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Trace Organic Contaminant Removal in Subsurface Flow Treatment Wetlands

by

Angela Nicole Stiegler

A dissertation submitted in partial satisfaction of the
requirements for the degree of
Doctor of Philosophy

in

Engineering - Civil and Environmental Engineering

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor David L. Sedlak, Chair
Professor Kara L. Nelson
Professor Laura N. Lammers

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Abstract

Trace Organic Contaminant Removal in Subsurface Flow Treatment Wetlands

by

Angela Nicole Stiegler

Doctor of Philosophy in Engineering - Civil and Environmental Engineering

University of California, Berkeley

Professor David L. Sedlak, Chair

Constructed wetlands are nature-based treatment systems that offer an attractive means of removing nutrients and trace organic contaminants from effluent-dominated surface water and treated wastewater due to their low cost and ability to provide additional benefits. One recently developed nature-based treatment system, the horizontal levee, consists of a gradually-sloped subsurface flow treatment wetland built on the seaward side of conventional storm control levees. Native vegetation grows on its surface and an underground treatment layer consisting of sand, gravel, and woodchips, removes nutrients and trace organic contaminants from wastewater effluent. Although the mechanisms controlling nitrate removal in the horizontal levee have been previously reported, ambiguity about the mechanisms through which trace organic contaminants are removed in horizontal levees may hinder their implementation due to uncertainty about how design and operational variables impact their performance.

Efforts to improve upon the design of horizontal levees require insight into contaminant removal mechanisms. Often, studies of constructed wetland performance have been limited to monitoring the concentrations of contaminants flowing into and out of them (i.e., a black box approach). In contrast, in this research, a combination of high-resolution porewater monitoring, sampling of wetland vegetation and sediments, and microcosm experiments were used to assess the relative contribution of different removal mechanisms. Results of this research are germane to other subsurface flow treatment systems, to natural riparian zones and wetlands of ecological importance, and to new applications of the horizontal levee technology (e.g., the treatment of reverse osmosis (RO) concentrate from potable water reuse).

To develop a mechanistic understanding of trace organic contaminant removal in horizontal levees (Chapter 2), a demonstration-scale experimental horizontal levee that received nitrified, treated wastewater from an activated sludge wastewater treatment plant was monitored over the course of 2.5 years. High-resolution sampling of porewater, soil and plants was paired with isotherms, microcosms, and one-dimensional transport modelling to quantify the contribution of sorption, transformation, and plant uptake to the removal of trace organic contaminants. Results indicated that persistent trace organic contaminants were removed primarily through biotransformation and that their removal was linked to redox conditions. In particular, sulfate- and Fe(III)-reducing conditions facilitated rapid transformation of sulfamethoxazole and carbamazepine, two contaminants that have proven difficult to remove

under aerobic and sub-oxic conditions. Transformation of relatively labile contaminants occurred in the presence and absence of nitrate in the wetland, implying that subsurface treatment systems that include a variety of redox conditions may effectively remove a broad range of trace organic contaminants via transformation reactions.

Although plant uptake and translocation did not contribute significantly to the mass balance of trace organic contaminants in the horizontal levee (Chapter 2), the presence of trace organic contaminants in plants may raise concerns about potential exposures to herbivorous and detritivorous wildlife and invertebrates. Results presented in Chapter 3 indicated that concentrations of the compound with the highest affinity to be taken up into the shoots of plants, carbamazepine, rapidly dropped in plants growing along the flow path and that concentrations varied widely among plant species. These results indicate that subsurface flow constructed wetlands could be designed to minimize plant uptake through careful selection of plants along the flow path or by minimizing the concentrations of trace organic contaminants in porewater. These design options may be particularly helpful if horizontal levees are used to treat wastewater that has higher concentrations of contaminants (e.g., RO concentrate generated by advanced wastewater treatment plants used as part of potable water reuse projects).

The plants in the horizontal levee grew under conditions similar to those found in natural riparian zones and wetlands. Therefore, monitoring results from the horizontal levee may provide insight into the fate of trace organic contaminants in ecosystems at the aquatic/terrestrial interface. Results suggested that uptake and translocation of carbamazepine may be a widespread and overlooked terrestrial exposure pathway because rivers in waster-stressed regions are often comprised primarily of wastewater effluent. Although riparian zones are small in area, they are disproportionately important for the survival of most terrestrial vertebrates, especially in arid regions. In addition, the concentrations found in plants in the horizontal levee may exceed levels that cause ecotoxicological effects. Considering these factors, research conducted in Chapter 3 indicated that there is a need to understand the extent of plant uptake as a terrestrial exposure pathway of wildlife to wastewater-derived trace organic contaminants.

Under the design and operating conditions employed at the experimental field site, the horizontal levee removes most contaminants from treated wastewater within approximately 20% of its length, implying that it may be able to process higher contaminant loads, such as those produced when RO is used to treat municipal wastewater effluent. Management of RO concentrate is a major barrier to the adoption of reuse in most water stressed regions due to a lack of access to suitable discharge infrastructure or receiving waters and the absence of low-cost and/or multi-benefit treatment systems. In Chapter 4, the ability of horizontal levees to remove nitrate and trace organic contaminants from RO concentrate was assessed. Results indicated that the horizontal levee removed nitrate and a diverse suite of trace organic contaminants via biotransformation at rates that exceeded those reported in other nature-based RO concentrate treatment systems on an areal basis. Because sulfate- and Fe(III)- reducing conditions were not reached until the last 5-7 meters of the system, persistent trace organic contaminants that are resistant to biotransformation under aerobic or anoxic conditions were not removed. However, if horizontal levees were designed to reach sulfate- and/or Fe(III)-reducing conditions when they are used to treat RO concentrate, they could provide an extremely effective means of removing nitrate and trace organic contaminants.

In loving memory of Virginia S. Linkenhoker

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CHAPTER 1. Introduction

1.1 Trace Organic Contaminants in Municipal Wastewater Effluent

Municipal wastewater treatment plants are designed to remove particulate matter, biochemical oxygen demand, and, in some cases, nutrients from wastewater.[1,2] However, the treatment processes employed at municipal wastewater treatment plants are not optimized to remove hydrophilic trace organic contaminants, such as pharmaceuticals, personal care products, pesticides, and industrial chemicals.[3] Therefore, these contaminants are often detected in municipal wastewater effluent.[4,5] In addition, many trace organic contaminants (e.g., carbamazepine and sulfamethoxazole) are persistent under most conditions found in the environment.[6–8]

Over the past few decades, considerable effort has been directed at understanding the ecotoxicological risks posed by trace organic contaminants in wastewater effluent. Results from this research indicate that continuous exposure of aquatic organisms to certain contaminants at concentrations found in municipal wastewater effluent can result in endocrine disruption, oxidative stress, behavioral alterations and genotoxicity.[9–12] These contaminants may also present risks to human health via contamination of drinking water[13] (e.g., during de facto water reuse[14,15]) or via uptake into edible food crops that are irrigated with recycled water.[16–18] These issues may become more widespread, as water scarcity increases and treated wastewater becomes more important as a water resource.[19]

In response to concerns about the discharge of wastewater-derived trace organic contaminants, engineers have developed technologies aimed at removing them from water. The most studied technologies for this purpose include chemical oxidation and adsorption processes.[20,21] However, these techniques are expensive and, in most cases, have been difficult to justify for the treatment of municipal wastewater effluents prior to discharge into the environment.[22] For example, ozonation coupled to biologically activated carbon filtration – the main approach pursued for this purpose in Switzerland – is expected to increase the cost of water treatment by approximately 10-20% in wastewater treatment plants that serve more than 80,000 people and up to 50% in smaller treatment facilities.[23] Also, transformation products formed during chemical oxidation of trace organic contaminants may be more persistent than their parent compounds and may pose risks to aquatic life.[20,24] Although adsorption-based processes do not form toxic transformation products, they are hindered by the need to regenerate sorbents frequently[25,26] and the inability to reliably remove hydrophilic contaminants.[27] Considering these drawbacks, new, low-cost treatment technologies are needed for the removal of trace organic contaminants from municipal wastewater effluents.

1.2 Nature-Based Treatment Systems

According to the International Union for the Conservation of Nature, nature-based solutions are "actions to protect, sustainably manage, and restore natural and modified ecosystems that address societal challenges effectively and adaptively, simultaneously providing human well-being and

biodiversity benefits".[28] Nature-based water treatment systems are a subset of nature-based solutions that improve water quality. Examples of nature-based water treatment systems include constructed wetlands, vegetated stormwater bioretention systems, managed riparian buffer zones, and grasslands irrigated with treated wastewater.[29] In particular, constructed wetlands are an attractive means of improving water quality because they remove multiple contaminant classes simultaneously (e.g., trace organic contaminants and nutrients) at a relatively low cost while providing other benefits (e.g., habitat creation, recreation). These benefits may make constructed wetlands more appealing than conventional engineered approaches for the removal of trace organic contaminants from treated wastewater.[30]

Surface flow constructed wetlands are characterized by water flowing through and above a sediment surface. Vegetated surface flow wetlands feature emergent macrophytes and remove trace organic contaminants via physical, chemical and biological mechanisms.[31] Emergent vegetation enhances biotransformation of trace organic contaminants and nutrient removal by increasing the surface area upon which microbial biofilms grow.[32] Also, emergent vegetation creates habitat for a variety of invertebrates and fish (e.g., used for shelter, food, egg attachment sites).[33] However, an abundance of macrophytes in surface flow treatment wetlands prevents trace organic contaminant removal via photochemical reactions[31,34] and may lead to the formation of preferential flow paths and hydraulic short circuiting.[32] Surface flow wetland designs that incorporate periodic mixing zones or the operation of multiple wetland treatment cells in series are less susceptible to short-circuiting.[31]

To improve the hydraulic performance of surface flow wetlands, engineers have recently tested unvegetated surface flow treatment wetland designs. For example, unit process open-water wetlands are shallow (i.e., approximately 30 cm deep), unvegetated wetlands with plug-flow hydraulics. The lack of emergent vegetation and high length to width ratio minimizes short-circuiting and the shallow water depth promotes photolysis of trace organic contaminants.[35,36] Due to abundant light penetration, a biomat consisting of photosynthetic diatoms and heterotrophic bacteria colonizes the bottom of the open-water wetland and removes nitrate and some trace organic contaminants via biotransformation.[37,38] However, this design does not provide macrophyte habitat benefits and may be subject to significant seasonal performance declines during winter.[37,39]

Subsurface flow constructed wetlands are designed to exhibit horizontal or vertical flow through porous media and feature hydrophytic plants. The insulating effect of plants[32] and sediment in these systems lowers seasonal performance variability caused by temperature fluctuations.[40] However, the amount of water that can be passed through subsurface flow wetlands is limited by the hydraulic conductivity of the sediments.[41,42] Regardless, subsurface flow constructed wetlands designs can remove nutrients and trace organic contaminants from municipal wastewater effluent. In addition, subsurface flow wetlands can be designed to provide high quality habitat for terrestrial wildlife.[43]

A major limitation for the implementation of treatment wetlands is the large land areas that they require. For example, at least 10 hectares of open-water wetlands would be needed to remove 90% of the mass of nitrate and a suite of trace organic contaminants from 1 MGD of wastewater effluent in temperate climates.[37,38] However, the area required to remove 90% of the mass of nitrate

from wastewater effluent can be an order of magnitude lower in subsurface flow wetlands than in open-water wetlands.[44] To lower the land area required for treatment, researchers have investigated ways of increasing hydraulic performance and contaminant removal rates in constructed wetlands.[45] For example, deep mixing zones built between vegetated portions can minimize hydraulic short circuiting in surface flow wetlands.[46,47] In addition, the use of high porosity media that increases hydraulic conductivity and minimizes clogging can improve subsurface flow wetland designs.[48] Approaches to increase contaminant removal rates have included subsurface aeration, effluent recirculation, hybridization with chemical oxidation processes (e.g., upstream ozonation), or stimulating microbial activity by applying electrical currents to the subsurface.[39,49–53] However, intensified constructed wetland designs that employ pumps, electrodes, or other equipment require more maintenance and may sacrifice other important benefits, such as habitat quality.[54]

Constructed wetlands may be able to overcome the limitation of large land area requirements by offering additional benefits that are valued by communities. For example, the North Texas Municipal Water District constructed an 800-hectare surface flow wetland system capable of treating 91 MGD of treated wastewater because the wetlands enabled reuse of water from the effluent-dominated Trinity River and their construction prevented the need to invest in other water supply infrastructure (e.g., reservoir construction).[55] This project also highlighted the value that the community placed on habitat and recreational benefits provided by wetlands. As another example, multi-stakeholder analyses show that regional decision makers in the San Francisco Bay Area (e.g., utilities and regulators) are willing to spend more on wastewater treatment projects if they can provide additional benefits like habitat creation and sea level rise adaptation.[56,57] Sea level rise adaptation is a particularly appealing benefit because engineered or nature-based solutions built to protect coastal communities and infrastructure will require large land areas and substantial capital investments regardless of the chosen approach.

1.2.1 Horizontal Levees

Horizontal levees are subsurface flow treatment wetlands that are designed to treat municipal wastewater effluent while providing habitat and protecting coastal infrastructure from the effects of sea level rise.[44,58] Horizontal levees consist of a gradually sloped wetland that is built on the seaward side of conventional levees located near coastal wastewater infrastructure (Figure 1.1 A conceptual rendering of a horizontal levee constructed adjacent to a municipal wastewater treatment plant. Adapted from the bay institute and kingcounty.gov. The sloped design is intended to attenuate wave action while providing land area for the managed retreat of coastal marsh habitat as it is inundated by rising sea levels. The subsurface has a layered design consisting of a surficial layer (typically <1 m thick) comprised of fine sediments overtop of a thin treatment layer of sand, gravel and woodchips (e.g., <0.5 m). The fine surface layers provide a suitable matrix for native plants to grow which creates high-value habitat and dissipates wave action. In the treatment layer, sand and gravel increase the hydraulic conductivity while woodchips provide organic carbon to increase the rate of microbial denitrification.[59] After plants establish on the surface, decaying plant material, roots, and exudates contribute labile carbon that supports subsurface microbial processes.[59]

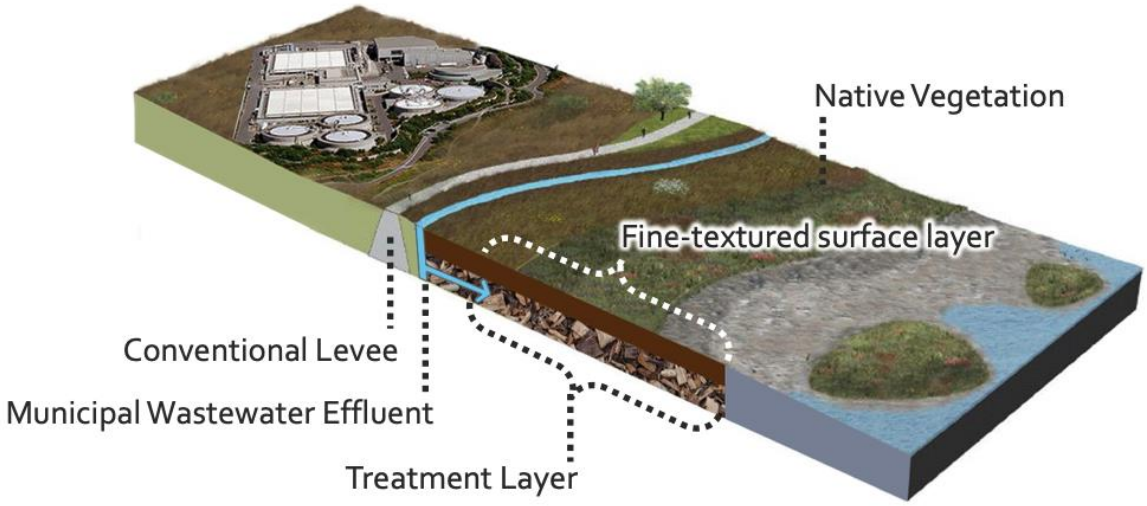


Figure 1.1 A conceptual rendering of a horizontal levee constructed adjacent to a municipal wastewater treatment plant. Adapted from the bay institute and kingcounty.gov

Previously published research on an experimental horizontal levee system indicated that nitrate is removed from treated wastewater mainly by denitrification within the first 5-10 meters of the subsurface along with most other wastewater-derived trace organic contaminants.[44,59,60] Specifically, monitoring results indicated that the experimental horizontal levee reduced the total mass of five trace organic contaminants by over 95% as the treated wastewater passed through the subsurface (Figure 1.2). Although these findings were encouraging, the amount of water that could be treated in the horizontal levee was limited by the system's design (i.e., the hydraulic head and hydraulic conductivity determined the flowrate); if too much water was introduced into the system, overland flow occurred, which substantially reduced the performance of the system.[44] Because contaminant removal was rapid, the horizontal levee was still able to remove nitrate in a significantly smaller area than other wetland designs when the inflow flowrate was reduced to avoid overland flow.[44] In addition, rapid contaminant removal suggested that the horizontal levee may be able to handle higher contaminant loads by treating more concentrated forms of wastewater (e.g., reject generated from reverse osmosis (RO) treatment of municipal wastewater effluent for water reuse).

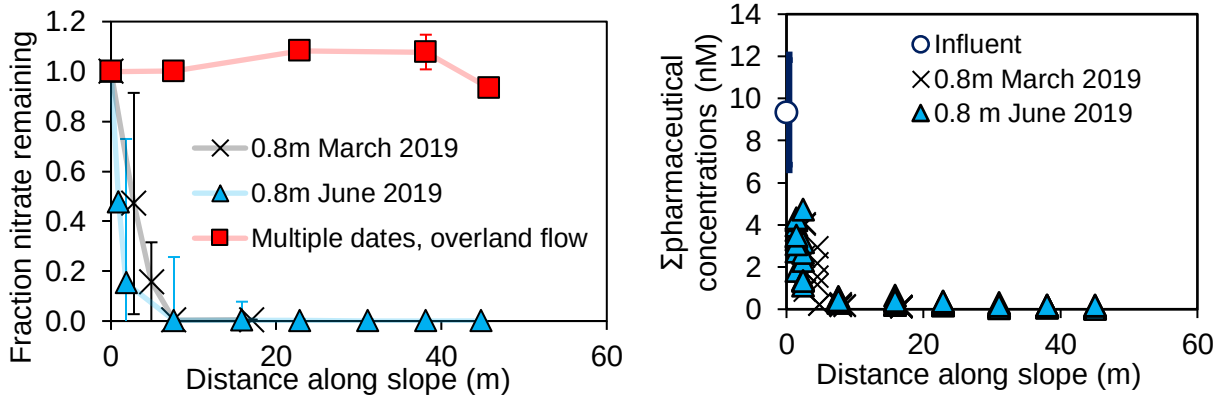


Figure 1.2 Removal of wastewater-derived contaminants in the horizontal levee subsurface. Adapted from Cecchetti et al 2020.[44]

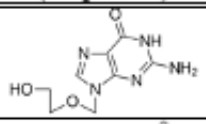
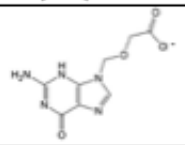
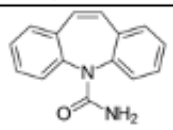
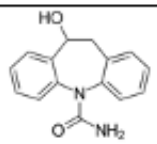
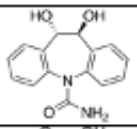
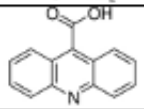
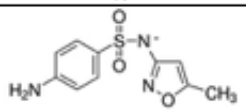
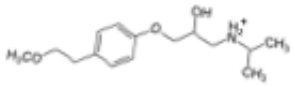
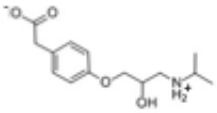
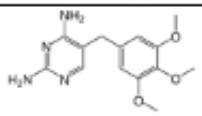
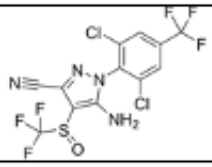
Although trace organic contaminant removal has been documented in experimental horizontal levees and other subsurface flow treatment systems,[44,61] their widespread adoption is hindered by a variety of factors including uncertainty about the relative importance of different simultaneous removal mechanisms and highly variable performance reported in the literature.[62,63] In the case of horizontal levees, differences in treatment performance among different trace organic contaminants as well as the mechanisms through which they are removed remain unclear.

