The rationale for the nine gene target panel was partly to characterize the genes that confer resistance to the commonly clinically prescribed antibiotics as identified in Government of Canada (2016c). This suite of gene targets was also selected in attempt to characterize genes which confer resistance to a range of antibiotic classes which have been previously detected within a municipal wastewater treatment plant by Szczepanowski et al. (2009). It should be noted that the nine gene targets are not an exhaustive nor comprehensive list of the genes which confer resistance to conventional antibiotics, and this is a limitation of the study. The gene target suite was quantified using TaqMan qPCR on a Bio-Rad CFX96 Touch system (Bio-Rad, Herculer, California, United States). The 16S ribosomal ribonucleic acid (rRNA) gene copies, which were determined to enable the calculation of the relative abundance of ARGs in the bacterial community, were quantified using SYBR Green qPCR. A complete description of the qPCR

methodologies is provided in Neudorf et al. (2017) and the primer and hydrolysis TaqMan probe sequences and cycling conditions are provided in Appendix B (Table B.1). The limit of quantification (LOQ) (copies/reaction) for each of the gene targets in the suite were: *int1* = 14, *mecA* = 69, *vanA* = 138, *sul1* = 12, *sul2* = 10, *qnrS* = 112, *ermB* = 14, *tetO* = 70, bla_{TEM} = 243, and $bla_{CTX\text{-}M}$ = 6, and 16S rRNA = 67,000. The limit of detection (LOD) was 5 copies/reaction (or 1 copy/mL for 500 mL sample volumes and 10 copies/mL for 50 mL sample volumes) determined according to McConnell (2017). The units of measurement for absolute abundance of gene target concentrations were determined in gene copies per mL of water or gram of sediment and presented as log transformed values.

2.2.6 Data Analysis

The relative abundance for each gene target, except for 16S rRNA, was calculated by dividing each gene target concentration at each sample location per each sampling event by the 16S rRNA concentration and by log transforming this result, which represented log (gene copies/16S rRNA genes). The relative abundance of the gene targets characterizes the proportion of the ARG target copy concentration in the total bacterial population represented by the 16S rRNA gene. The distribution of gene targets refers to the spread of individual absolute gene target concentrations, except for 16S rRNA, at each sample point within each of the two sites.

2.2.7 First-Order Rate Constant Determination

The first-order removal rate constants (k) are commonly used parameters in constructed treatment wetland design to describe the rates at which conventional wastewater contaminants are attenuated in wetlands. Numerous applications of this chemical reactor contaminant attenuation theory are summarized in Kadlec and Wallace (2009). Generally, the higher the value of k, the faster the rate of removal of that contaminant from the wetland (Hayward, 2013). The first-order rate constants for the gene targets were determined with a modified tanks-in-series (TIS) chemical reactor model parameterized with site-specific data according to the procedure detailed in Hayward and Jamieson (2015). The number of TIS determined from the dye tracer data were used to construct and parameterize the Microsoft Excel spreadsheet models which were used to determine the first-order rate constants. An example spreadsheet calculator template used to calculate the first-order rate

constants is presented in CWRS (2016). The areal first-order rate constants in units of metres per year (m/y) were adjusted to 20°C (k_{20}) according to the Arrhenius temperature correction equation and coefficients described in Hayward and Jamieson (2015). The temperature adjustment of k to 20°C is standard design practice for first-order rate constants to enable comparison to other treatment wetlands. The temperature correction coefficient of 1.07 was selected based on the assumption of doubling the rate of bacterial loss for a 10°C temperature rise (Chapra, 1997; Boutilier et al., 2009). Rate constants were determined only in cases when influent and effluent ARG concentrations from the wetlands were above LOQs. There were multiple model runs per site but solely the minimum k_{20}'s are presented in the results. All k_{20}'s were calculated from log transformed input gene concentrations.

2.2.8 Statistical Analysis

A principal components analysis (PCA) was conducted on the entire dataset (both wetlands combined) with the following parameters: *int1*, *mecA*, *sul1*, *sul2*, *tetO*, *blaTEM*, 16S rRNA, $CBOD_5$, TSS, VSS, TC, *E. coli*, TN, TAN, TP, WQIs, and eleven metals (aluminum (Al), barium (Ba), copper (Cu), iron (Fe), manganese (Mn), strontium (Sr), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na)). The PCA data was log-transformed and analyzed as a correlation matrix in SigmaPlot version 13.0 statistical software. For the PCA, the samples were un-pooled, except raw samples which were pooled for each site over the entire sample period, and the reference samples which were pooled for samples collected during the same season (e.g., spring and summer). Paired student t-tests (two-tailed) were used to assess whether the raw wastewater samples, and effluent and reference samples were significantly different with significance attributed at $p < 0.05$. In all instances, S.D. is an abbreviated form of standard deviation.

2.3 Results and Discussion

2.3.1 Hydraulics and Hydrology

The hydrology of both wetlands was strongly influenced by the seasonal changes in climate. High spring freshet flows occurred in late May to June, as the snow and ice which accumulated over the winter melted, with flows decreasing as the summer progressed into

July and August (Figures 2.4a and 2.4b). This finding is consistent with observations of seasonal hydrological changes in the WTA studied by Hayward et al. (2014). The surface flows at the wetland inlet in Sanikiluaq were generally much lower than the flows at the outlet throughout the study period (Figure 2.4a), with a maximum of 529 (n = 11 days discrete) and 4,612 m^3/d (n = 133 days continuous) for influent and effluent, respectively. A subsurface flow (i.e., seepage) area of approximately 1.3 ha was observed just downstream of the inlet, which in some locations had a depth of greater than 1.5 m. The subsurface flow was not quantified but likely accounted for some of the influent flow in Sanikiluaq. The measured flow rates for Sanikiluaq during the site visits were 196 m^3/d (n = 11 days discrete) for influent. The average effluent flow rates were 290 m^3/d (n = 133 days continuous) over the treatment season. Dilution was observed to range from 392 to 1,111% during the spring freshet study period in Sanikiluaq, which led to generally better water quality within the wetland and at the outlet. The hydraulic loading rates (HLRs) for Sanikiluaq ranged from 0 to 1.6 cm/d, with an average of 0.6 cm/d (n = 11 days discrete). These HLRs are relatively low, and well below typical engineering design criteria for HLRs for free water surface (FWS) wetlands, which range from 2.5 to 12.5 cm/d (Water Environment Federation, 2010). However, the HRT in Sanikiluaq during the spring freshet was determined to be 1.4 days from the dye tracer test, which is much shorter than the optimal 14 to 20 days for natural treatment wetlands (Alberta Environment, 2000; Kadlec & Knight, 1996). This may have been indicative of short-circuiting of effluent through the wetland. During the summer period, the HRT was longer (> 7 days) due to the subsurface flow area in Sanikiluaq and negligible inflow at the inlet. The number of TIS during the spring freshet for the entire wetland was determined to be high for FWS wetlands at 19 TIS; comparatively, for context Kadlec and Wallace (2009) reported a much lower average TIS of close to 4.1± 0.4 S.D. based on data from 35 constructed FWS wetlands. As the number of TIS approaches infinity, the hydraulic behavior of the wetland approaches plug flow with limited internal mixing (Kadlec & Wallace, 2009), which is not ideal from a treatment perspective. During the September trip, the wetland in Sanikiluaq behaved as a plug-flow reactor. This suggests that during both seasonal periods in Sanikiluaq there was considerable short-circuiting which is not conducive to treatment processes.

The hydrology of the Naujaat wetland system possessed some differences when compared to Sanikiluaq (Figure 2.4b). The minimum discrete inflow measurements were 305 m^3/d (*n* = 13 days discrete) and 0 m^3/d over the entire summer season (*n* = 79 days continuous). Discrete outflow measurements were 3,107 m^3/d (*n* = 13 days discrete) and an average of 1,117 m^3/d (*n* = 79 days) over the entire summer season. Periodically, inflow surpassed outflow on the same measurement day. This water deficit across the wetland may suggest that the two large ponds downstream of the mid-point in the Naujaat wetland likely acted as detention ponds, and evapotranspiration and seepage may have played a role in removal of surface water from the wetland. In Naujaat, maximum flows of 7,057 (*n* = 13 days discrete) and 6,004 m^3/d (*n* = 79 days continuous) were observed at the inlet and outlet, respectively. The HLRs over the treatment in season in Naujaat ranged from 0.8 to 17.5 cm/d, with an average of 8.5 cm/d (*n* = 13 days discrete), respectively. These HLRs were within the recommended for treatment wetlands for average but not high flow values. The results of the dye tracer tests demonstrated that similar to what was observed in the Sanikiluaq wetland, the HRT measured during the spring freshet was short (19.5 hours). The HRT in the wetland improved to 8 days later in the summer. The number of TIS determined within the wetland in Naujaat varied over the treatment season, with 17 TIS representative of the entire wetland during the spring, and 13 TIS representative of the system during August. These numbers suggest that there is plug flow-like behavior in the Naujaat wetland as well.

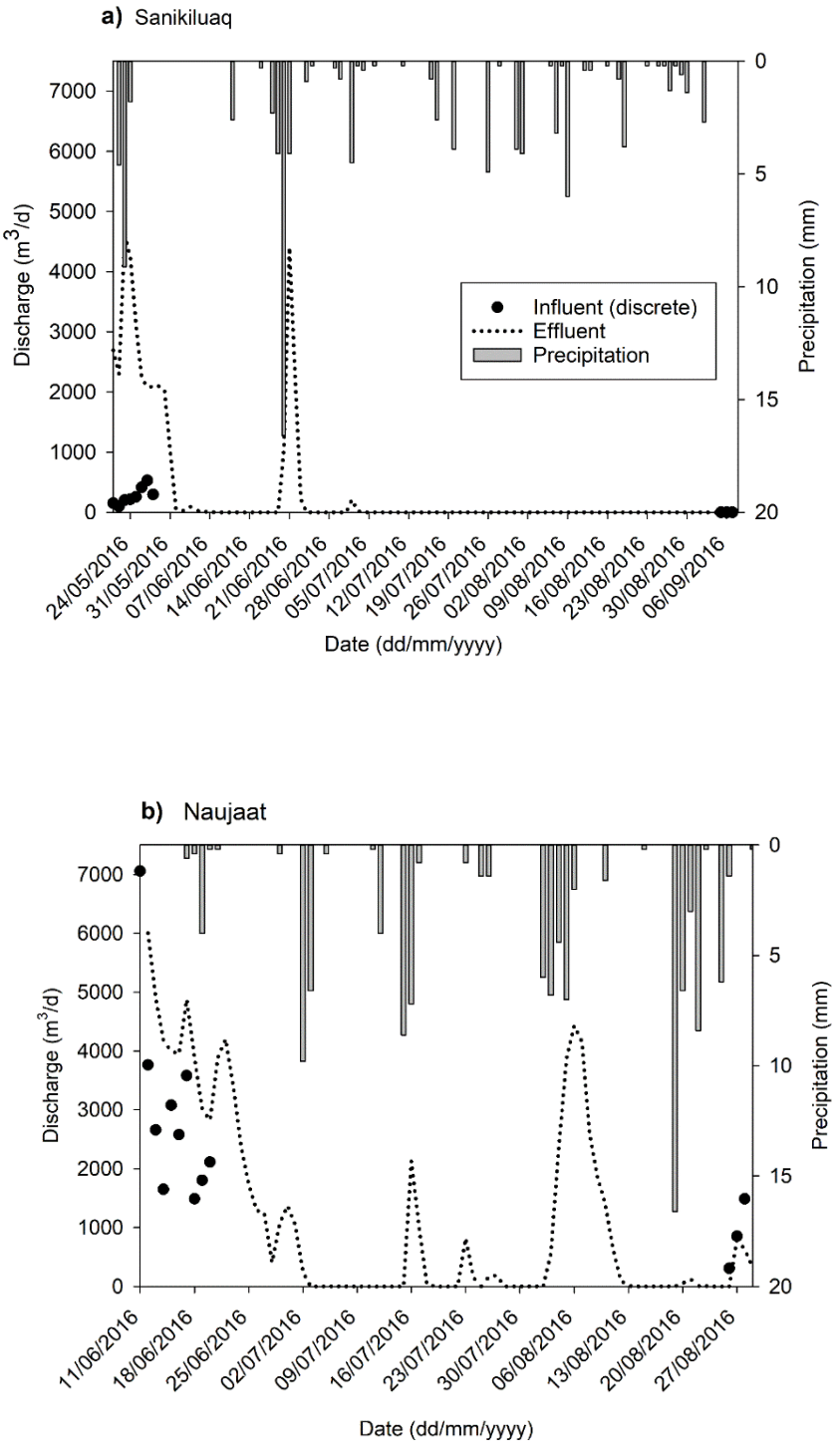

Figure 2.4 Hydrographs from a) the Sanikiluaq wetland; and b) the Naujaat wetland over the treatment season in 2016. The black dots represent the discrete influent measurements collected during the site visits.

2.3.2 General Water Quality

The sites both met their respective water licence requirements during the study period at their outlets, except for Naujaat for bacteria in the spring and pH in the summer (Table 2.1). At the wetland outlet in Sanikiluaq, average concentration reductions of 56% for $CBOD_5$, 20% for TSS, and 3.4 log for *E. coli* were observed during the spring freshet. During the summer, average concentration reductions of 96% for $CBOD_5$, 93% for TSS, and 3.9 log for *E. coli* were observed in the Sanikiluaq wetland. It can be noted that the effluent water quality generally improved as the treatment season progressed. These findings tend to corroborate with observations of seasonality in treatment performance observed in other tundra WTAs by Yates et al. (2012) and Hayward et al. (2014). The average percent reductions from inlet to outlet during the spring in Naujaat were 80% for $CBOD_5$, 48% for TSS, 0.04 log for *E. coli*; compared to an average increase of 523% for $CBOD_5$, 21% reduction for TSS, 5 log reduction for *E. coli,* and a 60% increase in NH_3-N during the summer. The elevated concentrations of organics and solids were likely due to algae accumulation that was noted near the wetland outlet at the end of the treatment season. Comparison between the two sites indicates that Sanikiluaq generally had lower effluent concentrations for contaminants in comparison to Naujaat. In summary, concentrations of most wastewater constituents decreased throughout both wetlands, except at times in Naujaat. Effluent water quality tended to improve over the course of the treatment season. Water samples collected at the outlet of both wetlands possessed elevated levels of organic material, nutrients, and fecal indicator bacteria as compared to reference wetlands (Table 2.1).

Table 2.1 Minimum, maximum, and mean concentrations of water quality parameters for raw wastewater, wetland influent and effluent, and from reference wetland sites obtained during the spring freshet (Spring), and end of the summer treatment season (Summer). Mean concentrations are displayed in parentheses and when no range is reported the samples had the same value.

Site	Sample location	Sample size (*n*)	$CBOD_5$ (mg/L)	TSS (mg/L)	*E. coli* (MPN/100 mL)	TN (mg/L)	NH_3-N[2] (mg/L)	TP (mg/L)	Temp. (°C)	DO (mg/L)	pH
Sanikiluaq	Water license requirement[3]		120	180	$1x10^6$						6 – 9
	Raw	6	189 – 387 (306)	160 – 280 (219)	$>6x10^4$ – $>1x10^6$	79 – 123 (107)	<0.2 – 4.2 (0.9)	8 – 13 (11)	16 – 25 (20)	0.7 – 8.5 (2.8)	7.3 – 8.1 (7.7)
	Influent (Spring)	2	5 – 13	2 – 13	$3.1x10^4$ – $6x10^4$	6 – 13	<0.2	1.4 – 1.7	1.2	0.3 – 0.7	7.4
	Effluent (Spring)	2	<4	4 – 8	10 – 27	0.6 – 1.2	<0.2 – 0.9	0.2	0.3 – 2.8	11.8 – 12.1	7.6
	Influent[4] (Summer)	2	23 – 250	72 – 93	$2.3x10^4$	20 – 26	0.7 – 3.0	3.8 – 4.2	11 – 12	15 – 22	8.5 – 9.1
	Effluent (Summer)	2	<6	1 – 11	<3	0.6	<0.2	0.3 – 0.7	7	10 – 11	7.9 – 8.1

[2] TN and NH_3-N were not determined for one of the two sample events in Naujaat during the spring period.

[3] Source: Nunavut Water Board (2015a). Specified in measurement units of BOD_5 (mg/L) and fecal coliforms in CFU/mL.

[4] The summer influent sample was collected directly from the lagoon due to no surface flow conditions at the inlet in Sanikiluaq.

Site	Sample location	Sample size (*n*)	$CBOD_5$ (mg/L)	TSS (mg/L)	*E. coli* (MPN/100 mL)	TN (mg/L)	NH_3-N^2 (mg/L)	TP (mg/L)	Temp. (°C)	DO (mg/L)	pH
	Reference	6	<4 – <6	1 – 32 (9)	<3 – <10	<0.4 – 0.6 (0.4)	<0.2	<0.05	3 – 11 (7)	9.6 – 13.6 (11.4)	7.7 – 8.2 (8.0)
Naujaat	Water license requirement[5]		80	70	$1x10^6$						6 – 9
	Raw	6	411 – 510 (462)	217 – 434 (324)	$4.1x10^6$ – $1.7x10^7$ ($1.2x10^7$)	84 – 149 (131)	0.9 – 3.5 (1.9)	10 – 19 (16)	19 – 25 (22)	4.5 – 6.7 (5.6)	7.6 – 8.1 (7.8)
	Influent (Spring)	2	85 – 125	44 – 57	$9.5x10^5$ – $2.4x10^6$	33 – 40	0.42	4.1 – 4.7	5	5.8 – 6.1	7.6 – 7.8
	Effluent (Spring)	2	17 – 24	9 – 44	$1x10^5$ – $1.7x10^6$	16 – 18	<0.2	2.1 – 4.1	4 – 6	1.6 – 9.7	7.4 – 7.6
	Influent (Summer)	2	7 – 17	48 – 64	$4.6x10^5$ – $1.1x10^6$	17 – 40	0.4 – 1.2	2.8 – 2.9	7 – 12	9 – 12	8.0 – 8.3
	Effluent (Summer)	2	6 – 139	40 – 48	3 – 9	6.4 – 7.1	0.5 – 1.9	1.4 – 1.5	7.1 – 8.6	20 – 29	9.6 – 9.7
	Reference	5	<2 – <6	4 – 7 (6)	<1 – 21	<0.4 – 0.7 (0.4)	<0.2	<0.05	1 – 8 (4)	10 – 14 (13)	7.2 – 7.9 (7.5)

[5] Source: Nunavut Water Board (2015b). Specified in measurement units of BOD_5 (mg/L) and fecal coliforms in CFU/mL.

2.3.3 Distributions of Gene Target Concentrations

The distribution of gene target absolute abundances and 16S rRNA absolute abundance in the raw wastewater and wetlands downstream of the wastewater disposal sites and reference wetlands are summarized in Figure 2.5. At both sites ARG concentrations were significantly higher in the spring in comparison to summer (p <0.05) (except at the outlet in Sanikiluaq), and this seasonal variation is linked to the hydrology of the wetlands.

This indicates that the spring poses the greatest risk with respect to presence of elevated ARG concentrations in the environment, which corresponds with trends observed from measurements of standard wastewater quality indicators in other WTAs by Hayward et al. (2014) and Yates et al. (2012). The raw wastewater quality between the two sites (n = 18 gene targets) for both sampling seasons were comparable with no significant differences in the distribution of gene target concentrations (paired t-test, t = 0.74, df = 8 p = 0.48). There was significant difference (p <0.05) in the distribution of gene target concentrations between the wetland outlet effluent and reference wetland sample locations during the treatment season in Sanikiluaq (paired t-test, t = 2.6, df = 17, p = 0.02). Likewise, in Naujaat there was a significant difference in the distribution of gene target concentrations between wetland outlet effluent and reference wetland sample locations during the treatment season (paired t-test, t = 6.6, df = 17, p <0.001). Therefore, the distribution of gene targets at both wetlands did not return to baseline concentrations by the wetland outlets.

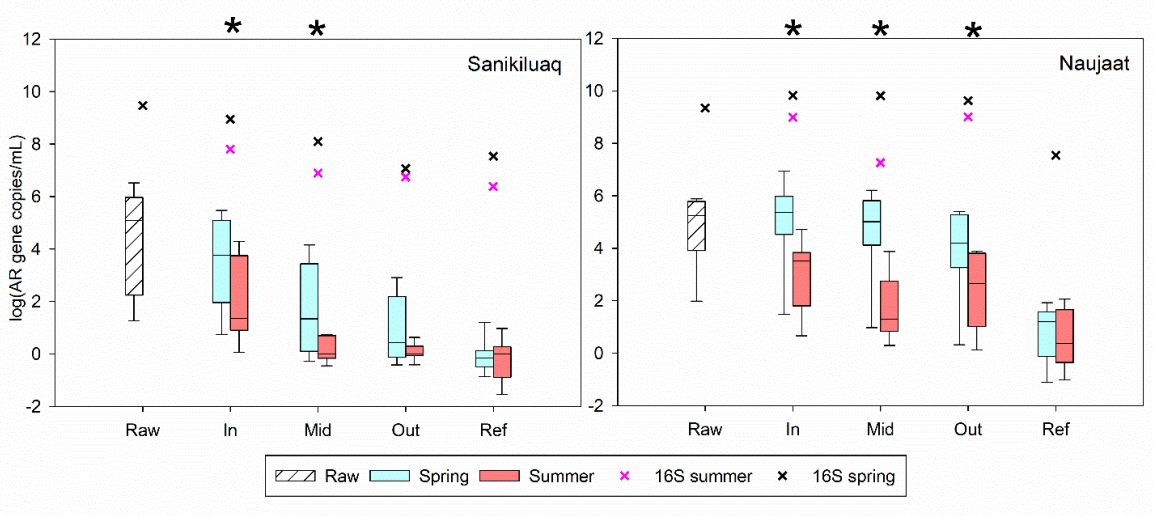

Figure 2.5 Distribution of absolute gene target abundances and 16S rRNA gene target absolute abundances at the sample locations in a) Sanikiluaq and, b) Naujaat. The stars on top x-axis are indicative of significant difference of distributions with paired student t-tests at p <0.05. The middle lines represent the mean values, the bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the distribution of gene targets. The raw data distributions were combined over the entire treatment season per site.

2.3.4 Absolute Abundance in Raw Wastewater

Absolute abundance of each individual ARG target in the raw wastewater is shown in Figure 2.6. The gene targets with the highest prevalence in the wetlands were the class I integrase gene *int1*, *sul1*, *sul2*, *qnrS, ermB, and tetO* in Naujaat. These findings agree with previous findings from Narciso-da-Rocha et al. (2014) and Gaze et al. (2011) who observed that *int1* is particularly prevalent in wastewater. In addition, the sulfonamides (to which *sul* type genes confer resistance) group of antibiotics is one of the most commonly found in wastewater (Davies & Davies, 2010). More recently, the mostly plasmid-borne *qnrS* have been documented to be prevalent in wastewater sources, where it may be a concern for horizontal gene transfer events (Rodriguez-Moraz et al., 2015). Although not shown in Figure 2.6, the gene *mecA* was present in the raw wastewater just slightly above the LOQ at 1.9 log gene copies/mL ± 0.3 S.D. and 2.0 log gene copies/mL ± 0.2 S.D. for Sanikiluaq and Naujaat, respectively. While, *vanA* was only observed above the LOQ once in one of the raw pump truck samples in Sanikiluaq.

Figure 2.7 shows the relative abundance of the distribution of ARGs in the raw wastewater were not significantly different between the two sites (paired t-test, t = 0.96, df = 8, p = 0.36). This could be attributed to similar strength and quality raw wastewater, and similar rates and types of clinical use of the antibiotics within the two hamlets. An exception to this trend can be observed with the *qnrS* gene target in Sanikiluaq, which was not present above LOQ in the raw wastewater.

2.3.5 Absolute Abundance in Wetlands

Absolute abundance for each individual ARG target at the sample locations within the wetlands is illustrated in Figure 2.6. The ARGs *ermB*, *tetO*, bla_{TEM}, and $bla_{CTX\text{-}M}$ were all below the LOQ (but above the LOD only in the spring excluding the reference site) in the water samples collected from the mid-point, outlet and reference wetlands in Sanikiluaq. In contrast, *ermB*, *tetO*, bla_{TEM}, and $bla_{CTX\text{-}M}$ were all present within the wetland during the spring period in Naujaat. At times there was limited log reduction in some ARGs as wastewater progressed through the wetland in Naujaat. The persistence of ARGs in Naujaat can be explained by the short HRT in the wetland in the spring, higher geographic latitude, and lower dilution at this site compared to Sanikiluaq. At both sites, the spring conditions in all instances produced the highest gene absolute abundances within the wetlands.

The ARGs *mecA* and *vanA* were generally at or below the LOQ levels in soil and water at both sites, therefore the individual plots for this ARG were not presented. However, it should be noted *mecA* was detected in low concentrations of 2.9 log gene copies per gram in the soil at the mid-point, and concentrations of 2.1 log gene copies per mL in the effluent and reference wetland water in Naujaat. *MecA* and *vanA* are often present on mobile genetic elements in chromosomal DNA instead of plasmids (Biavasco et al., 2007; Colomer-Lluch et al., 2011) and therefore the low prevalence in the study wetlands may be due to low persistence of the bacteria carrying these genes.

Comparison of the findings between Sanikiluaq and Naujaat with the findings from Chaves-Barquero et al. (2016) on the Cambridge Bay lagoon and WTA provides broader contextual analysis of ARGs in wetland settings in Nunavut as shown in Table 2.2. In terms of the *sul and tet* markers, the Cambridge Bay site had similar values to Naujaat in this

study during the spring. In terms of 16S rRNA markers, Cambridge Bay and Sanikiluaq were similar.

Table 2.2 Comparison of ARG absolute abundances in the wetland effluent from Cambridge Bay from Chaves-Barquero et al. (2016) and Sanikiluaq and Naujaat, Nunavut.

Gene target (log gene copies/mL)	Site				
	Cambridge Bay[7]	Sanikiluaq		Naujaat	
		Spring	Summer	Spring	Summer
Tetracycline resistance	3.8	0.6	-0.008	4.2	2.7
Sulfonamide resistance	5.6	2.9	-0.1	5.3	3.6
16S rRNA	7.3	7.1	6.7	9.7	9

Figure 2.7 shows a decline in the relative abundance of ARGs which was generally observed from the inlet to the outlet of the wetland in Sanikiluaq. In Naujaat, the relative abundances decreased to a lesser extent within the wetland. Furthermore, during the summer there were increases of relative abundance in Naujaat (0.2 and 1.3 log gene copies per 16S rRNA genes for *int1*, and *qnrS*, respectively), which indicated potential enrichment of these genes in the wetland during this period.

2.3.6 Absolute Abundance in Soil

The gene targets were also widely detected in the wetland soil samples (Figure 2.6). It should be noted that the soil samples were collected during the summer period. Although the water and soil concentrations are not directly comparable (colony forming unit (CFU); CFU/mL vs CFU/g), the fact that the soil concentrations were multiple orders of magnitude greater for some ARGs (e.g., *sul1, sul2, tetO, qnrS)* may suggest that the soil acts as a sink, or that the soil provides a favourable biofilm environment conducive to ARG carrying bacteria proliferation. Soil reservoirs for antibiotic resistance and ancient antibiotic resistance elements residing in soil environments is a field of current study and remains a field requiring further study to protect human health (Allen et al., 2010). There were

[7] Results from Barquero et al. (2016) study.

numerous instances whereby the gene absolute abundances were below the LOD and LOQ in the soil samples (Figure 2.6). At both sites, none of the soil samples contained levels of *bla*$_{CTX\text{-}M}$ above the LOQ despite it being quantified in the Naujaat water samples from the wetland. This may weakly infer that this ARG, commonly found among members of Enterobacteriaceae (Narciso-da-Rocha et al., 2014), may not be associated with the bacterial communities in the soil biofilms.

Figure 2.7 shows the relative abundance of the gene targets in soil which were generally much lower than observed with the water samples. This would be expected given the greater microbial biomass and genetic material in the soil samples compared to the water samples.

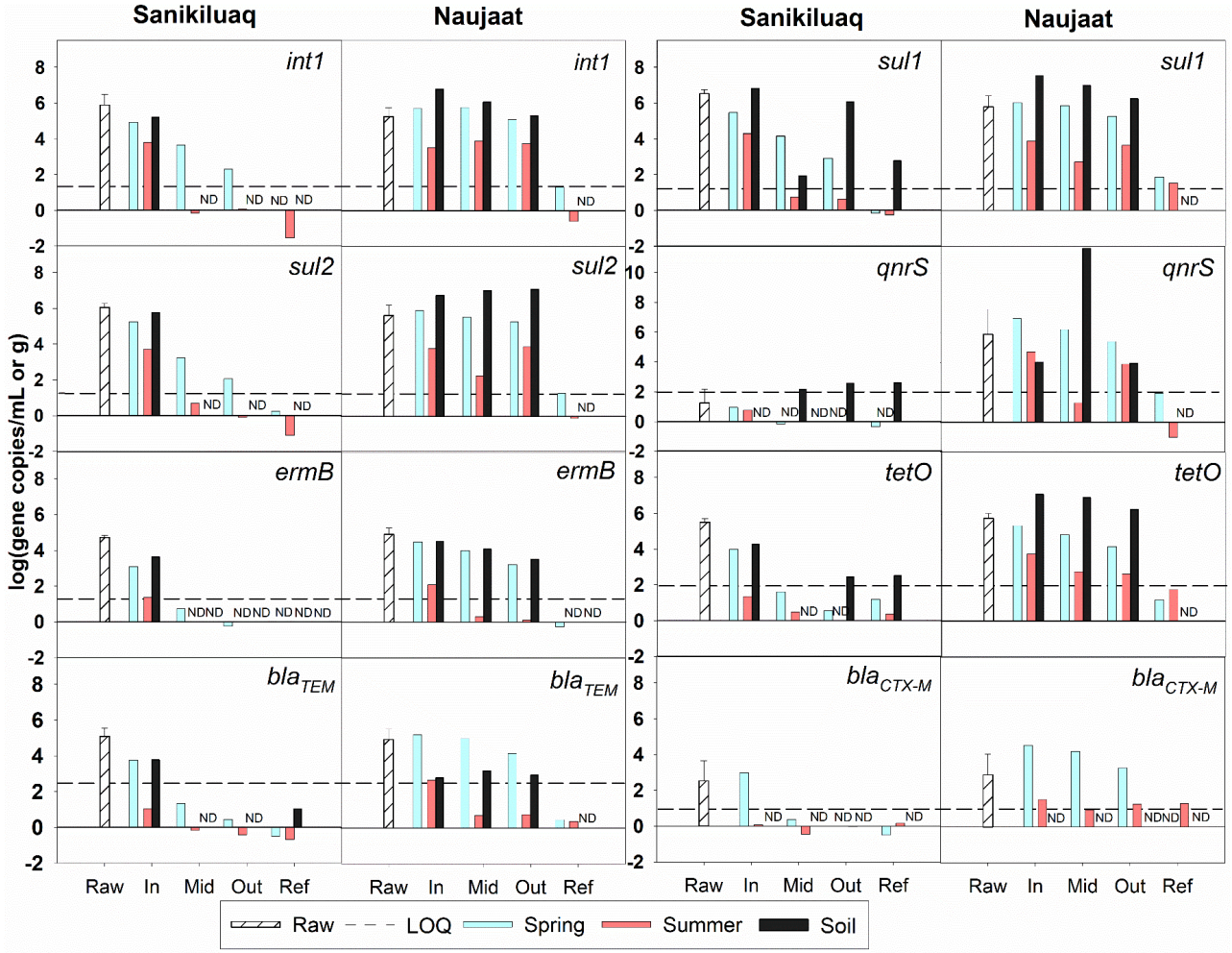

Figure 2.6 Absolute abundance of ARGs in the raw wastewater (raw), influent (In), mid-point (Mid) and effluent (Out) water and soil samples from the wetlands during spring and summer sampling periods.

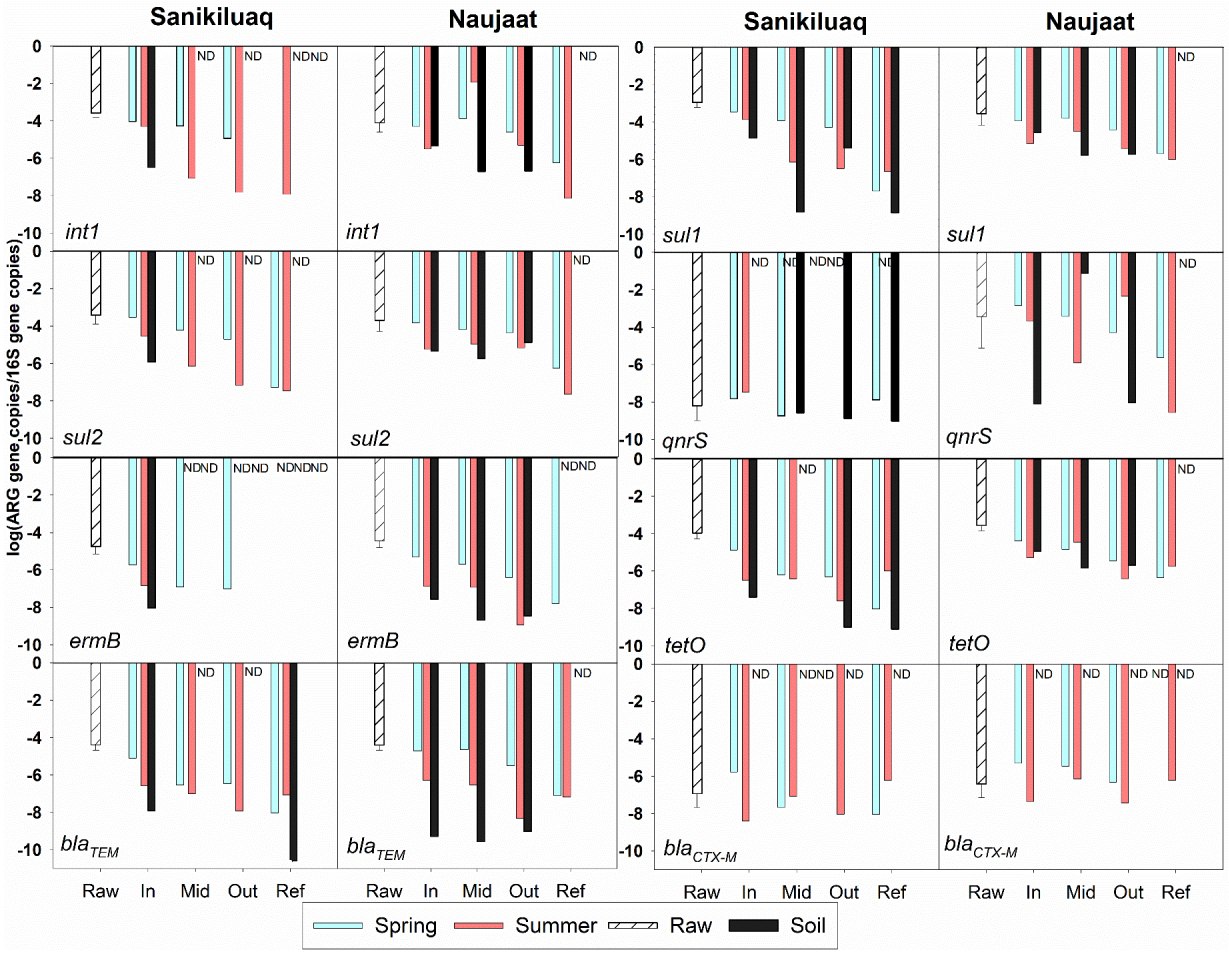

Figure 2.7 Relative abundance of ARGs in the raw wastewater, and soil and water, in the wetlands during spring and summer sampling periods. Note: as the dropdown bars become increasingly negative in the graphs, the gene target in question represents less of the overall proportion of genes in the bacterial population (i.e., is less enriched) .