Knowledge related to the relative importance of different removal mechanisms (i.e., sorption, plant uptake/translocation, and biotransformation) in horizontal levees could lead to the development of more efficient designs and insights that could be applied to other subsurface flow treatment systems. For example, a mechanistic understanding could be used to determine the conditions under which microbial communities are capable of biotransforming trace organic contaminants. This may allow for the design of subsurface flow treatment systems that achieve long-term and reliable contaminant removal in less space. This understanding could also ensure high levels of performance when subsurface flow treatment systems are applied to new wastewaters. Finally, mechanistic insights could be used to avoid unintended consequences associated with wildlife use of constructed wetland habitat.

1.2.2 Removal Mechanisms for Trace Organic Contaminants in Subsurface Flow Wetlands

The physicochemical properties of trace organic contaminants (Table 1.1) control their amenability to removal via different mechanisms. The contaminants selected for this research and/or their monitored metabolites are relatively hydrophilic and stable in conventional aerobic wastewater treatment processes (e.g., not amenable to biotransformation during activated sludge treatment or to hydrolysis reactions) and are therefore present in treated wastewater. These contaminants were also selected because they often pose risks to human and ecological health at concentrations found in municipal wastewater effluent,[64] and have been previously studied in other wetland and subsurface-flow treatment designs.[37,65,66]

Table 1.1 Physicochemical properties of the monitored trace organic contaminants.

Compound Name	Compound Structure (At pH 7.5)	pK_a	Charge at pH 7.5	$LogK_{ow}$	$LogD_{ow}$ @pH 7.5
Acyclovir		2.27 9.25	0	-1.56	-1.56
Carboxy-Acyclovir		~2.27 ~4 ~9.25	-1	<-1.56	-6.7
Carbamazepine		NA	0	2.45	2.45
10-OH Carbamazepine		NA	0	1.4	1.4
10-11, Dihydroxy Carbamazepine		NA	0	0.3	0.3
9-Acridine carboxylic acid		0.7	-1	3.1	-3.7
Sulfamethoxazole		1.6 5.7	-1	0.89	-1.2
Metoprolol		9.7	+1	1.88	-0.3
Carboxy-metoprolol		~4 ~9.7	0, zwitterion	-1.5	-5.1
Trimethoprim		7.12	0	0.91	0.78
Fipronil		NA	0	4.5	4.5

1.2.2.1 Sorption

Sorption removes dissolved chemicals from aqueous solution via adsorption – the accumulation of a chemical at the interface between two phases – and absorption – the partitioning of a chemical from one phase into another. In the subsurface, adsorption and absorption primarily result from the interactions between dissolved chemicals, water, and solid phases such as soil minerals or particulate organic matter. In the case of subsurface flow treatment wetlands, trace organic contaminants can sorb onto sediment particles, plant roots, particles or coatings of organic matter, or other media used in their construction (e.g., zeolite, light-expanded clay aggregate[67]). This behavior depends on the physicochemical properties of the contaminant of interest, aqueous matrix, and solid phase. Broadly, hydrophobicity and ionization of trace organic contaminants control their tendency for removal through sorption.

Hydrophobicity influences trace organic contaminant sorption because it is energetically unfavorable for a hydrophobic molecule to be dissolved in water. Therefore, hydrophobic compounds tend to aggregate and partition onto or into other hydrophobic (e.g., non-polar) media. A commonly used metric to predict the affinity of organic contaminants for organic phases in sediments is the octanol-water partitioning coefficient (K_{ow}). It is often assumed that only non-ionized organic contaminants undergo sorption via hydrophobic interactions, therefore the speciation of the contaminant under aqueous solution conditions can be used to calculate the equilibrium partitioning of the neutral species using a pH-dependent octanol-water distribution coefficient (D_{ow}). Contaminants with a higher D_{ow} have a higher affinity for sorption to organic phases. Of the contaminants selected for this research, we anticipated that carbamazepine, fipronil and trimethoprim would have the highest affinities for sorption via hydrophobic interactions due to their higher D_{ow}'s (Table 1.1).

Trace organic contaminants can also adsorb to surfaces via electrostatic interactions (i.e., the attraction between oppositely charged species). Because common soil minerals and organic material tend to bear negative surface charge at circumneutral pH,[68] cationic trace organic contaminants, such as metoprolol and trimethoprim, will exhibit a higher affinity for surfaces than anionic trace organic contaminants such as carboxy-acyclovir (Table 1.1). However, interactions between charged surfaces and contaminants are sensitive to aqueous solution chemistry (e.g., pH, the presence of competing or bridging ions).

Sediments are comprised of heterogeneous surfaces. As a result, sorption is often characterized empirically via isotherms. Despite the variety of mechanisms that can result in sorption of trace organic contaminants, at low sorptive concentrations, equilibrium concentrations are often predicted by a linear relationship between aqueous and particle-associated concentrations. The slope of the linear portion of the isotherm (i.e., the K_D value) can be used to predict contaminant fate and transport in subsurface systems. For example, contaminants with higher K_D values (e.g., hydrophobic or positively charged trace organic contaminants) tend to be retained to a greater degree via sorption and therefore exhibit longer breakthrough times in flow-through systems. Although sorption isotherms do not determine the mechanisms through which adsorption occurs, they do provide a basis for understanding the total mass of contaminants expected to adsorb onto sediments.

1.2.2.2 Plant Uptake

Removal by plants can be significant in systems in which trace organic contaminants that are amenable to plant uptake are not removed via other mechanisms, especially if a substantial portion of the water is transpired via plants as is sometimes the case in horizontal levees.[44] To enter plants, trace organic contaminants must first undergo root uptake via the apoplastic (in water along cell walls) or the symplastic (through plant cells by crossing cell membranes) route. Hydrophobic and ionized compounds tend to remain in plant roots[69] which limits the importance of plant uptake to the mass balance of these compounds because the mass of plant roots in the subsurface is often much lower than the mass of other sorbents. However, plant uptake can remove a substantial fraction of the mass of neutral compounds of intermediate hydrophobicity (e.g., log K_{ow} 2-4) because they are mobile in the evapotranspiration stream and can accumulate in leaves and other above-ground plant organs over time in systems with high transpiration rates.[69] Regardless, after uptake into roots and/or translocation to shoots, trace organic contaminants can be metabolized by plant enzymes.[70,71] After plant death and decay, trace organic contaminants in plant tissues can be released back into water or undergo transformation by enzymes present during decomposition.[72] Finally, plants roots may also impact contaminant removal by stimulating microbial communities in rhizospheres.[32,73]

1.2.2.3 Transformation Reactions

Biotransformation of trace organic contaminants mostly occurs due to coincidental transformation of trace organic contaminants that exhibit structural similarities to the substrates utilized by microorganisms in subsurface environments.[2] This is because trace organic contaminants are usually present at extremely low concentrations (i.e., <1 µg/L) and exist in the presence of other labile substrates. This limits evolutionary pressure on microorganisms to rely upon a specific trace organic contaminant for metabolic growth. The structure and function of microbial communities are sensitive to environmental conditions such as redox conditions, pH, temperature, and the availability of carbon or nutrients. Under anaerobic conditions, microbial communities must rely upon enzymes that do not make use of molecular oxygen to oxidize carbonaceous substrates.[74] These enzymes may be able to co-metabolize trace organic contaminants that are persistent under aerobic conditions. For example, trimethoprim and sulfamethoxazole, two compounds that are persistent under aerobic conditions, are removed rapidly in anaerobic treatment systems via biotransformation.[75,76] This suggests that exposing trace organic contaminants to a diversity of microbial communities growing under different redox conditions may lead to increased rates of contaminant transformation.[77]

Transformation of trace organic contaminants can also occur due to reactions with species produced by microbial activity. In subsurface environments, the reactive species are often produced by microorganisms due to their use of electron acceptors. For example, microbial communities that rely upon sulfate as a terminal electron acceptor typically produce HS^- or other reduced sulfur species (e.g., polysulfides) which are potent nucleophiles. Adsorbed and dissolved reduced sulfur species react with nitroaromatic compounds[78,79] and natural organic matter[80] through reduction and nucleophilic substitution reactions. Under Fe(III)-reducing conditions in

soils and sediments, Fe(II) adsorbed to Fe(III) oxyhydroxides can abiotically reduce sulfamethoxazole resulting in the formation of ring cleavage products.[81] Finally, sulfamethoxazole and other trace organic contaminants that contain aromatic amines can react abiotically with reactive nitrogen species produced during microbial denitrification.[82]

1.2.2.4 Distinguishing Among Possible Removal Mechanisms in Subsurface Treatment Wetlands

Determining the relative importance of different removal mechanisms in subsurface flow treatment systems is challenging because they occur simultaneously, and the rates of different processes can vary over time. For example, the rate of plant uptake of trace organic contaminants will vary seasonally in temperate climates due to higher plant growth and evapotranspiration rates in summer months when sunlight is abundant. In addition, during winter months, decaying plant material could be an internal source of trace organic contaminants if they are released from plant residues during decomposition. Seasonal temperature variability could also impact the rates of trace organic contaminant transformation. Adsorption is likely to be important during start-up periods when microbial and plant communities have yet to mature and when sediments have not yet reached equilibrium with respect to inflow concentrations of trace organic contaminants. Regardless, research to determine the roles of different mechanisms over long operation times is essential to inform subsurface flow treatment wetland designs.

Despite the potential benefits of understanding the mechanisms responsible for trace organic contaminant removal in subsurface flow wetlands, most available information is limited to observations of concentrations of trace organic contaminants in water flowing into and out of these systems.[83,84] Methods that could be used to determine the predominant removal mechanisms include: high-resolution porewater sampling, sampling of multiple media (e.g., plants, soil, and water), sampling after long periods of operation, pairing field studies with microcosm studies and fate and transport modeling, quantifying transformation products, and metagenomics and metatranscriptomics to relate contaminant removal to specific microbial activities.

Due to similarities in conditions (e.g., flow of treated wastewater through porous media), results from mechanistic research on managed aquifer recharge and riverbank filtration systems provide insights that could be applied to horizontal levees. For example, the primary mechanism through which hydrophilic trace organic contaminants are removed in these systems is via transformation reactions.[65] Therefore, removal rates depend on the presence and activity of microbial communities, which are sensitive to parameters such as redox conditions, the nature of available organic carbon, and other factors. In addition, results from this research suggest that trace organic contaminants that are extremely persistent in other biologically-based treatment systems (e.g., carbamazepine) could be removed in subsurface systems that employ anaerobic redox conditions.[74] However, the role that specific anaerobic terminal electron acceptors (e.g., sulfate, Fe(III)) play in the removal of these persistent compounds remains unclear. In addition, anaerobic conditions are often undesirable in managed aquifer recharge or riverbank filtration because water that has passed through anaerobic conditions may contain elevated concentrations of iron and sulfur which are undesirable in drinking water. However, anaerobic conditions may be less objectionable in the treatment of municipal wastewater effluent prior to discharge into surface water.

1.3 Research Objectives

Although prior research indicated that horizontal levees remove trace organic contaminants from municipal wastewater effluent, differences in the treatment performance among individual compounds as well as the mechanisms through which they are removed are unclear. In the absence of a mechanistic understanding, the design of horizontal levees to achieve specific treatment goals within the land area available for their construction is challenging. In addition, uncertainties about the relative importance of different removal mechanisms raise questions about the long-term use of horizontal levees due to the possibility that their performance might diminish or that they could act as attractive nuisances if wildlife is exposed to wastewater-derived trace organic contaminants within them.

A mechanistic understanding could provide insights that lead to improved horizontal levee design and performance while avoiding unintended consequences. To achieve this, we assessed the removal of trace organic contaminants from municipal wastewater effluent through a combination of high-resolution porewater, soil and plant sampling in an experimental horizontal levee. We combined field sampling with microcosm experiments and models of contaminant fate and transport to determine the conditions under which trace organic contaminants were removed. Findings related to plant uptake of trace organic contaminants in horizontal levees were translated to effluent-dominated riparian zones – areas of significant ecological value in semi-arid climates. Finally, the potential for using horizontal levees to treat RO concentrate from water reuse projects – a new application for the technology – was assessed.

Objective 1: Evaluate the ability of a horizontal levee to remove trace organic contaminants from treated wastewater and assess the relative importance of different removal mechanisms

In 2017, a demonstration-scale, experimental horizontal levee was built to test the efficacy of different soil types and plant communities for the removal of contaminants from treated wastewater. Samples of inflow, outflow and porewater were used to evaluate system performance. Multi-media sampling, analysis of water quality parameters related to redox conditions and biogeochemistry along with the analysis of transformation products and microcosm experiments were used to document performance, removal rates and seasonal effects and to gain insight into removal mechanisms. Monitoring of the system after upgrades to the treatment plant that supplies treated effluent to the field site yielded additional insights about the importance of redox conditions to contaminant removal. The findings from this objective allowed for a comparison between trace organic contaminant removal in horizontal levees to other nature-based treatment systems. Results were also used to provide design insights for the removal of extremely persistent trace organic contaminants and for the treatment of RO concentrate.

Objective 2: Assess the extent of plant uptake and translocation of trace organic contaminants in a horizontal levee and translate findings to other effluent-dominated ecosystems

Plant uptake and translocation of trace organic contaminants was assessed in the demonstration-scale field site using spatial, species-specific, and temporal plant sampling methods. Because conditions in the horizontal levee are similar to those found in effluent-dominated riparian zones,

results from this objective were translated to effluent-dominated ecosystems – a common and ecologically important feature of semi-arid regions such as the Southwestern United States. Results from this research highlighted an overlooked terrestrial exposure pathway that should be considered when predicting ecotoxicological risks associated with municipal wastewater effluent in effluent-dominated streams.

Objective 3: Evaluate the suitability of horizontal levees for the treatment of reverse osmosis (RO) concentrate from municipal wastewater reuse projects

In 2019, the source water for one of the twelve wetland cells of the horizontal levee test facility was converted from wastewater effluent to RO concentrate from a nearby municipal wastewater reuse facility. The fate of trace organic contaminants and nitrate in the RO concentrate cell was compared to an identically constructed wetland cell that received municipal wastewater effluent. We determined the annual mass of contaminants removed from wastewater effluent and RO concentrate per unit area in the horizontal levee and compared values to other recently tested nature-based RO concentrate treatment systems. Results highlighted that horizontal levees outperform other nature-based RO concentrate treatment systems. In addition, findings from this objective supported conclusions drawn in Objective 1 about the importance of redox conditions to the removal of persistent trace organic contaminants in horizontal levees.

CHAPTER 2. Persistent Trace Organic Contaminants are Rapidly Removed Through Biotransformation Under Sulfate and Fe(III)-Reducing Conditions in a Field-Scale Subsurface Treatment Wetland

The following chapter is adapted from a co-authored manuscript with permission from Cecchetti, A.R., Scholes, R.C, and Sedlak, D.L. Persistent Trace Organic Contaminants are Rapidly Removed Through Biotransformation Under Sulfate and Fe(III)-Reducing Conditions in a Field-Scale Subsurface Treatment Wetland. *Manuscript to be submitted for publication in Environmental Science & Technology.*

Abstract

Subsurface treatment systems, such as constructed wetlands, riverbank filtration and managed aquifer recharge systems offer an attractive means of removing trace organic contaminants derived from municipal wastewater effluent because they are relatively inexpensive and provide additional water quality benefits. To assess the processes through which trace organic contaminants are removed, a suite of pharmaceuticals and their metabolites were studied in a demonstration-scale subsurface treatment wetland that has been receiving nitrified effluent from a municipal wastewater treatment plant since 2017. Concentrations of trace organic contaminants and their major metabolites in wetland porewater, sediment, and plants rapidly declined along the flow path throughout the monitoring period. Mass balance calculations, analysis of transformation products, microcosm experiments, and one-dimensional transport modeling suggested that contaminant removal was primarily attributable to biotransformation. Monitoring of the system after treatment plant upgrades eliminated nitrate from the water flowing into wetland indicated that relatively biodegradable trace organic contaminants, such as acyclovir and metoprolol, were rapidly transformed with or without nitrate acting as a terminal electron acceptor. Trace organic contaminants that are normally persistent in biological treatment systems (e.g., sulfamethoxazole and carbamazepine) were not removed until Fe(III)- and sulfate-reducing conditions were achieved. Minor structural modifications to trace organic contaminants (e.g., hydroxylation) altered the pathways and extent of their biotransformation under different redox conditions. These results indicate that subsurface flow treatment systems can be designed to remove persistent trace organic contaminants via biotransformation if they are operated in a manner that results in Fe(III)- and sulfate-reducing conditions without sacrificing their ability to remove trace organic contaminants that are transformed under aerobic or anoxic conditions.

2.1 Introduction

The treatment processes employed at municipal wastewater treatment plants are not optimized to remove hydrophilic trace organic contaminants (i.e., micropollutants), such as pharmaceuticals, personal care products, urban use pesticides and industrial chemicals. Therefore, these chemicals are often present in wastewater effluent at concentrations that pose risks to downstream ecosystems and drinking water supplies.[4,5,13] Removal of hydrophilic contaminants that are not readily transformed in wastewater treatment plants represents a significant challenge because engineered processes that rely upon oxidation (e.g., effluent ozonation) and physical separation (e.g., activated carbon filtration) are expensive.[22]

Under some conditions, passage of water containing persistent trace organic contaminants through the subsurface can be an effective means of treatment. For example, a few studies indicate that sulfamethoxazole and carbamazepine were removed in anaerobic soil treatment systems and anaerobic membrane bioreactors despite their persistence in aerobic environments.[62,85–87] Under these conditions, microorganisms utilize different enzymes than those typically present during conventional activated sludge treatment which may promote biotransformation of wastewater-derived trace organic contaminants.[88,89] Even so, subsurface flow treatment systems are not typically designed to reach anaerobic conditions due to low levels of labile organic carbon in surface water and treated wastewater and because sulfide and Fe(II), which are produced under anaerobic conditions, are undesirable in applications intended to augment drinking water supplies. In addition, there are concerns that the removal of trace organic contaminants that are typically removed under aerobic conditions will be hindered in systems that employ anaerobic conditions.

The horizontal levee is a subsurface flow treatment wetland that includes a porous, woodchip-amended underground treatment layer and a vegetated surface layer covered with decomposing wetland plants to supplement labile organic carbon and enhance the rate of nitrate removal from wastewater effluent. Prior research showed that an experimental horizontal levee removed nearly all of the nitrate in wastewater effluent within the first five meters of the 45-meter long treatment zone via denitrification.[44,59,60] After nitrate was depleted, analysis of redox-active species suggested the presence of Fe(III)- and sulfate-reducing conditions in a spatiotemporal order dictated by thermodynamics.[59] However, the role that specific terminal electron acceptors (e.g., nitrate, sulfate, Fe(III)) play in the microbially-induced anaerobic or anoxic transformation of persistent trace organic contaminants in horizontal levees is unclear.

To test the hypothesis that the redox conditions in horizontal levees create conditions that are conducive to the biotransformation of persistent trace organic contaminants, we analyzed a suite of trace organic contaminants and common transformation products in porewater samples, sediments and plant tissues collected at different distances from the inlet of a field-scale experimental horizontal levee that continuously received effluent from an adjacent municipal wastewater treatment plant. Field measurements were complemented by microcosm experiments conducted in freshly collected sediments with and without nitrate supplied as a terminal electron acceptor and fate and transport modeling.

2.2 Methods

2.2.1 Field Site

Samples were collected from 9 hydraulically-isolated, parallel wetland cells in a 0.7-ha experimental horizontal levee.[44] These wetland cells were built to test the performance of various planting regimes and surface substrate textures with respect to treatment efficacy and plant growth (Figure 2.1). Three "fine" wetland cells were built with clay loam surface sediments (e.g., an approximately 80-cm thick sediment layer) planted with native meadow vegetation.[44,90] The six remaining cells were built with sandy loam surface sediments planted with either meadow vegetation (i.e., "coarse") or willow trees (i.e., "willow"). Surface sediments in all wetland cells were underlain with a 20-cm thick treatment layer comprised of sand, gravel, and woodchips located between 80-100 cm below the surface. Beneath the treatment layer, compacted clay lined with a geotextile filter fabric prevented water percolation into groundwater (Figure A.1). Photos of the field site can be found in section D1.1 of the Appendix.

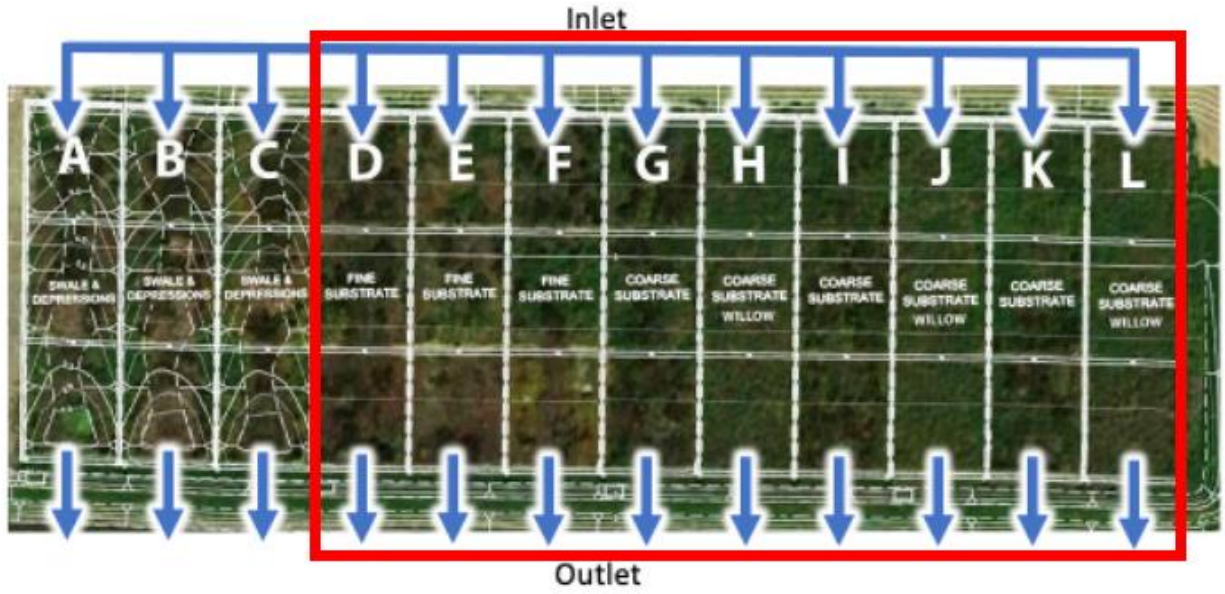


Figure 2.1 Satellite imagery of the horizontal levee overlaid by topographic contours showing parallel wetland cell configurations. Cells D-L were monitored in this study and are highlighted by the red box. Cells D-F were built with fine surface sediments. Cells G-L were built with coarser surface sediments. All cells were planted with a native plant palette typical of wet meadows except for cells H, J, and L which were planted with willow trees (*Salix spp.*).

Nitrified effluent from an activated sludge wastewater treatment plant was continuously fed into the subsurface treatment layer of the wetland cells starting in April of 2017. In August of 2020, the wastewater treatment plant underwent upgrades to achieve biological nutrient removal. This resulted in a decrease in the concentration of inorganic nitrogen entering the horizontal levee inflow, with nitrate dropping from approximately 30 mg-N/L to <1 mg-N/L starting in the summer of 2021. Total nitrogen also dropped from approximately 40 mg-N/L to 1.5 mg-N/L.

2.2.2 Sampling

According to previously published methods,[44] inflow and outflow samples were collected at approximately monthly intervals from the wetland cells between August 2018 and May 2019. During the summer (i.e., June-July) of 2019, inflow, outflow and porewater samples from 5 to 8 locations were collected in all 9 wetland cells. An additional set of samples was collected from fine-textured wetland cells in the winter (i.e., March) of 2019. In June of 2022, samples were collected from a representative fine wetland cell to compare performance after nitrate concentrations dropped in the water flowing into the horizontal levee. Inflow and outflow samples were collected using a Masterflex E/S portable water sampler (Cole-Parmer) after purging at least two well volumes from the sampling well where appropriate. Porewater samples were collected from approximately 0.9 meters below the sediment surface into Luer-Lock BD syringes using stainless steel PushPoint sediment porewater samplers (MHE Products) attached to PharMed® BPT tubing (Cole-Parmer). The first 50-100 mL of extracted porewater (i.e., approximately two to four times the volume of the porewater sampling apparatus) was discarded prior to sample collection. All samples were immediately filtered through 0.7-µm glass fiber filters into 50-mL polypropylene centrifuge tubes and stored on ice until their return to the laboratory.

In the summer of 2018, sediment was collected from surface sediments and from the sand, gravel, and woodchip treatment layer approximately 10 meters from the inlet of cell G (i.e., coarse wetland cell planted with meadow vegetation) using a "4-inch stony soil auger" (AMS, Inc., American Falls, ID, USA) to assess sorption of trace organic contaminants to the sediment and treatment layer materials. Upon returning to the lab, gravel larger than 0.5 cm was removed and the remaining sediment was allowed to air-dry at room temperature for over 48 hours. 5 g of air-dried sediment was portioned into 50-mL centrifuge tubes and 25 mL of a solution containing 10 mM $CaCl_2$ and increasing concentrations of trace organic contaminants was added to each tube. The centrifuge tubes containing sediment and water were placed on a rotary shaker and allowed to equilibrate for 24 hours prior to sample collection. Isotherms, which were run in triplicate, were conducted at concentrations that extended beyond the concentrations entering the wetland for most compounds. Distribution coefficients were compared to field-derived and microcosm-derived distribution coefficients calculated by pairing porewater concentrations with extractable sediment concentrations.

2.2.3 Microcosm Experiments

Microcosm experiments were performed using fresh sediments collected from the subsurface treatment layer. Sediments from the treatment zone of the nitrate-reducing region and the sulfate-reducing region were placed in plastic containers without headspace, stored on ice and transported back to the lab. Porewater that would later be spiked with trace organic contaminants and terminal electron acceptors for use in the microcosms was collected from approximately 3 meters from the outlet of the wetland. Porewater was collected at each sampling location and paired with sediment extractions prior to the microcosm experiments to determine the initial background mass of trace organic contaminants in the microcosms.

In the lab, approximately 30 g of well-homogenized sediments of each type were subsampled into pre-weighed 20-mL glass scintillation vials, after removing gravel larger than 0.5 cm from the sediment. Approximately 2 mL of a spiked-wetland porewater solution containing the corresponding terminal electron acceptor and a mixture of the trace organic contaminants (approximately 50 µg/L of each) without any residual solvent was added to each vial. The time between sediment collection and initiation of the experiment was approximately 10 hours.

Microcosms were incubated in the dark at room temperature (23ºC +/- 0.7ºC). Porewater was collected in triplicate by sacrificially sampling microcosms. Incubations lasted 2 days in the nitrate-reducing microcosms and over 28 days in the sulfate-reducing microcosms. Trace organic contaminants were extracted from microcosm sediments collected before and during incubation. A detailed sampling and experimental plan for the microcosm experiments can be found in section A1.2 of the Appendix.

2.2.4 Analytical Methods

Trace organic contaminants, redox-active species and water quality parameters were analyzed using previously published sample-processing and analytical methods.[44] Carbamazepine metabolites were analyzed using methods described in section B1.2 of the Appendix. Briefly, water samples were syringe-filtered through 0.7-µm glass fiber filters in the field and held on ice prior to analysis. Field-filtered subsamples were diluted 1:10 into 1% nitric acid and analyzed for redox-active metals using ion coupled plasma mass spectrometry. Immediately upon their return to the lab, water subsamples were syringe-filtered through 0.22-µm nylon filters and analyzed using ion chromatography. Trace organic contaminants were analyzed in subsamples that were filtered through a 0.22-µm nylon filter using stable-isotope dilution liquid chromatography mass spectrometry (LCMS).[44] A list of all analytes included in this research (Table A.3) as well as quality control/quality assurance protocols and additional analytical details (Section B1.2 of Appendix B) are provided in the Appendix. All porewater and outflow concentrations were normalized by the ratio of chloride in the sample to chloride in the influent to correct for evapotranspiration.[39,44]

Subsamples of freshly collected sediment used in microcosm experiments were extracted with a modified version of a procedure used for extractions of plant material (Chapter 3). Briefly, trace organic contaminants were extracted from 12 g of homogenized, wet sediment using 12 mL of a 3:5 methanol:Milli-Q water solution via sonication and shaking for 30 minutes each. The process was repeated with 7.5 mL of a 1:1 methanol:Milli-Q water solution and combined extracts were filtered through a 0.2-µm polyethersulfone membrane prior to analysis via LCMS. Results of spike-recovery tests for soil extractions can be found in section A1.3 of the Appendix.

2.2.5 Mass Balance Estimation Process

A mass balance was performed assuming that the mass of contaminants entering the wetland could be accounted for by the mass that exited the wetland plus the mass that was sorbed onto sediments, remained in the porewater, was taken up by plants, and was transformed through microbially-mediated and abiotic processes. The mass balance was informed by data reported elsewhere, such

as inflow flowrates,[44] annual plant biomass production,[60] and concentrations of trace organic contaminants measured in plant tissues (Chapter 3). Data from isotherm experiments (Figure A.4), estimates of evapotranspiration and concentrations in water samples were also used in the mass balance calculations. Details about the mass balance calculations are provided in section A2.1 of the Appendix.

Additional information about methods, such as the delineation of redox zones, estimates of hydraulic retention time, and one-dimensional transport modeling to predict contaminant breakthrough times due to sorption can be found in section A2 and A3 of the Appendix.

2.3. Results and Discussion

2.3.1 Contaminant Removal and Mass Balance

Concentrations of trace organic contaminants, including compounds that have proven to be particularly persistent in wastewater treatment plants, riverbank filtration systems and surface flow wetlands (e.g., carbamazepine and sulfamethoxazole) consistently decreased by over 70% in the horizontal levee in all of the wetland cell types (Figure 2.2). Carboxy-metoprolol, a metabolite of metoprolol and atenolol that is present in wastewater effluent and possibly produced within the horizontal levee, was not completely removed during some sampling events.

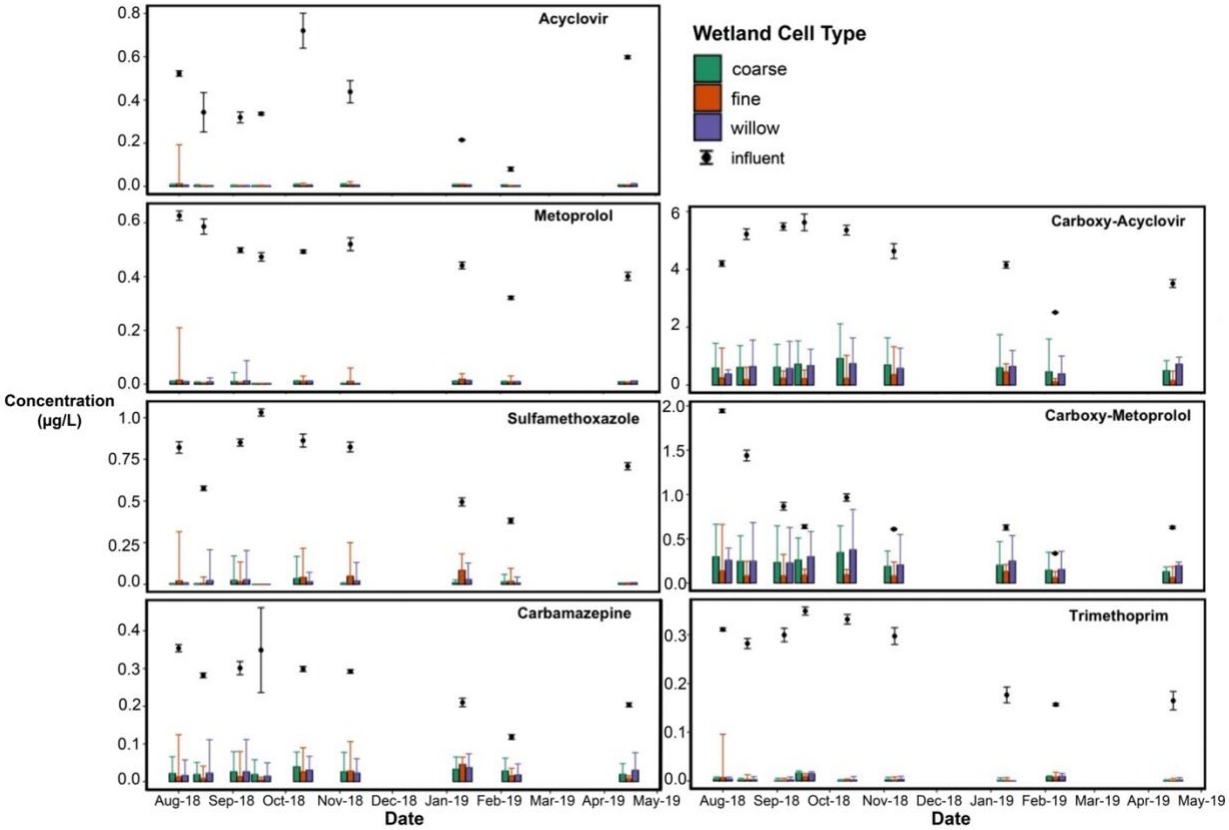


Figure 2.2 Median influent (dots) and effluent (bars) concentrations of a suite of trace organic contaminants in coarse, fine and willow horizontal levee cells (bars, n = 6-9; dots, n = 4-6) over an eight-month monitoring period.

Concentrations of trace organic contaminants measured in the inflow to the horizontal levee decreased by as much as 50% during winter months likely due to increased seasonal rainfall that often dilutes the sewage flowing into the wastewater treatment plant that serves as the inflow to the wetland system. Additional details about seasonal removal rates and the performance of different wetland cell types are presented in section A4.2 the Appendix.

The possible mechanisms of trace organic contaminant removal in the horizontal levee wetland cells included sorption to sediment, plant uptake, and transformation reactions (i.e., biotransformation and abiotic reactions including hydrolysis and reduction by metal- and sulfide-containing minerals). The relative importance of these different removal mechanisms was estimated by quantifying trace organic contaminants in wetland porewater, sediment, and plants over time, and comparing measurements to predictions based on the physicochemical characteristics of individual chemicals (Table 1.1). In addition, laboratory experiments designed to isolate different transformation processes, were utilized.

Analysis of data from sorption isotherm experiments (Figure A.4) suggested that trace organic contaminant removal could not be explained by partitioning to sediments. For example, assuming that sediments were at equilibrium with measured porewater concentrations, predictions made with isotherm data from the treatment layer (i.e., K_D values, Table A.5) indicated that

sorption accounted for less than 12% of the mass of all compounds that entered the wetland between August 2018 and July 2019 other than trimethoprim and metoprolol (Figure 2.3). For metoprolol and trimethoprim, less then 30% of the inflow mass could have been removed by sorption. (Appendix A2.1 for calculations)

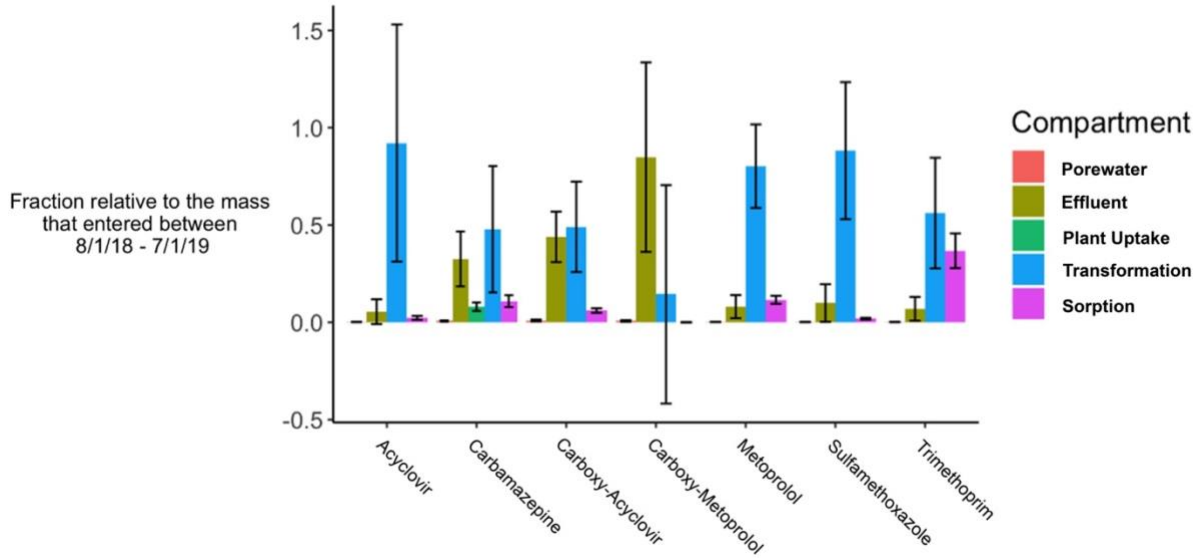


Figure 2.3 Mass balance results for monitored trace organic contaminants that entered the wetland between August of 2018 and July of 2019. The mass fraction in porewater, effluent, plant uptake and sorption wetland compartments were determined based on measurements whereas removal via transformation was estimated by subtraction.

To predict contaminant breakthrough, estimates of hydraulic retention time made with a lithium bromide tracer[44] were validated over the full length of the wetland by monitoring chloride concentrations when the water source of one wetland cell was converted to reverse osmosis (RO) concentrate generated from municipal wastewater reuse (e.g., the experiment conducted in Chapter 4) (Figure A.3 Increases in fraction remaining for Cl^- relative to the final fraction remaining of Cl^- during the transition from wastewater effluent to RO concentrate.). Using this information along with sorption data, we predicted that breakthrough (i.e., the occurrence of effluent contaminant concentrations that were at least 50% of the inflow concentrations) should have occurred for sulfamethoxazole, acyclovir, and carbamazepine within 8 to 12 months of introducing municipal wastewater effluent into the subsurface wetland, which was 4-8 months prior to the date when the first samples were collected. The two compounds with the highest affinities for the sediments in the sand and gravel layer, trimethoprim and metoprolol, were estimated to have travelled approximately 6 to 9 meters and 20 to 30 meters, respectively, by the time that the porewater samples were collected if sorption controlled their removal. Although complete breakthrough was not expected for these compounds, we conclude that sorption was only of modest significance for metoprolol and trimethoprim because their concentrations decreased by over 90% within the first 5 meters of the wetland cells (Figure 2.4). (See Appendix A2.5 for calculations)

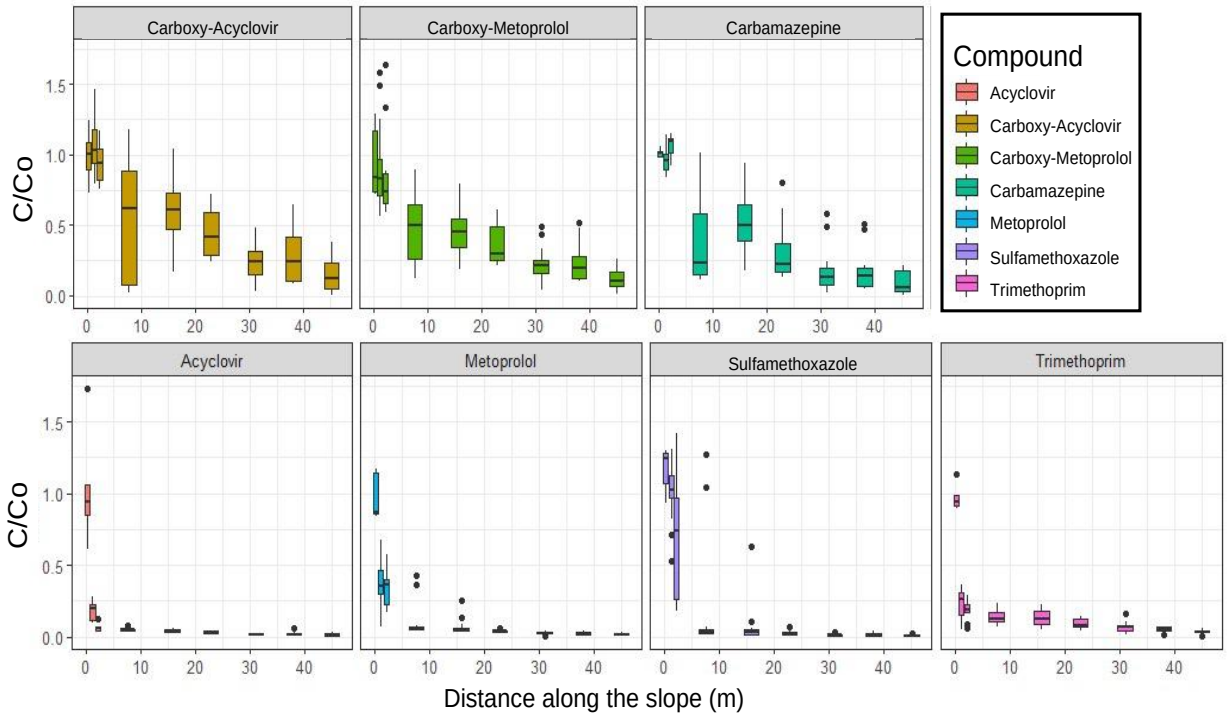


Figure 2.4 Fraction remaining of a suite of trace organic contaminants in porewater samples collected along the horizontal levee slope in all fine, coarse, and willow cells. At each sampling location n = 10-18. Samples were collected in June and July 2019.