2.3.7 Environmental Resistome

The absolute abundance of the gene targets in water were observed to be below the LOQ within the reference wetlands, with the exception of *int1*, *sul1*, *sul2*, and $bla_{CTX\text{-}M}$ in Naujaat; however the concentrations were close to the LOQ (Figure 2.6). These ARGs that were observed in the water of the reference wetland in Naujaat were also detected in much greater absolute abundances within both the raw wastewater and within the treatment wetland, which would suggest that there is selective pressure associated with the wastewater, which may contribute to the proliferation of naturally occurring ARGs. In three cases ARGs were detectable at low levels in the soil of the reference wetlands, including *sul1*, *qnrS*, and *tetO* in Sanikiluaq, which may also suggest these genes are naturally present in the soil, and potentially not originating from anthropogenic sources at the reference sites. The reference wetlands generally had the lowest relative abundance for all samples at both study sites (Figure 2.7).

Recent research has begun to assess the possibility of anthropogenic impacts on AMR in remote and seemingly un-impacted marine and terrestrial environments. Anthropogenic impacts on global ARG distribution was investigated in remote Arctic marine waters by Tan et al. (2017), where the human mitochondrial gene target *Hmt* was found in remote high artic marine sediment and correlated with elevated relative abundance of ARGs. Zhang et al. (2018a) studied ARGs in relatively un-impacted (glacial soil and permafrost) and anthropogenic environments (river sediment), where it was observed that there was greater abundance and diversity in the anthropogenic environment, than the relatively un-impacted sites. With the far-reaching global spread of AMR, it is important to develop an understanding of the ancient resistome as baseline for change monitoring and the reference sites in the tundra wetlands provide an example of a relatively un-impacted environment. In addition, sites with naturally occurring ARGs could contribute to the proliferation of ARGs given influxes of nutrients and environmental changes over time from anthropogenic impacts.

2.3.8 Correlations Between ARGs and Water Quality Indicators (WQI)

The PCA results with the WQIs and gene targets is presented in Figure 2.8a and illustrates that the gene targets were positively correlated with each other. Some gene targets were

excluded from the PCA due to low or inconsistent detection levels across the hamlets (*mecA*, *vanA*, *qnrS*, *ermB*, and $bla_{CTX\text{-}M}$). Total coliform, *E. coli,* VSS, and Zinc were the other water quality parameters that were the most strongly correlated with the gene targets. This positive correlation between ARGs and total coliforms and *E. coli* is expected because the bacteria often carry the ARGs within their cellular structure in a selective environment, therefore a high number of bacteria and accordingly ARGs would be anticipated in wastewater streams. The correlation of ARGs with organic matter may also be related to elevated nutrients for the bacteria to consume and hence proliferate. The positive correlation of ARGs with zinc may be attributed to co-selection of ARGs and zinc which has been observed by Pal et al. (2015). Many of the other wastewater contaminants including TN, Cu, TAN, TP, Al, Fe, TSS, $CBOD_5$, and Mn had weak positive correlations with the gene targets which indicated that their persistence in the wetlands followed similar trends. Overall, the results of the PCA indicated that fecal indicators, such as *E. coli*, and organic matter—in the form of VSS—are possible indicators for elevated ARG levels downstream of municipal wastewater sources in tundra wetlands. Figure 2.8b shows the PCA scores plot from which it can be qualitatively noted that there were seasonal differences in the gene targets and WQI results, with the spring and summer sample scores grouped separately. Differences in concentrations based on the distance from the wastewater source were also observed, with sample scores of similar qualities grouped together.

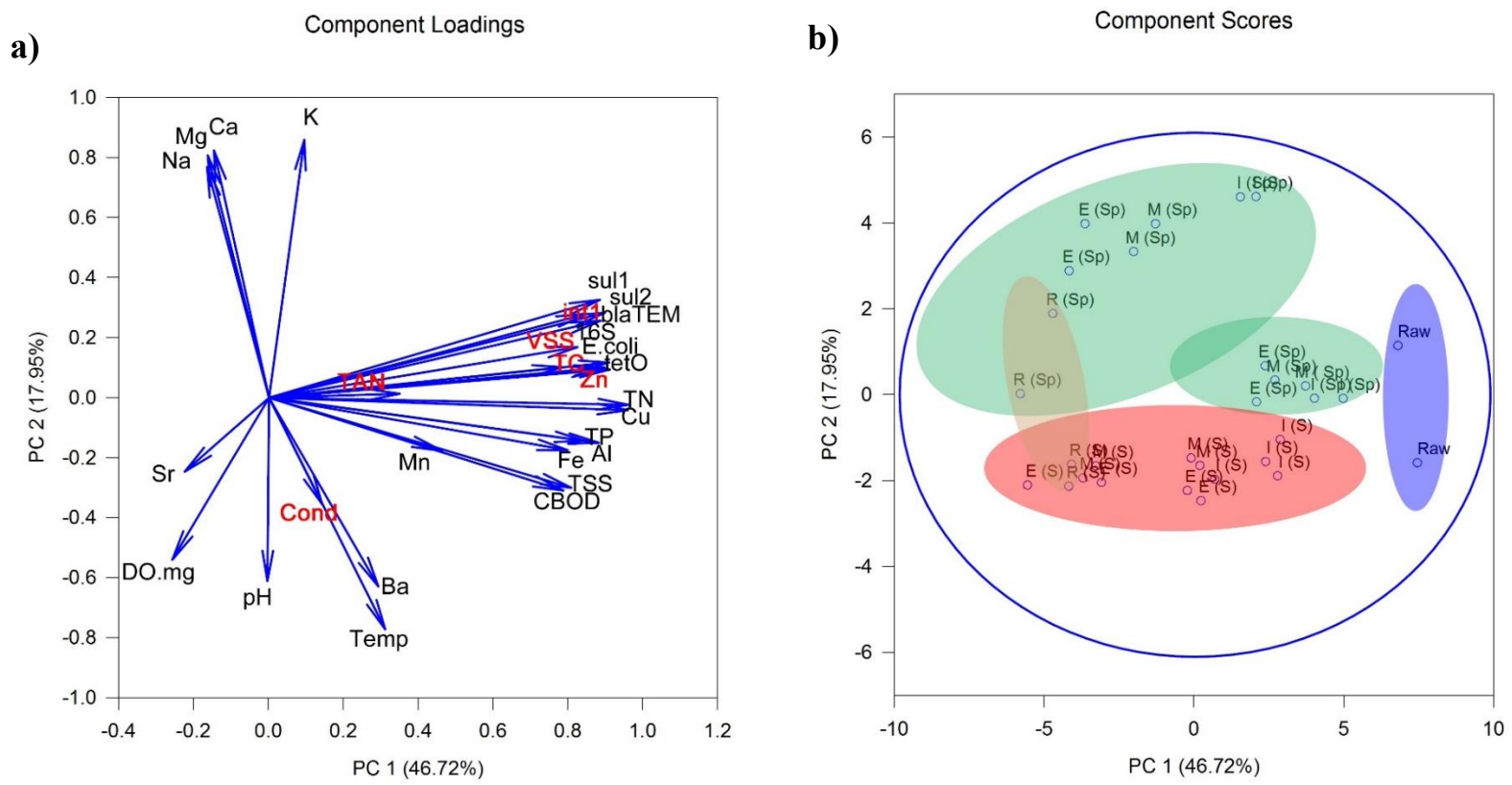

Figure 2.8 a) Loadings plot of the PCA results, use of red text is for contrast; and (b) scores plot of the gene targets and WQI PCA results. Ellipses denote scores groupings of spring samples (green), summer samples (red), raw truck samples (blue), and reference samples (orange). Abbreviations indicate influent (I), mid-point (M), effluent (E), reference (R), spring (Sp), and summer (S).

2.3.9 First-Order Rate Constants of ARGs

The first-order rate constants determined for the gene targets in the wetlands are summarized in Table 2.3. These k_{20} values incorporate the HRT of the WTA in their derivation calculation and discrete measurements of gene targets. Further details on the derivation of the k_{20} values are presented in Table S4 of Appendix D. It should be noted that the k_{20}'s for a few of the gene targets (including *mecA* and *vanA*) were not determined (denoted by n/a in Table 2.3), due to absolute abundances being below LOD or below the LOQ within the wetlands or dilution from external hydrologic contributions. Overall, the first-order rate constants determined for the gene targets fell within the 40th to 95th percentile (ranging from 52 m/y for *sul1* to 1,549 m/y for *int1*) for Sanikiluaq, and within the 50th to 95th percentile (ranging from 81 m/y for *sul1* to 1,954 m/y for 16S rRNA) for Naujaat, compared to fecal coliforms measured in a group of FWS constructed wetlands compiled by Kadlec and Wallace (2009). The k_{20}*'s* were variable and unique for each gene target. This is a preliminary attempt at the assessment of first-order rate constants for ARG and other gene targets in wetlands impacted by municipal wastewater and further study should be conducted, especially due to the wide variability in k_{20}'s, to assess whether these compare to other cold climate treatment wetlands. These first-order rate constants are important parameters to inform design of passive treatment wetlands for ARG specific removal, as they describe the rates at which the wetlands attenuate the ARGs assessed and allow for sizing requirement calculations to inform the design process.

Table 2.3 First-order rate constants (k_{20}) for the absolute abundances of the gene targets and *E. coli* in the wetlands.

Site		*int1*	*sul1*	*sul2*	*qnrS*	*ermB*	*tetO*	bla_{TEM}	bla_{CTX-M}	16S rRNA	*E. coli*
Sanikiluaq	k_{20} (m/y)	1549	52	428	n/a[b]	n/a	n/a	n/a	n/a	n/a	1592
	Percentile[8] (%)	95	40	90	n/a	n/a	n/a	n/a	n/a	n/a	95
Naujaat	k_{20} (m/y)	203	81	108	106	1117	254	157	473	1954	146
	Percentile (%)	80	50	60	60	95	80	70	90[9]	95	70

[8] Percentiles were where the k_{20}'s from this study fell in comparison to a distribution of k_{20} values for fecal coliforms from n = 47 wetland years for 23 wetlands FWS constructed wetlands summarized by Kadlec and Wallace (2009). The k_{20}'s from Kadlec and Wallace (2009) are based on calculation using nominal HRT.

[9] The k_{20}'s were not determined for *ermB*, *tetO*, bla_{TEM} and bla_{CTX-M} in Sanikiluaq because the ARG concentrations within the wetland were below LOQ. The k_{20} for 16S rRNA could not be determined for Sanikiluaq due to dilution from external hydrologic contributions.

2.4 Conclusions

This study demonstrated that the measured suite of nine ARGs were elevated above reference conditions at the wetland outlet at both sites, except for *mecA* and *vanA*. It was hypothesized that the hydrology of the systems would play a large role in the concentrations and spatial distribution of the ARGs within the wetlands, and this was supported by the data. Notably, the relatively short HRTs (< 2 days) during the high flow periods of the spring freshet produced the period with the highest ARG absolute abundance concentrations despite increased dilution effects during that time due to snowmelt. This spring period can be viewed as a worst-case scenario for ARG exposure risk within tundra wetlands impacted by municipal wastewater originating from continuous discharge systems. Overall, Sanikiluaq had improved levels of ARGs in comparison to Naujaat which may also have been linked to hydrology and latitude differences. Elevated levels of ARGs in soil samples in comparison to the water samples collected in the summer period illustrated that the soils could either retain ARGs from a period of higher concentrations in the water, or may provide an environment conducive to proliferation of bacteria that may carry the ARGs. The preliminary first-order rate constants were widely variable within the wetlands ranging from 52 m/y to 1,954 m/y ($\bar{x}$ = 587 m/y) depending on the specific gene target. This study has provided the first assessment of ARG concentrations in tundra wetlands over an entire treatment season, to our knowledge the first assessment of the kinetics of ARG removal in these unique wetland systems, and comparative characterization of ARGs in un-impacted tundra wetlands.

CHAPTER 3 LATERAL FLOW SAND FILTERS ARE EFFECTIVE FOR REMOVAL OF DETERMINANTS OF ANTIMICROBIAL RESISTANCE FROM DOMESTIC WASTEWATER

Abstract[10]

The ability of lateral flow sand filters, used as on-site wastewater treatment systems, to treat for removal of ARGs, ARB and other relevant genetic markers (HF183, 16S rRNA, and *int1*) was assessed. Municipal wastewater was settled in a septic tank prior to loading into six pilot-scale lateral flow sand filters comprised of three different sand media types, at 5 and 30% slopes. The sand filters were sampled bi-weekly for: 9 ARGs and 3 other complimentary gene markers (*sul1*, *sul2*, *qnrS*, *tetO*, *ermB*, bla_{TEM}, $bla_{CTX\text{-}M}$, *mecA*, *vanA*, *int1*, HF183, 16S rRNA), and conventional microbial and water quality indicators, from July to November in 2017, and four times in the summer of 2018. The sand filters were observed to attenuate 7 of the ARGs to mostly below 2 log gene copies per mL. Log reductions ranging from 2.9 to 5.4 log were observed for the removal of absolute abundances of ARGs from septic tank effluent in 5 of the 6 sand filters. The fine-grained filter on the 5% slope did not perform as well for ARG attenuation due to hydraulic failure. The apportionment of cell-associated versus cell-free DNA was determined for the gene markers and this indicated that the genes were primarily carried intracellularly. Average log reductions of ARB with resistance to either sulfamethoxazole, erythromycin, or tetracycline were approximately 2.3 log CFU per mL within the filters compared to the

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septic tank effluent. This field study provides in-depth insights into the attenuation of ARB, ARGs, and their genetic compartmentalization in variably saturated sand OWTS. Overall, this type of OWTS was found to pose little risk of antimicrobial resistance contamination spread into surrounding environments when proper hydraulic function was maintained.

3.1 Introduction

Antibiotic resistance has become a leading threat to global public health as treatable pathogenic microbial infections have acquired resistance to conventional antibiotics (WHO, 2014). Anthropogenic practices, including the use of clinical and agricultural antibiotics and antimicrobial product usage, can encourage the proliferation of AMR by introduction of selective pressure on bacteria (Davies & Davies, 2010; Kolář et al., 2001). A hot spot for AMR development is in municipal wastewater treatment systems where trace amounts of antibiotics taken within the general population are only partially metabolized, which leads to the development of AMR in bacterial communities within wastewater process streams (Munir et al., 2011). Antibiotic resistance in bacteria results from the expression of ARGs, acquired as MGEs via horizontal gene transfer or as mutations via vertical transmission (Depardieu et al., 2007). Quantification of abundances of ARB, ARGs and MGEs in WWTPs and receiving surface water environments have been conducted (Rizzo et al., 2013). ARG concentrations are typically reduced within many WWTPs; however, they persist in surface water systems downstream of effluent discharges (Freeman et al., 2018; McConnell et al., 2018a). Understanding the environmental dimension of AMR is important to enable the prediction of the spread of ARGs and AMR pathogens downstream of hot spots (Berendonk et al., 2015).

Removal, or conversely breakthrough, of ARGs and ARB within passive on-site wastewater treatment systems and variably saturated subsurface environments is less extensively studied. Despite this, antimicrobial products which encourage proliferation of AMR have been observed in septic tank effluent from OWTS (Conn et al., 2010). Improperly treated wastewater in OWTS could pose a risk of bacterial contamination of surrounding drinking water resources (Crane & Moore, 1984). Approximately 15% and 20% of the population uses OWTS for provision of wastewater treatment in Canada and

the United States, respectively. (Statistics Canada, 2015; EPA, 2018). OWTS are the second most frequent source of fecal contamination of groundwater in the United States (Carroll et al., 2005). These can be a source of contamination for groundwater and adjacent surface water systems if they are not properly maintained. They may not be effective for attenuation of some types of contaminants of emerging concern such as pharmaceuticals and personal care products (Schaider et al., 2017). OWTS are often recommended to improve sanitation in developing nations due to relatively low cost, low maintenance requirements, and technical feasibility (WWAP, 2017). Contamination of groundwater with vectors of AMR from OWTS may be considered an issue of increased concern due to elevated reported susceptibility of developing regions to AMR (Ashbolt et al., 2013). While AMR prevalence in conventional centralized WWTPs is becoming increasingly better characterized; there remains a knowledge gap in the efficacy of low-tech treatment options to reduce risk of AMR contamination for developing countries (Bürgmann et al., 2018).

Treatment of ARGs with subsurface flow filter media has been studied by Anderson et al. (2015). The authors observed that ARGs and ARB associated with sulfonamide and tetracycline resistance adsorbed and persisted on the filter media, posing challenges for media disposal at the end of the filter life cycle (Anderson et al., 2015). Rural OWTS and municipal WWTPs were compared in China for ARG removal by Chen and Zhang (2013). The authors observed 1 to 3 log removal for ARGs in centralized WWTPs, but less effective removal for ARGs in rural OWTS; potentially due to lower overall abundances of ARGs in OWTS (Chen & Zhang, 2013). The removal performance of ARGs in a horizontal subsurface flow constructed wetland was studied by Nõlvak et al. (2013). ARG removal rates were higher in the wetland than observed in conventional WWTPs. ARG carrying microorganisms interacted with the wetland biofilm media; however, the exact attenuation mechanisms were not identified (Nõlvak et al., 2013).

The ARGs which encode for AMR may be present intracellularly, as cell-associated ARGs, or extracellularly, as cell-free ARGs. Biologically active DNA may be transported in saturated soil environments with limited degradation, due to advective transport and reduced efficacies of inhibitory DNA nucleases (Poté et al., 2003). Cell-free DNA (e.g., extracellular DNA) can persist in soil environments for periods of up to several years

(Pietramellara et al., 2009). Characterization of cell-associated versus cell-free ARGs was recently identified by Zhang et al. (2018b) within a WWTP in China. Cell-associated ARGs were observed to decrease and cell-free ARGs increased as effluent progressed through the treatment train suggesting that the cell-free ARGs may persist and spread potential AMR contaminants in receiving environments. This is only a public health threat if the environmental DNA is taken up and becomes integrated into the genome of viable bacterial hosts that are pathogenic.

This study was undertaken to characterize the risk posed by OWTS in terms of introducing contaminants of AMR into water resources. The objectives were to assess attenuation of ARGs and ARB in lateral flow sand filters, which are an alternative to conventional septic fields, but exemplify similar physical filtration and biological treatment mechanisms. Sub-objectives for this study included an assessment of whether sand filter design factors (grain size and filter slope) affect treatment performance. The apportionment of cell-associated versus cell-free ARGs was quantified to assess whether the cell-free ARGs can penetrate through the filter more easily than cell-associated ARGs. This study provides a comprehensive assessment of an array of design configurations of OWTS for attenuation of AMR contamination, with a range of ARGs, other complimentary gene markers, ARB, and assessment of the genetic compartmentalization of ARGs.

3.2 Material and Methods

3.2.1 Sand Filters Description

The experimental facility used in this study was located at the Bio-Environmental Engineering Centre (BEEC) in Truro, Nova Scotia, Canada. Six lateral flow sand filters (SFs) were installed at BEEC in 2004 and were constructed as per the *Nova Scotia Environment On-Site Sewage Disposal Technical Guidelines* (Nova Scotia Environment, 2013; Sinclair et al., 2014). The BEEC withdraws municipal wastewater from the Village of Bible Hill sewage collection line, which is then pumped into a septic tank multiple times daily. A pump is programmed to periodically dose the sand filters with septic tank effluent on a sub-daily basis via a flow splitter box and gravel distribution trench. The flow of effluent within the filters has been characterized as primarily tension saturated flow

(Sinclair et al., 2014). Three different sand types were used in the construction of the filters, consisting of fine, medium, and coarse-grained sand; with saturated hydraulic conductivities of approximately 2.7×10^3 (SF1 and SF4), 6.3×10^3 (SF2 and SF5), and 1.2×10^4 cm/d (SF3 and SF6), respectively. The saturated hydraulic conductivities (K_s) of the three sand filter medias were assessed using ASTM D2434 - 68(2000) standard test method (ASTM, 2000). These tests were conducted on relatively clean sand samples collected from the top of the sand filters. Two slopes were assessed at 5 and 30%; design guidelines specify slopes ranging from 3 to 30% (Nova Scotia Environment, 2013). The grain size distributions are presented in the Appendix A (Figures A.10, A.11, and A.12). Each sand filter including the gravel distribution trench was fully lined on the sides and bottom with a high-density polyethylene (HDPE) liner. The tops of the SFs were covered with filter fabric overlain by approximately 0.6 m of topsoil. The SFs were constructed as per the dimensions illustrated in Figure 3.1. The effluent from each of the SFs was collected in a heated sampling building where each filter had a separate calibrated tipping bucket gauge for flow measurement. The influent dosing rate was set by a programmable logic controller (PLC) to emulate a domestic household use with peaks in flow at 8 am and 7 pm (Figure A.17 in Appendix A). The number of bucket tips were logged on a 30-minute frequency with a Campbell Scientific CR510 data logger (CSI, Logan, Utah, United States).

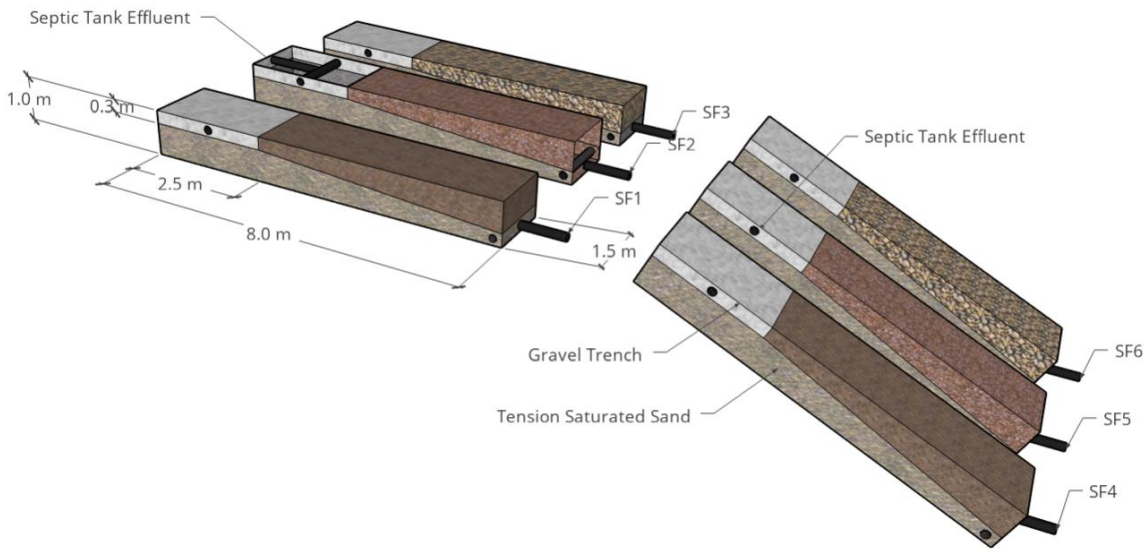

Figure 3.1 Schematic of the sand filter experimental layout (not to scale). Sand filter (SF)1 and SF4 filter media consist of fine-grained sand, SF2 and SF5 are medium grained sand, and SF3 and SF6 are coarse grained sand. SF1 – SF3 are on a 5% slope and SF4 – SF6 are on a 30% slope.

Average air temperatures near Truro were 18°C in July 2017 and ranged from a minimum of 12 to a maximum of 25°C; during November averaged 3°C, and ranged from -2 to 9°C. During July 2018, air temperatures near Truro averaged 21°C, and ranged from 14 to 27°C (Government of Canada, 2018).

3.2.2 Water Sampling

All water samples were analyzed within 24 hours, except for antibiotics, which were analyzed within a one week holding time. Water samples for metals analysis were acidified with nitric acid to below pH 2 and store chilled for up to six months prior to analysis. The sampling collection schedule is summarized in Table 3.1 below.

Table 3.1 Summary of sampling schedule and details for Raw, STE and sand filters 1 -6. A double check mark indicates two samples collected per day.

Date sampled (dd/mm/yyyy)	Parameters				
	Conventional	Antibiotics	Metals	ARGs & gene target	ARB
20/05/2017		√			
05/07/2017	√	√	√	√	
18/07/2017	√	√	√	√	
09/08/2017	√		√	√	
22/08/2017	√	√	√	√	
05/09/2017	√		√	√	
18/09/2017	√		√	√	
01/10/2017	√	√	√	√	
06/11/2017	√		√	√	
18/06/2018		√[11]			
16/07/2018	√	√	√	√	√√
23/07/2018	√	√	√	√	√√

3.2.3 Conventional Analysis

Water samples were collected from: the raw wastewater directly off the Bible Hill line as it discharged to a catch basin (1), the dosing box receiving effluent from the septic tank (1), and the filter effluent from each of the six (6) SFs. The HRT of the dosing box is approximately one day, and the HRT of the septic tank is a minimum of two days. A total of eight (8) sample events were conducted on approximately a bi-weekly basis from July 5 to November 6, 2017, and analyzed for conventional wastewater parameters, as well as a suite of ARGs and associated AMR genetic markers. Four (4) additional sets of samples were collected during a two-day intensive sampling event that was conducted during a dry weather period on July 16 and July 23, 2018 to assess for daily-scale temporal variability. The ARG results were pooled for each day of this intensive sampling event (n = 4) for individual sample locations resulting in two (2) additional samples sets for a total of ten (10) sample points. The intensive sample results were pooled due to low observed daily variability in concentrations as demonstrated in the results of the intensive sampling that are summarized in Table B.10 of the Appendix B. During these two intensive sample event

[11] SF5 was not analyzed on this date.

days additional microbial parameters including ARB, and cell-associated and cell-free DNA were characterized. However, the ARB data collected during the intensive sampling event were not pooled.

Water samples were collected in sterilized 1L plastic sample bottles and transported in coolers on ice to the analytical laboratory at Dalhousie University in Halifax, Nova Scotia, Canada. General water quality indicators of temperature, dissolved oxygen, specific conductance, and pH were made *in situ* for each sample collection event with a YSI600 handheld water quality sonde (YSI Inc., Yellow Springs, Ohio, United States). The sonde was calibrated as per manufacturer's specifications. Conventional wastewater quality parameters that were analyzed for each sample included five $CBOD_5$, TSS, *E. coli*, TN, TAN, and TP. These parameters were measured in accordance with standard methods (APHA, 2012). Total coliform and *E. coli* were enumerated with membrane filtration and Millipore mColiBlue24 broth® as per the standard instructions (Hach Company, Loveland, Colorado, United States). Quantification of a suite of 21 metals was conducted for all water samples with ICP-MS in accordance with APHA (2012).

3.2.4 Antibiotic Analysis

The samples were analyzed for a suite of antibiotics once a month at Acadia University in Nova Scotia, Canada. These included: amoxicillin, cefaclor, cefprozil, cefdinir, levofloxacin, ciprofloxacin, azithromycin, clindamycin, clarithromycin, and triclocarban. Samples were filtered by using 13 mm disposable syringe filters with the pore size of 0.1 µm and Nylon filter media. The pH was adjusted to 2.5 ± 0.1 with 5 M formic acid. Solid phase extraction (SPE) was conducted using an Agilent 1290 Flexible Cube. The Flexible Cube was configured with a single piston pump, a 10-port switching valve, and two SPE cartridges to improve sample throughput. This method required 500 µL of sample with the draw and injection speed of 100 µL per minute. The solvent gradient started with 90% of 0.1% formic acid and 10% methanol for 4 minutes, which was increased to 100% methanol by 25 minutes for the remainder of the sample run. The maximum flow gradient was 100 mL per minute. The quantification and quality assurance and quality control was conducted according to the methodology described in McConnell et al. (2018b).

3.2.5 Genetic Analysis

Approximately 25 mL of the raw wastewater and septic tank effluent (STE) water samples were filtered through a 0.45 µm pore size filter using a Millipore Vacuum Manifold and sterilized magnetic filtration funnels. Likewise, a measured volume of approximately 400 mL was filtered for the SF effluent. The DNA retained on the filters from the water samples was extracted with Qiagen DNeasy Powersoil Kits (Qiagen Inc., Toronto, Ontario, Canada). Following filtration, each filter was immediately placed in a Powerbead tube and subsequent processing steps were followed in accordance with manufacturer's specifications. qPCR was used to enumerate the gene copy numbers of the following suite of gene markers: *int1*, *sul1*, *sul2*, *mecA*, *vanA*, *qnrS*, *ermB, tetO,* and bla_{TEM} and $bla_{CTX\text{-}M}$. The nine ARG markers were selected to represent the genes that confer resistance to the common clinically prescribed antibiotics as identified by the Government of Canada (2016). The *int1* gene was analyzed because it is commonly associated with MGEs and genes which confer resistance to antibiotics (Gillings et al., 2015). The HF183 is a *Bacteroides* 16S rRNA gene marker that is human-specific and is used to measure human fecal pollution in water environments (Seurinck et al., 2005); it was included in the gene scan to assess its utility as an indicator marker of elevated presence of ARGs. The HF183 gene marker was assessed as per the methodology described by McConnell et al. (2018a). The gene marker suite was quantified using TaqMan qPCR on a Bio-Rad CFX96 Touch system (Bio-Rad, Herculer, California, United States). The bacterial 16S rRNA gene copies were enumerated for each sample with SYBR Green qPCR (Applied Biosystems Inc., Beverly, Massachusetts, United States). A comprehensive description of the qPCR method development is found in Neudorf et al. (2017). The primer and hydrolysis TaqMan probe sequences and cycling conditions are provided in Appendix B (Table B.1).

The LOD was 5 copies per reaction (or 0.6 copies per mL for ARGs in the SF effluent samples; 0.3 copies per mL for 16S rRNA in the SF effluent; 0.2 copies per mL for HF183 for in the SF effluent; 10 copies per mL for ARGs in the raw wastewater and STE; 5 copies per mL for 16S rRNA in the raw wastewater and STE; 2.5 copies per mL for HF183 in the raw wastewater and STE). The LOQs were calculated as per the following Equation 1:

$$LOQ = ((gene\ copies/reaction)/(vol.of\ DNA\ per\ reaction) * total\ DNA\ extraction\ vol.))/(vol.of\ sample\ filtered) \quad \text{Eq. [1]}$$

The total volume of DNA extracted from each sample was 100 µL. The number of gene copies per reaction were determined from a standard curve. A ten-fold dilution series of each of the gene standards were used to construct the standard curves for the qPCR. The LOQs were determined as the last point on the standard curves that could be accurately quantified.

3.2.6 Cell-Associated and Cell-Free DNA Analysis

Cell-associated and cell-free DNA was enumerated for a small sub-set of the samples collected in July 2018 according to a slightly modified version of a procedure introduced and described by Zhang et al. (2018b). The workflow for the total, cell-associated, and cell-free DNA isolation is outlined in Figure A.15 and was modified from Zhang et al. (2018b). The total DNA of the raw wastewater and STE water samples were prepared for analysis by combining 20 mL of each sample, 2 mL of 3 M sodium acetate and 44 mL of 100% ethanol in a sterilized Erlenmeyer flask. The total DNA of SF samples were prepared for analysis by combining 100 mL from each sample with 10 mL of 3 M sodium acetate and 220 mL of 100% ethanol in a sterilized Erlenmeyer flask.

The cell-free DNA of the water samples was prepared for analysis by filtering each sample through a 0.45 µm pore size filter. Basically, genes passing through filtration are assumed to be cell-free. The filter for these samples was discarded and the filtrate retained in a sterilized Erlenmeyer filter flask. A total of 20 mL of raw wastewater and STE filtrate were combined separately with 2 mL of 3 M sodium acetate and 44 mL of 100% ethanol in a sterilized Erlenmeyer flask. The cell-free DNA of SF samples were prepared for analysis by combining 100 mL of each filtrate sample, 10 mL of 3 M sodium acetate and 220 mL of 100% ethanol in a sterilized Erlenmeyer flask.

All Erlenmeyer flasks containing the sample solutions were placed in a freezer at –20°C overnight to encourage DNA precipitation. The samples were processed for cell-associated DNA by the same procedure as described above, whereby the assumption is that all DNA

retained on the 0.45 μm pore size filter is representative of cell-associated DNA. In other words, the genes retained on the filter were assumed to be cell-associated genes.