Additional evidence for the limited role of sorption was obtained by measuring trace organic contaminants in sediments collected approximately 5 meters and 20 meters from the inlet of the wetland in March 2021 (i.e., 47 months after the system started receiving wastewater effluent) and in microcosm experiments. Field-derived and microcosm-derived K_D values were either similar to or considerably lower than isotherm-derived K_D values, suggesting that the mass balance estimates based upon laboratory isotherms provided a conservative estimate of the contribution of sorption to the removal of trace organic contaminants (Table A.5).

Analysis of concentrations of trace organic contaminants in wetland vegetation indicated that plant uptake, translocation and in-planta transformation also did not account for a significant fraction of the observed removal of trace organic contaminants. Among the compounds present in the wetland porewater, those that are uncharged and of intermediate hydrophobicity (i.e., log K_{ow} ranging from 2 to 4) are most susceptible to plant uptake and translocation via the xylem.[69,91] Typically, only a small fraction of the trace organic contaminants present in the porewater entered the plants because they were partially or completely excluded from the transpiration stream due to difficulty bypassing the casparian strip to enter the xylem. Among the contaminants monitored in this study, carbamazepine is expected to have the highest levels of uptake due to its physicochemical characteristics (Table 1.1) and persistence in the horizontal levee porewater (Figure 2.4). Also, carbamazepine and its metabolites were the only monitored contaminants that were consistently detected in wetland plant samples (Chapter 3). Therefore, we assumed that plant uptake and translocation was negligible for all contaminants apart from carbamazepine.

Using carbamazepine plant concentrations measured in various plant species reported in Chapter 3, estimates of in-planta metabolism[70,71] and biomass measurements,[60] we estimated that plant uptake and translocation removed less than 12% of the carbamazepine entering the horizontal levee subsurface (Figure 2.3, Appendix A2.1 for calculations). This estimate is similar to estimates of plant uptake and translocation of carbamazepine made in other soil-plant systems.[18]

The majority (i.e., >60%) of the mass of trace organic contaminants that was removed in the subsurface cannot be accounted for by sorption and plant uptake (Figure 2.3). Given the large array of reactions that could take place in the horizontal levee, the observed relationships between porewater chemistry and contaminant removal described in the next section, and the removal trace organic contaminants in microcosm experiments (Section A4.3 of the Appendix) we concluded that microbial and chemical transformation reactions accounted for the remainder of the mass of trace organic contaminants. The reactions that could be important in the subsurface include microbial biotransformation (e.g., anaerobic co-metabolism by enzymes[81,85,92–94]) and abiotic reactions involving reactive species that are formed in situ (e.g., HS^-, $Fe[II]_{adsorbed}$).[80,81] Transformation reactions could result in the formation of persistent transformation products of trace organic contaminants with unknown toxicity. Regardless, evidence for the importance of redox conditions for transformation of trace organic contaminants in the wetland is presented in the following sections.

2.3.2 Redox Conditions

The observed relationship between contaminant fate and concentrations of redox-active inorganic solutes provided evidence for the importance of transformation processes in the subsurface. Redox conditions have a significant impact on microbial communities and the formation of reactive chemical species in the wetland sediments through which the porewater flows. Because diverse microbial communities exhibit different capacities for contaminant removal, microbially-driven transformation of trace organic contaminants in the horizontal levee is likely to exhibit distinct spatial variations.[89,95]

Results of prior research showed that spatial redox zones developed in the horizontal levee subsurface: Oxygen and nitrate present in the treated effluent flowing into the wetland served as the main terminal electron acceptors and were depleted within the first 5 meters of the flow path; sulfate in the treated effluent was reduced between 5 and 30 meters from the inlet, and Fe(III)-reducing conditions started at about 5 meters and extended throughout the entire length of the horizontal levee due slow microbial reductive dissolution of Fe(III)-containing minerals in the subsurface media used to construct the wetland (Figure 2.5).[59]

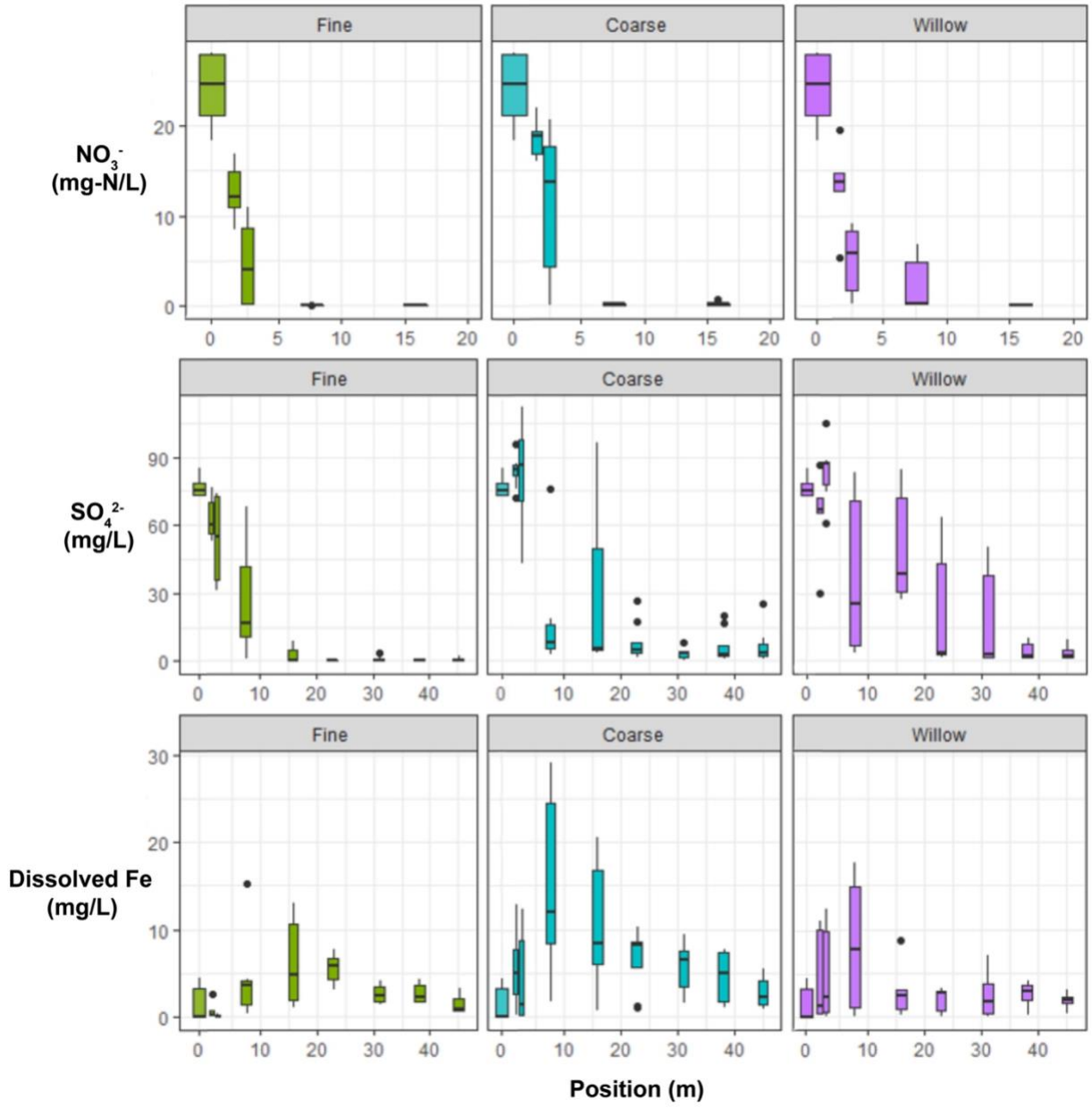


Figure 2.5 Redox conditions as indicated by the concentrations of nitrate, dissolved iron in field-filtered samples, and sulfate in all three wetland cell types- redrawn from Cecchetti et al.[59]

Porewater data from all nine of the monitored wetland cells showed that a subset of the trace organic contaminants (i.e., acyclovir, metoprolol, sulfamethoxazole, and trimethoprim) were transformed primarily within the first eight meters of the inlet (i.e., the zone where oxygen and nitrate were typically the most energetically favorable terminal electron acceptors; Figure 2.4). Other contaminants (i.e., carbamazepine, carboxy-acyclovir, and carboxy-metoprolol) were not transformed to an appreciable extent in the first eight meters. Instead, these compounds were transformed within the zone where sulfate and Fe(III) served as the main terminal electron acceptors.

In five of the wetland cells that did not exhibit spatial overlap between the nitrate- and sulfate-reducing zones, estimates of the fraction of each contaminant removed in each zone supported initial observations about redox-dependent transformation. Results indicated that both conditions contributed to the transformation of some compounds (e.g., metoprolol) whereas others were only appreciably transformed under oxic and nitrate-reducing (e.g., trimethoprim, acyclovir) or sulfate- and Fe(III)-reducing conditions (e.g., sulfamethoxazole, carbamazepine; Figure 2.6).

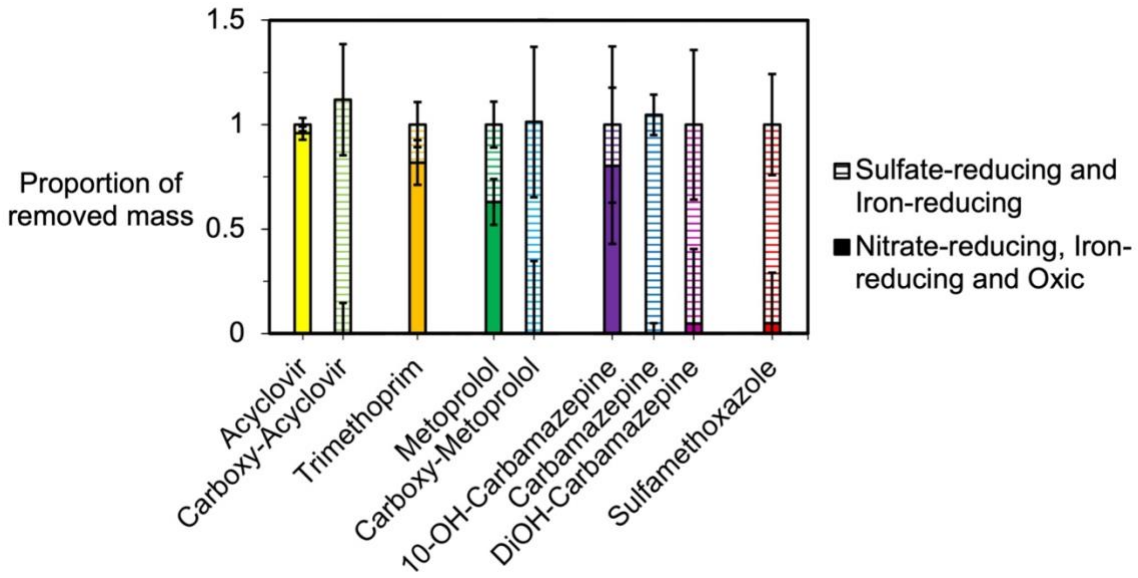


Figure 2.6 Mean mass removed in each redox zone (n=10 for each contaminant) relative to the total mass removed from the inflow for a suite of trace organic contaminants. Error bars represent standard deviations and incorporate variability among wetland cells. Wetland cells in which sulfate- and nitrate-reducing conditions did not overlap were included in this analysis (wetland cells F, G, H, J and L). Some bars do not sum to unity due to small concentration increases observed in the nitrate-reducing zone.

Spatially-resolved porewater sampling from individual wetland cells provided additional evidence for the importance of sulfate-reducing conditions to the transformation of persistent trace organic contaminants such as carbamazepine (Figure 2.7). For example, carbamazepine concentrations dropped concurrently with sulfate concentrations (i.e., in willow cells, the sulfate-reducing zone and carbamazepine removal extended over longer distances than in other wetland cell types).

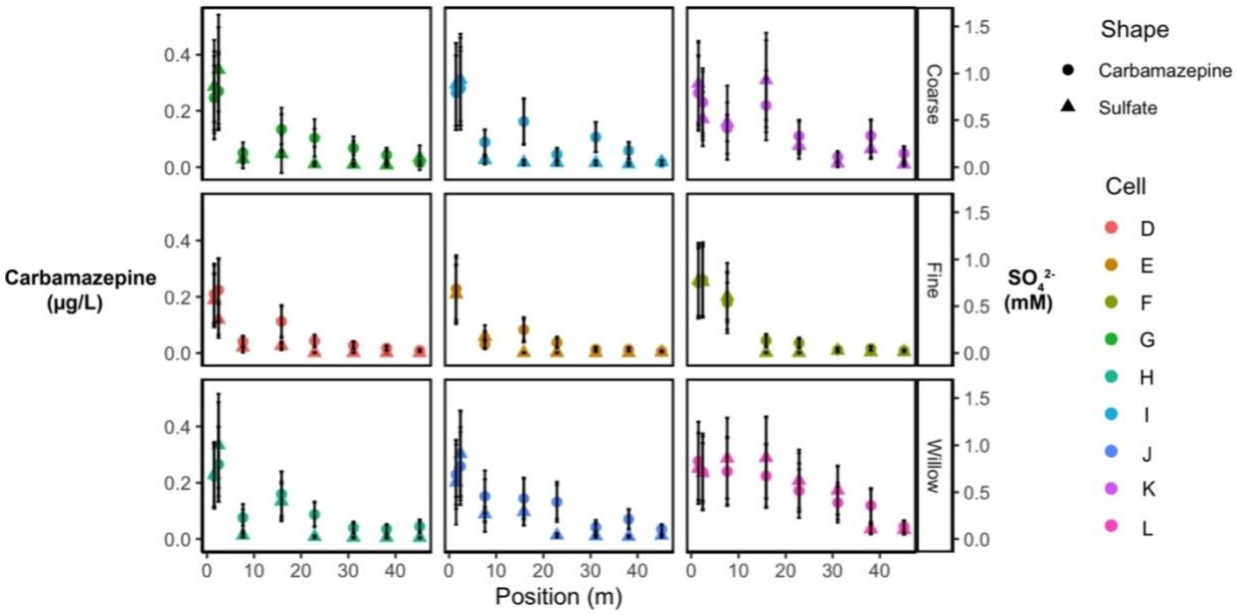


Figure 2.7 Mean concentrations of carbamazepine (dots) and sulfate (triangles) in the influent and coarse, fine, and willow cells along the flow path. Error bars represent the range of measured concentrations at each sampling location.

The strength of the correlation between concentrations of trace organic contaminants and dissolved terminal electron acceptors can partially reflect the importance of the prevailing redox conditions for trace organic contaminant transformation. For example, there were strong correlations ($R^2 > 0.6$) between concentrations of recalcitrant trace organic contaminants (i.e., carbamazepine, carboxy-acyclovir and carboxy-metoprolol) and sulfate concentrations in porewater samples collected from all wetland cells (Figure 2.8). In contrast, dihydroxy-carbamazepine exhibited much weaker correlations with sulfate concentrations ($R^2 = 0.38$), suggesting that sulfate-reducing conditions were less important to the removal of this compound despite decreasing concentrations of the compound observed in the sulfate- and Fe(III)-reducing zone (Figure 2.8, Figure 2.10). In addition, sulfamethoxazole concentrations were correlated to both nitrate and sulfate concentrations suggesting it was transformed under both conditions. However, this correlation could also mean that Fe(III)-reducing conditions played an important role in sulfamethoxazole transformation because Fe(III) reduction overlapped with both nitrate- and sulfate-reducing conditions and rapid sulfamethoxazole transformation has been observed under Fe(III)-reducing conditions previously.[81]

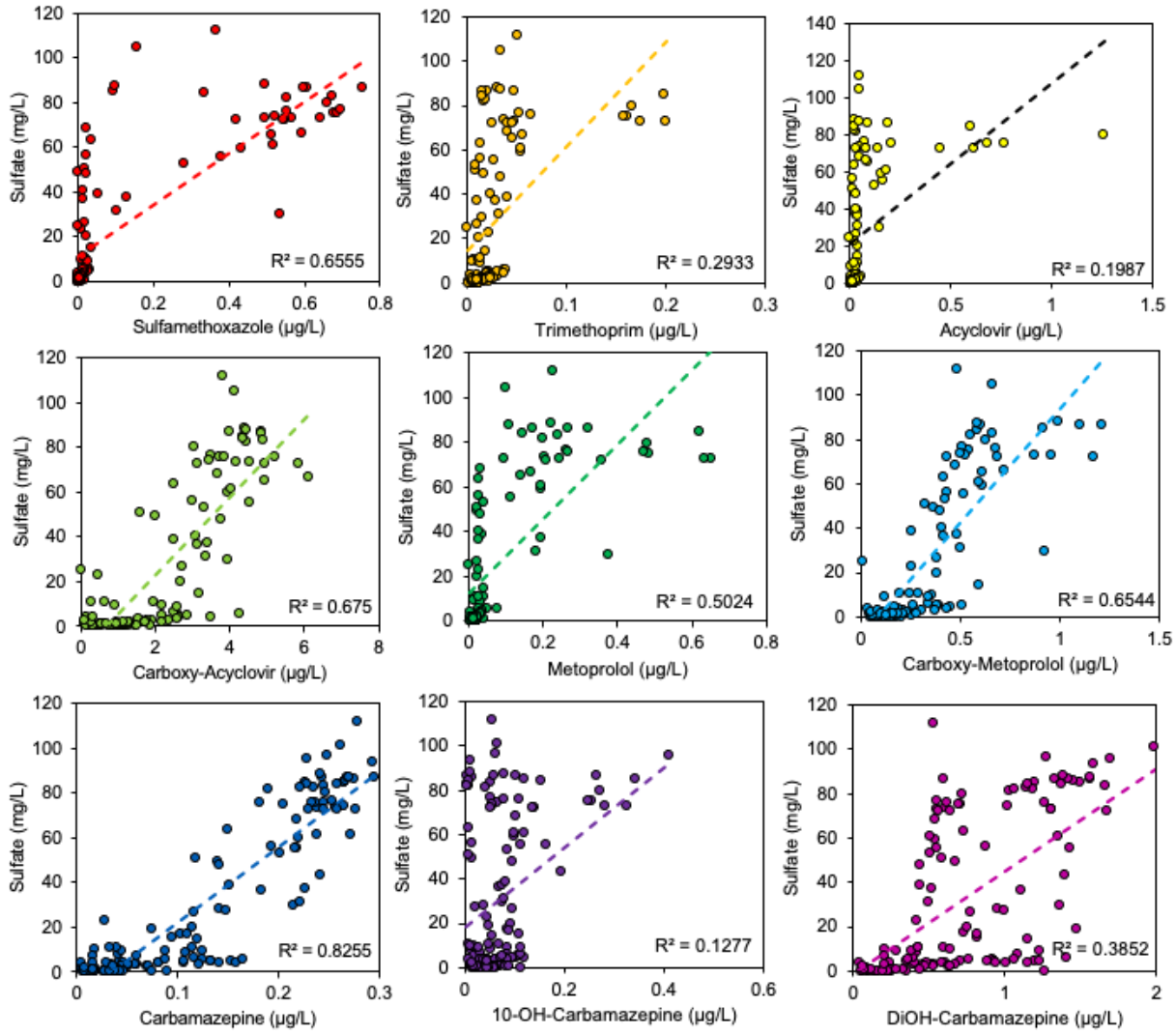


Figure 2.8 Concentrations of sulfate versus the concentrations of trace organic contaminants.

Concentrations of some compounds that are typically less resistant to transformation (e.g., acyclovir, metoprolol) exhibited a strong correlation ($R^2 > 0.6$) with nitrate concentrations, suggesting that nitrate-reducing conditions may have played a role in their transformation (**Figure 2.9**). However, metoprolol concentrations were correlated to both nitrate and sulfate concentrations suggesting it was transformed under both nitrate- and sulfate-reducing conditions in the field. 10-OH carbamazepine concentrations were not correlated to nitrate or sulfate concentrations despite its rapid transformation in the horizontal levee. This suggested that aerobic conditions likely played an important role in its transformation because it was removed primarily between the inflow and the first sampling point where both oxygen and nitrate served as the most energetically favorable terminal electron acceptors (0-1.5 meters, Figure 2.10).