All the samples in the Erlenmeyer flasks for total DNA and cell-free DNA were processed the next day by centrifuging each sample in multiple (~8) 50 mL sterilized conical plastic centrifuge Falcon tubes at 10,000 × *g* for 10 minutes. The pellet of the centrifuged samples was retained while the eluent was decanted. The pellet was concentrated from repeated centrifugation steps, whereby the pellet from the first tube was transferred to the subsequent falcon tube by repeated rinsing with a pipette and vortexing to dislodge the pellet and transferred into the final falcon tube. As each sample was processed the pellet from each falcon tube was gradually concentrated into a final falcon tube. The final falcon tube was centrifuged again at 10,000 × *g* for 10 minutes. The eluent was decanted and discarded leaving the relatively unsuspended and concentrated pellet in the final falcon tube. The final concentrated pellet was then carefully transferred with a pipette to a Powerbead tube by using the solution C1 from the PowerSoil DNA extraction kit to repeatedly wash the pellet from the falcon tube to the Powerbead tube. Once the pellet was transferred, the DNA was extracted as per described above.

3.2.7 Antibiotic Resistant Bacteria Enumeration

Total bacteria and antibiotic resistant bacteria in the raw wastewater, STE, and SFs samples from July 16 and July 23, 2018, were enumerated on agar plates containing no antibiotics (*i.e.,* total bacteria, control) and concentrations of either 50 mg/L sulfamethoxazole, 50 mg/L erythromycin, or 10 mg/L tetracycline (Mao et al., 2015). A spot plating method was used where three 20 μL drops (for a total volume of 60 μL) of serially diluted raw wastewater, STE, and SF effluent samples were placed on tryptone soy agar (TSA, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) plates, with or without each antibiotic at the defined concentrations, and incubated at 30°C for 24 hours. After incubation, the number of colonies were counted and recorded as log CFU per mL.

3.2.8 Sodium Bromide Tracer Tests

Sodium bromide (NaBr) tracer tests were conducted on the SFs on July 30, 2018 during a dry weather period. The tracer response curves for the tracer tests are illustrated in Figure

A.16. A mass of 7.5 g of bromide (Br^-), which equates to 9.675 g of NaBr, was diluted in 10 L of water and injected into each of the SFs. A portable automatic water sample model 6712 (Teledyne ISCO, Lincoln, Nebraska, United States) was used to collect the SF effluent samples over the duration of the tracer tests which lasted 16.5 days. The concentration of Br^- in the filter effluent samples was analyzed with an Orion 5 star meter (Thermo Scientific, Beverly, Massachusetts, United States) and a gel-filled combination ion selective electrode probe BR43-0001 (Analytical Sensors & Instruments, Inc., Sugar Land, Texas, United States). The bromide electrode probe was calibrated according to manufacturer's instructions and ionic strength adjuster was added to the samples prior to measurement of bromide concentrations. Hydraulic retention times were calculated with the Simpson's 1/3 rule according to the numerical integration methodology described in Fogler (2010).

3.2.9 Statistical Analysis

One-way analysis of variance (ANOVA) tests were performed on the absolute abundances of ARGs from the SF effluent over the study period to assess statistical difference at $p < 0.05$. A Shapiro-Wilk normality tested normality with the non-normality assigned at $p < 0.05$. The Brown-Forsythe method assessed for equal variance with significant differences in variances assigned at $p < 0.05$. When the assumption of normality was not met, a Kruskal-Wallis ANOVA on ranks was performed with significant difference between treatments assigned at $p < 0.05$. A Tukey test was performed to assess significant differences between SF effluent absolute abundances and significance attributable at $p < 0.05$. The same statistical analysis was performed on the relative abundances of ARGs with addition of the raw wastewater and STE sample data. Throughout, $\bar{x}$ denotes mean of the sample. The potential for correlations between ARGs and other water quality indicators was of interest to assess whether there are water quality indicators associated with ARGs. To address this, a PCA was conducted on the 10-sample dataset with gene marker concentrations, conventional wastewater indicators, and metals concentrations in the raw wastewater, STE, and sand filter effluent. The metals that were excluded from the analysis included selenium (Se), silver (Ag), cadmium (Cd), antimony (Sb), cesium (Ce), and uranium (U), due to most measurements being below the detection limit (see Tables B.5 – B.9 for metals data). The PCA data was log-transformed and analysed as a correlation

matrix. The statistical analysis was performed with SigmaPlot version 13.0 statistical software (Systat software, Inc., San Jose, California, United States).

3.3 Results and Discussion

3.3.1 Conventional Parameters

The sand filters had moderate removal of the conventional wastewater parameters that were analyzed (Table 3.2). The average removal efficiencies for the filters ranged from 99 – 100% for $CBOD_5$, 91 – 100% for TSS, 5.2 – 6.7 log for *E. coli*, 27 – 37% for TN, and -1 – 60% for TP (negative value indicates net phosphorus production), which compared well with findings on this specific system by Wilson et al. (2011). Wilson et al. (2011) reported removal efficiencies of: 97 – 98% for $CBOD_5$, 82 – 97% for TSS, 4.3 – 5.2 log reduction for *E. coli*, 41 – 57% for TN, and 44 – 93% for TP. Wilson et al. (2011) attributed the primary removal mechanisms to physical filtration processes from the sand media and biological removal processes within the biological zone (*i.e.*, biological mat) at the interface of the gravel distribution trench and the sand filter media. The slight improvement in $CBOD_5$, TSS, and *E. coli* removal efficiencies may be attributed to a matured biological zone over the past 7 years. Development of a biological mat is characterized by a physical clogging of the pores in the distribution interface of a soil-adsorption system; formation of this zone begins within the first few months of the operation of the soil-adsorption system and gradually reaches an equilibrium (Beal et al., 2005).

Table 3.2 Summary of conventional wastewater parameter results presented as mean values ± standard deviation (*n* = 10).

Sample description	$CBOD_5$ (mg/L)	TSS (mg/L)	*E. coli*[12] (CFU/100mL)	TN (mg/L)	TAN (mg/L)	TP (mg/L)	Temp. (°C)	DO (mg/L)	pH	Specific conductance (µS/cm)
Raw	343 ± 138	295 ± 141	$3.4x10^6$ ± $3.5x10^6$	46 ± 23	45 ± 20	6.5 ± 2.9	15.9 ± 1.5	4.5 ± 2.7	7.4 ± 0.2	1247 ± 114
STE	219 ± 148	182 ± 128	$3.3x10^5$ ± $5.8x10^5$	54 ± 24	62 ± 26	9.6 ± 9.7	17.1 ± 1.2	2.8 ± 2.2	6.6 ± 0.1	1501 ± 261
SF1	2 ± 1	25 ± 20	1 ± 12	30 ± 6	0.1 ± 0.1	2.6 ± 3.2	16.5 ± 1.8	8.1 ± 1.2	6.1 ± 0.4	1242 ± 237
SF2	2 ± 1	5 ± 10	1 ± 0.6	30 ± 4	0.2 ± 0.3	3.3 ± 1.3	16.3 ± 1.8	10.3 ± 1.3	6.6 ± 0.2	1341 ± 70
SF3	2 ± 1	2 ± 2	1 ± 10	29 ± 6	0.1 ± 0.1	5.8 ± 5.0	16.2 ± 1.8	10.1 ± 1.2	6.4 ± 0.3	1336 ± 88
SF4	2 ± 1	1 ± 1	1 ± 2	33 ± 9	0.1 ± 0.1	1.7 ± 1.0	16.6 ± 1.7	9.9 ± 1.3	6.0 ± 0.2	1308 ± 233
SF5	2 ± 1	4 ± 3	4 ± 7	31 ± 5	0.1 ± 0.1	4.3 ± 3.5	16.0 ± 1.5	10.1 ± 1.1	6.0 ± 0.3	1226 ± 224
SF6	2 ± 1	3 ± 3	19 ± 339	29 ± 10	0.2 ± 0.5	6.6 ± 7.3	16.1 ± 1.7	9.9 ± 1.0	6.3 ± 1.2	1333 ± 88

[12] *E.coli* data is presented as geometric means.

3.3.2 Hydraulic Characterization of Filters

The HRTs of the sand filters are summarized in Table 3.3. SF1 had the longest HRT (~8 days), given that this filter had the lowest hydraulic conductivity, and was on a shallow slope; however, the deviation from the other filters based on the tracer response curve (Appendix A Figure A.16) warranted further examination. To investigate this, SF1 was partially excavated to the interface between the gravel trench and the sand media where the biological mat resided. SF1 was found to be partially clogged, with saturated conditions and ponded water within the biological mat (see Figure A.14a). The finer grain size and low slope may have increased its vulnerability to failure. Saturated conditions in OWTS have been known to present a higher risk of conveyance of pathogens and ensuing human exposure (Beal et al., 2005). Average flows from each filter over the study period ranged from 108 to 152 L per day (See Figure A.18 for the hydrographs over the study period).

Table 3.3 Summary of hydraulic characteristics of the sand filters determined from the bromide tracer tests.

Filter ID	Grain size	Slope (%)	HRT (days)	Mass recovery (%)	Time to peak (hrs)	Variance (dimension less)
SF1	Fine	5	8	150[13]	154	0.16
SF2	Medium	5	4	73	36	0.79
SF3	Coarse	5	5	79	42	0.73
SF4	Fine	30	6	102	60	0.36
SF5	Medium	30	4	89	36	0.75
SF6	Coarse	30	3	86	30	0.84

3.3.3 Raw Wastewater and Septic Tank Effluent

3.3.3.1 Absolute and Relative Gene Abundances

All the gene markers were present in the raw wastewater and STE, with absolute abundances well above the LOQs for all gene markers except for *vanA* and *mecA* (Figure 3.2). The vancomycin resistance gene, *vanA,* and in most samples the methicillin resistance

[13] The mass recovery for SF1 was overestimated likely due to hydraulic failure of the filter and preferential flow in this filter (see Figure A.16).

gene, *mecA* were close to or below the LOQs. These two ARGs were not plotted in Figures 3.2 and 3.3, due to low levels. The most abundant ARGs within the raw wastewater were *ermB* ($\bar{x}$ = 6.5 ± 0.7 log gene copies/mL), *qnrS* ($\bar{x}$ = 6.2 ± 0.4 log gene copies/mL), and *tetO* ($\bar{x}$ = 5.7 ± 0.4 log gene copies/mL). Overall, the septic tank removed minimal amounts of the gene markers from the effluent stream. Therefore, the most abundant ARGs within the STE were *ermB* ($\bar{x}$ = 5.9 ± 0.8 log gene copies/mL), and *sul1* ($\bar{x}$ = 5.2 ± 0.6 log gene copies/mL), *tetO* ($\bar{x}$ = 5.1 ± 0.3 log gene copies/mL). These ARG abundances in the STE were comparable in order of magnitude to raw and primary treated wastewater from other studies (Czekalski et al., 2012; McConnell et al., 2018b). In comparison to raw wastewater samples, there was no significant enrichment of ARGs in the STE (Figure 3.3). The highest relative abundances of ARGs in the STE were *ermB* ($\bar{x}$ = -2.5 ± -2.3 log gene copies for *ermB*/16S rRNA), and *sul1* ($\bar{x}$ = -3.4 ± -3.4 log gene copies for *sul1*/16S rRNA).

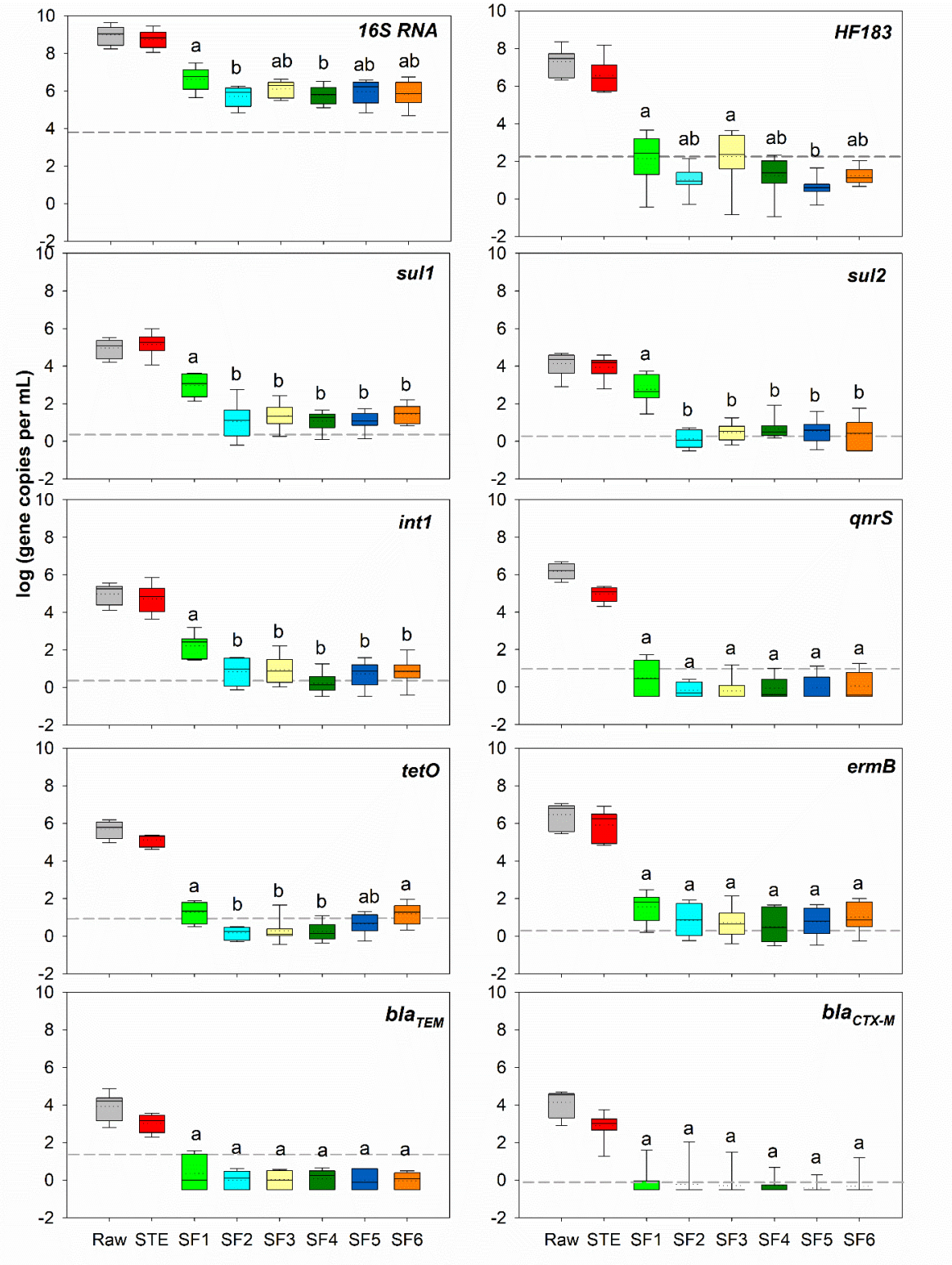

Figure 3.2 Absolute abundances of gene markers in the raw wastewater, septic tank effluent (STE), and sand filter (SF) 1 – 6 for the duration of the study (*n* = 10). The middle lines represent the median values, the dotted lines represent the means, the

bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the gene concentrations. The dashed line represents the limit of quantification for the sand filter effluent. Difference in letters denotes significant difference of the gene absolute abundances at $p < 0.05$ for the Tukey test. The raw wastewater and septic tank effluent samples were not analyzed statistically as the differences in sand filter performance were of primary interest.

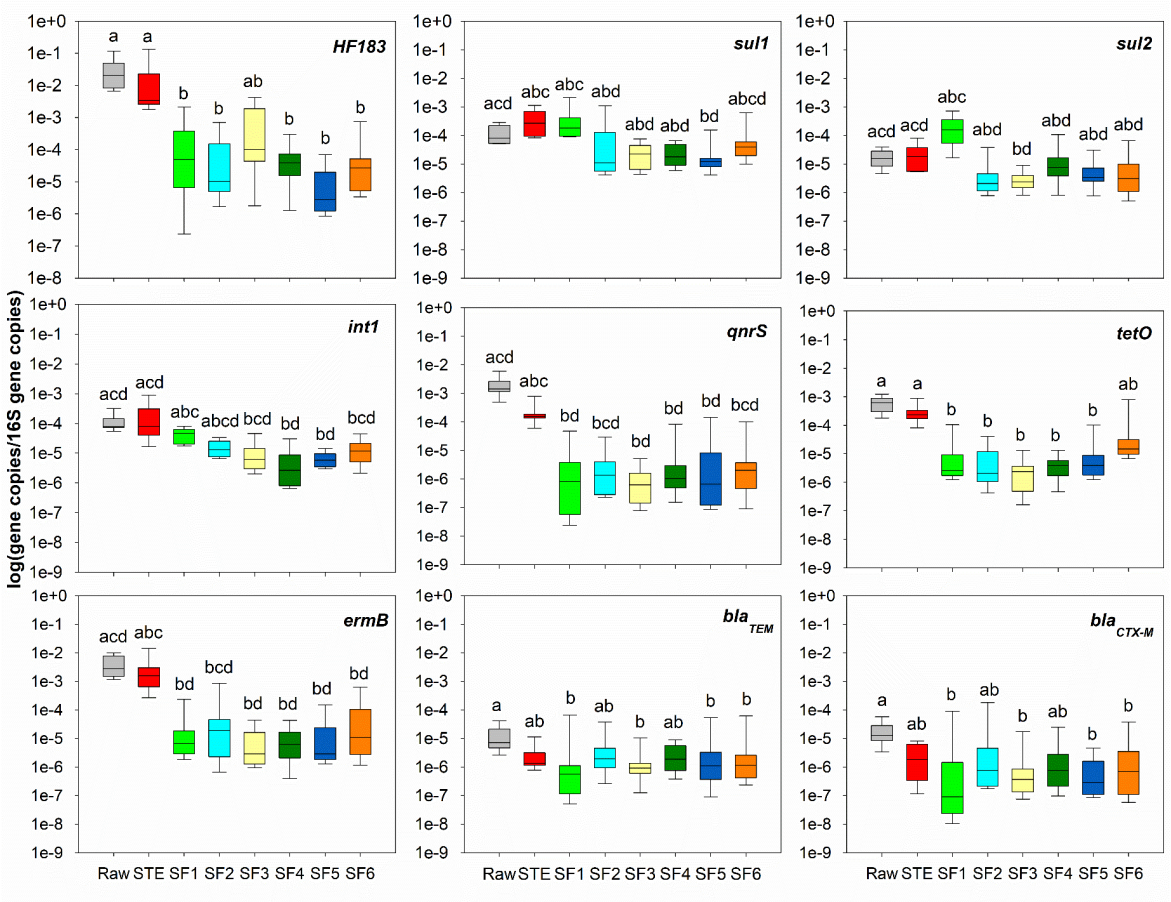

Figure 3.3 Relative abundances of gene markers in the raw wastewater, septic tank effluent (STE), and sand filters (SF) 1 – 6 for the duration of the study (n =10). The middle lines represent the median values, the dotted lines represent the means, the bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the gene concentrations. Difference in letters denotes significant difference between the gene absolute abundances at $p < 0.05$ for the Tukey test.

3.3.4 Filter Gene Marker Removal Performance

The sand filters performed effectively for the removal of ARGs from the STE as demonstrated with the absolute abundances illustrated in Figure 3.2. There were few significant differences between the filters apart from SF1, which removed significantly ($p < 0.05$) lower amounts of the ARGs. The effluent from all sand filters contained medians below LOQ levels for *qnrS*, bla_{TEM}, and *mecA*, and below LOD for *vanA*. No seasonal trends in ARG abundances were observed over the study period.

Treatment of the STE in the SFs 2 – 6 resulted in the following average absolute removal of ARGs: 2.6 to 3.0 log removal for 16S rRNA, 4.3 to 6.0 log removal for *HF183*, 3.7 to 4.1 log removal for *sul1*, 3.3 to 3.8 log removal for *sul2*, 3.8 to 4.5 log removal for *int1*, 4.9 to 5.2 log removal for *qnrS*, 3.9 to 4.9 log removal for *tetO*, 4.9 to 5.4 log removal for *ermB*, 2.9 to 3.0 log removal for bla_{TEM}, and 3.1 to 3.3 log removal for $bla_{CTX\text{-}M}$.

Due to the decreased performance of SF1, it was considered separately from the aforementioned ranges with absolute removals of: $\bar{x}$ = 2.1 log removal for 16S rRNA, $\bar{x}$ = 4.4 log for *HF183*, $\bar{x}$ = 2.2 log removal for *sul1*, $\bar{x}$ = 1.2 log removal for *sul2*, $\bar{x}$ = 2.5 log removal for *int1*, $\bar{x}$ = 4.5 log removal for *qnrS*, $\bar{x}$ = 3.9 log removal for *tetO*, $\bar{x}$ = 4.4 log removal for *ermB*, $\bar{x}$ = 2.7 log removal for bla_{TEM}, and $\bar{x}$ = 3.0 log removal for $bla_{CTX\text{-}M}$. The lack of difference in the absolute abundances of the gene markers between the sand filters (except for SF1) suggested that the grain sizes in the three different sand mediums and two different slopes had little effect on the removal of the gene markers. It should be noted that the effective size (D_{10})—which is the diameter that 10% of the material is finer than—of the three sand medias ranged from 0.12 – 0.18 mm. Therefore, the particle sizes of the media were similar, which may have contributed to similar gene removal efficiencies.

As noted above, the exception was SF1, which effected significantly ($p < 0.05$) less removal for *sul1*, *sul2*, and *int1* than all the other sand filters, resulting in levels that were well above the LOQ for these three ARGs. Likely, the decrease in attenuation of ARGs in SF1 was due to the suspected hydraulic failure of the filter. From an engineering perspective, this

may suggest that an OWTS like SF1 with low hydraulic conductivity configurations on a shallow slope may present greater risk of failure and ARG breakthrough as they age.

The HF183 markers were generally below the LOQ except for SF1 and SF3 (Figure 3.2). This contrasts with the trends in some of the ARGs, for instance *sul1* and *ermB* have median absolute abundances consistently above the LOQs. Therefore, the utility of HF183 as an indicator for elevated ARGs associated with human fecal contamination may be useful, but not all encompassing.

In general, the relative abundances of the gene markers in filter effluent were significantly lower than in the raw wastewater and STE (p <0.05; Figure 3.3). Some exceptions to this trend were evident, which included SF1 showing significant (p <0.05) enrichments of *sul1*, *sul2*, and *int1* compared to the majority of the other sand filters. This enrichment of gene markers in SF1 is likely attributable to the hydraulic failure of this filter, which affected treatment performance. Overall, the relative abundances of the gene markers in the sand filter effluent represented a small percentage of the overall 16S rRNA gene abundances. These results suggest minimal gene marker enrichment when comparing the effluent samples, except for SF1 for *sul1*, *sul2*, and *int1*. Persistence of *sul* genes have also been reported in other types of wastewater treatment systems (McConnell et al., 2018b; Gao et al., 2012).

3.3.5 Antibiotic Resistant Bacteria

The treatment train was analyzed for ARB twice on July 16 and July 23, 2018, respectively (Figure 3.4). Bacteria that were resistant to antibiotics that were plated separately (*i.e.*, sulfamethoxazole, erythromycin, tetracycline) were present at comparable magnitudes ranging from 1.6 to 2.8 log CFU/mL in the SF effluent, down from levels of ~5 log CFU/mL in the raw wastewater. *E. coli* counts for the same sample events were low (< 1.2 log CFU/100mL) for the SF effluent, which indicated that the bacteria carrying the resistance to these antibiotics were likely different species than *E. coli*. *Sul* and *erm* which confer resistance to sulfamethoxazole and erythromycin were detected with absolute abundances above the LOQs in the sand filter effluent. This suggests that a portion of the *sul* and *erm* genes in the effluent would be associated with live bacteria. The qPCR analyses

of a subset of the ARB colonies confirmed the presence of the relevant ARG markers (*sul1* and *sul2*, *ermB,* and *tetO*). It should be noted that the bacteria resistant to the three separate antibiotics represented almost the same amount as the total heterotrophic bacteria. This suggests that the bacteria that were cultivated were potentially intrinsically resistant to the antibiotics as opposed to having acquired resistance. In other words, mechanistically the bacteria that were cultivated may have been resistant via cellular structure or functionality, as opposed to resistance expression that has been acquired via horizontal gene transfer mechanisms (i.e., transformation, transduction or conjugation). In addition, actual quantification of true ARB with culture-based methods such as done for Figure 3.4 is not possible due to the fact that growth conditions are not favorable to all the bacteria in the samples that were plated.

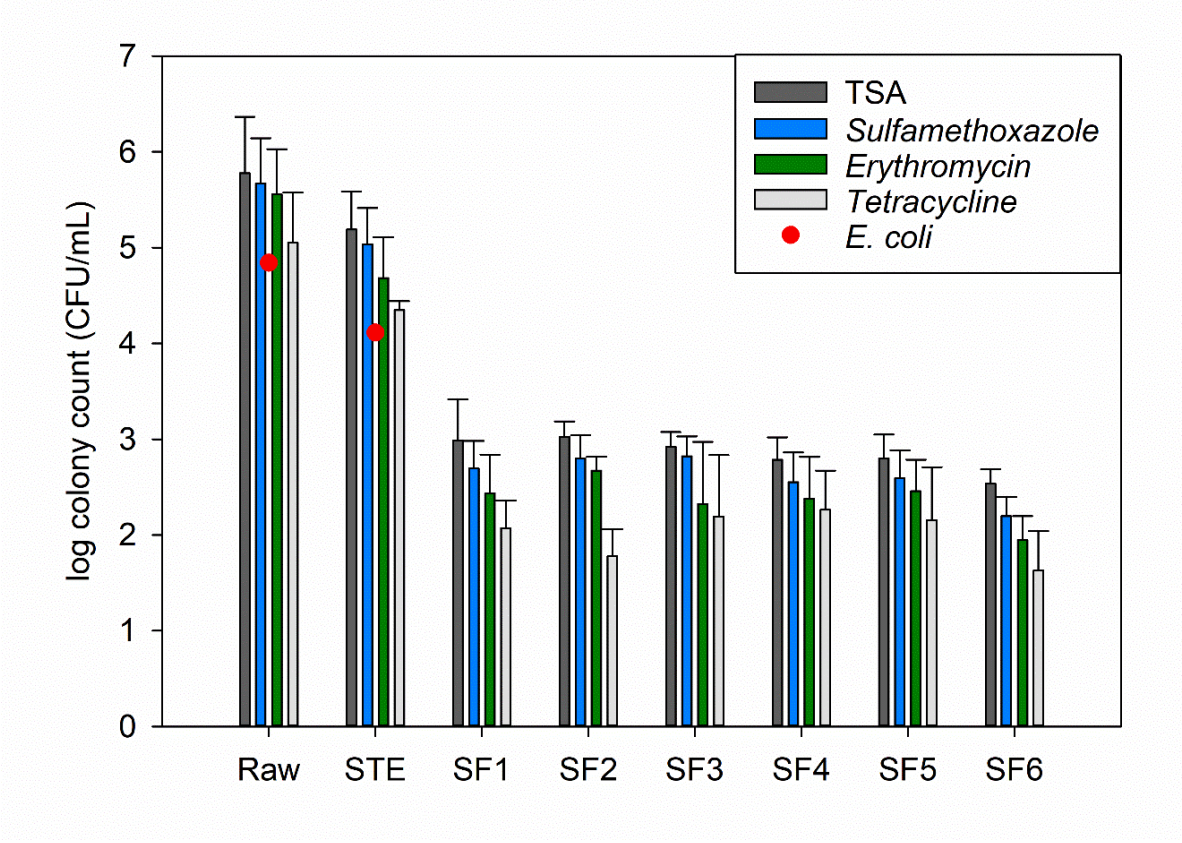

Figure 3.4 Geometric mean of total and antibiotic resistant bacteria in samples of raw wastewater, septic tank effluent (STE), and sand filter (SF) effluents (sampled on July 16 and 23, 2018, *n* = 4). The error bars represent one standard deviation. The *E. coli* is presented as the geometric mean of two samples collected on July 16 and 23, 2018. The *E. coli* concentrations for the SF effluent were all below 1 CFU/mL.

3.3.6 Correlations Between Gene Markers and Water Quality Parameters

There was a positive correlation between the gene markers and the conventional wastewater quality indicators (Figure 3.5a). *E. coli* showed a positive correlation to the gene markers, which is anticipated as bacteria such as *E. coli* can house selected gene markers intracellularly. Several heavy metals were positively correlated with the gene markers, which included chromium (Cr), Ba, and Cu, which may indicate co-selection for resistance to metals and ARGs in bacteria. Chromium and copper were elevated in the raw wastewater and STE which may have been an artifact of metals originating from household plumbing fixtures. Co-selection of ARGs and heavy metal resistance genes in municipal wastewater have been observed by Di Cesare et al. (2016), and specifically co-selection of tetracycline and copper was observed by Amachawadi et al (2013). Na, Mg, Ca and K were observed to be negatively correlated with the gene markers and conventional wastewater quality indicators. This inverse relationship may be explained by relatively lower concentrations observed for these cations in the raw wastewater and STE, and an elevated concentration in the sand filter effluent. Calcium carbonate ($CaCO_3$) dissolution is characteristic of septic field environments as a buffer for NH_4^+ oxidation, which results in increased Ca^{2+} concentrations in the effluent, and other major cations may also exhibit similar mineral dissolution, or cation exchange reactions (Wilhelm et al., 1994). The scores plot in Figure 3.5b shows the overall difference in concentrations between the raw wastewater and STE samples; which were generally grouped together, and the sand filter samples which were clustered together, except for SF1. This confirms the degree of system characterization (n = 10 sample events) was adequate to capture variability in water quality.

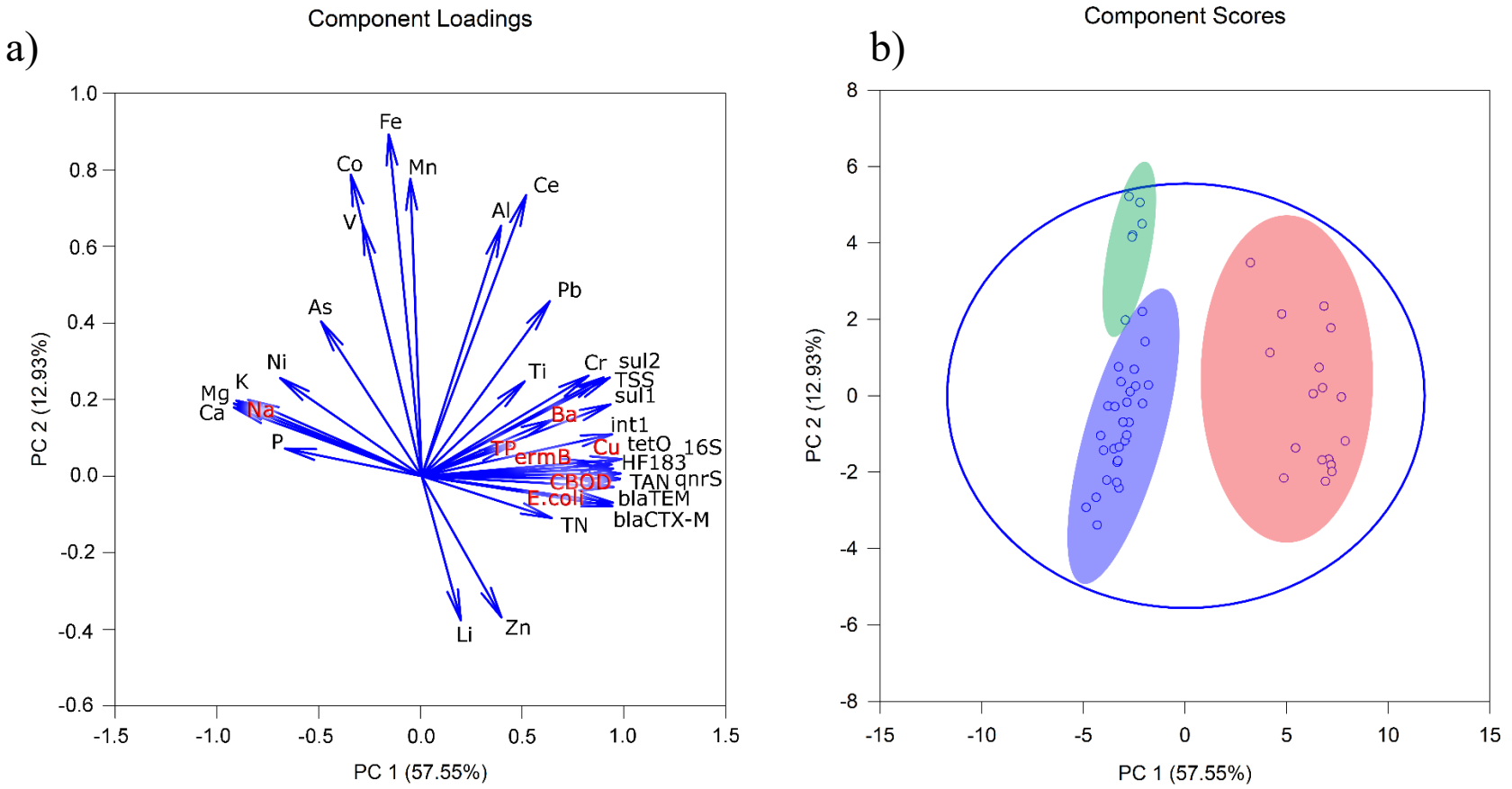

Figure 3.5 Principal component analysis (PCA) of the gene marker concentrations and water quality indicators along the treatment train. This illustrates the: a) loadings plot of the gene markers and other parameters (*n* = 10 sample events), use of red text is for contrast; and b) scores plot of the PCA results of the wastewater sampling. Ellipses denote groupings of scores of sand filters 2 – 6 (blue); sand filter 1 (green); and the raw wastewater and septic tank effluent samples (red). The numbers in brackets represent the percentage of variance described in the dataset by the first and second components.