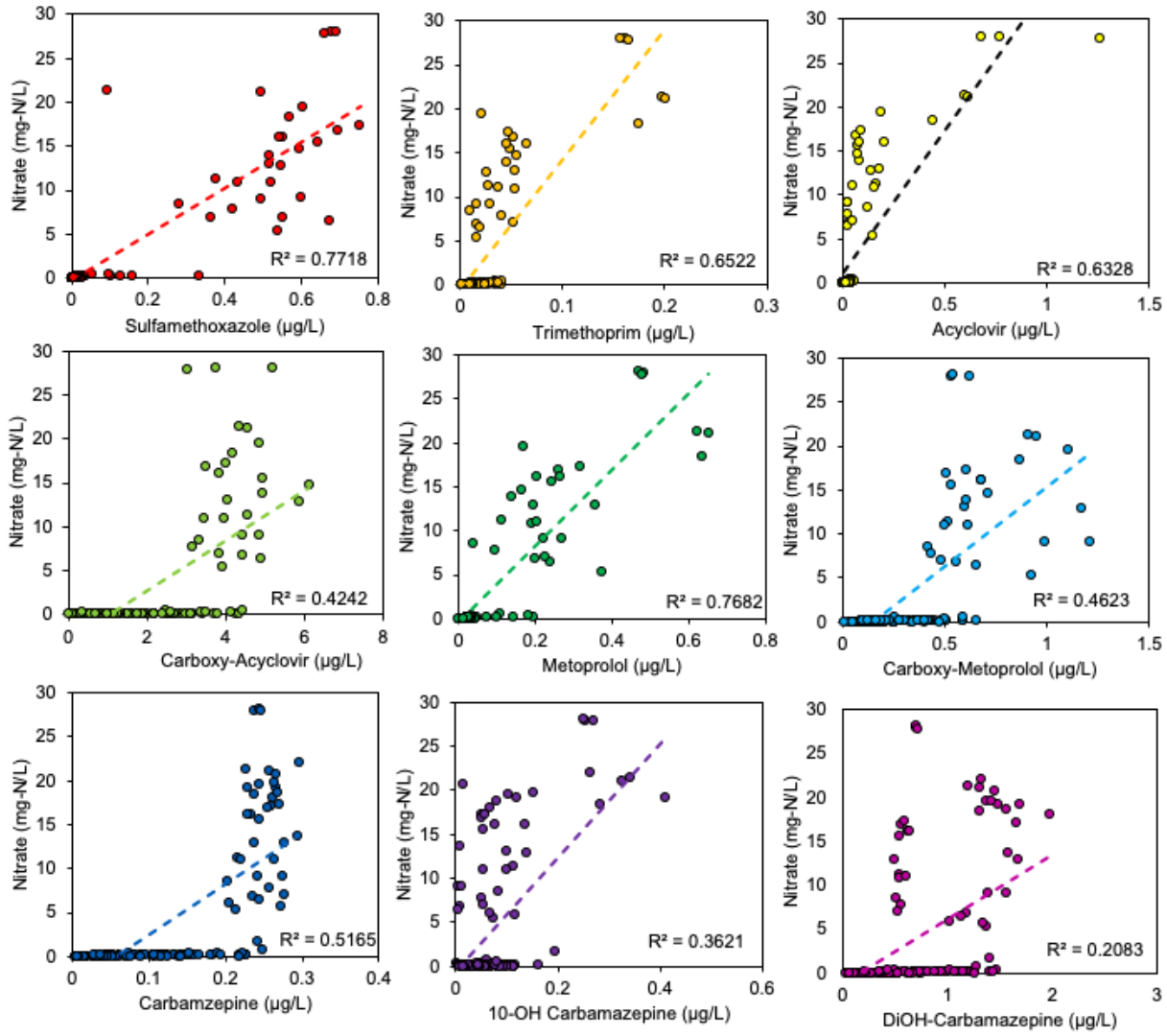


Figure 2.9 Concentrations of nitrate versus the concentrations of trace organic contaminants.

Analysis of concentrations of carbamazepine and its metabolites in porewater collected at different locations in the wetland suggested that subtle structural modifications (e.g., hydroxylation) altered the rates and pathways of carbamazepine transformation under different redox conditions (Figure 2.10). For example, concentrations of 10-OH carbamazepine decreased in the aerobic and nitrate-reducing zone in the field as concentrations of 9-acridine carboxylic acid—a transformation product that has been observed in aerobic sand filters[96]—was formed. After 9-acridine carboxylic acid was formed, it was rapidly removed under sulfate- and Fe(III)-reducing conditions. Despite 10-OH carbamazepine transformation under both nitrate- and sulfate-reducing conditions in microcosms, 9-acridine carboxylic acid was only formed in the nitrate-reducing microcosms which suggested that the 10-OH carbamazepine transformation pathway shifted under different redox conditions (Figure A.12). In contrast to the behavior of 10-OH carbamazepine, concentrations of carbamazepine decreased exclusively under sulfate- and Fe(III)-reducing conditions and dihydroxy-carbamazepine concentrations slowly declined over all redox conditions.

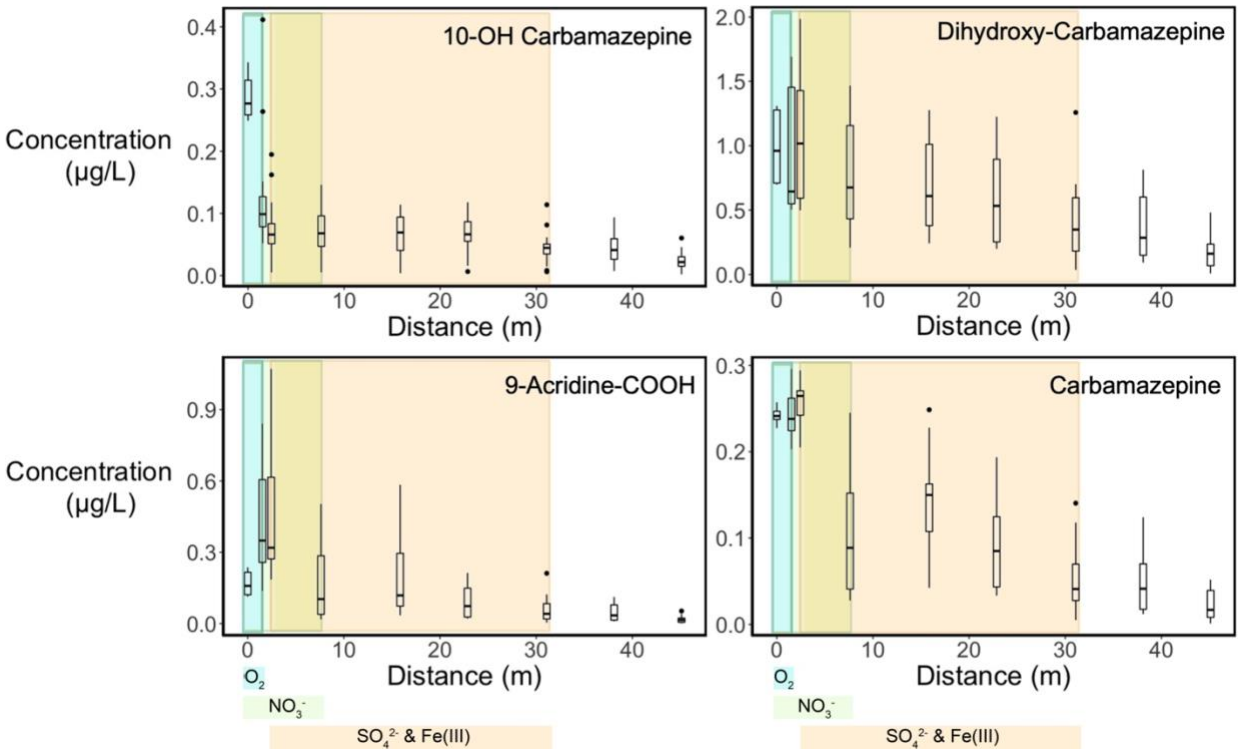


Figure 2.10 Concentrations of carbamazepine and related hydroxylated forms of carbamazepine (DiOH-carbamazepine and 10-OH Carbamazepine) and their known transformation product (9-Acridine carboxylic acid) in the horizontal levee subsurface. Approximate redox zones are shaded in the plot and in the bars below the plot (Distance = 0 m boxplot n = 4, each porewater boxplot n = 18)

These results suggested that hydroxylation of the 10-11 olefinic bond in carbamazepine influenced the pathways through which carbamazepine and its metabolites were transformed under different redox conditions (Figure A.16). We suspected that transformation was sensitive to modifications to carbamazepine at the 10-11 olefinic bond specifically because it is the electron-rich site of abiotic and biotic reactions in carbamazepine, such as oxidation by ozone[97] and enzymatic metabolism in humans.[98] In addition, forms of carbamazepine that are hydroxylated at the 10,11 bond are more readily biodegraded than carbamazepine itself under aerobic conditions.[88,96]

Additional evidence for the importance of nitrate- and sulfate-reducing conditions for the removal of trace organic contaminants was obtained through analysis of porewater data collected before and after the treatment plant that supplied water to the wetland was upgraded and through microcosm experiments (Microcosm results are found in Section A4.3 of the Appendix). Under conditions in which wastewater effluent entering the horizontal levee contained extremely low concentrations of nitrate (i.e., < 250 µg-N/L), acyclovir, sulfamethoxazole and metoprolol were transformed over similar distances when compared to their transformation in the presence of nitrate, which agreed with microcosm results (Figure 2.11, Figure A.13, Figure A.14).

Interestingly, carboxy-metoprolol transformation changed after inlet nitrate concentrations dropped; concentrations decreased for the first two meters before increasing to levels above inlet concentrations after sulfate-reducing conditions were reached, concurrently with a decrease in metoprolol concentrations. Removal of this increased concentration of carboxy-metoprolol occurred slowly over the remainder of the wetland where Fe(III)-reducing conditions were prevalent. This change in the behavior of carboxy-metoprolol, a metabolite of metoprolol, suggested that although metoprolol transformation was possible under both nitrate and sulfate-reducing conditions, the transformation pathway may have shifted. However, research into the anaerobic transformation pathways of metoprolol as well as atenolol, another compound that can be biotransformed to form carboxy-metoprolol, are needed to contextualize these results. Trimethoprim concentrations were significantly lower in the inflow to the wetland after nitrate concentrations declined, which we anticipated because trimethoprim removal is enhanced in treatment plants that employ anoxic biological nutrient removal,[75,76] but this prevented our ability to interpret the impacts of upstream treatment upgrades to its transformation from field data in the wetland.

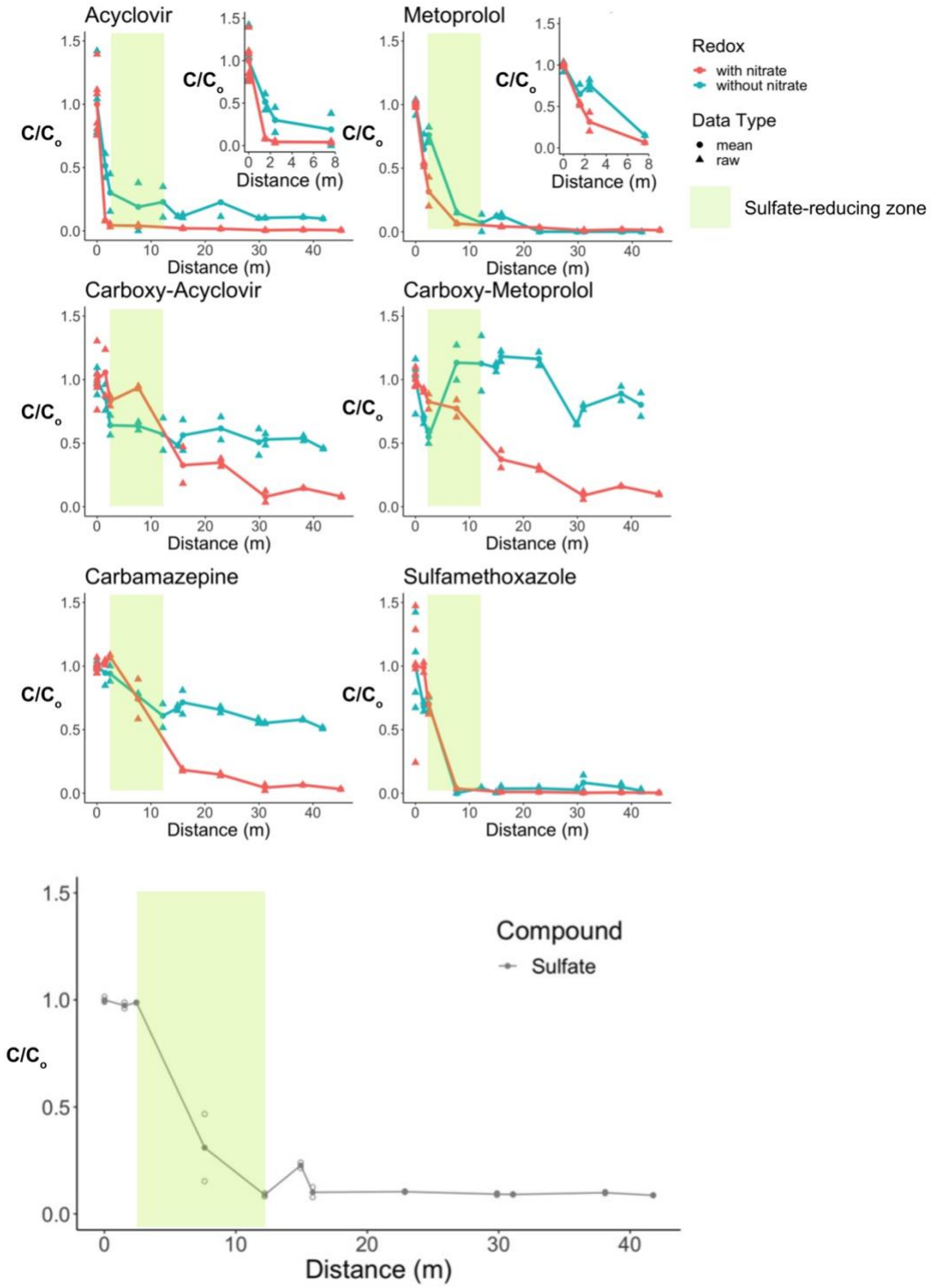


Figure 2.11 Fraction remaining of trace organic contaminants and sulfate in porewater collected from the same wetland cell before and after wastewater treatment plant upgrades lowered nitrate concentrations flowing into the wetland.

Some contaminants that were previously transformed under sulfate-reducing conditions exhibited performance differences after treatment plant upgrades eliminated nitrate from the horizontal levee inflow (Figure 2.11). For example, after treatment plant upgrades, carboxy-acyclovir transformation primarily occurred in the first 2 meters prior to the onset of sulfate-reducing conditions. Also, carbamazepine was not completely transformed after treatment plant upgrades (e.g., approximately 50% removal post upgrades versus >80% removal pre-upgrades), but its removal still exclusively occurred in the sulfate-reducing zone.

Decreased sulfate concentrations could have contributed to the performance decline measured for contaminants that were only transformed under sulfate-reducing conditions because rates of microbial sulfate reduction are sulfate-limited below concentrations of approximately 96 mg/L.[99,100] The concentration of sulfate in the inflow to the horizontal levee at the time of porewater sampling was approximately 70 mg/L in 2019 (i.e., pre-upgrades) versus approximately 40 mg/L in 2022 (i.e., post-upgrades). It is unlikely that treatment plant upgrades caused this decline because biological nutrient removal processes do not typically remove appreciable amounts of sulfate.[101] Variability in the concentrations of sulfate in the wastewater effluent has also been observed previously (Figure A.17) and could possibly be caused by changes in the source water used as the drinking water supply for the community served by the wastewater treatment plant during drought years.[102] A lack of nitrate flowing into the wetland could have also lowered internal sources of sulfate in the subsurface because autotrophic sulfide-driven denitrifiers regenerate sulfate during the wintertime in horizontal levees.[59] Regardless, the lower amounts of sulfate in the water flowing through the system at the time of sampling could have limited the activity of sulfate-reducing microbial communities in the subsurface which would lower the transformation of compounds that required sulfate-reducing conditions.

The persistence of carboxy-acyclovir in the sulfate-reducing zone post-treatment plant upgrades suggested that differences other than redox conditions may have impacted trace organic contaminant removal after treatment plant upgrades. For example, changes in the nature of the organic carbon in the treated effluent could have impacted treatment performance because the form of carbon available to microbial communities can impact their ability to transform trace organic contaminants. Similarly, the nature and supply of organic carbon from woodchips and/or plant roots likely changed over the course of the monitoring period,[103] which could have contributed to performance differences. Subsurface systems that employ low concentrations of readily biodegradable dissolved organic carbon or more persistent forms of carbon (e.g., lignocellulose, organic carbon remaining after wastewater is subjected to microbial processes) can elicit the use of different enzymes by microorganisms and can lead to faster rates of trace organic contaminant transformation compared to those fed with readily assimilable carbon (e.g., lactate).[104–107] Although the concentration of total organic carbon flowing into the wetland was consistent before and after treatment plant upgrades (i.e., approximately 8 mg C/L), the carbon that persisted in the treated effluent after plant upgrades was exposed to anoxic and aerobic treatment processes whereas prior to upgrades the wastewater-derived carbon was only exposed to aerobic treatment processes. This change could have led to the removal or transformation of carbon sources that were important for microbial communities that remove trace organic contaminants under sulfate- and Fe(III)-reducing conditions in the horizontal levee subsurface. However, more research is needed to elucidate the role of carbon for the measured performance differences.

2.3.3 Comparison to Other Treatment Systems

Using data on pore water concentrations and hydraulic residence times, we estimated half-lives for various trace organic contaminants as a basis to compare the horizontal levee performance to other treatment systems (see section A2.3 of the Appendix for details). However, this analysis greatly simplifies the removal processes occurring in each system by assuming that they could scale up or down depending on the volume of water needing treatment or that identical half-lives apply to wastewaters with differing compositions or upstream treatment processes.

Prior to the upgrade of the wastewater treatment plant, the half-lives for trace organic contaminants that are typically persistent in biological treatment systems (e.g., carbamazepine and sulfamethoxazole) were less than 10 days in the horizontal levee, which is considerably shorter than median values reported for other systems (Figure 2.12). The horizontal levee outperformed all approaches except for carbamazepine removal in an anaerobic riverbank filtration system[86] and sulfamethoxazole removal from a managed aquifer recharge system that treated water with a relatively low BDOC.[108] In all cases in which rapid carbamazepine removal was reported (i.e., half-life less than ten days) anaerobic conditions were observed, adding support to our observation that sulfate-reducing conditions result in faster carbamazepine removal.