3.3.7 Apportionment of Cell-Associated and Cell-Free Gene Markers

The enumeration of total, cell-associated, and cell-free DNA for each gene marker within the treatment train are presented in Table 3.4. Cell-associated DNA represented the greatest apportionment of DNA for all the gene markers and throughout the treatment train. All cell-free DNA observed in the analysis were either below LOQ or LODs. The raw wastewater and STE had negligible apportionment of cell-free DNA, and the sand filters contained cell-free DNA levels below the LODs, including the poorly performing SF1. This indicates that the gene markers that were measured throughout the treatment train resided primarily inside bacterial cells (*i.e.*, intracellularly). This finding indicates that this type of treatment system is at low risk of spreading cell-free ARGs.

Table 3.4 Summary of average gene marker concentrations in the total, cell associated, and cell-free DNA fractions for raw wastewater, septic tank effluent (STE) and sand filters (SF) 1 – 3 samples collected on July 16 and July 23, 2018. Bolded numbers indicate absolute abundances above the LOQ, italicized numbers are below the LOQ, <DL means are below the detection limit.

	Sample ID														
	Raw			STE			SF1			SF2			SF3		
Gene marker	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free
	Log gene copies per mL														
16S rRNA	**9.5**	**8.5**	*1.4*	**9.2**	**8.0**	*1.1*	**6.8**	**6.4**	<DL	**6.3**	**5.3**	*0.8*	**6.4**	**5.7**	<DL
HF183	**6.7**	**6.4**	*0.6*	**5.8**	**5.6**	*0.5*	*2.1*	<DL	<DL	**3.0**	**2.3**	<DL	**2.6**	*1.1*	<DL
sul1	**5.1**	**4.3**	*1.1*	**4.5**	**4.0**	*1.2*	**3.0**	**2.4**	<DL	**1.3**	<DL	<DL	**1.9**	<DL	<DL
sul2	**4.5**	**3.8**	<DL	**4.1**	**2.8**	*1.0*	**3.0**	**2.4**	<DL	<DL	<DL	<DL	**1.2**	<DL	<DL
int1	**5.6**	**4.5**	*1.1*	**4.1**	**3.6**	<DL	**2.7**	**1.7**	<DL	<DL	<DL	<DL	**1.5**	<DL	<DL
qnrS	**6.1**	**5.7**	<DL	**5.0**	**4.2**	<DL	**3.6**	<DL	<DL	**1.7**	<DL	<DL	**2.5**	<DL	<DL
tetO	**5.4**	**5.0**	<DL	**5.0**	**4.6**	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
ermB	**6.1**	**5.6**	<DL	**5.2**	**4.9**	<DL	**1.3**	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
bla_{TEM}	**3.3**	**2.9**	<DL	*2.4*	*2.1*	<DL	*1.1*	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
$bla_{CTX\text{-}M}$	**4.0**	**3.0**	<DL	**2.6**	**2.0**	<DL	**1.2**	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

	Sample ID														
	Raw			STE			SF1			SF2			SF3		
Gene marker	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free	Total	Cell-associated	Cell-free
mecA	*1.7*	*1.0*	<DL	*1.5*	<DL	<DL	**1.0**	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
vanA	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

3.3.8 Antibiotics

The antibiotic data is in Table B.11 of Appendix B. All the antibiotics were detected at least once in the treatment train during the study period. However, during many sample events, several of the antibiotics were not detected. An exception was clindamycin, which was often present in detectable concentrations within the treatment train. In clinical settings, the *erm* gene can confer resistance to clindamycin as well (Levin et al., 2005). Figure 3.2 shows that *ermB* was often present above LOQ in the effluent of all the sand filters. Intermittingly, all the antibiotics except for azithromycin, were detected in the sand filter effluents. The chemical stability of antibiotics varies, and some are quick to degrade, which may explain absence in the effluent. There was no direct relationship between antibiotics and ARGs because of the ephemeral nature of the presence of the antibiotics in the influent. The bacteria within the septic tank and biological mat acquire resistance through repeated intermittent exposure over time.

3.4 Conclusions

This study demonstrated lateral flow sand filters help to reduce the risk of AMR contamination from OWTS when the hydraulics are properly functioning. Most of the ARGs assessed were removed to below 2 log gene copies per mL for absolute abundance. Grain size of the filtration media or filter slope had no observable impact on the efficacy of the removal of ARGs except for SF1. The exception of SF1 was due to partial hydraulic failure of the system as evidenced by clogging and water retention on the biological mat. In SF1, significantly ($p < 0.05$) less removal of *sul1*, *sul2* and *int1* were observed in comparison to the other sand filters and therefore elevated ARGs passed through into the filter effluent. This highlights the need for inspection and maintenance of these types of OWTS as they age.

ARGs were mostly found to be present intracellularly in the bacteria as opposed to extracellularly. A few hypotheses are offered for this intracellularly heavy partitioning of ARGs is that there is limited cell lysis within the system, degradation of extracellular DNA by nucleases within the system, and potential sorption and metabolism of extracellular DNA in the biological mat zone. This type of OWTS system poses low risk of cell-free

DNA breakthrough and subsurface transport. ARB, resistant to either sulfamethoxazole, erythromycin, or tetracycline, were observed to undergo an average of 2.3 log reduction across the sand filters. Of importance, the ARB were present in the sand filter effluent with counts ranging from 1.6 to 2.8 log CFU per mL. Concurrently, these samples generally contained non-detectable levels of *E. coli*. Therefore, sole reliance on *E. coli* as an indicator may be inadequate to capture the risk of releasing AMR pathogens from mal-functioning OWTS.

Future research would be useful to characterize the filter biological mat, specifically examining ARGs and microbial community structure using metagenomics. This would enable further understanding and potential optimization of the biological mat attenuation mechanisms in filtration technology development. Understanding of fate of ARGs in saturated environments would also be useful for further characterization of risk to groundwater resources.

CHAPTER 4 PRELIMINARY MODELING OF ATTENUATION OF ANTIMICROBIAL RESISTANCE DETERMINANTS IN LATERAL FLOW SAND FILTER WASTEWATER TREATMENT SYSTEMS

Abstract

The transport and attenuation of ARGs and ARB in lateral flow sand filters receiving primary treated municipal wastewater were simulated with HYDRUS 2D. Three lateral flow sand filters, possessing different sand media, were monitored for their treatment of ARGs and ARBs over a 6-month period to guide the modeling approach. Hydraulic tracer tests were conducted with bromide on each of the filters and the tracer response data was used to calibrate the hydraulics within the model. Determinants of antimicrobial resistance included *sul1*, *tetO*, and *ermB* ARGs, and bacteria resistant to sulfamethoxazole (50 mg/L), tetracycline (10 mg/L), and erythromycin (50 mg/L). Total microbial levels were assessed through measurements of 16S rRNA gene copy numbers and total heterotrophic bacteria. Bench scale experiments were performed to determine the first order degradation rates and partition coefficients of each gene target and bacteria type. These degradation rates and partition coefficients were used to parameterize the models. The preliminary model overpredicted the effluent genes likely due to inadequate characterization of the role of the biological mat in attenuation processes. In most scenarios, the calibrated models were able to successfully simulate the attenuation and transport of the ARB. The modeling of these removal processes may be further improved with better characterization of the biological mat layer, and further understanding of gene transfer rates in this type of environmental system. This study demonstrates a novel application of porous media fate and transport modeling of determinants of AMR. This type of modeling approach could help assess risk of AMR contamination of groundwater systems.

4.1 Introduction

Antimicrobial resistance has become a threat to global public health as previously treatable infectious microorganisms have acquired resistance to currently developed antimicrobials (Michael et al., 2014; Ventola, 2015; WHO, 2014). Development of AMR in bacteria occurs as a result of selective pressure from exposure to antimicrobials in clinical and environmental settings (Kolář et al., 2001). Various anthropogenic practices can encourage the proliferation of AMR, such as in municipal wastewater treatment systems, where trace amounts of antimicrobials consumed by the general population can contribute to the development of AMR in resident bacterial communities (Nagulapally et al., 2009; Munir et al., 2011). The prevalence of antibiotic resistance development in environmental settings can be tracked with ARGs, which are typically carried within ARB. A direct relationship between ARGs elevated above background abundances and heavy antimicrobial use has been observed in the environmental dimension (Vikesland et al., 2017). ARGs are considered as an emerging contaminant of concern due to their ability to transfer genes vertically and horizontally to increase resistance among microorganisms (Sanderson et al., 2016). The 'One Health' approach for combatting AMR specifies assessment of the facets of AMR interconnection between clinical incidence and the environmental dimension. As part of this strategy, the need for research into the development of technologies to attenuate agents of AMR from wastewater environments and prevention of dissemination into the environmental dimension was identified (Bürgmann et al., 2018; Larsson et al., 2018).

To continue to build on this understanding of the fate of ARGs in the environmental dimension, the risk of subsurface transport of ARGs, both in the vadose and saturated zones, warrants research. Echoing this, a review by Bondarczuk et al. (2016) identified that research into the relationship between soil properties and AMR development is needed to facilitate risk assessments of the exposure routes through soil from land based agricultural and waste management practices. A commonly used and low-technology option for rural regions and developing nations is the use of OWTS. OWTS have been identified as a potential major contributor to groundwater contamination, especially when poor hydraulic functioning is observed (Carroll et al., 2005). Hayward et al. (2019) demonstrated that lateral flow sand filtration—which is a non-conventional type of OWTS—when used as a

secondary treatment stage following a septic tank, was effective in attenuation of a target panel of ARGs. These types of sand media filters are characterized by unsaturated flow conditions after periodic dosing of effluent into a distribution trench following a settling step in a septic tank (Wilson et al., 2011). This hydraulic behavior is similar to the unsaturated percolation characteristics of other types of OWTS soil adsorption systems (Siegrist et al., 2000).

HYDRUS is a commonly used software package for simulation of microbial transport processes in unsaturated flow conditions (Šejna et al., 2014). Several studies have simulated the transport of bacteria, viruses, and colloids with HYDRUS including, but not limited to, Morales et al. (2014; 2015), Balkhair (2017), Jiang et al. (2010), and Gargiulo et al. (2008). HYDRUS is based on two fundamental governing equations describing solute and water movement in variably and fully saturated porous media. The governing equation used in the numerical computation of variably saturated fluid flow is Richard's equation, as given by Šimůnek et al. (2006) :

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x_i}\left[K\left(K_{ij}^{A}\frac{\partial h}{\partial x_j} + K_{iz}^{A}\right)\right] - S \qquad \text{Eq. [2]}$$

Where: θ is the volumetric water content [L^3L^{-3}], h is the matric potential (pressure head) [L], S is a sink term [T^{-1}], x_i *(i = 1,2)* are the spatial coordinates [L], t is time [T], K_{ij}^{A} are components of a dimensionless anisotropy tensor $K^{A,}$ and K is the unsaturated hydraulic conductivity function [LT^{-1}]. HYDRUS also incorporates the advection-dispersion equation (ADE) into the numerical code to describe contaminant transport. The governing flow and transport equations are numerically solved using the Galerkin-type finite element schemes which are solved with various matrix algebra methods (Šimůnek et al., 2012). Contaminant fate processes such as degradation and sorption can also be simulated. HYDRUS has two different options for modeling microbial contaminant sorption. One is an attachment-detachment module with two types of kinetic deposition sites (Šimůnek et al., 2006; Gargiulo et al., 2008; Morales et al., 2014). The other is a linear partitioning module, which was chosen for this study due to the ability to experimentally measure partition coefficients in bench scale sorption studies.

The objective of this study was to conduct a preliminary assessment of the use of HYDRUS 2D for the prediction of ARG and ARB attenuation in OWTS lateral flow sand filters. The modeled effects of different sand filter media on the fate and transport of ARGs and ARB were assessed and compared to field observed results. To reach the study objective, the sorption and degradation characteristics of key ARGs and ARB were characterized with bench-scale experiments using different sand filter media types and two air temperatures of 4°C and 15°C. This study provides insights into the attenuation mechanisms of ARGs and ARB in variably saturated porous media, and is a novel screening-level attempt to model subsurface transport and attenuation of ARGs and ARB with commercially available software. Development of modeling tools, or adoption of existing tools, to simulate the fate and transport of these emerging contaminants of concern can contribute to a better understanding of the environmental dimension of AMR. This understanding will enable quantification of the potential public health risk posed by exposure to these emerging microbial contaminants, and identification of mitigation strategies for AMR proliferation in environmental settings.

4.2 Materials and Methods

4.2.1 Lateral Flow Sand Filter Description

The lateral flow sand filters that are the focus of this study were built at an experimental pilot-scale facility located at the BEEC in Bible Hill, Truro, Nova Scotia, Canada. The BEEC facility withdraws municipal wastewater from the Town of Bible Hill into a centralized catch basin and then distributes the raw wastewater via pumping to a septic tank with a retention time of 2 to 4 days for solids settling. Septic tank effluent is then periodically dosed into a flow splitter, which doses the experimental lateral flow SFs on a sub-daily basis. These lateral flow SFs have been studied for treatment performance of ARGs and ARB removal by Hayward et al. (2019). These lateral flow SFs were previously modeled with HYDRUS 2D for phosphorus attenuation by Sinclair et al. (2014). Transport of ARGs and ARBs in three different sand filter media types was simulated and compared to field observed measurements presented in Hayward et al. (2019).

Figure 4.1 illustrates three of the SFs from the Hayward et al. (2019) paper which were selected for the HYDRUS modeling. These were representative of fine (SF4), medium (SF5), and coarse (SF6) grained sand filter media, on a 30% slope. The SFs had K_s values of 2.7 x10^3, 6.3 x10^3, and 1.2 x10^4 cm/d for SF4, SF5, and SF6, respectively. The *Ks* values were determined from ex-situ samples according to ASTM method D2434-68 (ASTM, 2000). The dimensions of the lateral flow SFs were 8 m x 1.5 m in length and width in plan view, respectively. Each filter has a gravel distribution trench of 2.5 m in length and 0.3 m in depth, which receives effluent from the flow splitter box, and distributes the influent into the sand filter media. The sand media layer was 0.5 m in depth below the distribution trench and 0.3 m in depth at the outflow pipe. The effluent flow within the sand media is characterized as tension saturated flow (Sinclair et al., 2014). The bottom and sides of each SF was lined with a HDPE liner. The tops of the filters were overlain by filter fabric and 0.6 m of topsoil. The effluent from each filter was collected in a downgradient heated building. Flow rates were recorded by individual tipping bucket gauges located in the building and logged on a 30-minute frequency with a Campbell Scientific CR510 data logger (CSI, Logan, Utah, United States).

4.2.2 Field Observed Hydraulic Tracer Tests

Hydraulic tracer tests were conducted at the BEEC on July 30, 2018, during a period of no precipitation. The hydraulic tracer tests were performed with NaBr. A mass of 7.5 g of bromide (Br^-) was injected into each of the three SFs, and the effluent concentration of the Br^- was measured with a gel-filled combination ion selective electrode probe BR43-0001 (Analytical Sensors & Instruments, Inc., Sugar Land, Texas, United States). The HRT of each of the tracer response curves was calculated using the Simpson's 1/3 rule according to the numerical integration methodology described in Fogler (2010). An example of this methodology is provided in Table S1 in Appendix D.

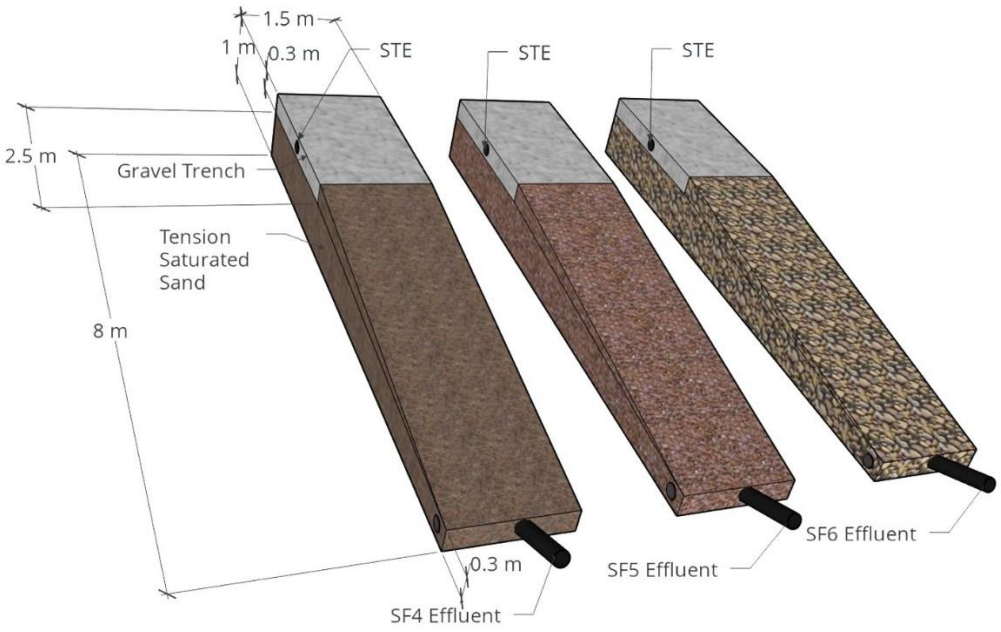

Figure 4.1 3D view of the three lateral flow sand filters SF4 (fine grained), SF5 (medium grained), and SF6 (coarse grained), on a 30% slope, which receive septic tank effluent (STE) into gravel distribution trenches.

4.2.3 Microbial Techniques

Four gene targets were selected to be the focus of the modeling: of 16S rRNA, *sul1*, *tetO*, and *ermB*. The three ARG gene targets were selected based on their elevated concentrations in comparison to the other genes in a suite of 9 ARGs that was monitored within the on-site wastewater treatment system at the BEEC (Hayward et al., 2019). The 16S rRNA gene target was selected as a proxy indicator of the total amount of heterotrophic bacteria in the effluent. The DNA on each type of sand media was extracted to provide an initial concentration of 16S rRNA, *sul1*, *tetO*, and *ermB* gene targets using qPCR. The concentrations of genes in the sand media and the eluent were reported as gene copies per gram and mL, respectively. The detailed qPCR methodology for enumeration of the gene targets is described in Hayward et al. (2019). Liquid samples for DNA extraction were filtered through a 0.45 µm pore size filter using a Millipore Vacuum Manifold and sterilized magnetic filtration funnels. The DNA retained on the filters and from the soil samples (sub-samples of 0.25 g) was extracted with the Qiagen DNeasy Powersoil Kits

(Qiagen Inc., Toronto, Ontario, Canada) according to the methodology described in Hayward et al. (2019).

Numbers of total heterotrophic bacteria and ARB were assessed through plating on agar plates as a complimentary suite of AMR contaminants and to assess the ARB attenuation predicted in the modeling. Total heterotrophic bacteria were selected as a control indicator for bacteria because we were using a standard culture-based method and wanted to have a consistent agar throughout the three different antibiotic plating applications. The selective antibiotics for quantification of ARB were sulfamethoxazole, tetracycline, and erythromycin; of which *sul1*, *tetO* and *ermB*, express resistance to correspondingly. The total heterotrophic bacteria and ARB were enumerated on tryptone soy agar (TSA, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) plates containing no antibiotics, and concentrations of 50 mg/L of sulfamethoxazole, 10 mg/L of tetracycline, or 50 mg/L of erythromycin. The concentrations of antibiotics were selected based on experiments conducted by Mao et al. (2015). Total heterotrophic bacteria and ARB in liquid samples were enumerated using a spot plating method; whereby, a known volume from suitable 10-fold dilutions in peptone saline (PS, 1 g/L peptone, 8.5 g/L NaCl) of the sample was applied to each plate in discrete drops and incubated at 30°C for 24 hours. The resulting colonies were enumerated, and counts reported as CFU per mL. Total heterotrophic bacteria and ARB in sand samples were enumerated by using a sterile metal scoop to collect approximately 1 g of sand, which was then placed in a 15 mL sterile falcon centrifuge tube with 9 mL of PS, vortexed for 2 min, spot plated on the different agars and enumerated as described above.

4.2.4 Sample Collection

The sorption and degradation experiments used the three filter media types from the pilot scale experimental facility (i.e., fine (SF4), medium (SF5) and coarse grained (SF6) sand filter media). Sand samples were collected from the SFs on December 21, 2017 at the BEEC, and were collected from approximately mid way along the length of the filters. The sand was collected by extending a clean and sterilized Dutch auger from the ground surface to the barrier filter fabric which separated the topsoil from the filter media. An access hole was cut in the filter fabric with an exacto-knife. A sand sample of approximately 2 kg was

collected from the top of the filters. STE from the BEEC was sampled and transported chilled at 4°C to the Dalhousie University laboratory in Halifax, Nova Scotia, Canada. Within 24 hours, the STE was centrifuged in 50 mL sterile plastic falcon tubes for 15 min at 1,000 x g to separate the solids from suspension. Approximately 50 mL of the liquid from the centrifuged STE was volumetrically measured, filtered, and the DNA extracted.

4.2.5 Sorption Experiment

The sorption experiment was conducted to characterize the partition coefficients (K_d) of each of the ARGs and ARBs of the three media types. A wet sand mass of 32 g of fine, medium, and coarse sand was placed into 100 mL sterile glass Erlenmeyer flasks. Each flask was spiked with a known volume (50 mL) of the centrifuged STE, covered with an aluminum foil cap, and placed on a shaker table at ambient room temperature (20-22°C). The shaker table was set at a mixing rate of 275 rpm for 1 h to allow for an equilibrium between the solid and liquid phases to be established (Mankin et al., 2007). Each sand type was tested in duplicate. A 100 mL sterile control flask with 50 mL STE and no added sand was used to quantify potential sorption of ARGs to the glass beaker surface. After shaking, the eluent from each flask was poured off into a sterile plastic falcon tube. The eluent samples were then centrifuged for 15 min at 1,000 x g to separate the suspended solids from the solution. The volume of the liquid portion of the eluent sample was measured, and DNA extracted and analyzed for the four gene targets pre- and post shaking. Total heterotrophic bacteria and ARB in the pre-shaking and post-shaken centrifuged eluent were enumerated by spot plating the eluent as per the methodology described above. The gene target analysis and enumeration of the total heterotrophic bacteria and ARB in the liquid of the sand-free control beaker pre- and post shaking was also conducted.

A linear isotherm was used to describe the sorption of 16S rRNA genes, ARGs, total heterotrophic bacteria and ARB onto the sand particles as per Equation 3.

$$S = K_d C \qquad \text{Eq. [3]}$$

Where: S is the number of 16S rRNA genes, ARGs or number of bacterial cells (measured as number of gene copies, or colony forming units, CFU) sorbed per gram of sand (gene

copies/g or CFU/g), K_d is the partition coefficient (mL/g), and C is the concentration of 16S rRNA genes, ARG or number of bacterial cells (gene copies/mL or CFU/mL) in the liquid phase. A single batch sorption experiment was used to determine the K_d values which has limitations, this was done because the relatively low concentrations of ARGs and difficulties in obtaining meaningful data with small sand masses. Therefore, a mass of 32g was experimentally determined to be a threshold under which the K_d's were not well characterized.

4.2.6 Degradation Experiment

The degradation experiment was conducted to characterize appropriate degradation rates, k (d^{-1}), to describe how select ARGs and ARBs degrade at two different temperatures of 4 and 15°C. Small sterile Petri dishes were filled with 7 g of sand; which was sufficient sand to cover the bottom of the Petri dishes with a <2 mm thick layer of sand. Approximately 1.6 mL of the eluent from the centrifuged STE was added to the Petri dishes and sand. The amount of centrifuged STE added to the sand was determined as the minimum amount required to wet the sand but avoid freely draining conditions. The intent was to simulate tension-saturated sand conditions to best represent the sand media conditions present within the effective treatment area of a lateral flow sand filter.

A control plate was prepared for each sand type and temperature with the addition of 1.6 mL of distilled water to a Petri dish filled with 7 g of sand. The weight of the filled Petri dishes was recorded. As the experiment progressed, additional sterile distilled water was added to the Petri dishes to compensate for evaporation. All samples were stored at 4 or 15°C in the dark for the duration of the experiment. The experiment used sacrificial samples in duplicate. Subsequently, the DNA was extracted, and ARB cultured from the sand samples on day 0 and after 1, 3, 6, 14, and 21 days, as per the method described above. On each sampling day, DNA was also extracted from approximately 25 mL of the centrifuged STE sample stored at 4°C. This was done to monitor ARG and ARB degradation in the STE without the sand media influence.

A first order degradation rate was selected to approximate the degradation of the ARGs and ARB based on Equation 4.

$$lnC_t = lnC_0 - kt \quad \text{Eq. [4]}$$

Where: C_t is the concentration at time t (gene copies per mL or g; or CFU/mL), C_0 is the initial concentration (gene copies per mL or g; or CFU/mL), k is the first order rate constant and t is time (days). Throughout, k was calculated by calculation of the slope of the linear regression of the ln[C/C_0] as the independent variable versus time as the dependent variable plot. Generally, the degradation demonstrated linear behavior until at least day 6; rates were calculated using only the linear sections of the curves.

4.2.7 HYDRUS Modeling

4.2.7.1 Model Limitations

The modeling approach taken in this thesis should be considered a 'screening-level' approach to modeling gene targets and bacteria in lateral flow sand filters. The approach that was taken is a very simplified representation of the transport processes occurring in this type of porous media filtration system. The simplified approach considered only first order degradation and adsorption, because as a first attempt it is better to start with a simplified model. The reader must recognize that the processes governing attenuation of genes and bacteria in variably saturated media is more complex than this preliminary assumption. More discussion of next steps in modeling are discussed in the recommendations section in Chapter 5.

4.2.7.2 Model Set-Up

Three 2-dimensional SF models were established in HYDRUS 2D/3D version 2 (PC-Progress, Prague, Czech Republic) with the same side view dimensions as shown in Figure 4.1. The boundary conditions of each SF were as shown in Figure 4.2. A 3-cm thick biological mat layer was included in all the models initially based on observations of this zone during exhumations of the sand filters conducted and described by Hayward et al. (2019). The biological mat layer was black in color, high in organics, and observed to have formed at the interface of the gravel distribution trench and sand filter media (See Figure A.14 for a photograph). However, the biological mat layer was only retained in SF4 and SF6 after the calibration of the hydraulics of the model to the observed tracer response curve. In other words, the models were initially run with biological mat layers, and this

was discarded during the calibration for SF4 and SF6 (e.g., no biological mat for those two filters). HLRs were selected as the average of the loading rates measured in the field for the duration of the tracer tests, which were: 124, 67, and 100 L/d for SF4, SF5, and SF6, respectively. The HLR for SF5 was changed to 124 L/d during the calibration routine. The reason for this change was that the models were calibrated during the tracer test simulations and the HLR in SF5 was low to match the field observed data during this period. There may have been potential for error in the tipping bucket gage measurement of flow during this period. Therefore, this change was justified because the average field measured HLR onto the SFs from July 5, 2017 to July 24, 2018 was 152, 148, and 130 L/d, for SF4, SF5, and SF6, respectively.

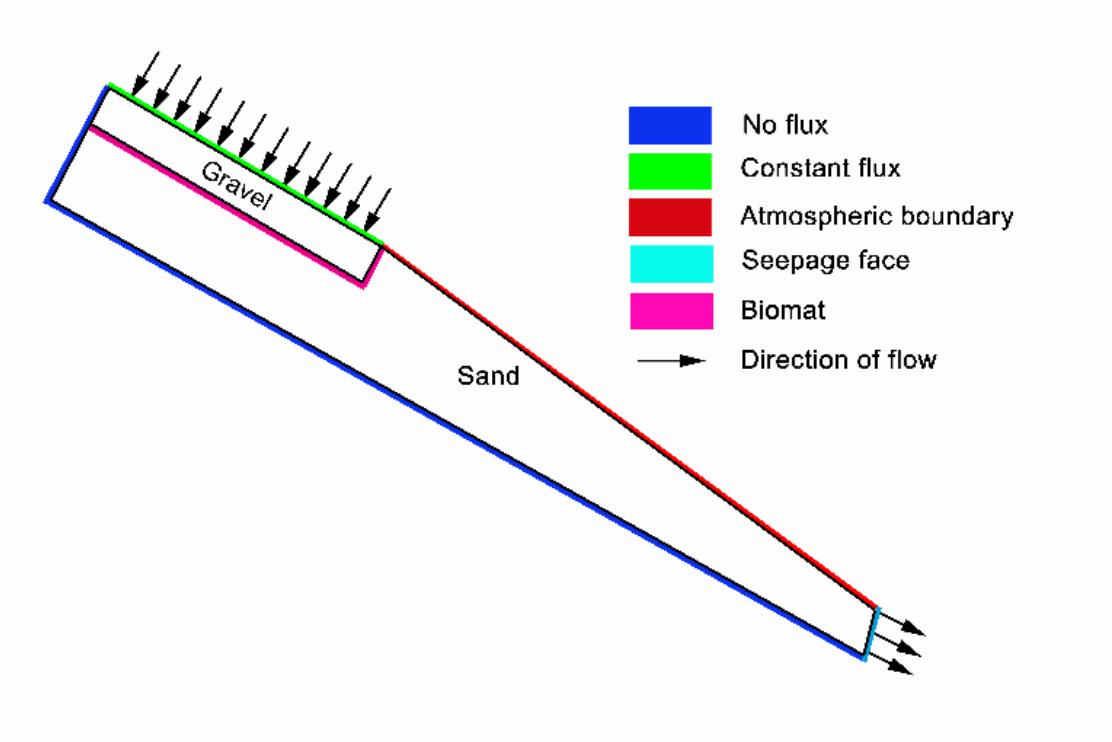

Figure 4.2 The boundary conditions of each of the lateral flow sand filters modeled in Hydrus 2D. The dimensions are shown in Figure 4.1.

The hydraulic and advection-dispersion parameters used in the models were primarily based on those initially specified and selectively calibrated by Sinclair et al. (2014). A finite element (FE) mesh size of 2.5 cm and a finer mesh of 1 cm along the biological mat was specified for SF4 and SF6; this was based on initial work done by Sinclair et al. (2014).

The FE mesh size for SF5 was selected as 4 cm, with a finer mesh size of 1 cm, along the interface of the sand and gravel. The FE mesh generator function built into HYDRUS was used to generate each of the FE meshes.

Each model was set up to run for 25 days with an initial boundary condition of a pressure head of -1000 cm applied to the constant flux boundary to initiate rapid initial infiltration into the sand filters. The models were run in steady state conditions to obtain a steady state inflow and outflow rate as previously optimized by Sinclair et al. (2014). The moisture contents from the final time step of each of the initial steady state model runs were imported as the initial moisture content boundary condition for each of the simulated tracer model runs.

The standard solute transport module was selected with a single porosity model with the modified van Genuchten hydraulic model, assuming no hysteresis. A Crank-Nicholson time weighting scheme and the Galerkin finite element space weighting scheme was specified. For the solute transport module, tortuosity and the Millington and Quirk method were specified. A third type solute flux boundary condition was applied to the flux boundaries to ensure conservation of mass for the simulations.

4.2.7.3 Model Calibration

The hydraulics of each of the three SF models were first calibrated by simulating the conservative movement of bromide (Br^-) tracer through each filter. A pulse of 0.75 mg/cm^3 of Br^- was applied to the gravel layer over 0.08 days for SF4 and SF5, and 0.07 days for SF6, which was equivalent to a total mass of 7.5 g of Br^-. Various hydraulic and advection-dispersion parameters were calibrated as denoted with the square brackets in Table 4.1. The parameters that produced a simulated tracer response curve, which most closely matched the field observed tracer response curve measured in terms of HRT and peak concentration, were selected as base models for all subsequent modeling.

Table 4.1 Assumed and calibrated hydraulic and advection dispersion parameterization values for the three lateral flow sand filters (SFs) (SF4, SF5, and SF6) (values adapted from Sinclair et al., 2014). Values in the square brackets were calibrated to enable matching of the simulated data to the field observed data.

Parameter	Units	SF4				SF5		SF6			
		Gravel	Horizontal biological mat	Vertical biological mat	Sand	Gravel	Sand	Gravel	Horizontal biological mat	Vertical biological mat	Sand
Hydraulic loading rate	cm/d	3.31				[3.31]		3.72			
Residual water content, Θ_r,	cm^3/cm^3	0.056	0.09	0.07	0.026	0.056	0.027	0.056	0.09	0.07	0.023
Saturated water content, Θ_s,	cm^3/cm^3	0.15	0.3	0.35	0.375	0.15	0.365	0.15	0.3	0.35	0.373
Fitted parameter, α,	1/cm	0.145	0.005	0.006	0.145	0.145	0.145	0.145	0.005	0.006	0.145
Fitted parameter, *n,*	—	1.92	2.68	2.68	2.68	1.92	2.68	1.92	2.68	2.68	2.68
Saturated hydraulic conductivity, K_s, (cm/d)		$1.44x10^6$	1.5	2.16	[5000]	$1.44x10^6$	6328	$1.44x10^6$	[900]	[900]	$[1.5x10^4]$
Pore connectivity, *I*, (dimensionless)		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fitted parameter, Θ_m	cm^3/cm^3	0.15	0.3	0.35	0.375	0.15	0.365	0.15	0.3	0.35	0.373
Fitted parameter, Θ_a	cm^3/cm^3	0.05	0.09	0.07	0.026	0.05	0.027	0.05	0.09	0.07	0.023
Water content for K_k, Θ_k	cm^3/cm^3	0.15	0.3	0.35	0.075	0.15	0.109	0.15	0.3	0.35	0.075
Unsaturated hydraulic conductivity, K_k	cm/d	$1.44x10^6$	1.5	2.16	0.411	$1.44x10^6$	16.07	$1.44x10^6$	[150]	[220]	[100]

Parameter	Units	SF4	SF5	SF6							
		Gravel	**Horizontal biological mat**	**Vertical biological mat**	**Sand**	**Gravel**	**Sand**	**Gravel**	**Horizontal biological mat**	**Vertical biological mat**	**Sand**
Bulk media density, p_b	g/cm^3	1.3	1.25	1.25	1.3	1.3	1.3	1.3	1.25	1.25	1.3
Longitudinal dispersivity, D_L	cm	[2.5]	[2.5]	[2.5]	[2.5]	[1.0]	[1.0]	[0.25]	[0.25]	[0.25]	[0.25]
Transverse dispersivity, D_T	cm	[0.125]	[0.125]	[0.125]	[0.125]	[0.125]	[0.125]	[0.05]	[0.05]	[0.05]	[0.05]
Diffusion coefficient, D_w[14]	cm^2/d	0.523	0.523	0.523	0.523	0.523	0.523	0.523	0.523	0.523	0.523
Biological mat thickness	cm	-	3	3	-	[0]	[0]	-	[3]	[3]	-

[14] Halved value from Addiscott (1982).

4.2.7.4 Simulation of ARG and ARB Attenuation

The 16S rRNA and ARG data presented in Hayward et al. (2019) were collected on an approximate bi-weekly basis between July 5 and November 6, 2017, and on July 16 and July 23, 2018 (n = 10). An average of the absolute abundances of gene targets observed in each of the SFs during this sampling period for 16S rRNA, *sul1*, *tetO*, and *ermB* were used as influent concentrations (gene copies per mL) for the solute concentration boundary conditions for simulation of ARG attenuation. The average of the influent ARB concentrations (CFU per mL) of the samples collected on July 16 and July 23, 2018 (n = 4) were used as influent concentrations. These were the pulse application solute concentration boundary conditions for simulation of ARB resistant to sulfamethoxazole (50 mg/L), tetracycline (10 mg/L), or erythromycin (50 mg/L), as well as for total heterotrophic bacteria. The quantification of field observed ARG and ARB concentrations are described in detail in Hayward et al. (2019). Table 4.2 summarizes the average field observed influent and effluent values for each of the sand filters from that study, and the influent values were used as input for each calibrated HYDRUS model.

The experimentally determined partition coefficients and first order degradation rates were applied for each respective microbial target and the models were run using steady-state flow conditions. The first order rate constants for the dissolved phase (μ_w) for each target was set to be equivalent to the first order rate constant of the solid phase (μ_s) throughout. The sorption properties of all layers in the model were homogenously applied as the values obtained from the sorption experiment from the sand media. The ARGs and 16S rRNA genes were modeled together as four separate contaminants over a 50-day simulation period for each of the SFs using first order degradation constants representative of the decay at 4°C and 15°C. The ARB resistant to the three different antibiotics and total heterotrophic bacteria were modeled together as four separate contaminants over a 50-day simulation period for each of the SFs at 4 and 15°C. The simulated effluent values for the 16S rRNA, ARGs, ARB, and total heterotrophic bacteria were then compared to field observed effluent concentrations. A diffusion coefficient of 0.131 cm^2/d was selected for simulation of the 16S rRNA and ARGs based on an approximation of single stranded RNA molecules by Yeh and Hummer (2004). A diffusion coefficient of 1.814 cm^2/d was used

for simulation of the heterotrophic bacteria and ARB based on characterization of the diffusion coefficient of the multi-drug resistant *Pseudomonas aeruginosa* reported by Kim (1996).

4.2.8 Statistical Analysis

ANOVA tests were performed to assess for statistical difference between multiple groups of data. The Shapiro-Wilk normality test was used to assess for normality with non-normal data specified at $p < 0.05$. The Brown-Forsythe method was used to assess for equal variances and a Kruskal-Wallis ANOVA on ranks was performed and significant differences was attributable at $p < 0.05$. A Tukey test was conducted to assess for differences between groups of data with significance assigned at $p < 0.05$. All statistical analysis were conducted with Sigmaplot 14.0 commercial software (Systat Software, Inc., San Jose, California, United States).

Table 4.2 Field observed gene and bacteria influent and effluent concentrations used to parameterize and validate the SFs models from Hayward et al. (2019). The 16S rRNA and ARGs were based on the arithmetic mean of *n* = 10 samples and the total heterotrophic bacteria and ARB were based on the geometric mean of *n* = 4 samples.

Solute	Units	Average influent concentration	Average effluent concentration SF4	Average effluent concentration SF5	Average effluent concentration SF6
16S rRNA	log gene copies/mL	8.7	5.8	6.0	5.8
sul1	log gene copies/mL	5.2	1.1	1.1	1.4
tetO	log gene copies/mL	5.1	0.4	0.6	1.2
ermB	log gene copies/mL	5.9	0.5	0.7	1.0
Total heterotrophic bacteria	log CFU/mL	5.2	2.8	2.8	2.5
Sulfamethoxazole (50 mg/L) ARB	log CFU/mL	5.0	2.5	2.6	2.2
Tetracycline (10 mg/L) ARB	log CFU/mL	4.3	2.3	2.2	1.6
Erythromycin (50 mg/L) ARB	log CFU/mL	4.7	2.4	2.5	1.9

4.3 Results and Discussion

4.3.1 Gene and Bacteria Attenuation Characteristics

Tables 4.3 and 4.4 summarize the results of the degradation experiments for the gene targets and bacteria. Only the linear portions of the decay curves were used to produce the first order degradation rate constants. The first order degradation rates of the gene targets extracted from the sand are shown in Table 4.3. The bacteria decayed generally at variable rates with minimal temperature dependency (Table 4.4). There was no significant difference between the first order degradation rates of the 16S rRNA and ARGs for the three sand media types (one-way ANOVA, $F(2,21) = 1.0$, $p = 0.39$). Likewise, there was no significant difference between the first order degradation rates for total heterotrophic bacteria and ARB for the three sand media types (Kruskal-Wallis one-way ANOVA, $H = 0.64$, $df = 2$, $p = 0.73$).

The assumption of first order rate kinetics for gene target and bacteria attenuation may not be wholly accurate in all cases. This study used only the linear sections of the $\ln(C/C_0)$ versus time curves for determination of the first order rate constants of the gene targets and bacteria. The complete dataset from the degradation and sorption experiments is tabulated in Tables S9 – S11 in the supplemental spreadsheet as described in Appendix D. In addition, the first order degradation curves are presented in Figures A.19 to A.34 of Appendix A. It should be noted that in some instances, portions of the $\ln(C/C_0)$ versus time curves were not linear and displayed biphasic rate kinetics, which is characterized by a high degradation rate initially followed by slowed decay. This display of biphasic rate kinetics is now hypothesized as a behavioral survival characteristic for some bacteria residing in relatively low density bacterial environments and exposed to depleting resources; whereby, many bacteria initially die-off, while some bacteria are able to starve themselves and persist for longer periods of time, and then die-off at a slower constant rate (Phaiboun et al., 2015). A similar trend in gene degradation may likely be expected in some cases, as most genes in the effluent from these sand filter systems were characterized as being housed intracellularly (Hayward et al., 2019).

The results of the sorption experiment are summarized in Table 4.5. There was little difference between partition coefficients for ARGs versus ARB. The one-way ANOVA on the sand filter media type indicated no significant difference between partition coefficients ($F(2,9) = 3.0$, $p = 0.10$). The exception was between medium and coarse sand for the ARB, with the medium sand having lower partition coefficients than coarse sand (Kruskal-Wallis one-way ANOVA, $H = 6.5$, $df = 2$, $p = 0.03$).

Table 4.3 First order degradation rates (*k*) for 16S rRNA, *sul1*, *tetO*, and *ermB* with fine, medium, and coarse sand and without sand (STE) at 4 and 15°C.

		Gene targets							
		16S rRNA		*sul1*		*tetO*		*ermB*	
Sand media type	Temp. (°C)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)
STE (no sand)	4	0.18	0.97	0.14	0.98	0.35	0.97	0.30	0.97
Fine	4	0.27	0.82	0.41	0.96	0.28	0.91	0.29	1.00
	15	0.15	0.94	0.15	0.99	0.18	0.98	0.07	0.93
Medium	4	0.13	0.99	0.31	0.99	0.30	0.96	0.16	0.99
	15	0.18	0.93	0.16	0.97	0.30	0.99	0.21	0.85
Coarse	4	0.23	0.99	0.29	0.99	0.35	0.96	0.41	0.93
	15	0.16	0.94	0.18	0.90	0.27	1.00	0.32	1.00

Table 4.4 First order degradation rates (*k*) for total heterotrophic bacteria (on TSA) and ARB with resistance to sulfamethoxazole (50 mg/L), tetracycline (10 mg/L), or erythromycin (50 mg/L) in STE (no sand) and fine, medium, and coarse sand stored at 4 and 15°C for 21 d.

		Bacteria							
		TSA		*Sulfamethoxazole*		*Tetracycline*		*Erythromycin*	
Sand media type	Temperature (°C)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)	*k* (d^{-1})	R^2 (adjusted)
STE (no sand)	4	0.22	0.92	0.04	na[15]	0.07	0.95	0.06	0.82
Fine	4	0.10	0.97	0.13	0.96	0.17	na	0.06	0.82
	15	0.10	0.83	0.46	na	0.17	0.99	0.13	0.94
Medium	4	0.32	na	0.06	0.98	<DL	na	0.32	na
	15	0.09	0.75	0.18	na	<DL	na	0.16	na
Coarse	4	0.15	1.00	0.42	na	0.08	na	0.07	na
	15	0.09	0.96	0.27	na		na	0.06	na

[15] na denotes non-applicable due to only two data points being used to obtain the rate constant.

Table 4.5 Partition coefficients (K_d) for the genes and bacteria in the three sand medias.

Parameter	Fine sand	Medium sand	Coarse sand
	K_d (mL/g)		
16S rRNA	0.313	0.083	0.290
sul1	0.236	0.153	0.179
tetO	0.125	0.076	0.540
ermB	0.677	0.094	0.712
Total heterotrophic bacteria	0.044	0.032	0.105
Sulfamethoxazole (50 mg/L) ARB	0.096	0.075	0.145
Tetracycline (10 mg/L) ARB	0.113	0.073	0.431
Erythromycin (50 mg/L) ARB	0.173	0.073	0.339

4.3.2 Model Calibration

The best fits of the simulated models to the field observed tracer response curves, which represent the Br^- effluent concentrations discharging from each of the SFs, are shown in Figure 4.3. The simulated tracer arrival was delayed compared to the field observed data. The likely reason for this delayed arrival of simulated Br^- is due to the boundary condition type specified. Whereby, the solute flux type boundary does not immediately apply an initial C_o concentration to the boundary upon commencement of the simulation. This creates a delay in the breakthrough curve of the Br^- compared to a time zero injection in a field setting. As a result, the peak concentration and HRT values of the field observed, and simulated tracer response curves, were used to select the closest fit for subsequent modeling. The residence time distribution metrics for the field observed and best fit simulated results are shown in Figure 4.3. The arrival times of the simulated tracer response curves were truncated by 1.24, 1.96, and 1.33 days to account for the lag between simulation time zero and tracer application at the boundary condition as shown in Figure 4.3. The truncation was required due to the lag time between the solute tracer pulse and accuracy of the initial C_0 concentrations with the solute flux boundary type condition application. The simulated HRTs were developed based on the un-truncated tracer response curves.

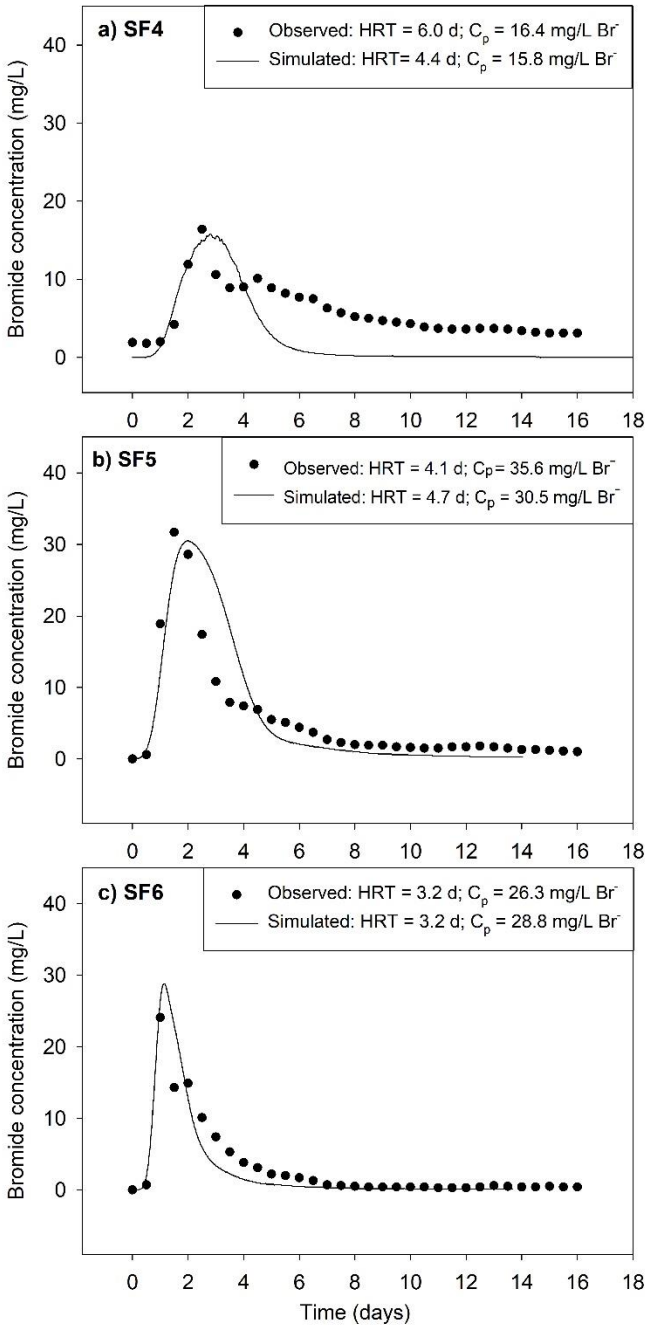

Figure 4.3 The effluent tracer response curves of observed versus truncated simulated bromide (Br^-) concentrations for 7.5 g Br^- mass injections for the sand filters (SF). The peak Br^- concentration of the curve is denoted by C_p.

4.3.3 Modeling Results

4.3.3.1 Gene Targets

Figure 4.4 shows the gene target model results with the field-observed average effluent concentrations and the simulated effluent concentration from each of the three sand filters. The field observed and simulated 16S rRNA had comparable effluent concentrations. There was no difference in field observed and simulated absolute abundances for all filters and gene targets apart from *sul1* at 15°C. Generally, the modeled ARG effluent concentrations overestimated all ARG targets for SF5, *sul1* for SF6 (Figure 4.4b), and *tetO* for SF4 (Figure 4.4c). This may be due to the model considerations pertaining to the biological mat layer. Notably, SF4 and SF6 were modeled with a 3 cm thick biological mat, whereas, SF5 had no representation of this zone. The model results may be improved by attempting to characterize the sorption and degradation properties of the ARGs in the biological mat layer. It is hypothesized that the biological mat layer may provide a mechanism for removal of ARGs that required further characterization to improve the representation of this layer in the model. The simulated results with the decay rate at 4°C more closely matched the observed data for *sul1*. Of note, in all cases except with *tetO* and *ermB* for SF6, the model results produced effluent concentrations that were higher than observed; which, from a risk quantification perspective is preferable because the modeled effluent concentrations are conservative.

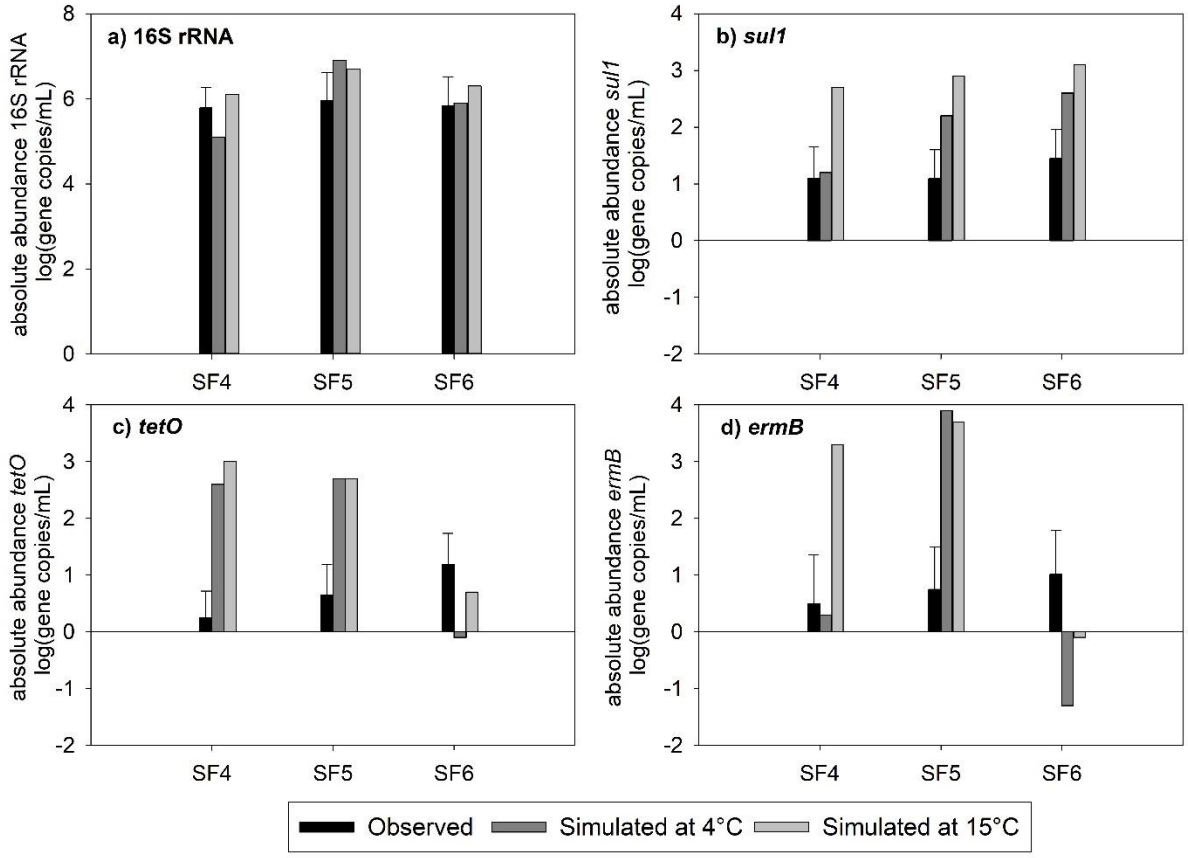

Figure 4.4 Results of the field observed ARG effluent concentrations and the modeling simulations for each sand filter (SF) with a decay rate at 4 and 15°C. The observed data bar represents the mean value of field observed data and the error bar represents one standard deviation of n = 10 samples.

4.3.3.2 Bacteria

The results of the total heterotrophic bacteria and antibiotic resistant bacteria modeling are illustrated in Figure 4.5. Overall, the model results compared well with the field observed results. No differences between the field observed and simulated data were notable for the bacteria resistant to 50 mg/L of sulfamethoxazole (Figure 4.5b), 10 mg/L of tetracycline (Figure 4.5c), or 50 mg/L of erythromycin (Figure 4.5d). The simulated results for bacteria cultivated on the TSA, representative of total heterotrophic bacteria, were greater than the field observed data. Despite this difference, the simulated results were still close in value with an average difference for all three filters of less than 1 log for heterotrophic bacteria.

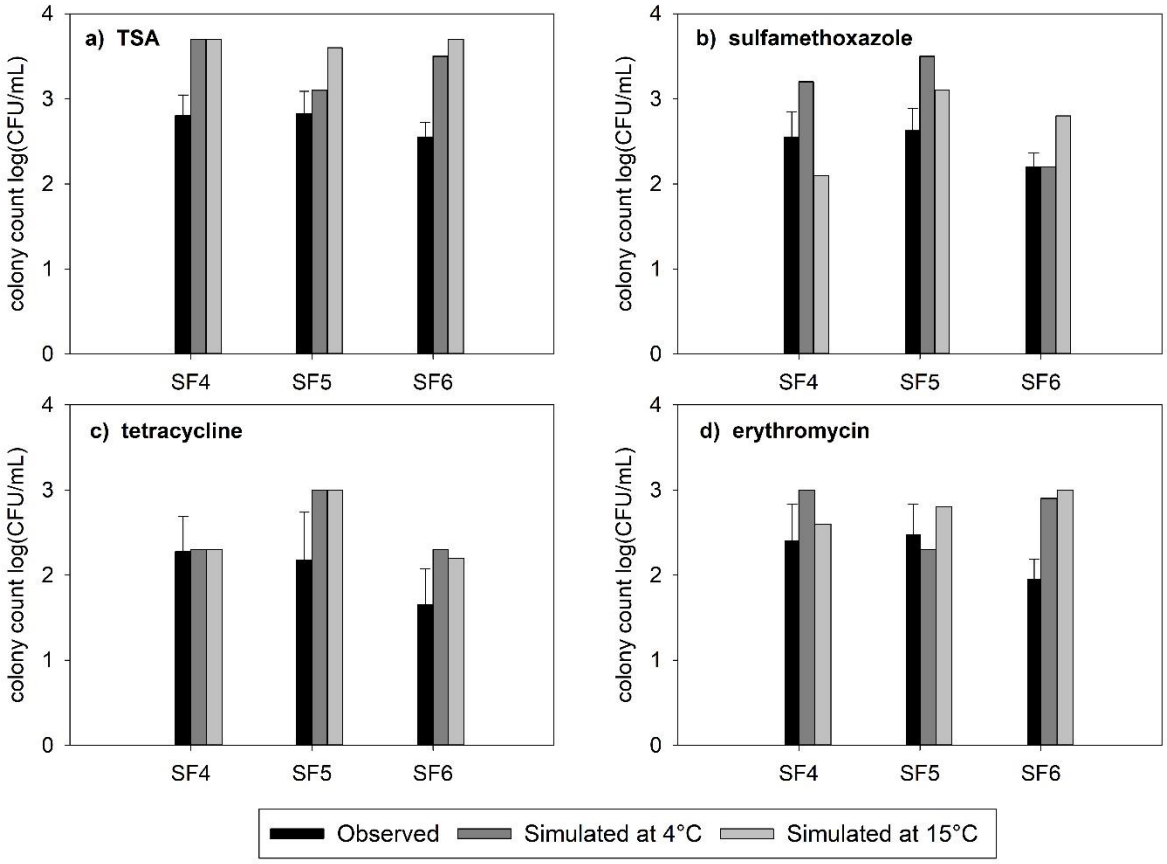

Figure 4.5 Results of the simulations of sand filter (SF) effluent concentrations of: a) total heterotrophic bacteria (TSA); and bacteria resistant to b) 50 mg/L sulfamethoxazole; c) 10 mg/L of tetracycline; and d) 50 mg/L of erythromycin. The model results represent bacterial decay rates at 4 and 15°C compared to field observed data for each sand filter (SF). The bar graphs represent the mean of the field observed data with the error bars representative of one standard deviation (*n* = 4 samples).

4.4 Discussion of the Model Approach

The preliminary approach used for representation of the 16S rRNA, ARG, total heterotrophic bacteria, and ARB in the lateral flow sand filters was simplified with the representation of bacterial degradation as a first order kinetic reaction in all cases. This screening-level approach produced simulated effluent concentrations from each sand filter which were in most cases comparable to the field observed results. Figures 4.6 and 4.7

shows the field observed versus simulated values for the gene targets and bacteria. These plots demonstrate that the modeling approach used was adequate to represent attenuation of bacteria in the lateral flow sand filters with an r^2 of 0.98. However, the gene target simulation was weaker with only an r^2 of 0.79. Other authors such as Balkhair (2017) and Morales et al. (2015) have used the attachment – detachment module of HYDRUS to simulate bacteria attenuation in porous media, which specifies first order attachment and detachment coefficients of the bacteria. This module was not explored for this study; however, future work could be done to characterize the attachment and detachment rates from the biological mat surface with laboratory bench-scale experiments.

The analytical results could be used to validate the simulated results using the attachment – detachment module to explore the role of the biological mat in gene and bacteria attenuation. The role of horizontal gene transfer has been identified as a major contributor to the evolution and spread of AMR in the environment (Martinez, 2008). HGT is defined as the transmission of genetic information via transformation, transduction or conjugation between bacteria (Founou et al., 2016). Future modeling efforts should attempt to characterize the rates of ARG transfer via HGT, with consideration of mobile genetic elements, and vertical gene transfer from cell division.

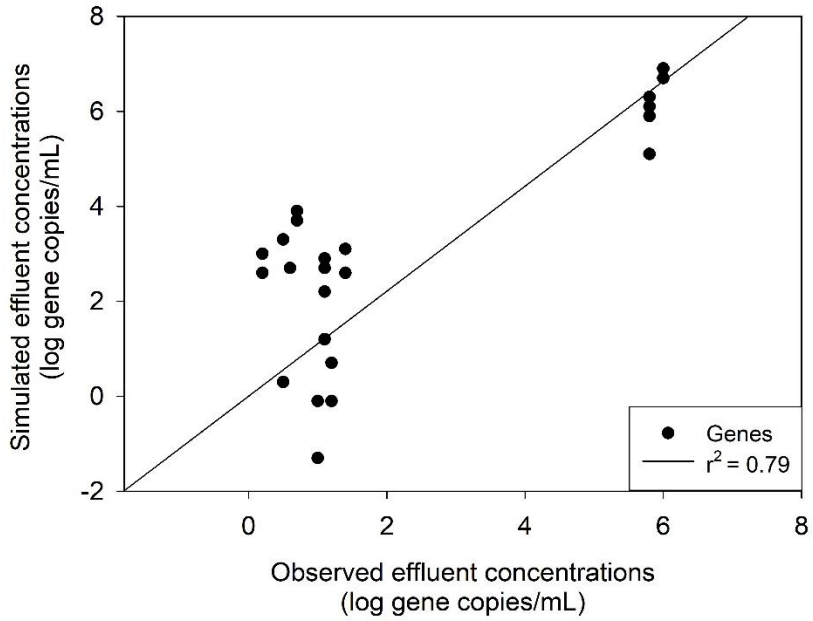

Figure 4.6 Observed versus simulated gene target concentrations.

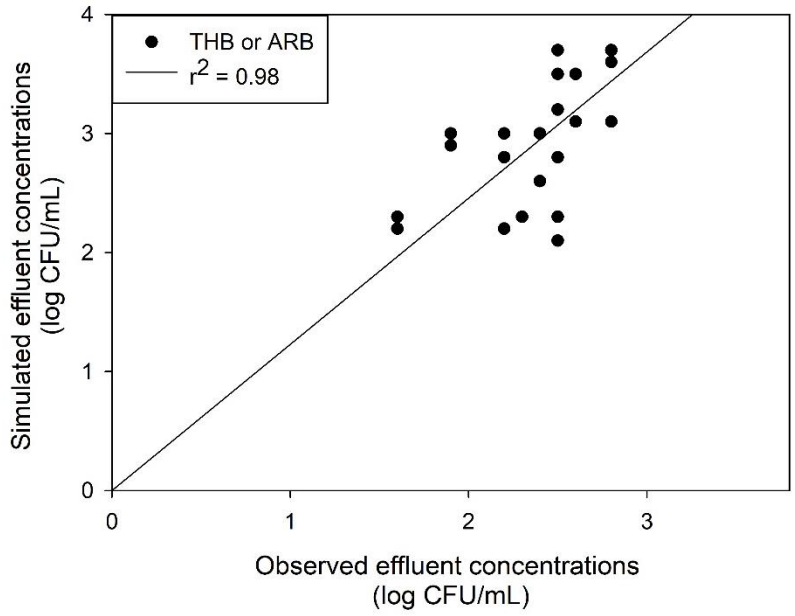

Figure 4.7 Observed versus simulated total heterotrophic bacteria and ARB concentrations.

4.5 Conclusions

In summary, the preliminary HYDRUS 2D model was effective for the simulation of the transport and attenuation of ARGs and ARB in lateral flow sand filters. The preliminary models tended to overpredict the gene targets, and this is hypothesized to be due to the inadequate representation of the role of the biological mat. The models produced little difference in field observed and simulated data for the ARB. This serves as a proof of concept that this type of physically based model can be used as a tool to characterize transport and attenuation of genes and ARB for reduction of antimicrobial resistance in hydrogeological settings, but more work is needed to refine the approach. The preliminary model at times overestimated effluent ARG concentrations and this could be potentially improved with a better understanding of the attenuation properties of the biological mat layer at the sand and gravel interface.

There were no significant differences in the degradation rates or partition coefficients between the three sand filter media types; except for between the medium and coarse-grained sand for the ARB partition coefficients, whereby the medium grained sand had lower partition coefficients. The first order rate constants derived at 4°C were slightly better than 15°C to simulate the gene target effluent concentrations.

Bench-scale experiments as per described within this study could be used to characterize other common soil types to enable prediction of the dissemination of AMR contaminants from anthropogenic and agricultural sources in the near-surface environment. Further work is recommended to characterize the role of the biological mat in the attenuation of agents of antimicrobial resistance. The biological mat is hypothesized to play a key role in attenuation of the ARGs and ARB and its sorption and degradation characteristics are not well understood. A better understanding of the role of biological mat may enable optimization of wastewater treatment technologies to target and treat for contaminants of AMR. In addition, capabilities for model prediction of rates of horizontal gene transfer should be developed.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The thesis was structured to answer specific research questions related to the fate of AMR determinants in terrestrial environments. Specifically, the research involved field-based studies of full-scale municipal wastewater treatment systems in the Canadian Arctic, pilot-scale experimental studies at a passive domestic wastewater treatment facility, complementary laboratory bench-scale experiments, and numerical modeling. The following section reviews the major contributions of the research. This section is structured to show how the studies addressed each of the original research questions.

Research Question 1: Persistence of Determinants of Antimicrobial Resistance Downgradient of Rural and Remote Sources

The fate and transport of determinants of AMR were studied in two wetlands in Nunavut, Canada. These two tundra wetlands were located downgradient of passive municipal wastewater treatment systems consisting of wastewater stabilization ponds (e.g., sewage lagoons) in two geographically remote communities. While some studies have examined AMR hot spots in urban environments, at the time of this study, there had been limited research conducted in remote Canadian communities that have high clinical incidence of AMR infections (Daley et al., 2014). The tundra wetlands receive primary treated effluent from the wastewater stabilization ponds during the summer months, and provide ancillary treatment before discharge into marine receiving environments, which are often used recreationally and for sustenance fishing by the members of the communities (Hayward et al., 2014). These tundra wetland treatment areas represent natural terrestrial environments that are variably saturated. Indicators of AMR were tracked within the two wetlands by analysing for determinants of AMR (i.e., ARGs and *int1* gene) and subsequently comparing results to background concentrations of the AMR indicators from reference wetlands with limited anthropogenic impacts.

The results from this study demonstrated that concentrations of determinants of AMR were higher than the reference concentrations in both wetlands. The wetland hydrology strongly

affected the persistence and concentrations of AMR discharging into the marine receiving environment. Generally, during the spring freshet, the flows in the wetlands were elevated due to meltwater runoff. This decreased the HRT of the wetlands (< 2 days) and led to a higher risk period for dissemination of determinants of AMR in the environment. To the best of the author's knowledge, this study was the first to characterize the first order rate constants to describe ARG attenuation in wetlands used for wastewater treatment.

Research Question 2: Characterization of the Environmental Resistome

Understanding the environmental resistome is important for assessing potential hot spots from anthropogenic sources in environmental settings. The arctic environment is one potential environmental setting where the environmental resistome may be impacted to a lesser degree by anthropogenic activities. The environmental resistome in the Canadian arctic was partially characterized at the two study sites of Sanikiliuaq and Naujaat, Nunavut. Consultation with local community members was conducted to attempt to locate areas of the tundra which are relatively un-impacted by anthropogenic activities. Concentrations of determinants of AMR were determined with a target panel of ARGs and complementary genes from the soil and water in the reference tundra wetlands.

Results demonstrated that some determinants of AMR were elevated above detection limits for some key gene targets. This suggests that even in relatively un-impacted geographically isolated regions, there is evidence for either (i) ancient AMR in the soil and water microbiome, or (ii) far-reaching anthropogenic impacts even in unsuspecting environments. This baseline characterization of determinants of AMR in remote and geographically isolated arctic environments is an important contribution for future AMR studies.

Research Question 3: Performance of Alternative Wastewater Treatment Technologies for Removal of Antimicrobial Determinants

Alternative and passive technologies for municipal and domestic wastewater treatment in rural and remote areas include the use of on-site wastewater treatment systems. The potential for these OWTS to create hot spots of AMR in the environment have not been well studied. To explore this question, a pilot-scale experimental facility located in Nova

Scotia, Canada, was used to test the effectiveness of lateral flow sand filters receiving septic tank effluent for treatment of determinants of AMR. The influent and effluent from six lateral flow sand filters were tested for determinants of AMR ten times from 2017 to 2018.

Results showed that the lateral flow sand filters were generally effective at attenuating 7 ARGs to below 2 log gene copies per mL. Bacteria resistant to either sulfamethoxazole, erythromycin, or tetracycline were reduced by approximately 2.3 log CFU per mL compared to influent concentrations from the septic tank. Grain size of the sand media used in the filters and the slope of the filters (5 and 30%) were observed to have little to no effect on treatment performance of the filters. There was one exception, which was the fine-grained sand filter positioned on a 5% slope, which was susceptible to hydraulic failure. The well-functioning filters posed low risk of AMR contamination to the surrounding subsurface environment. It should be mentioned that there remains AMR contaminants that could persist at relatively at low levels in the vadose and saturated zones downgradient of these types of treatment systems.

Research Question 4: Mechanisms and Rates of Attenuation of Determinants of Antimicrobial Resistance in Porous Media

The intracellular and extracellular compartmentalization of ARGs is an important consideration when conceptualizing the attenuation and transport mechanisms of ARGs (Yuan et al., 2019). The risk of cell-free ARGs persisting after advanced municipal wastewater treatment and potentially contributing to elevated AMR in the environmental resistome was recently identified as an issue (Zhang et al., 2018b). The quantification of cell associated and cell-free ARGs was conducted for the influent and effluent of the lateral flow sand filters. This experiment demonstrated that the ARGs detected in the effluent from the sand filters were primarily housed intracellularly. Therefore, the risk of molecularly small and potentially mobile microbial contaminants associated with AMR is reduced with this type of on-site domestic wastewater treatment technology.