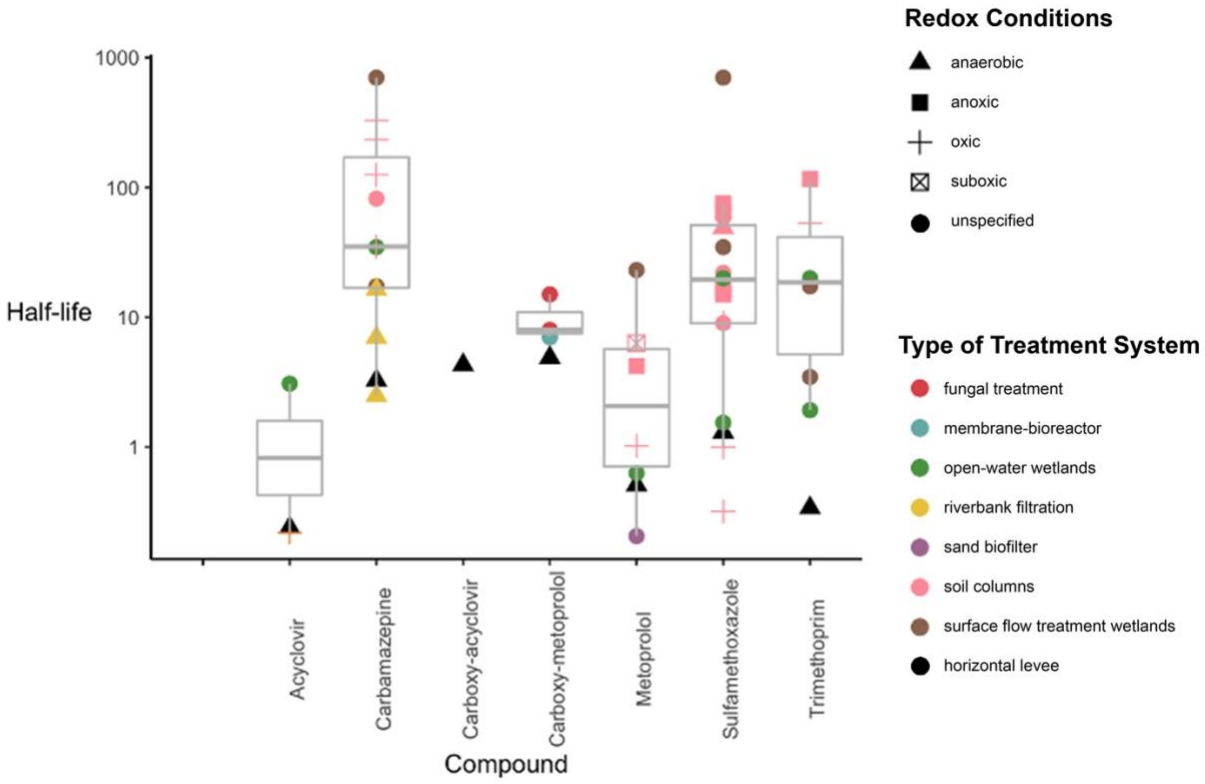


Figure 2.12 Half-lives for the monitored trace organic contaminants in the horizontal levee prior to upgrades made to the upstream wastewater treatment plant (black triangles) compared to other treatment systems (colored symbols). The half-lives for the other treatment systems are summarized in the grey box plots.[37,86,108–117] After treatment plant upgrades, half-lives for sulfamethoxazole, metoprolol, and acyclovir did not change. Removal of carbamazepine, carboxy-acyclovir, and carboxy-metoprolol varied spatially after treatment plant upgrades which limited the relevance of half-life predictions over the entire horizontal levee system under these conditions.

Aerobic or anoxic conditions can result in faster transformation of certain types of labile organic contaminants[53,118,119] due to higher microbial growth rates and greater energy yields from the use of oxygen and nitrate as terminal electron acceptors. However, comparisons of the horizontal levee half-lives with those reported in aerobic or anoxic treatment systems indicated that the horizontal levee did not exhibit slower removal of these contaminants. For example, half-lives of acyclovir and metoprolol in the horizontal levee were less than 1 day which is similar or better than those reported for other aerobic treatment systems (Figure 2.12).

2.4. Implications and Future Research

The horizontal levee can remove persistent trace organic contaminants via transformation in the subsurface, likely due to its relatively long hydraulic residence times coupled to anaerobic conditions (i.e., sulfate and Fe(III) reduction). Anaerobic conditions are rarely encountered in riverbank filtration or managed aquifer recharge systems due to limited amounts of assimilable carbon in treated wastewater or river water and concerns about sulfide or Fe(II) production in

drinking water applications. Although surface flow constructed wetland systems usually include vegetation that can serve as a source of assimilable carbon, sulfate-reducing conditions are often restricted to the sediments, where only a small fraction of the water flows. Surface flow constructed wetlands also do not typically achieve complete nitrate removal due to mixing issues (i.e., hydraulic short-circuiting) which inhibits sulfate reduction in these systems.[31]

Horizontal levees and other subsurface flow treatment wetlands could be particularly well-suited for trace organic contaminant removal via anaerobic transformation reactions because plants growing on the surface of the treatment system can serve as a source of organic carbon to the subsurface.[120] The decay of biomass on the ground surface and plant roots below ground may provide a means of maintaining anaerobic conditions for extended periods of operation without the need to supplement organic carbon periodically.[59] However, initial organic carbon supplements (e.g. woodchip addition) such as those employed in the horizontal levee could ensure anaerobic treatment mechanisms are prevalent prior to the establishment of plant roots. In addition, the availability of Fe(III) minerals in the media used in the subsurface and sulfate in the inflow should be considered when designing subsurface flow treatment systems intended to remove persistent trace organic contaminants.

The quality and quantity of carbon used by microbial communities capable of trace organic contaminant biotransformation under anaerobic redox conditions merits more research. In the horizontal levee, the forms of carbon available to microorganisms included dissolved organic carbon in wastewater effluent, organic carbon in decaying woodchips, decaying plants and plant root exudates.[59] The quality of the carbon in the wastewater effluent may have been influenced by the nature of the unit processes employed in the upstream treatment plant whereas the carbon from the decaying woodchips and wetland plants may vary over time or by plant species. The role that each of these organic carbon pools played in supporting a microbial community capable of removing recalcitrant trace organic contaminants is uncertain.

Although our findings suggested that sulfate reduction was important for the removal of certain recalcitrant trace organic contaminants like carbamazepine, we are unable to attribute removal exclusively to sulfate-reducing microorganisms because they often coexist with or rely upon other anaerobes in sediments and soils. For example, sulfate-reducers often form close associations with fermenting microorganisms or microorganisms that can break down solid forms of carbon, such as those found in woodchips.[121–123] The microbial community in the horizontal levee is comprised of a complex consortia of interdependent and/or competing organisms,[59] which implies that many members of the microbial community could be responsible for trace organic contaminant transformation. Research into the structure, function and activity of the microbial communities in anaerobic subsurface treatment systems and their links to contaminant removal (e.g., in microcosm experiments) is needed to further the design of these systems.

In addition to biotic reactions, abiotic reactions with microbially-generated reactive species can result in the transformation of trace organic contaminants under anaerobic redox conditions. For example, sulfamethoxazole can undergo ring cleavage through reactions with Fe(II) adsorbed onto Fe(III)-containing minerals that undergo reductive dissolution (e.g., geothite).[81] Although the concentration of dissolved sulfide species are likely to be low in the horizontal levee due to excess Fe(II), in Fe(III)-deficient sediment, sulfide species (e.g., HS^-, polysulfides) can act as potent

nucleophiles transforming organic compounds with electron-deficient moieties.[80] Regardless, anaerobic trace organic contaminant transformation pathways via biotic and abiotic processes remain understudied, limiting our ability to predict the mechanisms and products of contaminant transformation in this study. Future research should target the transformation pathways, kinetics, product identities and toxicity under anaerobic redox conditions.

Our results indicated that the experimental horizontal levee rapidly removed a suite of trace organic contaminants with a wide range of reported aerobic biodegradability. Trace organic contaminant removal was achieved through transformation reactions under anoxic and anaerobic (e.g. sulfate- and Fe(III)-reducing) conditions. However, similar contaminant removal was achieved with and without nitrate available as a terminal electron acceptor, demonstrating that nitrate reduction was not essential to the removal of trace organic contaminants. This is an important finding because nitrate is not present in all waters that might be fed into a horizontal levee. For example, the operators of wastewater treatment plants equipped with nutrient removal systems that discharge very low concentrations of nitrate (i.e., <1 mg-N/L) may still be interested in the removal of trace organic contaminants. The ability of subsurface treatment systems amended with carbon sources to remove trace organic contaminants that are labile or persistent under aerobic conditions (e.g., sulfamethoxazole and carbamazepine) via anaerobic transformation reactions implies that these systems may find wide application.

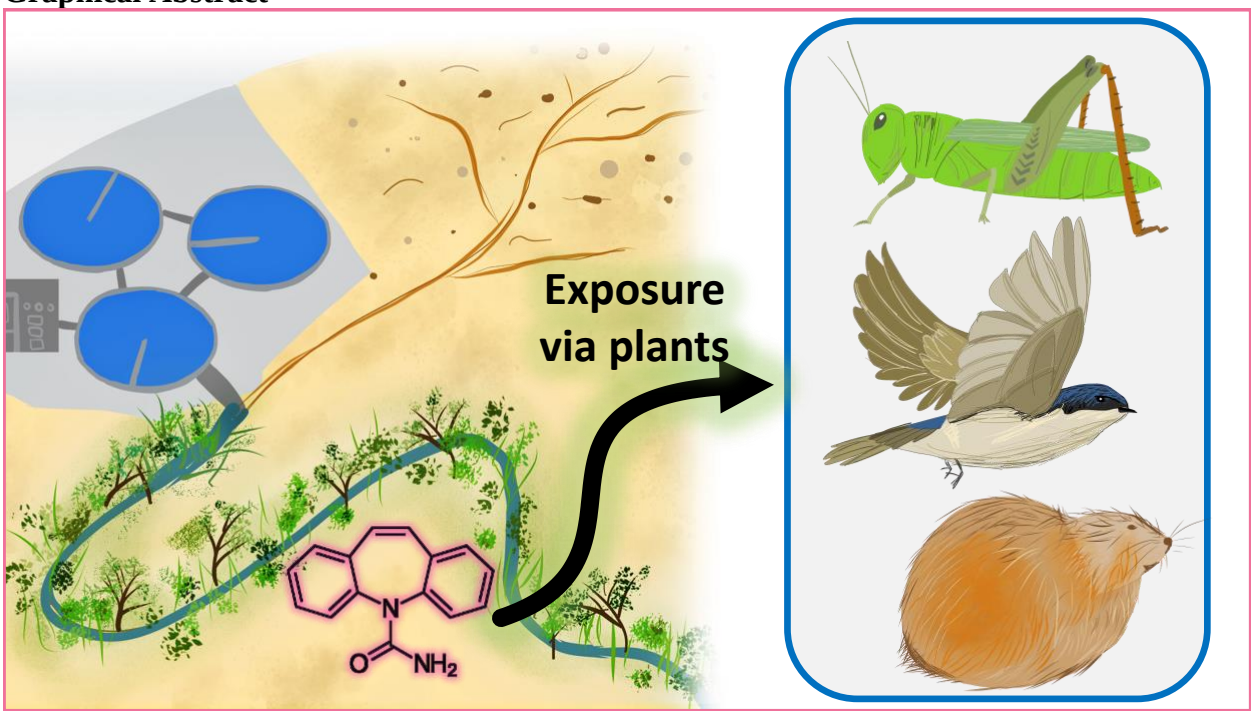
CHAPTER 3. Plant Uptake of a Trace Organic Contaminant in Effluent-Dominated Streams: An Overlooked Terrestrial Exposure Pathway

The following chapter is adapted from a co-authored manuscript with permission from Cecchetti, A.R., and Sedlak, D.L. Plant Uptake of a Trace Organic Contaminant in Effluent-Dominated Streams: An Overlooked Terrestrial Exposure Pathway. *Manuscript to be submitted for publication in Environmental Science & Technology Letters*

Abstract

Plant uptake and translocation represents an important means through which terrestrial organisms can be exposed to organic contaminants. In terrestrial systems adjacent to wastewater effluent-dominated streams (e.g., riparian corridors), insects, mammals and birds could be exposed to trace organic contaminants through this pathway, especially in arid regions where other water sources are scarce. Analysis of concentrations of the antiepileptic drug, carbamazepine, in plants grown under conditions that mimic effluent-dominated riparian zones indicate that concentrations in plant tissues exceed levels that may pose risks to herbivorous and/or detritivorous organisms. Carbamazepine uptake exhibited considerable inter-species variability and distinct spatial and temporal trends. It also showed evidence of in-planta transformation. Because effluent-dominated riparian zones serve as important habitats for terrestrial species in arid regions, plant uptake and translocation of wastewater-derived trace organic contaminants should be considered when assessing the potential ecotoxicological impacts of municipal wastewater effluent discharges.

Graphical Abstract



3.1 Introduction

In arid and semi-arid regions downstream of cities, municipal wastewater effluent is frequently the dominant source of water for rivers and streams. In the United States, municipal wastewater effluent makes up more than half of the flow in over 900 streams. During dry periods, nearly all of the flow in these streams is derived from treated wastewater.[14,15,124,125] Over the past two decades, considerable research has been conducted on the effects of wastewater-derived trace organic contaminants on aquatic organisms. Results suggest that continuous exposure to wastewater effluent can lead to endocrine disruption, oxidative stress, behavioral alterations and genotoxicity.[9–12] Despite the progress on understanding and managing these impacts, little attention has been directed towards the potential effects of wastewater-derived trace organic contaminants on terrestrial organisms.[126–128] Recently, pharmaceuticals were detected in emergent insects that developed in effluent-dominated streams, which highlights the potential for contaminant transfer from effluent-dominated water bodies to terrestrial ecosystems.[129,130]

Trace organic contaminants can undergo plant uptake and translocation when plants rely upon municipal wastewater effluent as their main water source. For example, plant uptake of trace organic contaminants has been well documented in agricultural systems employing treated wastewater for irrigation.[17,69,91,131–134] Results of this research indicate that uncharged compounds of intermediate hydrophobicity (i.e., log K_{ow} ranging from 2 to 4) are most readily taken up and translocated via the xylem. With the exception of relatively volatile compounds (e.g., TCE),[135] translocated trace organic contaminants and/or their metabolites tend to accumulate in leaves, stems, fruits, seeds, and pollen.[136–138]

In riparian zones, herbivory and detritivory result in transfers of energy, nutrients and potentially anthropogenic contaminants from aquatic to terrestrial systems.[139,140] In arid environments, riparian vegetation is particularly important to invertebrates and wildlife because it is a major source of food and/or water.[141] Considering this context, we hypothesized that uptake of trace organic contaminants by plants could create an important contaminant exposure pathway for terrestrial organisms in effluent-dominated riparian zones and wetlands. To test this hypothesis, we characterized plant uptake and in-planta transformation of carbamazepine in a constructed subsurface wetland system that mimics the conditions encountered in riparian zones and wetlands where water primarily flows through the subsurface. We selected carbamazepine as the target analyte because it is persistent under the typical conditions found in effluent-dominated streams[8] and because its plant uptake, in-planta metabolism, sequestration into different plant parts, and inter-species variability has been well characterized in agricultural systems and in some hydrophytic plants.[18,70,71,131,142–145] Because plant litter is an important energy source in aquatic and terrestrial foodwebs,[146] we evaluated the transformation of carbamazepine in decomposing plant litter. We also assessed the presence of sixteen other compounds (e.g., pharmaceuticals, pesticides, and their metabolites) in wetland plants.

3.2 Materials and Methods

The 0.7-ha field site consisted of twelve parallel subsurface wetland and riparian cells with two types of plant communities[44,90] and two sediment types (Figure 2.1). All cells were hydraulically isolated from each other and from groundwater (Figure A.1). Six of the twelve cells were planted with meadow vegetation (e.g., sedges and rushes) on fine sediments with or without swale topography. Six cells were planted on coarser sediments with meadow plants or riparian vegetation (i.e., willow trees). The three fine-sediment meadow cells without swales and the coarse-sediment riparian cells were included in this study. Nitrified municipal wastewater effluent from a neighboring activated sludge treatment plant was continuously released at a depth of about 1 meter below the surface near the entry to the wetland cells starting in April of 2017.[44,59,60]

Live plant samples were collected between June 2019 and June 2021. Horizontal transects were installed at approximately 0, 7.5, 15, 22.5, 30, 37.5, and 45 meters from the inlet perpendicular to the flow of water in three fine meadow wetland cells. Plant species were first characterized along each transect to determine approximate spatial abundance and distribution. Species with the greatest distribution and abundance (i.e., *B. maritimus*, *Juncus spp.*, *B. glutinosa* and *E. occidentalis)* were selected for collection. Along each transect, a spatially representative subset of these plants was collected as whole plant shoot samples in June 2019. *Juncus spp.* samples were collected using the same method again in June 2021. Individual willow trees (i.e., *S. lasiolepis)* were tagged in triplicate riparian cells at different distances along the flow path, and mature leaves were sampled from the same trees multiple times over the growing season. Samples were stored on ice prior to returning to the lab.

In November 2019, fresh *B. maritimus* and *Juncus spp.* samples were collected at the entrance of the fine meadow cells for litterbag experiments. After returning to the lab, *B. maritimus* and *Juncus spp.* were subsampled into pre-weighed 40 cm x 40 cm polypropylene mesh litterbags. Fresh litterbags were then returned to the site and placed on the sediment surface. Periodically, litterbags were sacrificially sampled.

In the laboratory, all plant parts (e.g., leaves, whole plant shoots, or litterbag samples) were manually cut into 1-2 cm pieces, homogenized, and shredded with a Cuisinart® DCG-12BC grinder. Homogenized plant samples were packed into 15 mL glass vials before freeze-drying. After freeze-drying was completed, plant samples were ground into a fine powder using a BioSpec Products mini-beadbeater and stored at -20 °C prior to extraction.

Solid-liquid extraction was performed using modified versions of published methods.[147] Briefly, 0.1 g of freeze-dried, powdered plant material was extracted in 4 mL of a 3:5 Milli-Q water:methanol solution via sonication and shaking. The supernatant was decanted and reserved. The process was repeated with 2.5 mL of a 1:1 Milli-Q:methanol solution. Combined supernatants were filtered through a 0.2 µm polyethersulfone membrane. An additional extraction step was performed to ensure that no residual organic contaminants could be detected, and the method was validated via spike-recovery tests (section B1.3 of the appendix).

Prior to analysis, extracts were diluted 1:2 in Milli-Q water and spiked with a mixture containing 5 ng of each internal standard compound (Table B.5). Carbamazepine and

carbamazepine metabolites were analyzed using liquid chromatography mass spectrometry on an Agilent 1260 HPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer. A matrix-matched calibration curve was prepared using extracts from unexposed *S. lasiolepis* leaves collected from mature trees that received rainwater and potable water on UC Berkeley's campus. Additional details about preliminary experiments performed in the winter of 2019, extraction methods, LC-MS/MS methods and the methods used to estimate wildlife dietary exposures can be found in section B1 of the appendix.

3.3 Results and Discussion

Carbamazepine and its main plant metabolites were frequently detected in plant residues (e.g., carbamazepine was detected and quantified in 81% and 62% of the samples respectively) (Figure B.1 Semi-quantitative detection of carbamazepine and other pharmaceuticals in live plants and dead plants collected in winter of 2019. Carbamazepine metabolites can be found in figure S4. **Note: These samples were quantified without matrix matching**. (At each sampling location, n = 3) and Figure B.4). Concentrations of carbamazepine in this study were in the same order of magnitude as levels found in field-grown agricultural plants that were irrigated with treated wastewater.[17] In addition, carbamazepine concentrations in the leaves and stems of most live meadow plants decreased along the water flow path; plant concentrations were inversely correlated with distance in the fine meadow cells ($p < 0.05$, Spearman's Rank) (Figure 3.1). For *Juncus spp., B. maritimus,* and *E. occidentalis,* median carbamazepine concentrations decreased by approximately 60% between the inlet and outlet of the wetland. *S. lasiolepis* leaves collected at 7.5 meters contained the highest concentrations of carbamazepine among the riparian cells with coarse sediment. The spatial trend of carbamazepine in meadow plants was consistent with concentrations measured in porewater samples taken along the water flow path; porewater carbamazepine concentrations decreased by about 50% within the first 15 meters of the wetland (Chapter 2).[148]

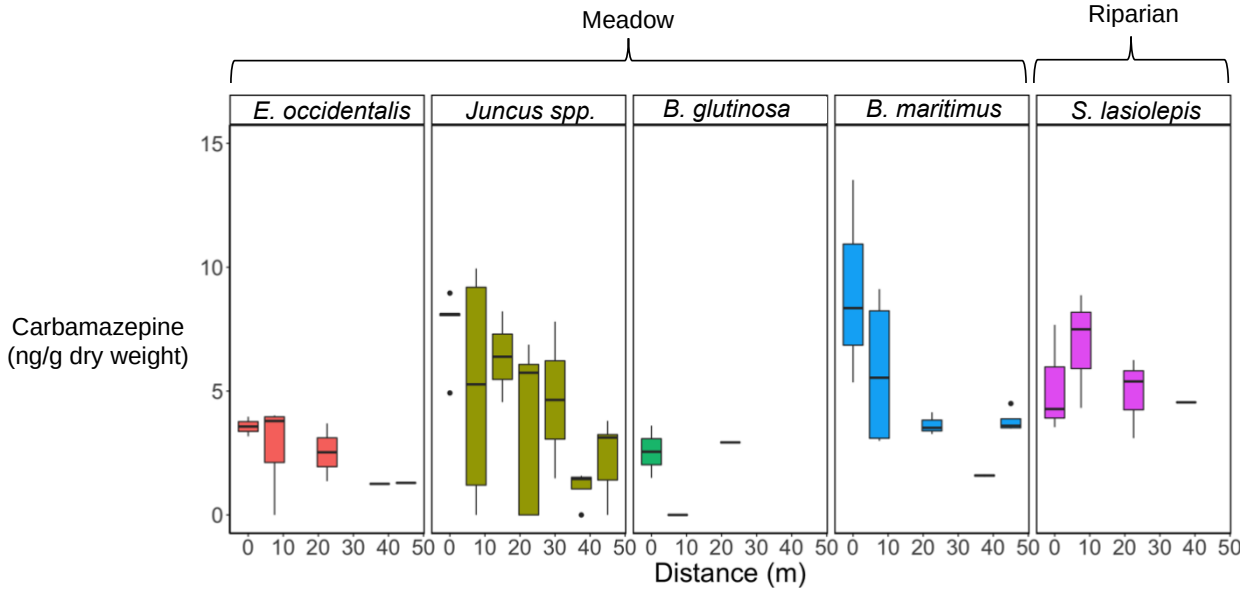


Figure 3.1 Carbamazepine concentrations in wet meadow and riparian plant species grown under field conditions. S. lasiolepis leaves were collected in December of 2019. Meadow plants were collected in June

of 2019 and 2021. In boxplots n = 3-5. Lines represent locations where n = 1. n is only less than three in cases where plant species were not located at all sampling distances.