Bench scale experiments were conducted for the characterization of the rates of attenuation of determinants of AMR in the near-surface terrestrial environment. This research

objective required quantification of the sorption and degradation properties of ARGs and ARB in porous media. The partition coefficients of *sul1*, *ermB*, and *tetO*, bacteria resistant to 50 mg/L of sulfamethoxazole, 10mg/L of tetracycline, and 50 mg/L of erythromycin, were characterized with the three sand media types used in the sand filter experiments. The first order degradation rates for these determinants of AMR were also quantified with sacrificial sample experiments conducted at 4 and 15°C. These partition coefficients and first order degradation rates showed little difference between the three sand media types, which explains why there was little difference observed in treatment performance between the pilot-scale filters.

Research Question 5: Simulation of Attenuation of Determinants of Antimicrobial Resistance with Conventional Reactive Transport Models

A screening-level simulation of determinants of AMR including select ARGs and ARB in the lateral flow sand filters was conducted with HYDRUS software. The preliminary models were parameterized and calibrated with data collected in pursuit of research questions 3 and 4. The simulated model results were verified against observed effluent concentrations for determinants of AMR from the pilot-scale experiment on the lateral flow sand filters. The preliminary HYDRUS model was capable of predicting effluent concentrations that were similar to field-observed results for the sand filters. Improvements may be made by better representing the attenuation properties of the biological mat layer.

5.2 Major Contributions

In brief, the major contributions of this thesis research were:

- Identified that surface water hydrology is intrinsically linked to AMR dissemination in the environment;
- Calculated first order rate constants for attenuation of ARGs in wetlands used for wastewater treatment;
- Documented the baseline environmental resistome of soil and water in Canadian arctic tundra environments;
- Assessed passive on-site wastewater treatment technology for removal of determinants of AMR and found that lateral flow sand filters treat adequately;
- Showed that the majority of ARGs detectable in the on-site wastewater treatment technology tested reside intracellularly;
- Provided one of the first characterizations of sorption and degradation properties of determinants of AMR in soil media; and
- Demonstrated to the best of the author's knowledge, the first attempt of using a computer model to simulate attenuation of ARGs and ARB in porous media wastewater treatment systems.

5.3 Recommendations

Recommendations for future work that have arisen from this research include:

5.3.1 Improvements to Tundra Wetland Treatment Areas

Typically, the tundra wetland treatment areas in the Canadian North are comprised of a 'natural' area of the tundra or taiga that has been shown to provide ancillary treatment of municipal wastewater following primary treatment (Hayward et al., 2014; Balch et al., 2018). Communities which rely on wetlands for provision of secondary treatment following settling in a wastewater stabilization pond, could benefit from small design improvements such as construction of flow diversion berms and baffles placed strategically to increase the hydraulic retention time of the wetland treatment areas. Other improvements include influent flow rate control measures and distribution pipes to apply the influent over a broader spatial expanse. This would improve the attenuation and decrease the risk posed by AMR contamination in receiving environments, which are used by residents for recreation and sustenance food. Research by Daley et al. (2019) showed through a QMRA that treatment wetland areas contributed the highest probability of acute gastrointestinal illness associated with a single exposure event by activities such as snowmobiling, riding all terrain vehicles (ATV), and transiting by foot across the wetlands. Engineering improvements to the wetland treatment areas would help to increase treatment and thereby decrease the risk of emerging contaminants such as ARGs and ARB from adversely impacting community health. Signage with maps of the delineated wetland treatment areas and receiving environments would be useful to warn residents of the extent of the treatment areas. This would help to reduce the risk of public exposure to spatial areas that have elevated risk of AMR hot spots.

5.3.2 Documentation of Baseline Environmental Resistome with Standardized Methods

Efforts are being made internationally to characterize the baseline ancient and current levels of ARGs and ARB in different compartments of the environment. This study characterized baseline levels in soil and water in arctic tundra wetlands. More research is needed to continue to characterize the current and historical baselines of determinants of

AMR in the environment (Rothrock et al., 2016). Furthermore, there is a need to standardize the key determinants and methods to analyze for AMR (Larsson et al., 2018). Ideally, a consensus-based approach could be established for each environmental compartment and adopted moving forward to standardize surveillance methods. This could involve a mixed method qualitative and quantitative approach using qPCR for key AMR indicators, metagenomic analysis for broad microbial overviews, and culture-based detection of ARB indicators.

5.3.3 Lateral Flow Sand Filtration

The research demonstrated that lateral flow sand filters are effective for attenuation of determinants of AMR. Therefore, the risk of contamination of groundwater resources is low for this type of on-site wastewater treatment technology. Efforts should be made to ensure that on-site wastewater treatment systems have properly functioning hydraulics to protect groundwater resources. Hydraulic failures of these systems were demonstrated to represent a high-risk scenario for AMR contamination of the vadose zone.

5.3.4 Mobilization and Attenuation of Determinants of Antimicrobial Resistance in Groundwater

The partition coefficients and first order degradation rates for determinants of AMR were determined experimentally for three sand types used as a filtration media for lateral flow sand filters. Quantification of the partition coefficients and first order degradation rates for other types of soil media would be worthwhile. These would be helpful parameters for refinement of plot-scale and watershed-scale contaminant fate and transport simulation and quantitative microbial risk assessments for AMR contamination. In some cases, the degradation of bacteria appeared to follow biphasic behavior, and this could be further explored to see if this kinetic relationship would be a better representation in numerical models. An emerging area of concern for public health is the risk of ARB and associated mobile genetic elements migrating into groundwater and further research is needed on the rates of horizontal gene transfer in groundwater settings (Bradford & Harvey, 2017). Modeling tools—such as HYDRUS used in this research—should be further explored for simulating attenuation and transport of determinants of AMR in the vadose zone and in groundwater environments. For instance, there are modules within HYDRUS which could

be used to simulate different compartmentalization of ARGs, such as the attachment-detachment module. Quantification of the rates of horizontal gene transfer and persistence of uptaken genetic elements in soil environments should also be conducted and incorporated into modeling frameworks.

5.3.5 Investigation of the Role of Biological Mats

The HYDRUS modeling indicated that using the partition coefficients and first order degradation rates derived from experiments on the sand filtration media led to overprediction of effluent concentrations of ARGs. A hypothesis for this observed discrepancy is that the field-scale systems have a biological mat at the interface of the gravel distribution trench and the sand media, which may provide favorable conditions for bacterial sorption and colonization. Degradation of ARGs by nucleases may occur at the biological mat more efficiently than elsewhere in the sand filters. Research into the adsorption characteristics, bacterial diversity, and role of the biological mat in attenuation of determinants of AMR warrants further investigation. Understanding the role of the biological mat in the attenuation of determinants of AMR could lead to improvements in wastewater treatment technology and improved prediction with computer models.

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APPENDIX A — SUPPLEMENTAL FIGURES

Figure A.1 Wetland treatment area in Naujaat, Nunavut looking upgradient towards inlet (Photograph taken on August 27, 2016).

Figure A.2 Wetland treatment area in Sanikiluaq, Nunavut taken from toe of inlet berm looking downgradient (Photograph taken on September 7, 2016).

Figure A.3 Stream gauging mid-point in Naujaat, Nunavut looking downgradient (Photograph taken on June 11, 2016).

Figure A.4 Water quality measurement in Sanikiluaq, Nunavut (Photograph taken on May 28, 2016).

Figure A.5 Soil sampling in Naujaat, Nunavut (Photograph taken on August 27, 2016).

Figure A.6 Reference wetland in Naujaat, Nunavut (Photograph taken on August 28, 2016).

Figure A.7 Mid-point looking downgradient towards the outlet in the wetland treatment area in Naujaat, Nunavut (Photograph taken on August, 28, 2016).

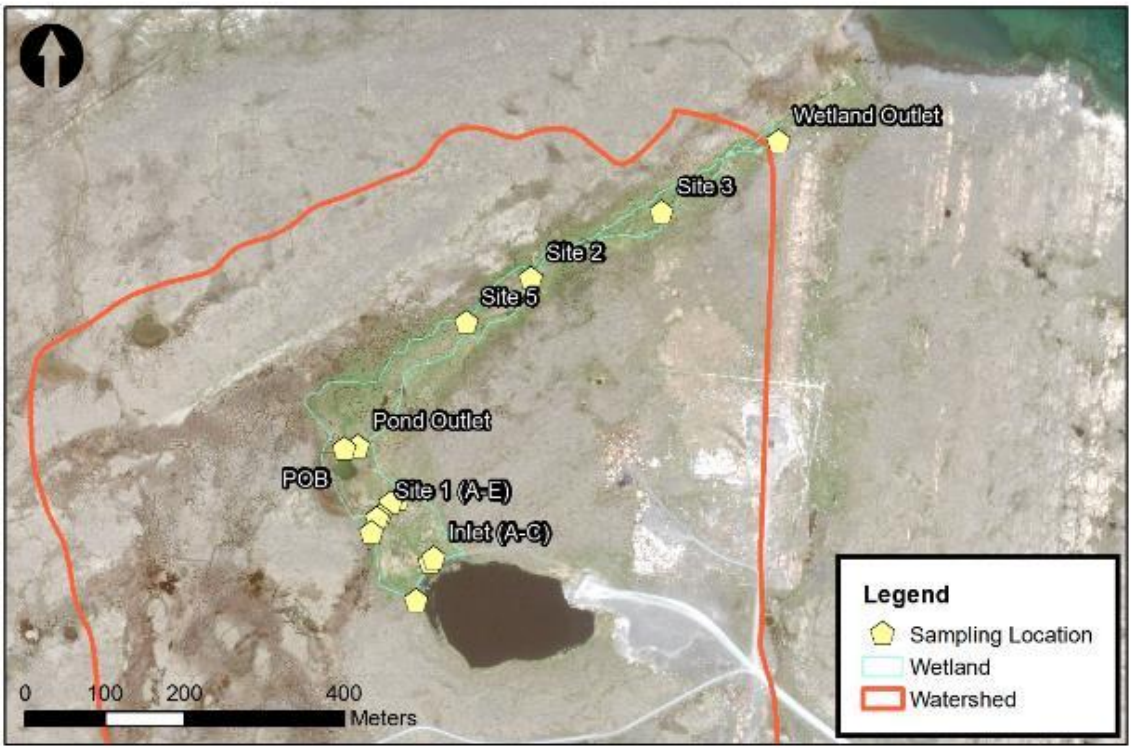


Figure A.8 Map of the detailed sampling locations within the wetland treatment area in Sanikiluaq. This corresponds with Tables S1 and S2 in Appendix D.

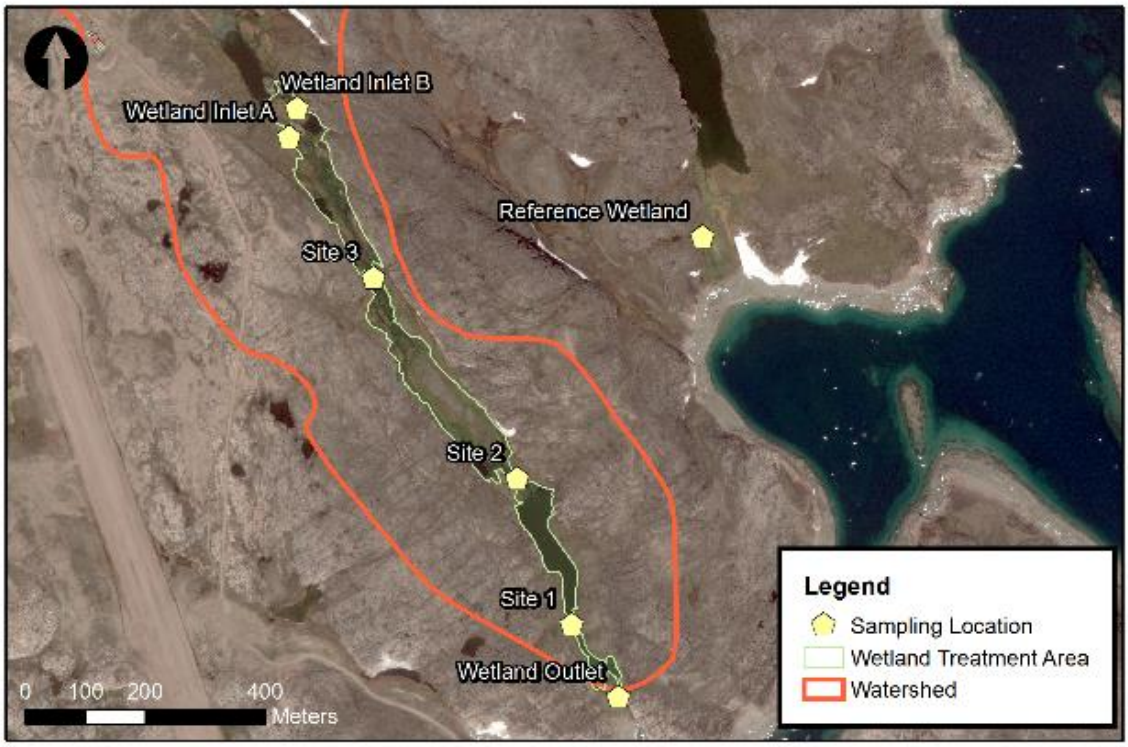
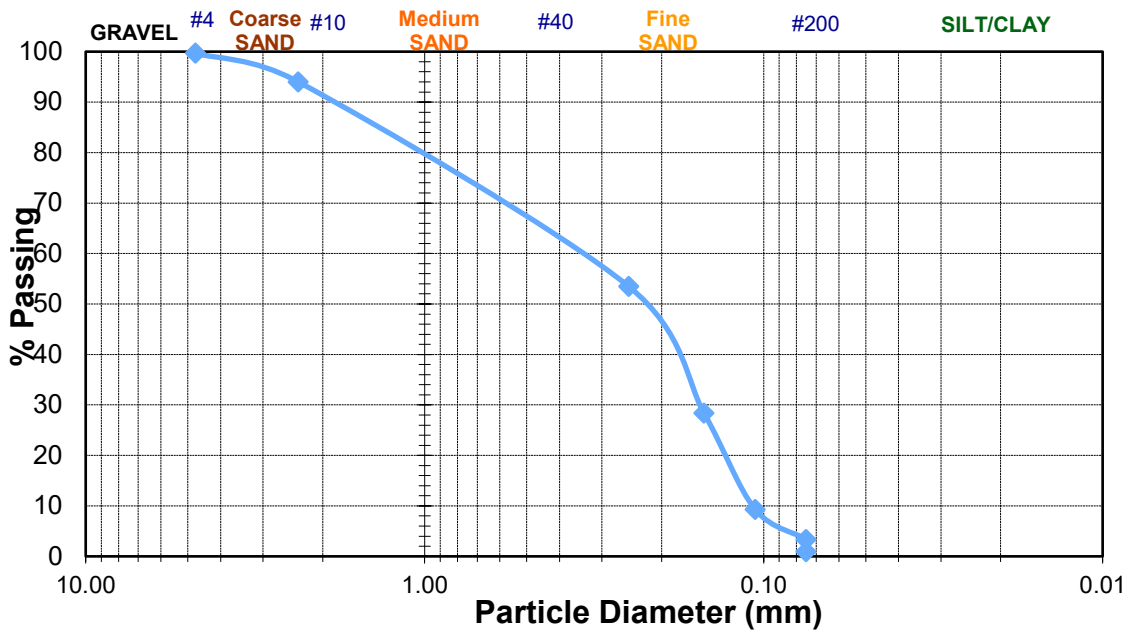


Figure A.9 Map of the detailed sampling locations within the wetland treatment area in Naujaat. This corresponds with Tables S1 and S2 in Appendix D.

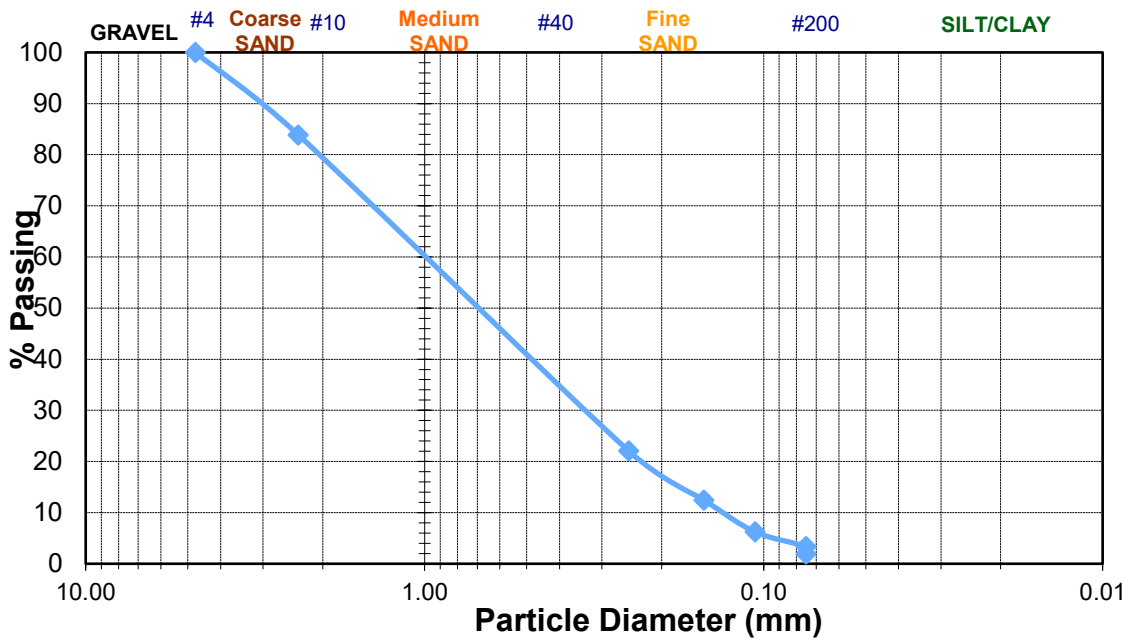


Grain Size Distribution Curve

Results:

% Gravel:	0.3	**D_{10}:**	0.12	**C_u:**	2.92
% Sand:	97.4	**D_{30}:**	0.16	**C_c:**	0.610
% Fines:	2.3	**D_{60}:**	0.35		

Figure A.10 Grain size distribution of fine-grained sand media used in sand filter 1 and sand filter 4.

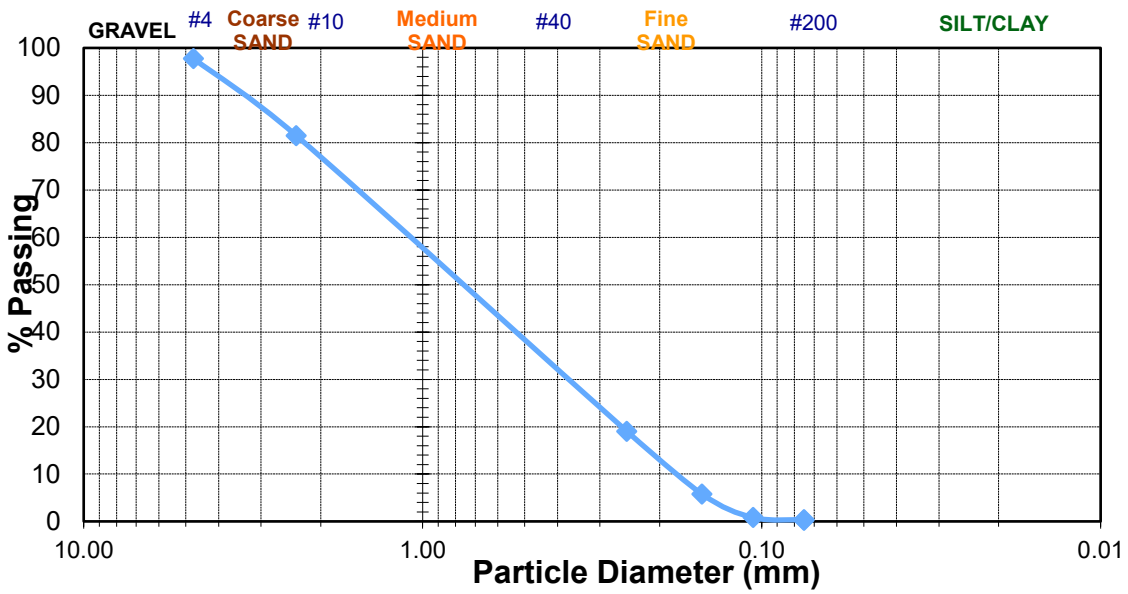


Grain Size Distribution Curve

Results:

% Gravel:	0.0	**D_{10}:**	0.14	**C_u:**	7.14
% Sand:	96.6	**D_{30}:**	0.35	**C_c:**	0.875
% Fines:	3.4	**D_{60}:**	1		

Figure A.11 Grain size distribution of medium-grained sand media used in sand filter 2 and sand filter 5.



Grain Size Distribution Curve

Results:

% Gravel:	2.2	**D_{10}:**	0.18	**C_u:**	8.89
% Sand:	97.4	**D_{30}:**	0.37	**C_c:**	0.475
% Fines:	0.4	**D_{60}:**	1.6		

Figure A.12 Grain size distribution of coarse-grained sand media used in sand filter 3 and sand filter 6.

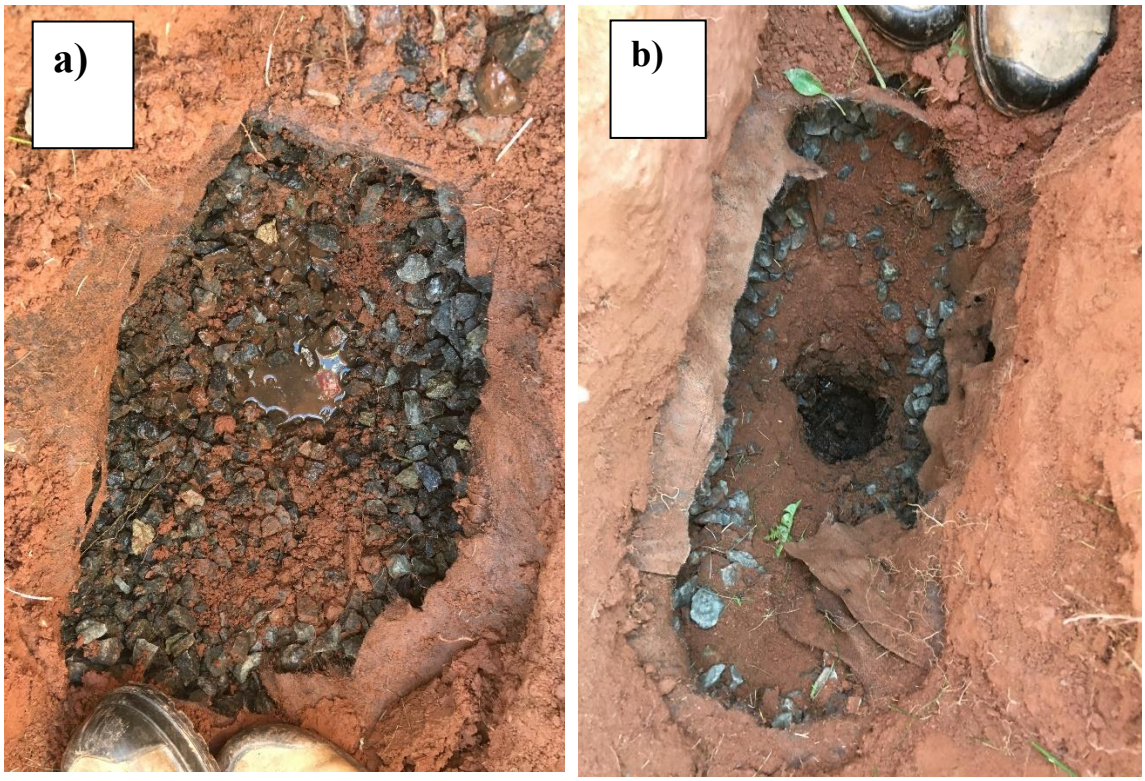


Figure A.13 a) The gravel distribution trench of sand filter 1 showing saturated conditions on top of the biological mat (Photograph taken on October 22, 2018), and b) The gravel distribution trench of sand filter 3 showing the core into the biological mat and unsaturated conditions (Photograph taken on October 1, 2018).

Figure A.14 Photograph of the biological mat on the gravel from the distribution trench of the lateral flow sand filter taken on October 15, 2018.

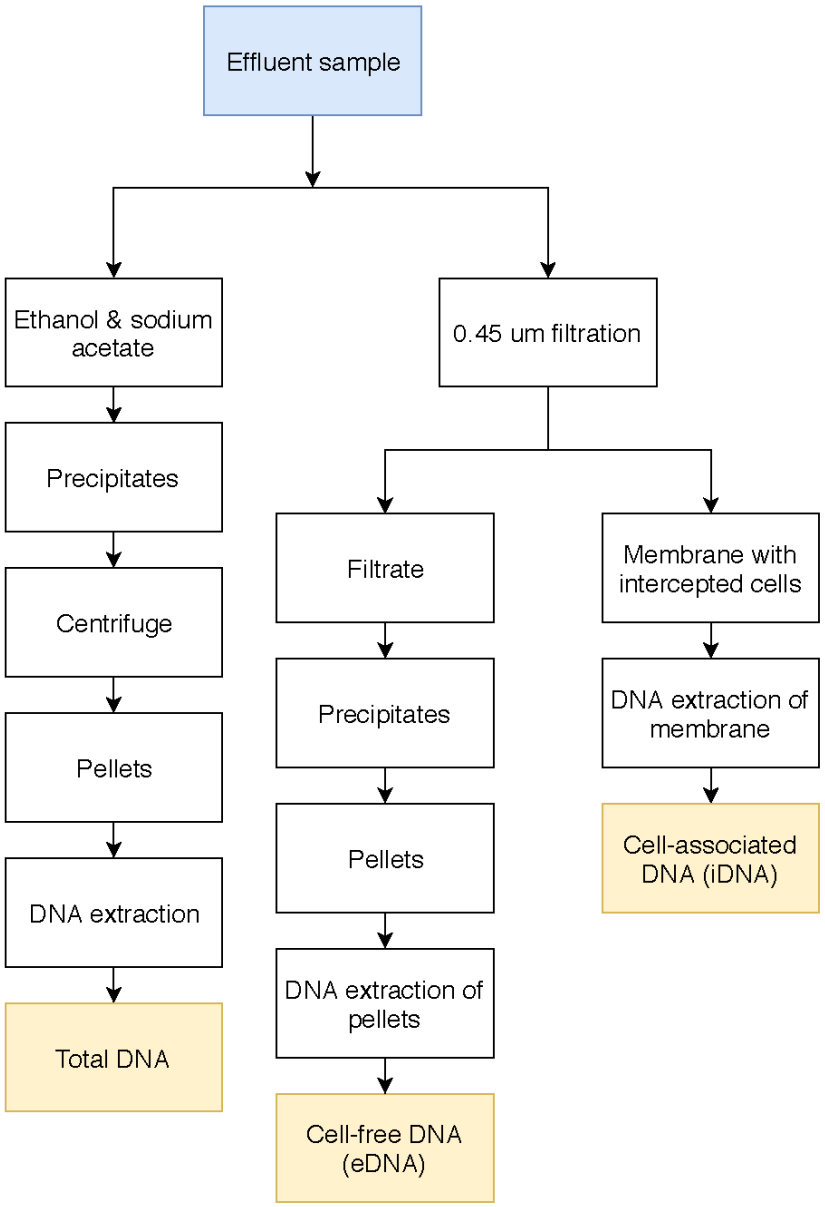


Figure A.15 Workflow for the total, cell-free, and cell-associated DNA extraction adapted from Zhang et al. (2018b).

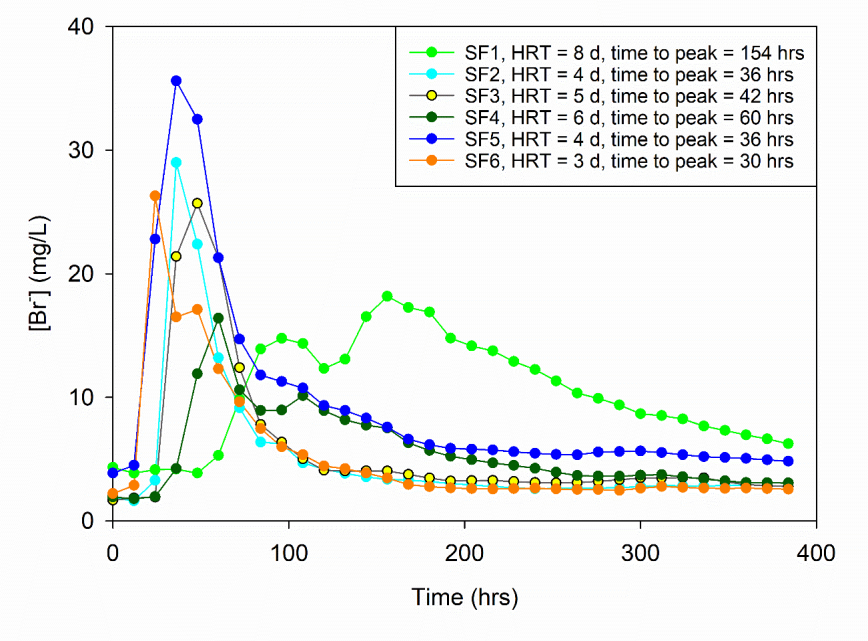


Figure A.16 Tracer response curves for the sand filter (SF)s bromide (Br-) tracer tests.

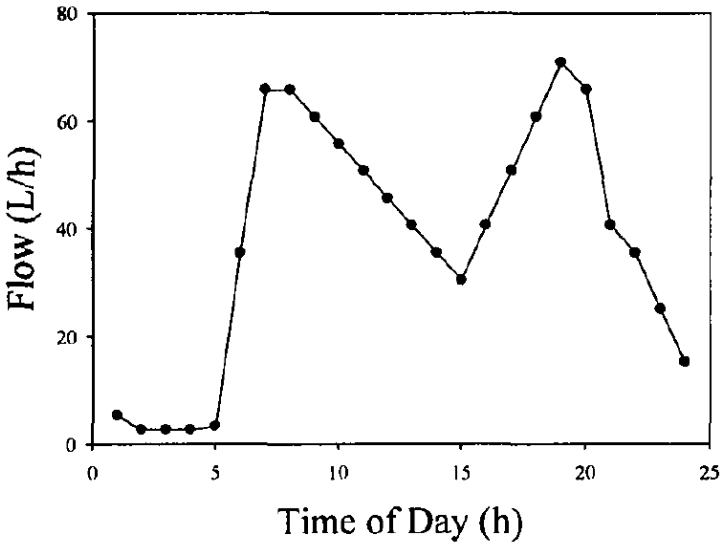


Figure A.17 The influent hydrograph of the dosing of the sand filters that is controlled by a programmable logic controller (PLC). The dosing regime was programmed to emulate a domestic septic system with peaks of use at 8 am and 7 pm (figure obtained with permission from Wilson et al. (2009)).

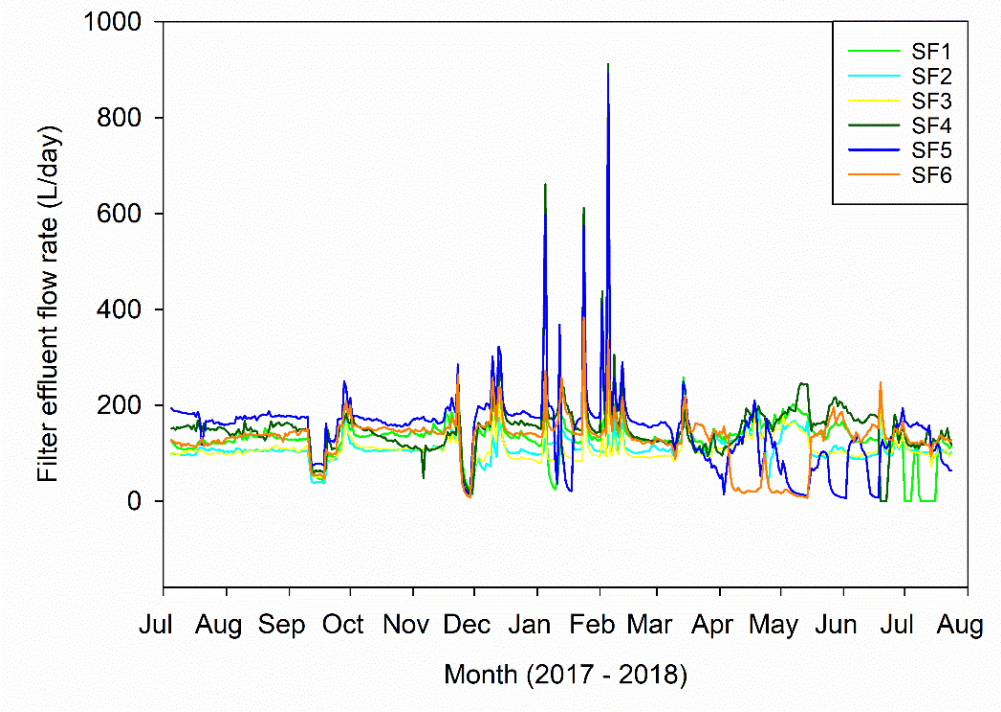


Figure A.18 Hydrographs of the sand filter effluent for the duration of the study period from July 2017 to July 2018.

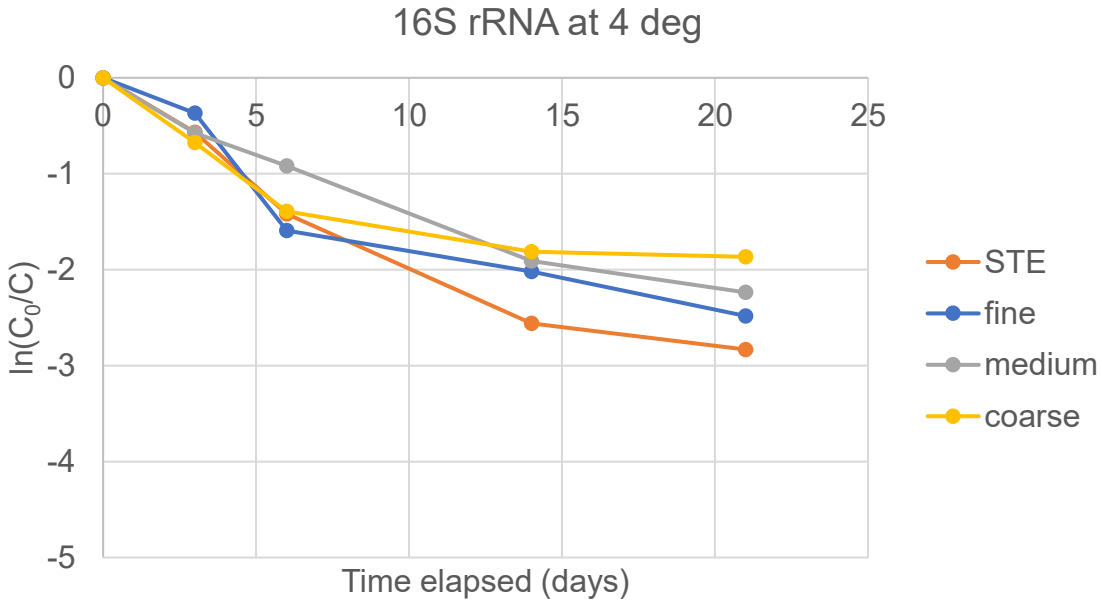


Figure A.19 First order degradation curves for 16S rRNA at 4 degrees Celsius.

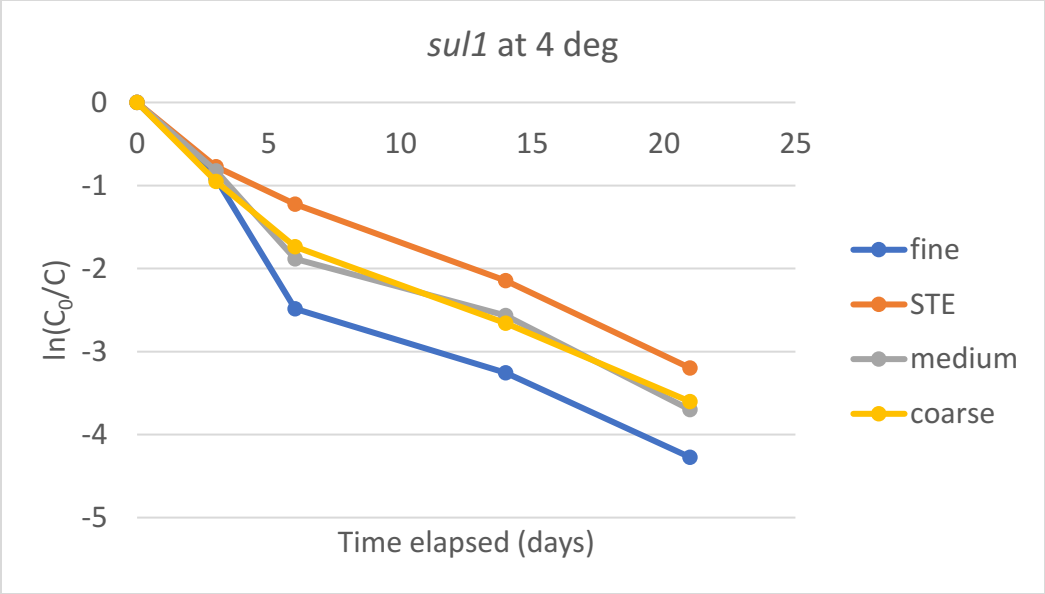


Figure A.20 First order degradation curves for *sul1* at 4 degrees Celsius.

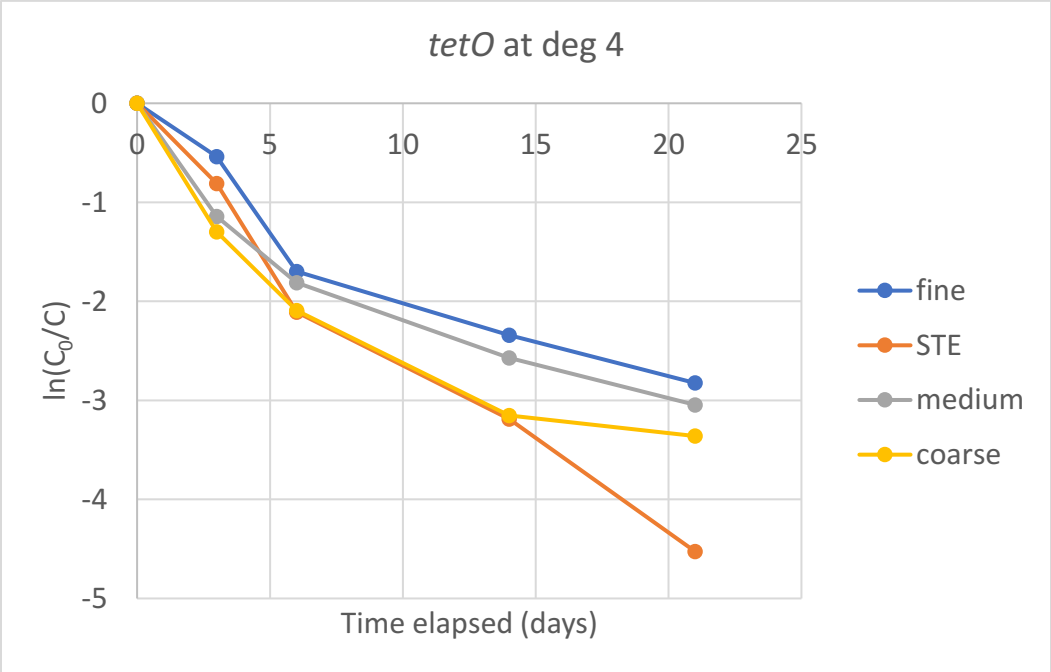


Figure A.21 First order degradation curves for *tetO* at 4 degrees Celsius.

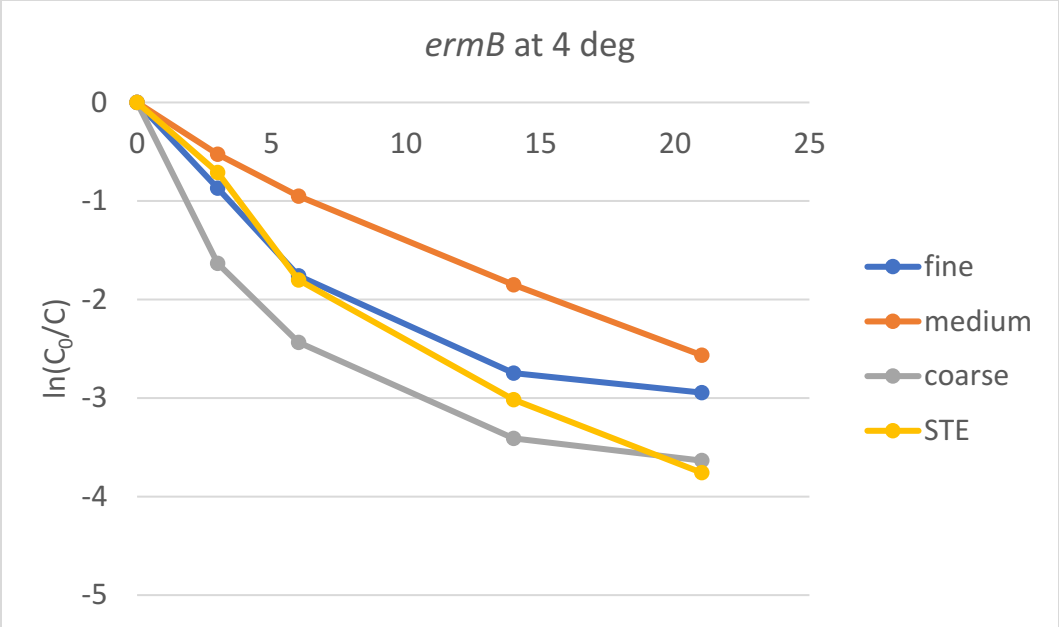


Figure A.22 First order degradation curves for *ermB* at 4 degrees Celsius.

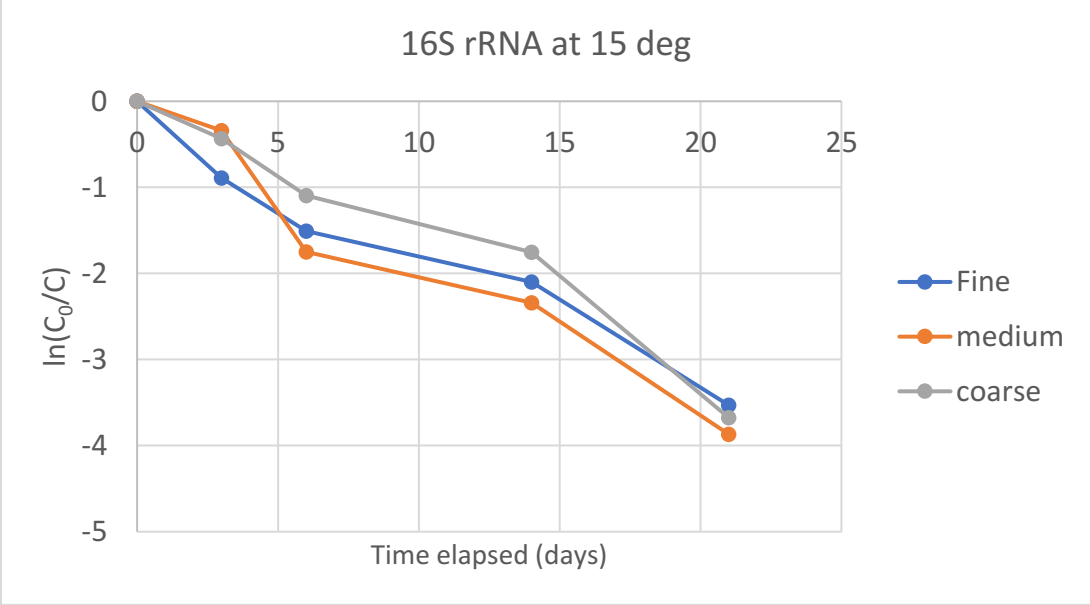


Figure A.23 First order degradation curves for 16S rRNA at 15 degrees Celsius.

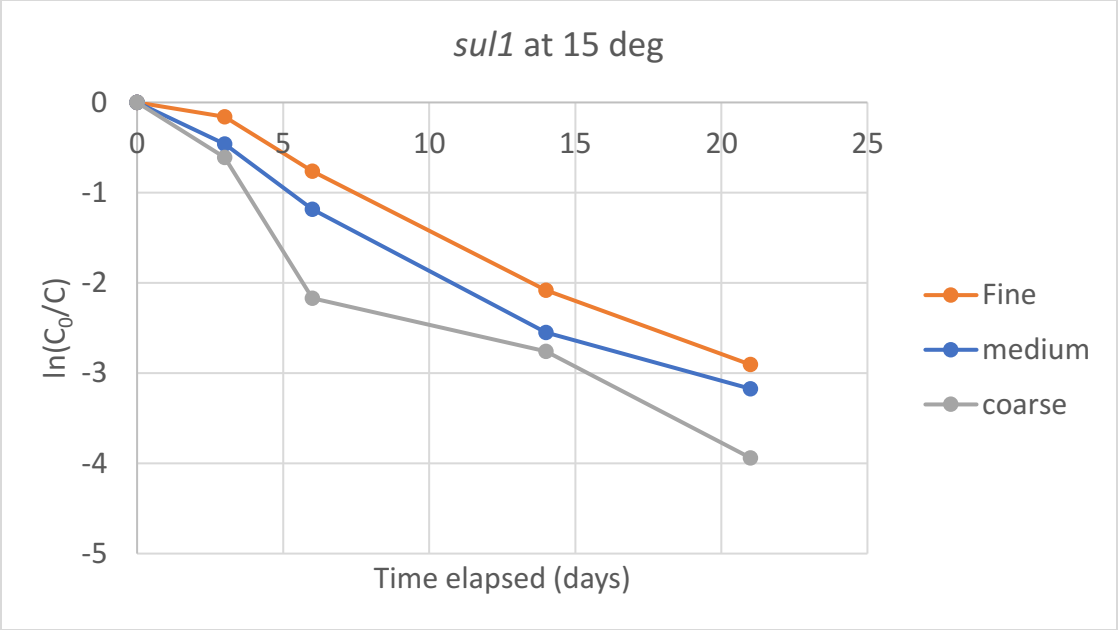


Figure A.24 First order degradation curves for *sul1* at 15 degrees Celsius.

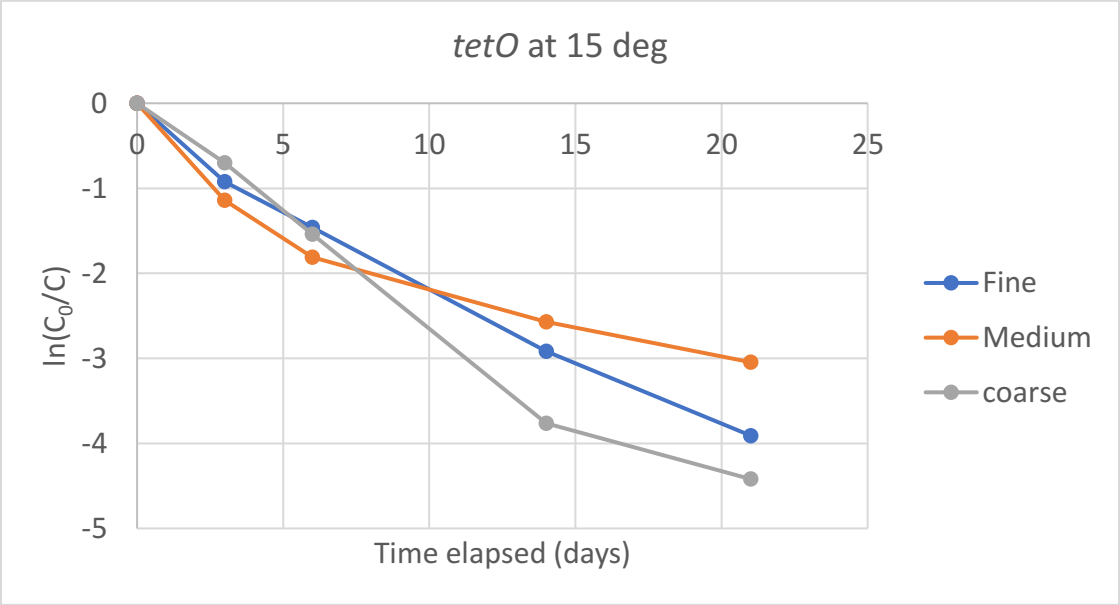


Figure A.25 First order degradation curves for *tetO* at 15 degrees Celsius.

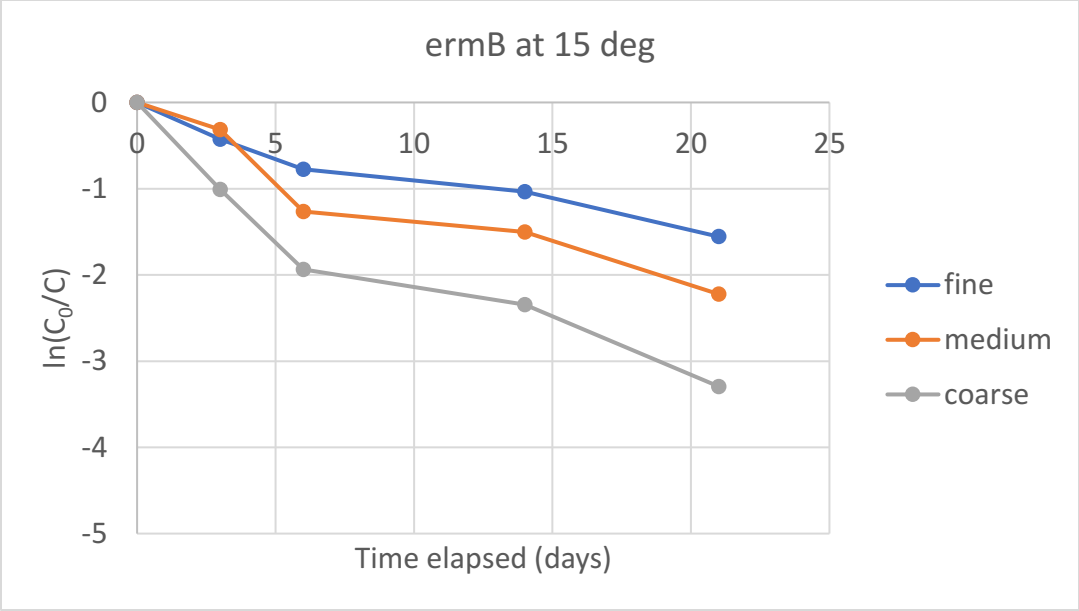


Figure A.26 First order degradation curves for *ermB* at 15 degrees Celsius.

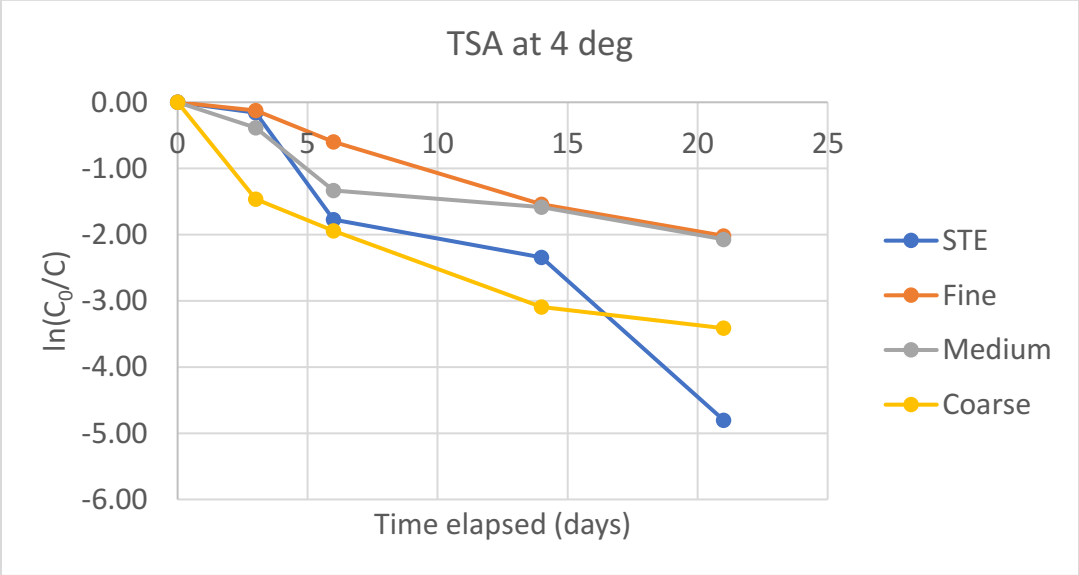


Figure A.27 First order degradation curves for total heterotrophic bacteria grown on TSA control plate at 4 degrees Celsius.

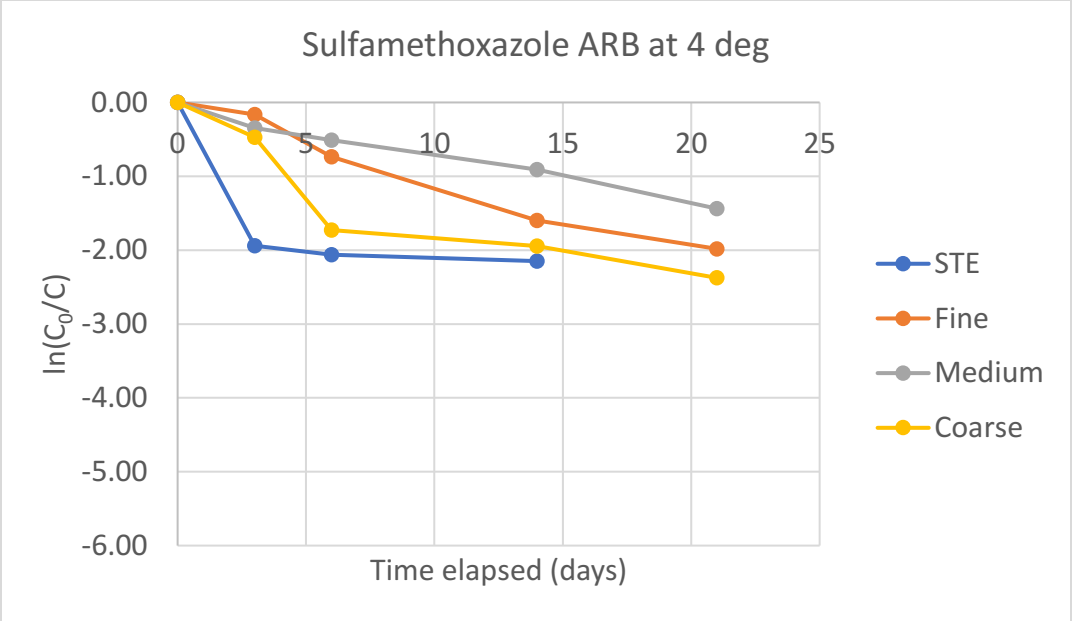


Figure A.28 First order degradation curves for bacteria resistant to 50 mg/L sulfamethoxazole at 4 degrees Celsius.

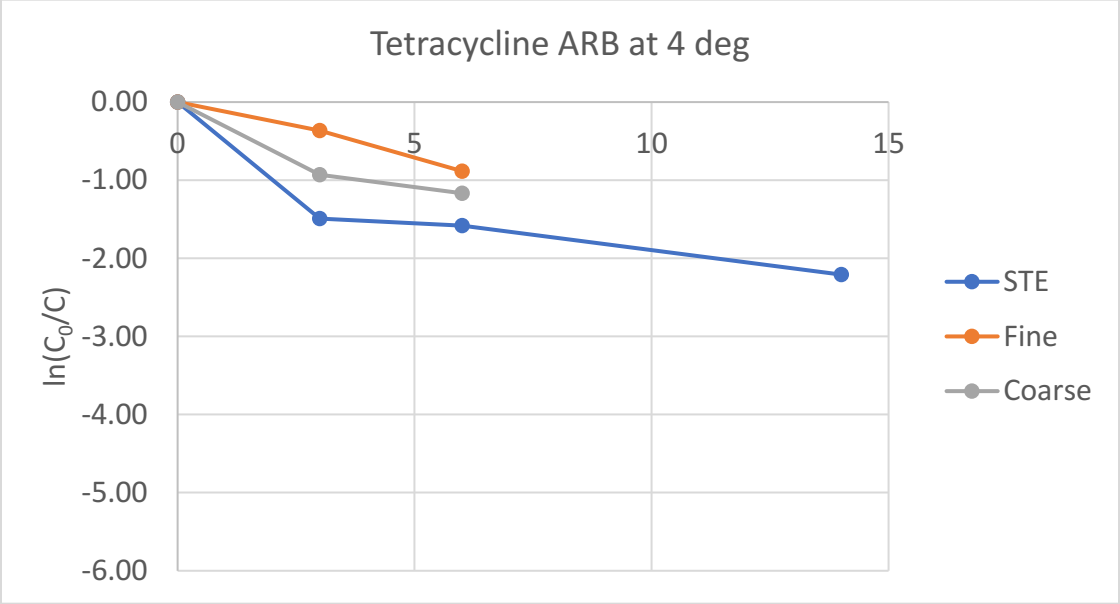


Figure A.29 First order degradation curves for bacteria resistant to 10 mg/L tetracycline at 4 degrees Celsius.

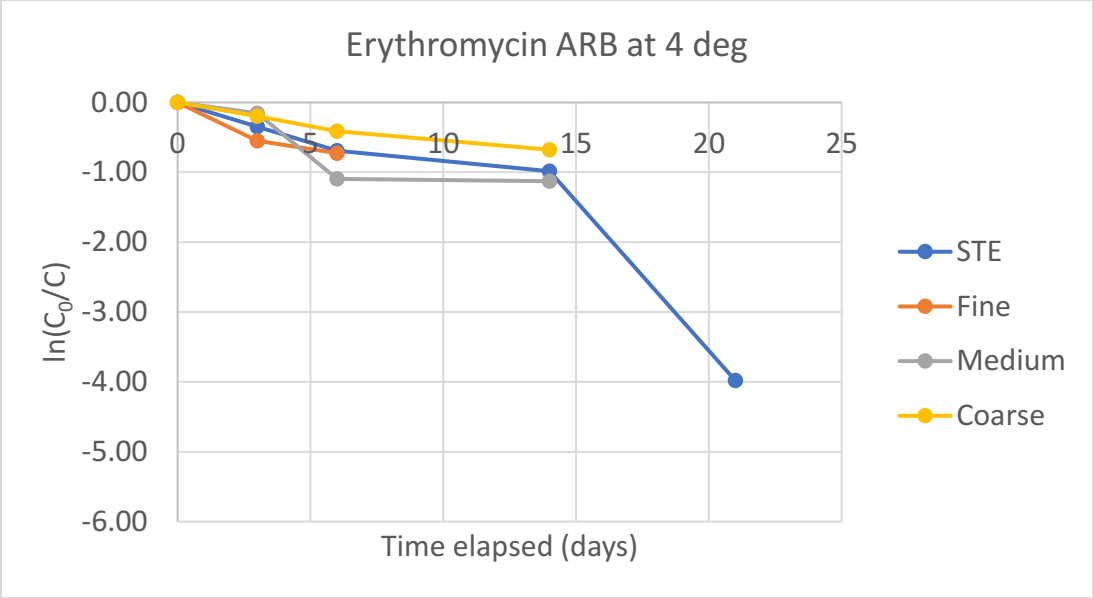


Figure A.30 First order degradation curves for bacteria resistant to 50 mg/L erythromycin at 4 degrees Celsius.

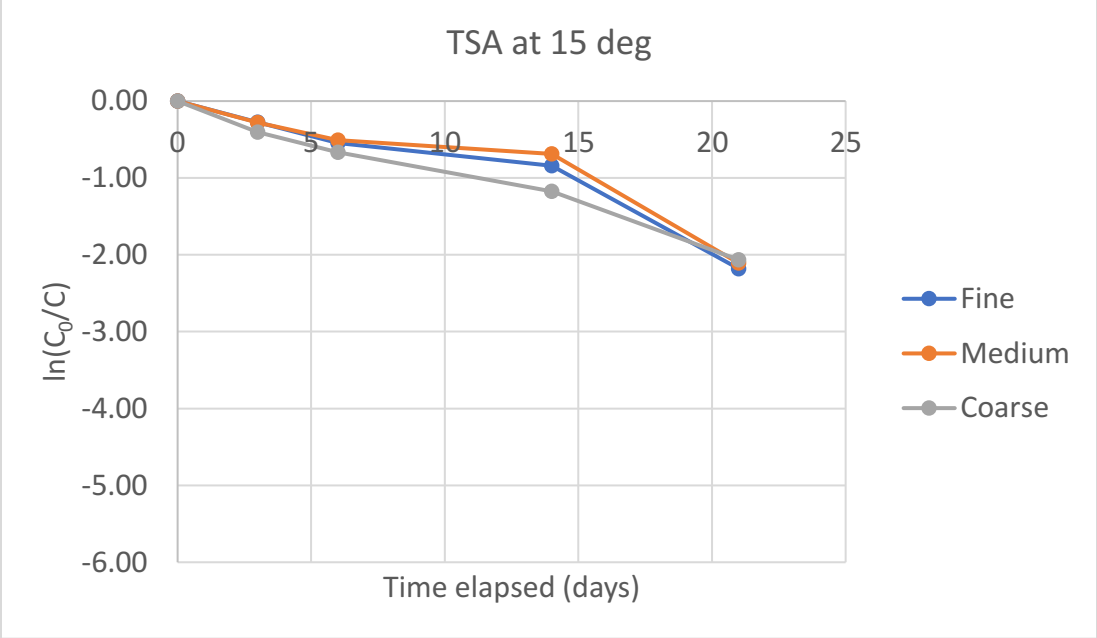


Figure A.31 First order degradation curves for total heterotrophic bacteria grown on TSA control plate at 15 degrees Celsius.

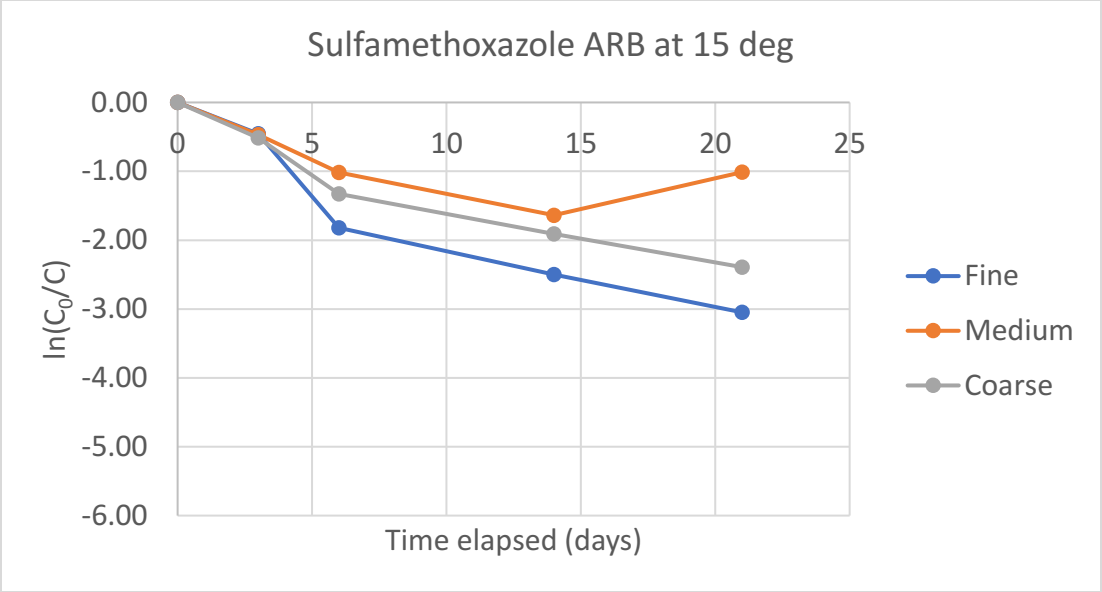


Figure A.32 First order degradation curves for bacteria resistant to 50 mg/L sulfamethoxazole at 15 degrees Celsius.

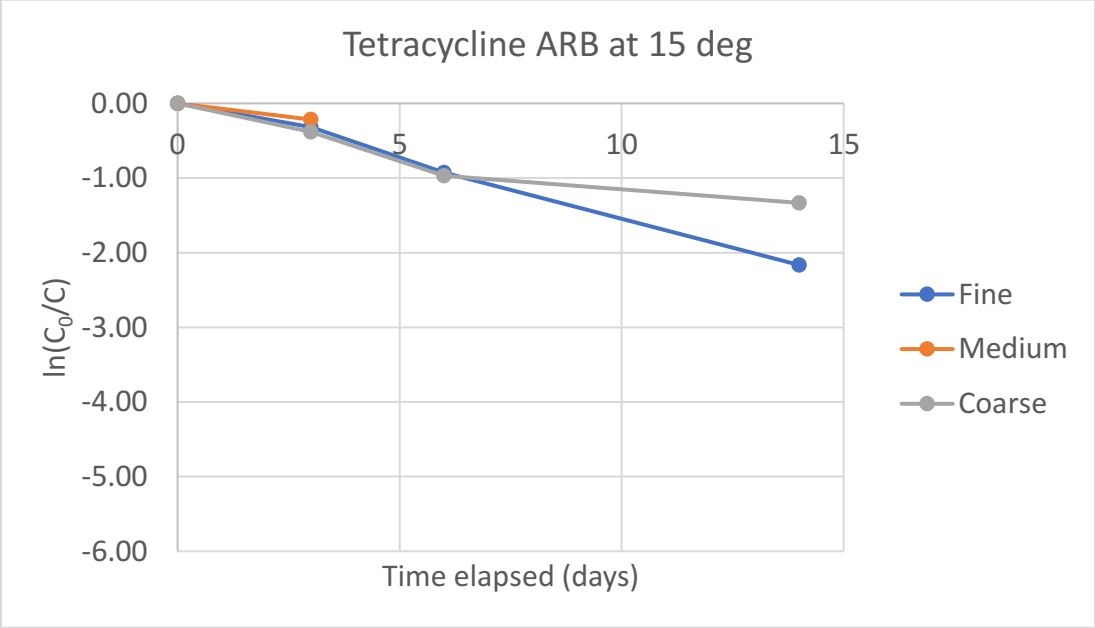


Figure A.33 First order degradation curves for bacteria resistant to 10 mg/L tetracycline at 15 degrees Celsius.

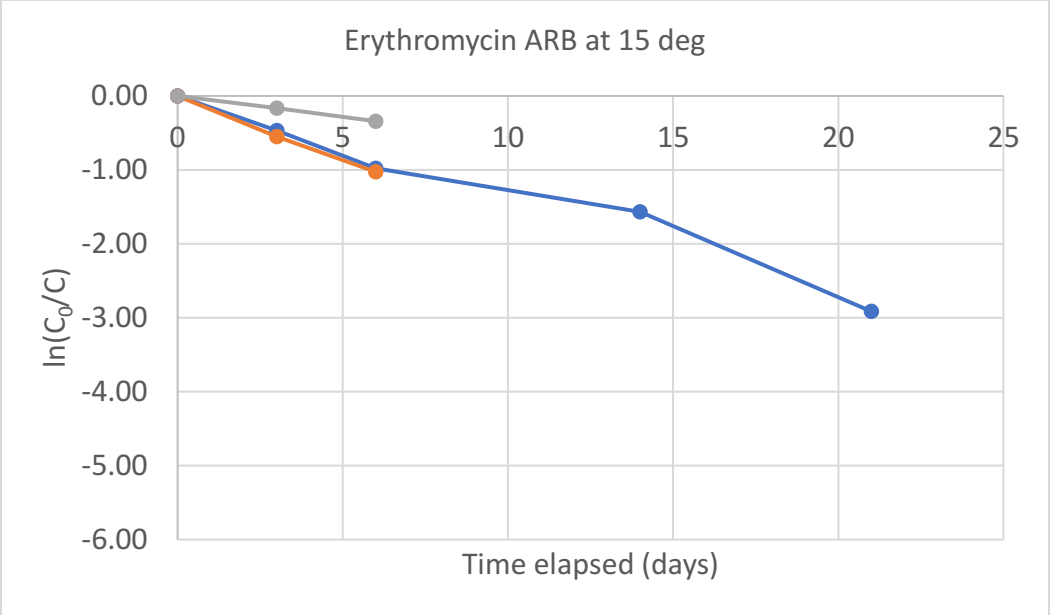


Figure A.34 First order degradation curves for bacteria resistant to 50 mg/L erythromycin at 15 degrees Celsius.