Plant species exhibited considerable variability in carbamazepine concentrations. At the start of the wetland, the median carbamazepine concentration in *Juncus spp.* and *B. maritimus* was approximately twice that of *B. glutinosa* and *E. occidentalis* (Figure 3.1). A similar or even greater degree of inter-species variability has been observed in agricultural plants (i.e., concentrations of carbamazepine in lettuce were approximately six times those found in spinach).[136] Inter-species variability was likely due to differences in root lipid content, development of the casparian strip, evapotranspiration rates, expression of carbamazepine-metabolizing enzymes, or other plant physiological factors.[143]

Carbamazepine gradually accumulated in *S. lasiolepis* leaves during the growing season. Mean carbamazepine concentrations in *S. lasiolepis* leaves from the same tree more than tripled between June and December of 2019 (Figure 3.2 & Figure B.2). Although temporal variations were not measured in other perennial plants, we anticipate that concentrations would also increase in those plants during the growing season because plants would continue to transpire carbamazepine-laden water throughout the entire season. Therefore, concentrations of carbamazepine in meadow plants may have been higher in September and October than at the time that samples were collected (i.e., June). Efforts to quantify wildlife exposures should account for seasonal variability because habitat use differs across seasons. For example, in the case of semi-aquatic mammals in riparian habitats, muskrats and beavers breed during late spring and winter respectively.[149,150]

A suite of carbamazepine plant metabolites was detected in all plant species (i.e., metabolites were detected in 12-42% of the plant samples), but these metabolites could not be quantified using the matrix-matched methods employed in this study due to higher limits of quantification for those compounds relative to carbamazepine (Table B.6 Limits of detection and quantification for carbamazepine and a suite of its metabolites in matrix-matched calibration curve.). However, we estimate that detectable carbamazepine metabolites were present at levels that were of a similar order of magnitude as carbamazepine, which is consistent with data from agricultural plants.[71] The presence of carbamazepine metabolites in plant tissues suggests that carbamazepine is transformed in plant tissues under field conditions because some of these metabolites (e.g., 10-hydroxy carbamazepine) were not observed in porewater more than 5 meters from the inlet (Figure 2.10), but were present in plants throughout the wetland. Also, some of the metabolites that were detected in the wetland influent and porewater (e.g., dihydroxy-carbamazepine) may not be as readily taken up by plants due to their higher hydrophilicity (log $K_{ow} < 1$).[70]

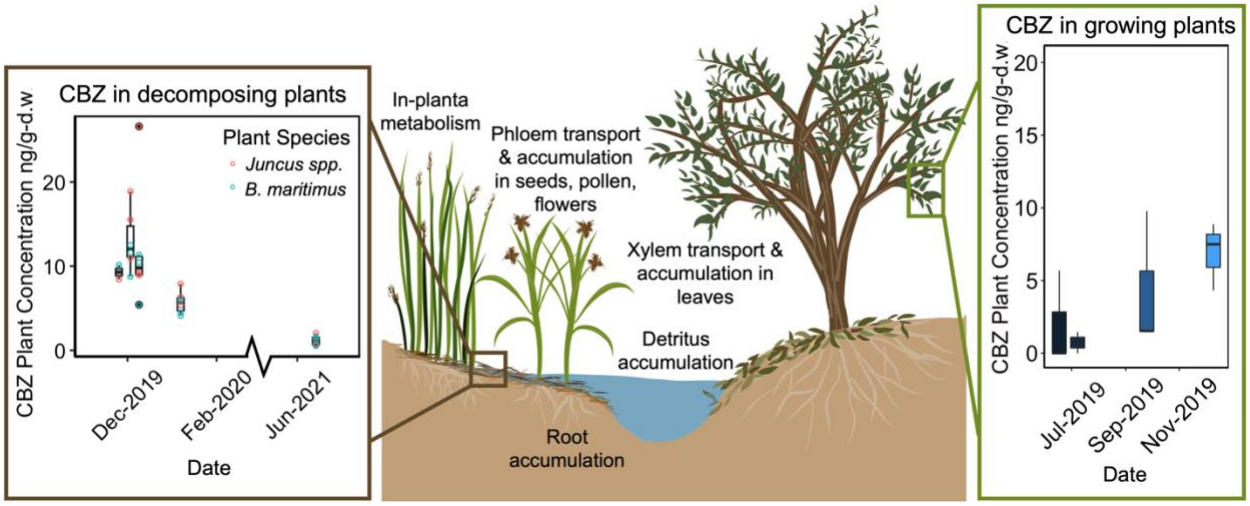


Figure 3.2 Visual rendering of a cross section of effluent-dominated riparian zones and wetlands. Major in-plant transport and transformation processes are labeled in the center. The left plot displays concentrations of carbamazepine over time in decomposing *Juncus spp.* and *B. maritimus* in litterbag experiments. The right plot shows concentrations of carbamazepine in *S. lasiolepis* leaves over the growing season. Leaf samples were collected from individual trees located 7.5 meters from the inlet.

Carbamazepine concentrations gradually decreased in dead *Juncus spp.* and *B. maritimus* exposed to field conditions in litterbags likely due to leaching from plant material or transformation reactions (left panel of Figure 3.2 and Figure B.3). In most decomposing plant samples, carbamazepine metabolites were below the limits of quantification, limiting our ability to attribute carbamazepine removal to transformation. However, a suite of carbamazepine metabolites was detected in senescing plants collected during preliminary experiments conducted in the winter of 2019 (Figure B.4). Regardless, carbamazepine removal from decomposing plants was slow (i.e., the carbamazepine half-life was greater than one-and-a-half months) and suggests that carbamazepine may linger for months in detritus layers (Figure 3.2). Due to seasonal growth cycles, detritus concentrations would likely be highest during winter months after plant senescence occurs or deciduous trees drop their leaves.

Plant uptake and translocation may expose detritivorous or herbivorous insects, birds, and mammals to carbamazepine because research on human exposure to carbamazepine in wastewater-irrigated food crops indicate that carbamazepine is bioavailable in plant tissues.[16] Using published estimates of daily dietary intake,[151] we predict that during the summer, herbivorous birds and mammals (e.g., Canada goose and muskrat) feeding exclusively on plants grown in a 0-7.5 meter strip adjacent to the water source would ingest an amount of carbamazepine ranging from one to seven times the acceptable daily intake (ADI) for human exposure (0.34 μg kg-body weight^{-1} d^{-1}, Figure B.5). Herbivorous insects can consume up to one-and-a half times their body weight per day, resulting in a weight-normalized dietary exposure that is higher than that received by birds and mammals.[152] Insects, birds, and mammals feeding on herbivorous insects might thus receive even higher doses of the compound.

The human ADI employs cancer as an endpoint, therefore data on other endpoints may be needed to evaluate risks to wildlife. Unfortunately, little is known about the impact of pharmaceuticals on terrestrial wildlife and invertebrates.[128] However, the insect *N. cinerea* (i.e., the speckled cockroach) exhibited behavioral shifts and oxidative stress when exposed to food containing carbamazepine at concentrations that were 39-88% of the levels observed in the plants collected near the inlet to the wetland in this study.[153]

Other aspects of terrestrial exposure pathways further complicate matters. Some organisms may receive higher or lower doses of carbamazepine through exposures to different parts of the plant (e.g., flowers, seeds, nectar, pollen, xylem sap, phloem sap) because wildlife and insects selectively feed on specific plant organs and transport of carbamazepine into different plant compartments varies widely. For example, many birds and some mammals feed primarily on the seeds of *Juncus spp*. and *B. maritimus* as opposed to their live stems and leaves.[154] Also, carbamazepine can contaminate pollen via plant uptake, as demonstrated by the measurement of carbamazepine in pollen from zucchini irrigated with treated wastewater amended with carbamazepine.[138] To provide insight into these exposure pathways, future efforts should include measurements of carbamazepine and other wastewater-derived contaminants in various wetland and riparian plant compartments.

Carbamazepine metabolism is also an important factor to consider because carbamazepine is rapidly metabolized by the CYP 450 family of enzymes to produce hydrophilic products. CYP 450 enzymes are prevalent in many terrestrial plants, invertebrates and vertebrates, but they may be less effective at low concentrations because carbamazepine metabolism is dose dependent in humans.[155] The main metabolite produced by CYP 450 enzymes, carbamazepine epoxide, is toxic and may be teratogenic in humans.[156] Similar metabolites may form if carbamazepine is transformed by enzymes present during decomposition (e.g., lignin-degrading enzymes).[72] Therefore, both herbivores and detritovores could be exposed to carbamazepine and its metabolites in plants.

Although carbamazepine was the focus of this study, other trace organic contaminants can be taken up into plants and translocated to above-ground tissues (Figure B.1).[133,157] For example, primidone and phenytoin – two relatively stable compounds – have been detected in cabbage and lettuce irrigated with treated wastewater.[136] Likewise, plant uptake and translocation could be an important entry pathway for short-chain per and polyfluoroalkyl substances that are persistent and bioaccumulative in terrestrial systems.[158–162] Finally, fipronil, a potent systemic insecticide that is present in treated wastewater at concentrations as high as 340 ng/L,[163] could undergo plant uptake and translocation because it is designed to be distributed throughout plants after application to seeds. Attempts to quantify fipronil in the plants from this study were hindered by high background levels of the compound detected in the willow leaves used for matrix-matching, low porewater concentrations, and relatively high limits of quantification for the compound. The willow leaves that were used for matrix-matching could have had elevated concentrations of fipronil because they were collected from a site that is exposed to urban runoff. However, when compared to the willow leaves used for matrix matching, fipronil was detected at higher concentrations in 37% of the plant samples collected from the wetland cells. Regardless, fipronil and several of its primary metabolites have been detected in the roots and shoots of emergent hydrophytes collected from a surface flow wetland treating water from a wastewater effluent-dominated river in southern

California.[164] The potential for terrestrial plants to be contaminated with fipronil is concerning because it is extremely toxic to invertebrates. In addition, plants growing in the presence of wastewater effluent would likely contain multiple trace organic contaminants which may pose additional risks to wildlife or insects due to mixture effects.

A wide array of plant species grows in effluent-dominated riparian zones and wetlands. As indicated by our data and data on crop uptake of contaminants, different species exhibit varying tendencies to take up carbamazepine and other trace organic contaminants.[136] Different species also play a different role in the diets of herbivores. For example, most insects are highly specialized and will only feed on a single plant species.[165] Thus, efforts to predict exposure to organisms in riparian zones would benefit from the application of species-specific data.

Because wastewater effluent accounts for an increasing fraction of the flow in water-stressed streams and rivers,[19] the importance of plant uptake as an exposure pathway for terrestrial organisms may increase in the future. In addition, riparian zones, wetlands, and other ecotones at the aquatic-terrestrial interface are more important than implied by their land area alone.[166] For example, 80% of terrestrial vertebrate species rely on riparian zones for food, water and shelter in the American west, even though they account for only 5% of the land area.[167] Riparian zones also support a majority of neotropical migratory birds and provide critical habitat corridors for the migration and dispersal of land-bound wildlife.[168] Although wastewater effluent can create or restore riparian habitats that otherwise would not exist,[125] these habitats could unintentionally expose organisms to contaminants. Considering this context there is an urgent need to document plant uptake of trace organic contaminants and its impacts on terrestrial wildlife and insects living and feeding in wastewater-impacted riparian zones and wetlands. These efforts will require environmental engineers and chemists to work collaboratively with ecologists and ecotoxicologists to ensure relevant ecotoxicity endpoints, receptors, and exposure pathways are wholistically assessed in important riparian ecosystems.[169]

CHAPTER 4. Removal of Nitrate and Trace Organic Contaminants from Reverse Osmosis Concentrate in Horizontal Levees: Nature-Based Treatment to Enable Water Reuse

A portion of this chapter is reproduced with permission from Scholes, R. C.; Stiegler, A. N.; Anderson, C. M.; Sedlak, D. L. Enabling Water Reuse by Treatment of Reverse Osmosis Concentrate: The Promise of Constructed Wetlands. *ACS Environ. Au* **2021**, *1* (1), 7–17. https://doi.org/10.1021/acsenvironau.1c00013.

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A portion of this chapter is adapted from a co-authored manuscript with permission from Scholes, R.C, Cecchetti, A.R., and Sedlak, D.L. Removal of Nitrate and Trace Organic Contaminants from Reverse Osmosis Concentrate in Horizontal Levees. *Manuscript to be submitted for publication.*

Abstract

As more cities experience water stress, potable water reuse will become more common. The use of reverse osmosis (RO) during advanced treatment of municipal wastewater results in a concentrated waste that can pose chronic ecotoxicity risks if discharged to surface waters or shallow coastal ecosystems. Most existing RO concentrate treatment technologies are cost prohibitive, but constructed wetlands hold promise because they have the potential to provide simultaneous treatment of nutrients, metals, and trace organic contaminants at a relatively low cost. They also can protect coastal infrastructure from sea-level rise, create habitat and provide recreational opportunities. A handful of water-stressed cities have already begun experimenting with constructed wetlands for RO concentrate treatment. However, further research could reduce the land area needed for treatment and increase the reliability of constructed wetland systems. We assessed the ability of a recently developed subsurface flow treatment wetland, a horizontal levee, to remove nitrate and trace organic contaminants from RO concentrate. Results of this research indicated that horizontal levees outperform other nature-based RO concentrate treatment systems with respect to their ability to remove nitrate and trace organic contaminants that are susceptible to biotransformation under aerobic and suboxic conditions. However, under the conditions in this study, the composition of RO concentrate prevented the system from achieving sulfate-reducing conditions which are important for the removal of persistent trace organic contaminants, such as carbamazepine. Methods to improve the design of subsurface flow treatment wetlands for RO concentrate treatment are proposed.

4.1 Introduction

Reverse osmosis (RO) has become one of the most widely adopted approaches for potable water reuse in the world.[170] When RO is used to treat municipal wastewater effluent, only about 80% of the water passes through the membrane. The concentrations of solutes in the remaining water (i.e., the RO concentrate) are typically 5 to 6 times higher than those that were present in the wastewater effluent. Prior research has indicated that the concentrations of contaminants in RO concentrate can exceed chronic ecotoxicity thresholds and may be unsafe to discharge into many surface waters.[39] Thus, management of RO concentrate represents a major barrier to the widespread adoption of RO-based municipal wastewater reuse, especially in locations lacking deep ocean outfalls or infrastructure that discharges into waterbodies where RO concentrate can be safely diluted.[171]

Existing treatment options for RO concentrate are limited and often require multi-step treatment trains to remove nutrients, trace organic contaminants, and metals. For example, ozone paired with biological activated carbon (O_3/BAC) has been adopted at full scale for removal of trace organic contaminants from municipal wastewater effluent.[20,21,172] Laboratory-scale studies indicate that it is also likely to be feasible for RO concentrate.[173,174] However, when applied to RO concentrate, ozonation can form toxic byproducts, such as bromate.[173] Furthermore, O_3/BAC cannot remove other important contaminants present in RO concentrate, such as metals and nutrients, unless an additional source of labile organic carbon (e.g., lactate, glycerol) is added to the BAC.[175] Biological processes employed to remove nutrients from wastewater might also remove trace organic contaminants from RO concentrate, but higher salt and contaminant concentrations will likely increase costs and decrease reliability.[176,177] Irrespective of performance, the added cost of treating contaminants in RO concentrate is likely to slow the spread of potable water reuse under conditions in which concentrate treatment is necessary.

In recognition of the need to develop more cost-effective and reliable RO concentrate treatment technologies, researchers have begun to investigate new treatment methods. Proposed approaches for removing trace organic contaminants from RO concentrate include advanced oxidation,[178] electrochemical treatment,[179,180] activated carbon adsorption,[181] photocatalysis and sonolysis.[182] However, substantial barriers exist prior to implementation of these technologies, including cost, energy intensity, the need for highly-trained operators, and the potential formation of toxic byproducts.[183]

Nature-based treatment systems may be an appropriate alternative for RO concentrate treatment because they are cost-effective, and well-designed nature-based systems have the potential to address multiple contaminant classes simultaneously.[184] Furthermore, wetland plants and microorganisms are well adapted to the elevated salinity and solute concentrations encountered in RO concentrate. Nature-based systems can also create public support for water recycling projects because they provide ancillary benefits (e.g., aesthetic features, habitat) that are valued by community members.[57]

4.2 Early-Stage Experiments on the Use of Constructed Wetlands for RO Concentrate Treatment

Although the ability of constructed wetlands to remove contaminants from wastewater effluent or effluent-impacted surface water in a cost-effective manner is well established,[112,113,185–189] the use of nature-based systems for treatment of RO concentrate is still in an early stage of development. Several cities are experimenting with nature-based RO concentrate treatment systems through projects that are motivated by a diverse set of factors (Table 4.1 Early constructed wetland projects for RO concentrate treatment. *The pilot wetland in Goodyear, Arizona treated RO concentrate from a brackish groundwater desalination facility).

Table 4.1 Early constructed wetland projects for RO concentrate treatment. *The pilot wetland in Goodyear, Arizona treated RO concentrate from a brackish groundwater desalination facility

Facility Name & Location	Project Driver	Project Description	Area/ Flow rate	Year	Performance Findings	Development Stage
Torreele Water Reuse Facility Koksijde, Flanders, Belgium	Savings associated with decreased nutrient discharges into the North Sea[190]	Subsurface flow sand bed planted with willow trees	28.5 m^2 250 L/h	2011	30% nitrogen removal No phosphorus removal Minimal BOD/TOC removal[190]	Moving to full-scale construction[195]
Silicon Valley Advanced Water Purification Center San Jose, California, USA	Decrease the loading of nitrogen and other contaminants into the San Francisco Bay	Floating Wetlands	48 m^2	2019	None yet	Study ongoing
		Subsurface flow wetland	583 m^2 204 L/h	2017	None yet	Results herein[44]
		Shallow, open-water wetland	400 m^2 1560 L/h	2017	28-47% nitrate removal (varied seasonally and by year)[193] 0-90% trace organic contaminant removal (varies by contaminant)[39]	Under consideration[39]
Groundwater Recovery Enhancement and Treatment (GREAT) program Oxnard, California, USA	Restoration of costal brackish wetland habitat through discharge of treated RO concentrate	Horizontal subsurface flow wetland, vertical subsurface upflow wetlands and surface flow wetlands/ponds	3155 L/h	2016	96% nitrate removal 93% Se removal[192]	Built at pilot scale[194]
Goodyear Bullard Water Campus* Goodyear, Arizona, USA	Restoration of riparian habitat along the Salt River. Preventing discharge of high salinity RO concentrate from brackish water desalination into municipal wastewater	Vertical flow wetlands followed by surface-flow marsh	125 m^2	2010	60-90% removal of arsenic, selenium, and chromium >90% removal of nitrate[191]	Moving to full-scale construction

Installation of a subsurface flow wetland system for treating RO concentrate from the Torreele Water Reuse Plant in Belgium was motivated by a desire to reduce permit fees associated with the discharge of nutrients and metals to the North Sea. In a pilot project that started in February 2011, RO concentrate was passed through a 28.5-m^2 wetland cell containing various species of willow trees (*Salix spp.*). During these experiments, a 30-35% reduction in the mass of phosphorus and nitrogen discharged by the system was observed. This load reduction would allow the Intermunicipal Water Company of Veurne-Ambacht (IWVA) to save a minimum of 20,000 €/yr in discharge fees if the wetland were to be scaled up to treat all of the RO concentrate from the reuse facility. The IWVA has designed a full-scale system and has applied for permits to construct a full-scale subsurface flow RO concentrate treatment wetland.