APPENDIX B — SUPPLEMENTAL TABLES

Table B.1 Quantitative PCR primer sequences and reaction conditions (McConnell, 2018a; Neudorf et al., 2017).

Target gene	Primers & Probes	Sequence (5'-3')	Conditions	Reference
16S rRNA	1369F 1492R	CGGTGAATACGTTCYCGG GGWTACCTTGTTACGACTT	95°C for 10 mins; 40 cycles of 95°C for 15s, 55°C for 30s, and 72°C for 30s	Suzuki et al., 2000
HF183	HF183-F HF183-R HF183-p	ATCATGAGTTCACATGTCCG CTTCCTCTCAGAACCCCTATCC CTAATGGAACGCATCCC	95°C for 6 min; 40 cycles of 95°C for 30s, 58°C for 30s, 72°C for 30s.	Haugland et al., 2010; Layton et al., 2013
ermB	ermBF ermBR ermBP	GGATTCTACAAGCGTACCTTGGA GCTGGCAGCTTAAGCAATTGCT FAM-CACTAGGGTTGCTCTTGCACACTCAAGTCBHQ-1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Böckelmann et al., 2009
mecA	mecAF mecAR mecAP	CATTGATCGCAACGTTCAATTTAAT TGGTCTTTCTGCATTCCTGGA FAM-CTATGATCCCAATCTAACTTCCACATACCBHQ-1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Böckelmann et al., 2009; Francois et al., 2003
tetO	tetOF tetOR tetOP	AAGAAAACAGGAGATTCCAAAACG CGAGTCCCCAGATTGTTTTTAGC FAM-ACGTTATTTCCCGTTTATCACGGAAGCG-BHQ-1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Böckelmann et al., 2009
bla*CTX-M*	*BLACTX-M* UP *BLACTX-M* LP CTX-probe	ACCAACGATATCGCGGTGAT ACATCGCGACGGCTTTCT FAM-TCGTGCGCCGCTG-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Colomer-Lluch et al., 2011

Target gene	Primers & Probes	Sequence (5'-3')	Conditions	Reference
bla$_{TEM}$	TEM UP TEM LP TEM Probe	CACTATTCTCAGAATGACTTGGT TGCATAATTCTCTTACTGTCATG FAM-CCAGTCACAGAAAAGCATCTTACGG-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Lachmayr et al., 2009
sul1	qSUL653f qSUL719r tpSUL1	CCGTTGGCCTTCCTGTAAAG TTGCCGATCGCGTGAAGT FAM - CAGCGAGCCTTGCGGCGG-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Czekalshi et al., 2012; Heuer et al., 2008
sul2	qSUL2_595f qSUL2_654r tpSUL2_614	CGGCTGCGCTTCGATTCGCGCGCAGAAAGGATT FAM - CGGTGCTTCTGTCTGTTTCGCGC-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Czekalshi et al., 2012; Heuer et al., 2008
qnrS	*qnrS* UP *qnrS* LP *qnrS* probe	CGACGTGCTAACTTGCGTGA GGCATTGTTGGAAACTTGCA FAM – AGTTCATTGAACAGGGTGA-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Colomer-Lluch et al., 2014
vanA	vanAF vanAR vanAP	CTGTGAGGTCGGTTGTGCG TTTGGTCCACCTCGCCA FAM-CAACTAACGCGGCACTGTTTCCCAAT-BHQ-1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Volkmann et al., 2004
int1	intI1-LC1 intI1-LC5 intI1-probe	GCCTTGATGTTACCCGAGAG GATCGGTCGAATGCGTGT FAM-ATTCCTGGCCGTGGTTCTGGGTTTT-BHQ1	95°C for 3 min; 40 cycles of 95°C for 15s, 62°C for 30s	Barraud et al., 2010

Table B.2 Limits of quantification, qPCR efficiencies, and R^2 values.

Gene target	LOQ (gene copies/reaction)	Log LOQ for Raw and STE (gene copies/mL)	Log LOQ for SFs (gene copies/mL)	Efficiency (%)	R^2
16S rRNA	67000	4.8	3.6	107	0.995
HF183	3630	3.3	2.1	90	0.997
ermB	13.8	1.4	0.2	93	0.999
mecA	69	2.1	0.9	91	0.993
tetO	69	2.1	0.9	87	0.987
$bla_{CTX\text{-}M}$	6.2	1.1	-0.1	87	0.997
bla_{TEM}	243	2.7	1.5	108	0.995
sul1	11.7	1.4	0.2	104	0.998
sul2	9.6	1.3	0.1	109	0.994
qnrS	112	2.4	1.1	112	0.998
vanA	138	2.4	1.2	96	0.992
int1	14.4	1.5	0.3	99	0.994

Table B.3 Summary of the absolute abundance of ARG data in water samples for Chapter 2.

Site	Season	Sample location	Sample date		Gene target (log gene copies/mL)										
				# of samples	*int1*	*mecA*	*sul1*	*sul 2*	*vanA*	*qnrS*	*ermB*	*tetO*	bla_{TEM}	bla_{CTX-M}	*16S*
Sanikiluaq	Averaged over season	Raw		6	5.88	1.95	6.52	6.04	0.78	1.26	4.72	5.50	5.09	2.54	9.47
	Spring	Influent	25-05-2016	1	4.91	1.01	5.66	5.67	1.35	1.38	3.45	4.30	4.08	3.44	9.05
			30-05-2016	1	4.96	0.46	5.29	4.83	n.d.	0.52	2.74	3.65	3.44	2.51	8.85
		Mid-point	25-05-2016	1	3.21	n.d.	4.00	4.19	n.d.	-0.34	1.48	2.17	1.84	0.74	8.19
			30-05-2016	1	4.09	0.55	4.30	2.26	n.d.	n.d.	-0.02	1.07	0.84	n.d	8.01
		Effluent	25-05-2016	1	2.44	0.83	3.13	2.84	n.d.	n.d.	0.55	1.24	1.07	n.d	7.50
			30-05-2016	1	2.18	0.00	2.69	1.27	n.d.	n.d.	1.04	0.13	0.21	n.d	6.65
		Reference		3	n.d.	0.86	0.15	0.24	n.d.	-0.34	n.d.	0.48	-0.50	0.50	7.54
	Summer	Influent	06-09-2016	1	4.16	0.43	4.53	3.66	n.d.	0.87	1.34	2.03	1.95	n.d	8.52
			08-09-2016	1	3.44	1.74	4.06	3.74	n.d.	0.65	1.43	0.68	0.16	0.14	7.09
		Mid-point	06-09-2016	1	0.29	0.90	0.48	0.42	n.d.	n.d.	n.d.	0.27	-0.50	1.04	6.69
			08-09-2016	1	0.01	0.48	0.99	0.99	n.d.	n.d.	n.d.	0.68	0.16	0.14	7.09
		Effluent	06-09-2016	1	n.d	1.15	0.23	1.01	n.d.	n.d.	n.d.	0.28	-0.82	0.04	5.50
			08-09-2016	1	0.16	0.12	1.49	0.82	n.d.	n.d.	n.d.	0.30	n.d.	n.d.	7.98

Site	Season	Sample location	Sample date		Gene target (log gene copies/mL)										
			# of samples	*int1*	*mecA*	*sul1*	*sul 2*	*vanA*	*qnrS*	*ermB*	*tetO*	bla_{TEM}	bla_{CTX-M}	*16S*	
		Reference		3	-1.54	0.97	-0.25	-1.09	n.d.	n.d.	n.d.	0.38	-0.67	0.17	6.38
Naujaat	Averaged over season	Raw		6	5.25	1.98	5.79	5.65	n.d.	5.89	4.90	5.78	4.95	2.92	9.34
	Spring	Influent	16-06-2016	1	5.77	2.10	6.20	6.42	n.d.	6.47	4.94	5.86	5.48	4.91	10.2
			21-06-2016	1	5.64	0.86	5.85	5.45	n.d.	7.42	4.04	4.88	4.96	4.22	9.41
		Mid-point	16-06-2016	1	5.34	0.64	5.47	5.30	n.d.	5.77	3.66	4.52	4.59	3.89	9.71
			21-06-2016	1	6.20	1.31	6.26	5.81	n.d.	6.65	4.37	5.20	5.44	4.58	9.92
		Effluent	16-06-2016	1	5.14	0.48	5.35	5.51	n.d.	5.47	3.46	4.36	4.21	3.54	9.86
			21-06-2016	1	5.01	0.17	5.19	5.04	n.d.	5.33	2.98	4.04	4.16	3.07	9.40
		Reference		3	1.30	-1.11	1.85	1.29	n.d.	1.93	-0.25	1.20	0.46	n.d.	7.55
Naujaat	Summer	Influent	29-08-2016	1	3.15	1.07	3.52	3.44	n.d.	5.78	1.66	3.50	2.25	0.97	8.60
			31-08-2016	1	3.87	0.27	4.24	4.14	n.d.	3.65	2.52	4.08	3.12	2.07	9.39
		Mid-point	29-08-2016	1	5.63	1.27	2.55	2.10	n.d.	1.02	0.09	2.60	0.84	1.34	7.31
			31-08-2016	1	2.13	0.65	2.89	2.46	n.d.	1.56	0.50	2.95	0.60	0.58	7.21
		Effluent	29-08-2016	1	3.87	1.74	3.73	3.87	n.d.	0.76	0.12	2.60	0.78	0.67	9.23
			31-08-2016	1	3.60	2.50	3.54	3.91	n.d.	7.02	0.13	2.70	0.73	1.91	8.78
		Reference		2	-0.60	2.06	1.54	-0.10	n.d.	-1.02	n.d.	1.79	0.38	1.32	7.54

Table B.4 Summary of absolute abundance of soils data in the wetlands (log gene copies/gram).

Site	Sample location	*int1*	*mecA*	*sul1*	*sul 2*	*vanA*	*qnrS*	*ermB*	*tetO*	bla_{TEM}	$bla_{CTX\text{-}M}$	*16S*
Sanikiluaq	Influent	5.20	n.d.	6.82	5.76	n.d.	n.d.	3.64	4.28	3.78	n.d.	11.68
	Mid-point	n.d.	n.d.	1.93	n.d.	n.d.	2.15	n.d.	n.d.	n.d.	n.d.	10.74
	Effluent	n.d.	n.d.	6.07	n.d.	n.d.	2.57	n.d.	2.45	n.d.	n.d.	11.45
	Reference	n.d.	n.d.	2.77	n.d.	n.d.	2.60	n.d.	2.52	1.04	n.d.	11.62
Naujaat	Influent	6.78	n.d.	7.53	6.76	n.d.	4.00	4.52	7.14	2.81	n.d.	12.09
	Mid-point	6.06	2.87	6.99	7.02	n.d.	11.65	4.08	6.94	3.20	n.d.	12.76
	Effluent	5.30	n.d.	6.25	7.10	n.d.	3.94	3.52	6.29	2.96	n.d.	11.98
	Reference	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.04

Table B.5 Summary of metals data.

					Metals concentrations (ug/L)						
Site	Season	Sample location	Sample date	# of samples	Aluminum (Al)	Antimony (Sb)	Arsenic (As)	Barium (Ba)	Beryllium (Be)	Bismuth (Bi)	Boron (B)
Sanikiluaq	Averaged over season	Raw		6	1611	1.0	1.2	24.3	<0.1	44.4	293
	Spring	Influent	25-05-2016	1	75	<1	<1.0	2.8	<2	<1	90
			30-05-2016	1	49	<1	<1.0	2.7	<2	<1	69
		Mid-point	25-05-2016	1	<10	<1	1.1	<2.0	<2	<1	85
			30-05-2016	1	<10	<1	1.4	2.1	<2	<1	79
		Effluent	25-05-2016	1	<10	<1	<1.0	2.6	<2	<1	52
			30-05-2016	1	<10	<1	<1.0	3.1	<2	<1	59
	Summer	Influent	06-09-2016	1	182	<0.5	1.0	5.6	<0.1	<1	307
			08-09-2016	1	26	<0.5	0.8	3.7	<0.1	<1	244
		Mid-point	06-09-2016	1	14	<0.5	1.6	5.9	<0.1	<1	212
			08-09-2016	1	8	<0.5	1.7	6.3	<0.1	<1	222
		Effluent	06-09-2016	1	8	<0.5	1.0	12.4	<0.1	<1	161
			08-09-2016	1	16	<0.5	0.9	16.5	<0.1	<1	259
	Averaged over season	Reference		6	54	<1	0.4	4.1	<2	<1	68
Naujaat	Averaged over season	Raw		6	1608	2.6	1.4	18.3	0.1	7.9	210
	Spring	Influent	16-06-2016	1	238	0.5	0.3	5.6	0.1	-	-
			21-06-2016	1	157	0.6	0.4	5.0	0.1	-	-
		Mid-point	16-06-2016	1	29	0.2	0.3	2.8	0.1	-	-
			21-06-2016	1	65	0.3	0.3	4.2	0.1	-	-

					Metals concentrations (ug/L)						
Site	Season	Sample location	Sample date	# of samples	Aluminum (Al)	Antimony (Sb)	Arsenic (As)	Barium (Ba)	Beryllium (Be)	Bismuth (Bi)	Boron (B)
		Effluent	16-06-2016	1	35	0.3	0.3	3.7	0.1	-	-
			21-06-2016	1	24	0.3	0.3	3.5	0.1	-	-
	Summer	Influent	29-08-2016	1	109	<0.5	0.4	7.2	<0.1	<1	147
			31-08-2016	1	106	<0.5	0.4	8.5	<0.1	<1	297
		Mid-point	29-08-2016	1	42	<0.5	0.3	6.1	<0.1	<1	100
Naujaat			31-08-2016	1	48	<0.5	0.3	6.8	<0.1	<1	105
		Effluent	29-08-2016	1	36	<0.5	0.3	6.7	<0.1	<1	102
			31-08-2016	1	41	<0.5	0.3	7.2	<0.1	<1	104
	Averaged over season	Reference		5	9	0.1	0.2	4.3	0.1	<1	<50

Table B.6 (Cont'd) Summary of metals data

				Metals concentrations (ug/L)						
Site	Season	Sample location	Sample date	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Lithium (Li)
				0.37	2.5	1.1	189.0	848	3.25	9.5
Sanikiluaq	Averaged over season	Raw		<0.20	<5	<1	20.0	390	<0.50	<10
	Spring	Influent	25-05-2016	<0.20	<5	<1	9.2	240	<0.50	<10
			30-05-2016	<0.20	<5	<1	4.5	100	<0.50	<10

				Metals concentrations (ug/L)						
Site	Season	Sample location	Sample date	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Lithium (Li)
		Mid-point	25-05-2016	<0.20	<5	<1	1.7	140	<0.50	<10
			30-05-2016	<0.20	<5	<1	1.3	<60	<0.50	<10
		Effluent	25-05-2016	<0.20	<5	<1	1.2	<60	<0.50	<10
			30-05-2016	0.04	<1	0.7	26.5	306	0.55	6.7
	Summer	Influent	06-09-2016	0.01	<1	<0.5	8.7	111	<0.20	6.9
			08-09-2016	<0.010	<1	0.5	2.8	188	<0.20	7.8
		Mid-point	06-09-2016	<0.010	<1	0.6	2.5	204	<0.20	8.1
			08-09-2016	0.02	<1	<0.5	2.2	48	<0.20	11.4
		Effluent	06-09-2016	0.03	<1	<0.5	2.6	135	<0.20	14.5
			08-09-2016	<0.2	<1	<0.5	2.1	232	<0.5	<10
Sanikiluaq	Averaged over season	Reference		<0.2	<1	<0.5	2.1	232	<0.5	<10
Naujaat	Averaged over season	Raw		0.10	0.5	0.3	42.8	350	0.80	2.4
	Spring	Influent	16-06-2016	0.10	0.5	0.2	27.9	467	0.60	2.2
			21-06-2016	0.10	0.1	0.1	9.7	302	0.30	2.1
		Mid-point	16-06-2016	0.10	0.3	0.2	17.2	518	0.40	2.1
			21-06-2016	0.10	0.3	0.2	13.3	310	0.30	2.1
		Effluent	16-06-2016	0.10	0.1	0.1	8.9	333	0.20	1.8
			21-06-2016	0.03	<1	<0.5	16.0	560	0.40	6.0
	Summer	Influent	29-08-2016	0.03	<1	<0.5	16.8	558	0.48	6.5
			31-08-2016	0.01	<1	<0.5	6.7	441	<0.20	5.2
		Mid-point	29-08-2016	<0.010	<1	<0.5	6.5	421	0.23	5.8
			31-08-2016	<0.010	<1	<0.5	4.2	278	<0.20	5.7

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)	Iron (Fe)	Lead (Pb)	Lithium (Li)
Naujaat		Effluent	29-08-2016	<0.010	<1	<0.5	4.4	263	<0.20	6.1
			31-08-2016	0.05	0.1	0.1	0.9	60	0.10	1.5
	Averaged over season	Reference		0.05	0.1	0.1	0.9	60	0.10	1.5

Table B.7 (Cont'd) Summary of metals data.

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Selenium (Se)	Silicon (Si)	Silver (Ag)	Strontium (Sr)
Sanikiluaq	Averaged over season	Raw		60	1.4	4.5	0.7	3430	0.1	371
	Spring	Influent	25-05-2016	130	<1	<2	<3	1200	<1	140
			30-05-2016	120	<1	<2	<3	870	<1	83
		Mid-point	25-05-2016	34	<1	<2	<3	920	<1	84
			30-05-2016	44	<1	<2	<3	710	<1	82
		Effluent	25-05-2016	3	<1	<2	<3	460	<1	69
			30-05-2016	1	<1	<2	<3	480	<1	88
	Summer	Influent	06-09-2016	183	<1	2.4	0.4	2240	0.1	289
			08-09-2016	162	<1	1.8	0.2	2020	<0.02	265
		Mid-point	06-09-2016	116	<1	2.8	0.2	2550	0.0	270
			08-09-2016	129	<1	2.8	0.1	2290	<0.02	271
		Effluent	06-09-2016	8	<1	1.6	0.2	1780	<0.02	529
			08-09-2016	41	<1	1.6	0.4	2110	<0.02	587
	Averaged over season	Reference		27	1.8	<2	0.2	1670	<1	151
Naujaat	Averaged over season	Raw		47	1.7	4.0	1.5	3120	0.2	35
	Spring	Influent	16-06-2016	27	0.9	1.2	0.5	-	0.1	31
			21-06-2016	31	0.6	1.3	0.5	-	0.1	37
		Mid-point	16-06-2016	24	0.5	0.7	0.5	-	0.1	28
			21-06-2016	30	0.4	1.2	0.5	-	0.1	34

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Selenium (Se)	Silicon (Si)	Silver (Ag)	Strontium (Sr)
		Effluent	16-06-2016	26	0.5	1.3	0.5	-	0.1	27
			21-06-2016	26	0.3	0.7	0.5	-	0.1	30
	Summer	Influent	29-08-2016	47	<1	1.8	0.4	2410	0.0	116
			31-08-2016	47	<1	1.9	0.3	2400	0.0	120
		Mid-point	29-08-2016	44	<1	1.5	0.2	2140	<0.02	92
			31-08-2016	48	<1	1.5	0.2	2360	0.0	96
		Effluent	29-08-2016	38	<1	1.2	0.2	2280	<0.02	93
			31-08-2016	37	<1	1.7	0.2	2710	0.0	102
Averaged over season		Reference			0.2	0.1	0.3	2140	0.1	23

Table B.8 (Cont'd) Summary of metals data.

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Uranium (U)	Vanadium (V)	Zinc (Zn)	Zirconium (Zr)
Sanikiluaq	Averaged over season	Raw								
	Spring	Influent	25-05-2016	<0.05	4.1	11.3	0.9	<5	298.0	2.4
			30-05-2016	<2	<2	<10	<1	<2	66.0	<5
		Mid-point	25-05-2016	<2	<2	<10	<1	<2	34.0	<5
			30-05-2016	<2	<2	<10	<1	<2	9.6	<5
		Effluent	25-05-2016	<2	<2	<10	<1	<2	<7	<5

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Uranium (U)	Vanadium (V)	Zinc (Zn)	Zirconium (Zr)
Sanikiluaq			30-05-2016	<2	<2	<10	<1	<2	<7	<5
	Summer	Influent	06-09-2016	<2	<2	<10	<1	<2	<7	<5
			08-09-2016	<0.05	<5	<5	0.9	<5	50.9	<0.5
		Mid-point	06-09-2016	<0.05	<5	<5	0.7	<5	24.4	<0.5
			08-09-2016	<0.05	<5	<5	1.2	<5	<5	<0.5
		Effluent	06-09-2016	<0.05	<5	<5	1.3	<5	<5	<0.5
			08-09-2016	<0.05	<5	<5	3.2	<5	<5	<0.5
	Averaged over season	Reference		<0.05	<5	<5	3.8	<5	<5	<0.5
Naujaat	Averaged over season	Raw		<2	<5	<10	1.4	<5	7.0	<5
	Spring	Influent	16-06-2016	0.1	<5	161.4	1.1	0.6	330.7	1.7
			21-06-2016	0.1	-	15.1	0.8	0.2	62.7	-
		Mid-point	16-06-2016	0.1	-	26.4	0.7	0.1	48.1	-
			21-06-2016	0.1	-	2.6	0.6	0.2	13.1	-
		Effluent	16-06-2016	0.1	-	8.7	0.7	0.2	26.9	-
			21-06-2016	0.1	-	2.4	0.7	0.2	19.1	-
	Summer	Influent	29-08-2016	0.1	-	2.1	0.6	0.2	16.0	-
			31-08-2016	<0.05	<5	5.0	2.0	<5	29.7	<0.5
		Mid-point	29-08-2016	<0.05	<5	<5	1.9	<5	30.6	<0.5
			31-08-2016	<0.05	<5	<5	3.0	<5	10.3	<0.5
		Effluent	29-08-2016	<0.05	<5	<5	3.0	<5	11.3	<0.5

			Metals concentrations (ug/L)							
Site	Season	Sample location	Sample date	Thallium (Tl)	Tin (Sn)	Titanium (Ti)	Uranium (U)	Vanadium (V)	Zinc (Zn)	Zirconium (Zr)
			31-08-2016	<0.05	<5	<5	3.3	<5	6.5	<0.5
Averaged over season		Reference		0.1	-	0.3	1.0	0.2	0.4	<0.5

Table B.9 (Cont'd) Summary of metals data.

				Metals concentration (ug/L)			
Site	Season	Sample location	Sample date	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Sodium (Na)
Sanikiluaq	Averaged over season	Raw		38024	23348	13681	161771
	Spring	Influent	25-05-2016	29000	9800	5400	58000
			30-05-2016	19000	6600	3200	33000
		Mid-point	25-05-2016	22000	9500	6000	48000
			30-05-2016	24000	11000	5700	56000
		Effluent	25-05-2016	16000	7400	2400	32000
			30-05-2016	21000	10000	2700	47000
	Summer	Influent	06-09-2016	58	26	16	168
			08-09-2016	54	25	16	169
		Mid-point	06-09-2016	67	34	6	234
			08-09-2016	73	36	7	256
		Effluent	06-09-2016	94	50	6	302
			08-09-2016	99	51	9	351
	Averaged over season	Reference		7224	1987	561	6622
Naujaat	Averaged over season	Raw		27	9	41	54
	Spring	Influent	16-06-2016	22	7	17	23
			21-06-2016	25	7	14	21
		Mid-point	16-06-2016	19	5	8	12
			21-06-2016	24	7	12	18

		Metals concentration (ug/L)					
Site	Season	Sample location	Sample date	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Sodium (Na)
		Effluent	16-06-2016	20	5	9	14
			21-06-2016	22	6	9	14
	Summer	Influent	29-08-2016	53	12	10	36
			31-08-2016	54	11	11	33
		Mid-point	29-08-2016				
		Effluent	31-08-2016	50	10	6	27
	Averaged over season	Reference		19	6	1	19

Table B.10 Minimum and maximum values of the samples collected twice daily on July 16 and 23, 2018 for Chapter 3.

	Raw		STE		SF1		SF2		SF3		SF4		SF5		SF6	
Parameter	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
16S rRNA	8.3	8.6	8.0	8.3	6.2	6.6	5.2	5.5	5.5	6.0	5.0	5.8	5.3	6.6	5.3	5.7
HF183	6.2	6.4	5.5	5.9	<LQ[16]	<LQ	<LQ	4.3	<LQ	4.6	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
sul1	4.2	4.5	4.0	4.5	2.1	2.7	<LQ	0.5	<LQ	1.3	<LQ	0.5	0.3	1.5	0.5	1.4
sul2	3.4	3.8	2.7	3.0	2.3	2.5	<LQ	<LQ	<LQ	0.7	<LQ	0.9	<LQ	1.3	<LQ	<LQ
int1	4.2	4.6	3.6	4.0	1.5	2.0	<LQ	0.4	<LQ	<LQ	<LQ	0.4	<LQ	1.2	<LQ	1.3
qnrS	5.6	5.8	4.1	4.5	<LQ	1.6	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
tetO	4.8	5.3	4.6	4.7	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
ermB	5.4	5.6	4.8	5.0	0.6	0.9	<LQ	2.4	<LQ	0.4	<LQ	0.3	<LQ	0.7	<LQ	0.7
bla_{TEM}	2.7	3.6	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
$bla_{CTX\text{-}M}$	2.7	3.4	1.1	3.1	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

[16] LQ – Limit of quantification

Table B.11 Summary of antibiotic data collected from the raw, STE, and sand filters for Chapter 3. It should be noted unless otherwise indicated that data is only shown for samples that had at least one detectable concentration of antibiotic. The full suite of samples was analyzed but many were below detection limits (<DL) and therefore not shown. The cells with numeric values are bolded for ease of viewing.

		Amoxicillin (ng/L)	Cefaclor (ng/L)	Cefprozil (ng/L)	Cefdinir (ng/L)	Levofloxacin (ng/L)	Ciprofloxacin (ng/L)	Azithromycin (ng/L)	Clindamycin (ng/L)	Clarithromycin (ng/L)	Triclocarban (ng/L)
	DL (ng/L)	**35**	**29**	**36**	**95**	**91**	**71**	**40**	**30**	**74**	**50**
Sample date (yyyy-mm-dd)	**Sample ID**										
2017-05-20	Raw	< DL	< DL	< DL	< DL	< DL	**176**	**51**	< DL	< DL	**75**
	STE	< DL	< DL	< DL	< DL	< DL	< DL	57	< DL	**84**	< DL
	SF2	< DL	< DL	< DL	< DL	< DL	**669**	< DL	< DL	< DL	< DL
	SF6	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**42**	< DL	< DL
2017-07-05[17]	All	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
2017-07-18	SF1	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**41**	< DL	< DL
	SF4	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**38**	< DL	< DL
	SF6	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**52**	< DL	< DL
2017-08-21	Raw	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**37**	**140**	< DL
	STE	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**48**	**104**	< DL
	SF1	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**58**	< DL	< DL

[17]All samples on this date were below detection limits.

		Amoxicillin (ng/L)	Cefaclor (ng/L)	Cefprozil (ng/L)	Cefdinir (ng/L)	Levofloxacin (ng/L)	Ciprofloxacin (ng/L)	Azithromycin (ng/L)	Clindamycin (ng/L)	Clarithromycin (ng/L)	Triclocarban (ng/L)
	SF2	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**53**	< DL	< DL
	SF3	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**39**	< DL	< DL
	SF4	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**39**	< DL	< DL
	SF5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**57**	< DL	< DL
	SF6	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**55**	< DL	< DL
2017-10-01	Raw	< DL	< DL	< DL	< DL	< DL	< DL	181	**137**	< DL	< DL
	STE	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**138**	< DL	< DL
	SF1	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**114**	< DL	< DL
	SF2	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**144**	< DL	< DL
	SF3	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**191**	< DL	< DL
	SF4	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**105**	< DL	< DL
	SF5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**111**	< DL	< DL
	SF6	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**159**	< DL	< DL
2018-06-18[18]	Raw	< DL	**90**	< DL	< DL	< DL	**180**	**89**	**69**	**92**	**198**
	SF1	< DL	**41**	**77**	< DL	< DL	**86**	< DL	**49**	< DL	**124**
	SF3	**74**	**166**	**322**	**120**	**144**	**221**	< DL	117	< DL	< DL
	SF4	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**89**	< DL
2018-07-16	STE	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**37**	< DL	< DL
	SF1	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**66**	< DL	< DL
	SF5	< DL	< DL	< DL	< DL	< DL	< DL	< DL	**35**	< DL	< DL
2018-07-23	SF4	< DL	< DL	< DL		< DL	< DL	< DL	**153**	< DL	< DL

[18] SF5 was not analyzed on this date.

APPENDIX C — COPYRIGHT PERMISSIONS

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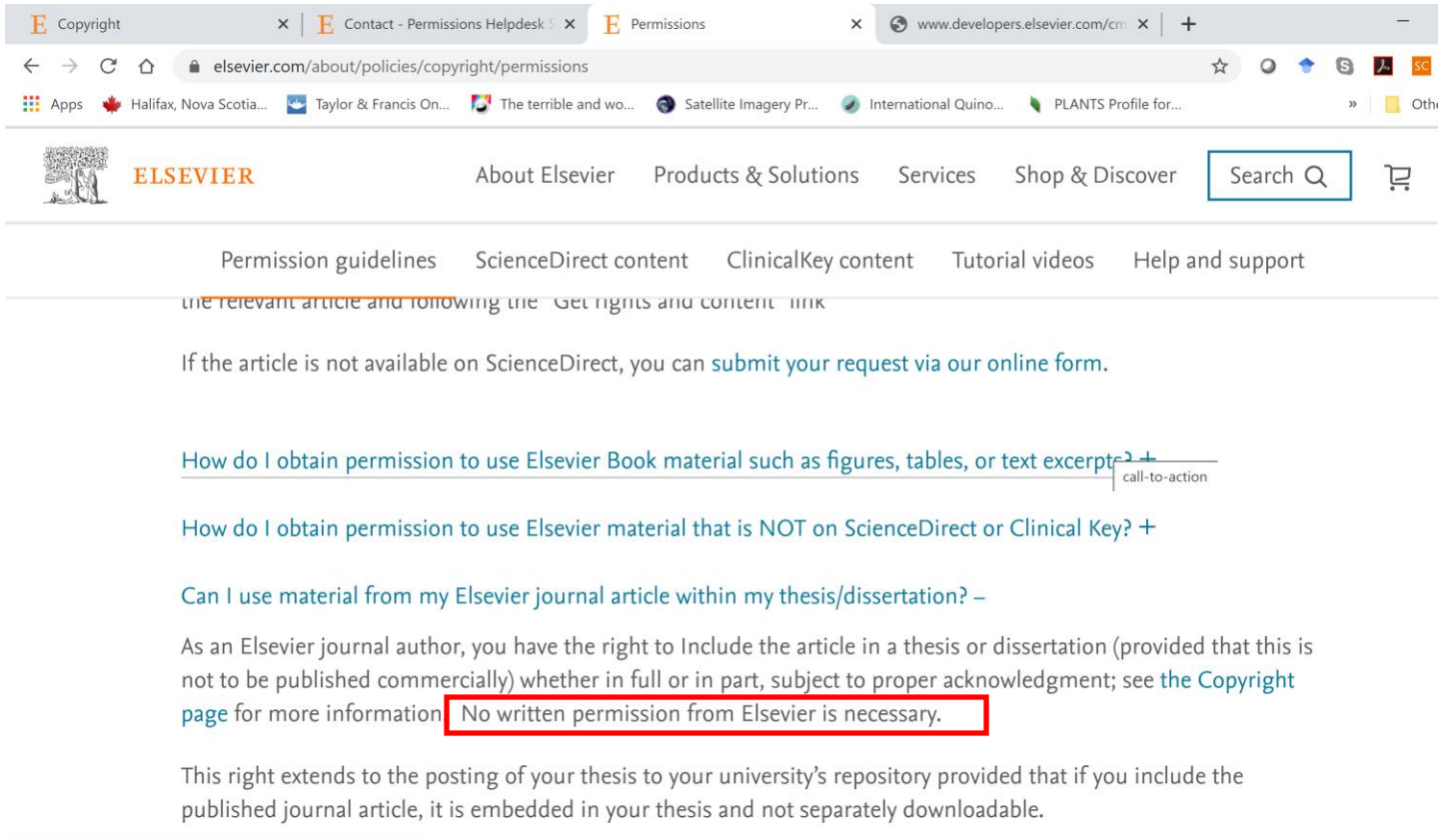

APPENDIX D — ELECTRONIC SUPPLEMENTAL INFORMATION

Additional supplemental information accompanies this thesis as a Microsoft Excel spreadsheet which presents HRT sample calculations for Chapter 2, 3 and 4, raw RWT tracer data, and TIS sample calculations from Chapter 2, raw gene target data from Chapter 3 and 4 and metals data. The data is also published in:

Hayward, J. L., Jackson, A. J., Yost, C. K., Hansen, L. T., & Jamieson, R. C. (2018). Fate of antibiotic resistance genes in two Arctic tundra wetlands impacted by municipal wastewater. *Science of the Total Environment*, *642*, 1415-1428.

Hayward, J. L., Huang, Y., Yost, C. K., Hansen, L. T., Lake, C., Tong, A., & Jamieson, R. C. (2019). Lateral flow sand filters are effective for removal of antibiotic resistance genes from domestic wastewater. *Water research*, *162*, 482-491.

Tables S1 – S4 correspond to Chapter 2.

Tables S1 and S5 – S8 correspond to Chapter 3.

Tables S1 and S9 – S11 correspond to Chapter 4.

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