In the San Francisco Bay, nature-based RO concentrate treatment is also under consideration as one of the region's main water suppliers (i.e., Valley Water) develops plans to build a potable water reuse system. Although the premise of potable water reuse was accepted by the regulatory agency and the local community, disposal of RO concentrate produced by the project in combination with a lack of mixing in the waterbody available for RO concentrate discharge (i.e., south San Francisco Bay) emerged as an issue that could prevent project permitting. In an effort to find a way to safely discharge the RO conceontrate, the utility responsible for the project is investigating the use of three different types of nature-based RO concentrate treatment systems.[190] A pilot-scale study of RO concentrate treatment using open-water unit process wetlands to remove nitrate and trace organic contaminants operated between 2017-2019.[39,191] Removal of nutrients, trace metals, and trace organic contaminants is now being studied in subsurface-flow (i.e., horizontal levees) and floating wetland systems, with the aim of identifying an efficient and cost-effective RO concentrate treatment method.

Another motivating factor for cities considering constructed wetland treatment of RO concentrate is habitat creation. The City of Oxnard, California, has identified habitat creation as a potential benefit associated with the discharge of RO concentrate from its advanced treatment plant. Although this coastal city has access to a deep ocean outfall for wastewater effluent discharge, they are considering the creation of a full-scale RO concentrate treatment wetland to create brackish wetland habitat.[192] To assess the feasibility of this approach, a pilot-scale wetland currently treats a small fraction of the RO concentrate generated at the advanced water purification center. The use of RO concentrate for habitat creation has also been employed by the city of Goodyear, Arizona. In this case, the city tested the use of RO concentrate from brackish groundwater desalination for the restoration of riparian habitat along the Gila River.[193] These projects highlight that habitat creation is not just a co-benefit, but may be a driver when selecting RO concentrate management options.

These initial studies suggest that nature-based treatment systems for RO concentrate management are attractive for multiple reasons: water quality benefits, cost savings, sea level rise adaptation, and habitat creation. However, the experiments conducted thus far do not provide a thorough understanding of the potential for removal of the full suite of contaminants of concern. For example, the fate of trace metals and/or trace organic contaminants have only been considered in a few studies despite the potential for these contaminants to cause adverse impacts in receiving waters. The data from this small set of pilot-scale projects is encouraging, but constructed wetlands are not yet considered a proven treatment technology for RO concentrate treatment. For nature-

based RO concentrate management to become more popular, additional research is needed to inform design, demonstrate performance, and decrease costs.

4.3 Removal of Trace Organic Contaminants and Nutrients from RO concentrate in Horizontal Levees

As indicated in the previous section, horizontal levees are under consideration in the San Francisco Bay area for treatment of RO concentrate because they efficiently remove contaminants from municipal wastewater effluent. For example, previously published research indicated that horizontal levees remove nitrate via denitrification within the first 10 meters of the inlet, or in a distance equivalent to approximately 20% of the total system length.[44] Research presented in Chapter 2 indicated that horizontal levees can remove a suite of trace organic contaminants including those that have proven to be difficult to remove in other types of biological treatment systems.

Considering this context, we hypothesized that the horizontal levee would remove a considerable fraction of the nitrate and trace organic contaminants in RO concentrate and that the higher initial concentrations would enable removal of the same mass of contaminants in a smaller area. However, treatment of RO concentrate in horizontal levees may alter the biogeochemistry and microbial activity in the subsurface of the treatment system. For example, higher concentrations of terminal electron acceptors, organic carbon, and other solutes could affect the ability of microorganisms in the treatment layer to remove contaminants from RO concentrate. It also could affect the growth of plants and alter the flow paths of water (e.g., by mineral precipitation).

To test the ability of horizontal levees to remove contaminants from RO concentrate, the source water for one horizontal levee wetland cell was converted from wastewater effluent to RO concentrate starting in early 2020. Periodically, contaminant removal in the RO concentrate cell was monitored and compared to an identically constructed wetland cell that received municipal wastewater effluent. Using the mechanistic understanding and methods developed in Chapter 2, we evaluated contaminant removal in the RO concentrate cell. We also compared the performance of horizontal levee cells treating RO concentrate to those treating municipal wastewater effluent and to the other nature-based RO concentrate treatment systems outlined in Section 4.2 (Table 4.1 Early constructed wetland projects for RO concentrate treatment. *The pilot wetland in Goodyear, Arizona treated RO concentrate from a brackish groundwater desalination facility). We used these results to identify potential approaches for the design of subsurface flow treatment systems that are better suited for RO concentrate treatment.

4.3.1 Methods

4.3.1.1 Sample Collection

In November of 2019, a single coarse-textured wetland cell from the experimental horizontal levee[44] (i.e., Cell K; see Figure 2.1) was gradually transitioned from operation with municipal wastewater effluent from the adjacent activated sludge treatment plant to RO concentrate from the

Silicon Valley Advanced Water Purification Center in San Jose, CA. This facility employs microfiltration and RO to effluent from the San Jose/Santa Clara Water Pollution Control Facility as described previously.[39,191] Twice weekly, RO concentrate was transported via truck to the Oro Loma Sanitary District in San Lorenzo, CA where the horizontal levee test facility was located. RO concentrate was held in two high-density polyethylene storage tanks with a total volume of approximately 17,000 liters. To minimize disturbances to the microbial community, the inflow to the wetland cell was gradually transitioned from wastewater effluent to RO concentrate; over the course of approximately two and a half months, the fraction of RO concentrate was increased until it reached 100% in January of 2020. Flow rates were set on the basis of calculations from prior research that indicated that 3.4 liters per minute prevented hydraulic short-circuiting via overland flow.[44]

Samples were collected from the inlet, from monitoring wells installed at approximately 15 and 30 meters, and from porewater samplers deployed at 3.0, 7.5, 12.0, 23.0, 38.0, and 42.0 meters from inlet of the monitored wetland cells (RO concentrate and wastewater effluent) approximately every 6-12 weeks between January 2021 and April 2022. Samples collected between January 2020 and January 2021 were obtained from the inlet, outlet and porewater samplers located between 3.0 and 12.0 meters from the inlet of the wetland. Porewater samples were collected in triplicate using either stainless steel PushPoint sediment porewater samplers or polyethylene SedPoint samplers (MHE products). The samplers remained in place between sampling rounds. Porewater was filtered through 0.7-μm glass fiber filters in the field and immediately stored on ice. The wastewater treatment plant that provided effluent to the wastewater effluent-receiving wetland cell underwent plant upgrades during the course of the experiment. Therefore, porewater samples collected from three identically constructed wetland cells operated during the summer of 2019 (i.e., the monitoring period discussed in Chapter 2) were also used for RO concentrate treatment performance comparisons.

4.3.1.2 Analytical Methods

A suite of water quality parameters was quantified using previously described methods including: electrical conductivity, pH, temperature, dissolved oxygen, total organic carbon, and total nitrogen.[44] A suite of anions and cations were also analyzed using previously described methods with a minor modification – samples from the RO concentrate wetland cell were diluted by a factor of six prior to analysis to ensure concentrations were within the calibration range.[44] A suite of trace organic contaminants was analyzed using a matrix-matched method as described elsewhere[39] with a minor modification - the liquid chromatography flowrate was set to 0.6 mL/min. LCMS parameters can also be found in the methods discussed in section B1.2 of the Appendix. All concentrations were normalized by the ratio of chloride in the sample to chloride in the inflow to correct for water loss via evapotranspiration.[39,44]

4.3.1.3 Mass Balances and Fate and Transport of Trace Organic Contaminants

Methods described in Chapter 2 were used to predict sorption breakthrough times using isotherm-derived distribution coefficients (i.e., K_D) and estimates of hydraulic retention time based upon increases in chloride concentrations that occurred during the transition of wetland cell K from

wastewater effluent to RO concentrate. We assumed that background concentrations of trace organic contaminants on the sediments within the treatment layer of the RO concentrate-receiving cell were sufficiently low to treat the system as an empty bed. This assumption was justified by the observed removal of most trace organic contaminants from wastewater effluent within the first 10 meters of the wetland cell as well as data indicating that the concentrations of trace organic contaminants entering the cell were 3-5 times lower than those that entered the cell after the inlet stream was switched to RO concentrate (wastewater effluent Figure 2.2, RO concentrate Figure C.4).

We used a similar approach to that described in Chapter 2 to estimate the mass of contaminants expected to be removed in the system via plant uptake. In accordance with results from Chapter 3, we assumed that carbamazepine was the only trace organic contaminant appreciably removed via plant uptake and translocation. To estimate the mass of carbamazepine taken up by plants, we assumed that concentrations in plants growing in the RO concentrate-receiving cell increased by the same factor as the concentrations entering the wetland (i.e., we assumed that plant uptake scaled with inlet concentrations, which increased by approximately a factor of five). We also assumed that the mass of carbamazepine entering plants was proportional to the amount of evapotranspiration in the cell, which was estimated from chloride increases in the porewater. Finally, we assumed that the distribution of plant species was similar in the RO concentrate-receiving cell and the wastewater-receiving cell. To offer a maximum case of anticipated plant uptake, we performed these concentrations based on the highest concentrations measured in plants growing in the wastewater effluent-receiving cells. See Appendix C1.2 for equations used to make plant uptake estimates in the RO concentrate cell.

The annual mass of each contaminant removed per unit area in wastewater cells was calculated using inflow and outflow data collected from the wastewater effluent-receiving cell(s) and the RO concentrate-receiving cell after sorption breakthrough was achieved (i.e., the 2018-2019 monitoring period discussed in Chapter 2 and the 2021-2022 monitoring period for the wastewater and RO concentrate wetland cell respectively). The 2018-2019 wastewater effluent monitoring period was the basis of these calculation because the wastewater treatment plant that provided treated effluent to the experimental horizontal levee underwent treatment plant upgrades during the monitoring period. The areas required for removal was estimated using porewater data collected in the summer of 2019 and November 2021-June 2022 in the wastewater effluent- and RO concentrate-receiving cells, respectively. All equations can be found in sections C1.3 of the Appendix.

4.3.2 Results

Because all dissolved species temporarily declined in the zone between 3-15 meters, and increased thereafter, porewater samples from this location were excluded from the analysis that follows (Figure C.1). Sampling at various distances along the edges of the RO concentrate cell conducted in January of 2022 indicated that water likely flowed around this zone (Figure C.2). Therefore, samples collected from the center of this area were likely within a zone of low permeability. More details about this zone can be found in section C1.1 of the Appendix.

Results of prior research indicated that redox conditions influenced the rate of biotransformation of trace organic contaminants and the removal of nitrate in the horizontal levee (Chapter 2) and other subsurface flow treatment systems.[37,61,65,189] As discussed in Chapter 2, terminal electron acceptors were naturally-present in the inflow (e.g., nitrate and sulfate) or in the media used to construct the wetland cells (e.g., Fe(III)-containing minerals). Concentrations of dissolved redox-active ions throughout the wetland differed substantially between the RO concentrate-receiving cell and the cells that received nitrified wastewater effluent in 2019 (Figure 4.1). Namely, nitrate-reducing conditions persisted over nearly the entire length of the RO concentrate-receiving cell (0-40 m), whereas nitrate was removed in the first 8 meters of the cells that received wastewater effluent. In addition, dissolved iron remained at low levels for the first 40 meters of the RO concentrate-receiving cell, suggesting that Fe(III)-reducing conditions were not reached until the last five meters of the cell, whereas Fe(III)-reducing conditions were prevalent throughout the wastewater effluent-receiving cells after approximately 5 meters from the inlet. These low levels of dissolved iron cannot be explained by the precipitation of $FeCO_{3(s)}$ because the precipitation of other carbonate minerals, such as $CaCO_{3(s)}$, would likely limit dissolved inorganic carbon concentrations to below levels of supersaturation for iron carbonate species under the conditions found in RO concentrate (i.e., $[Ca^{2+}] \approx$ 6mM, pH = 7-6.5). Sulfate exhibited results that were consistent with the observations made for nitrate and iron based on the sequential use of energetically favorable terminal electron acceptors: sulfate concentrations remained near those measured in the inlet throughout the RO concentrate-receiving cell, whereas sulfate reduction was prevalent in the zone between 5-30 meters of the inlet in the wastewater effluent-receiving cells.

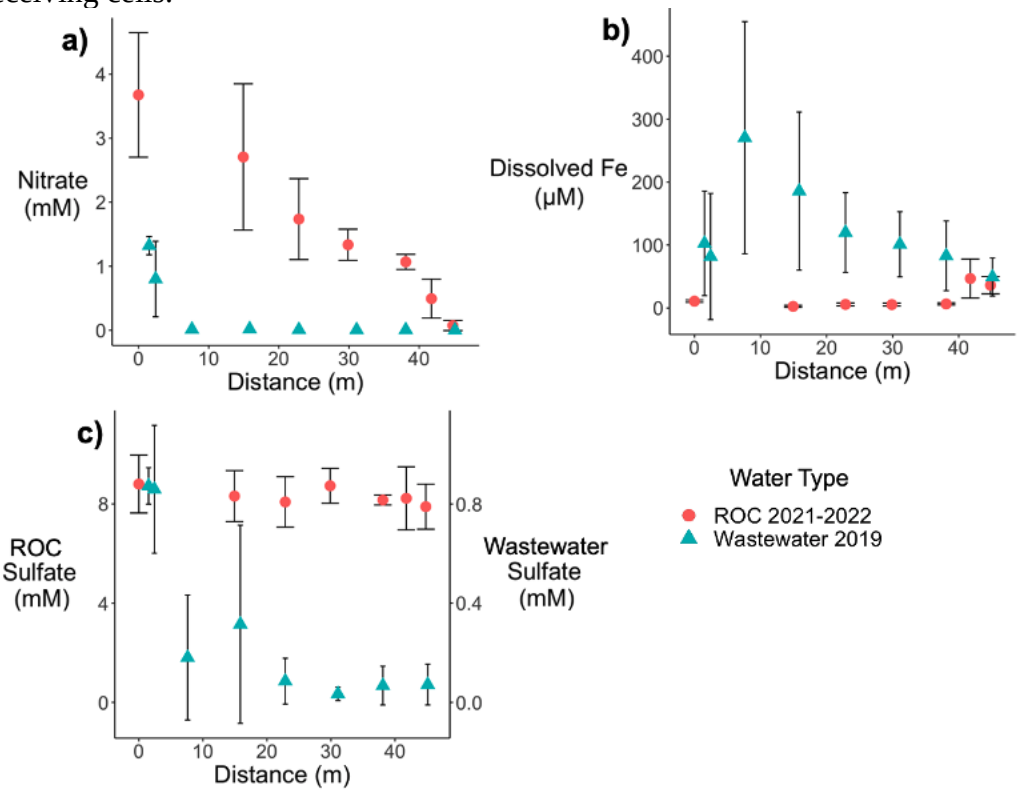


Figure 4.1 Redox conditions in porewater samples collected from the RO concentrate-receiving wetland cell collected between 2021 and 2022 (red circles, n = 12 at each location), and identically constructed

wastewater-receiving wetland cells collected in summer of 2019 (blue triangles, n = 6 at each location). Mean values are plotted with error bars representing +/- 1 standard deviation.

The results obtained in the RO concentrate- and wastewater-receiving cells are consistent with expectations based on the sequential reduction of terminal electron acceptors typical of flow-through subsurface systems. For example, denitrifiers may be able to outcompete Fe(III)- and sulfate-reducing microbial communities in presence of nitrate in the RO concentrate-receiving cell because nitrate is a more energetically favorable terminal electron acceptor.[194] In addition, nitrate is a potent inhibitor of sulfate-reducing microbial communities which could prevent sulfate concentrations from dropping in the RO concentrate-receiving cell.[195] Thus, the presence of nitrate throughout most of the horizontal levee slope and the lack of apparent Fe(III) or sulfate reduction in most of the subsurface indicated that redox conditions were poised at nitrate-reducing conditions through at least 90% of the RO concentrate-receiving cell. This stands in contrast to conditions in the wastewater effluent-receiving cell, where Fe(III)- and sulfate-reducing conditions prevailed in all but the first few meters near the inlet of the cell.

Although we expected nitrate to be rapidly removed in the RO concentrate-receiving cell, nearly the entire slope was required for complete nitrate removal. The longer-than-expected distances required for nitrate removal could be partially explained by differences in the composition of RO concentrate compared to wastewater effluent. For example, the higher concentrations of nitrate in the RO concentrate could have exceeded the ability of local microbial communities to denitrify incoming nitrate if carbon leaching rates from wood chips were insufficient. However, because nitrate concentrations were only about twice those found in the wastewater effluent, the increased supply of nitrate should only have increased the nitrate reducing zone by approximately a factor of two if carbon leaching rates were unaffected by the composition of RO concentrate compared to wastewater effluent.

Other differences in water quality (e.g., the nature of the organic carbon in RO concentrate versus wastewater effluent, increased salinity, lower pH and higher concentrations of other solutes) between RO concentrate and municipal wastewater effluent could have slowed the rates of microbial denitrification (Table 4.2). Slower denitrification rates were reported for open-water unit process wetlands treating RO concentrate relative to those treating municipal wastewater effluent and were attributed to the increased salinity of RO concentrate.[191] The addition of carbon amendments in open-water wetlands accelerated rates of denitrification despite salinity impacts. However, more research is needed to determine the role that salinity, carbon limitations, or the presence of nitrate reduction inhibitors play in the slower nitrate reduction rates observed in the RO concentrate horizontal levee cell.

Table 4.2 Average ± standard deviation of pH and electrical conductivity measured in the inflow to the wastewater effluent and RO concentrate cells during the monitoring period.

Inflow Water Type	pH	Electrical Conductivity (µS/cm)
Wastewater Effluent	7.48 ± 0.67	725 ± 80
RO Concentrate	7.00 ± 0.70	6061 ± 270

Lower primary productivity in the RO concentrate-receiving cell also may have played a role in the slower rates of denitrification because plant-derived carbon (e.g., roots and exudates) act as electron donors in horizontal levees.[59,60] For example, plant-derived sources were estimated to have increased the amount of carbon in the subsurface of the experimental horizontal levee by 22% during its first year of operation (See Figure D.1 and Figure D.2 for photos of above ground plant growth during the first year of operation).[59] The production of plant-derived carbon could have been particularly important in the RO concentrate cell because it had been operated for over 2.5 years with municipal wastewater effluent as the water source prior to the introduction of RO concentrate. During this period, the rates of labile organic carbon release from the woodchips may have declined, thereby increasing the importance of fresh sources of carbon from decaying roots and above ground biomass.[103]

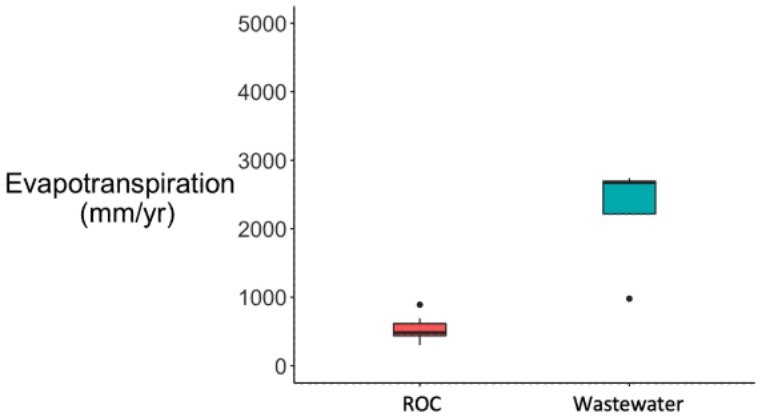


Figure 4.2 Evapotranspiration rates estimated in the wetland cell treating RO concentrate and the cell treating wastewater effluent (2021-2022) by tracking increases in conservative ion species (Chloride) in the subsurface of the horizontal levee. Chloride concentrations in the RO concentrate cell can be found in the Appendix (Figure C.3).

Evidence for lower primary productivity in the RO concentrate-receiving cell was provided by measuring evapotranspiration, because transpiration by plants represents 50-90% of the water loss measured in surface and subsurface wetlands.[196,197] In addition, transpiration is metabolically linked to photosynthesis and directly proportional to the dry matter produced by plants during the growing season.[198] Evapotranspiration was significantly lower in the RO concentrate cell when compared to the wastewater cells (Figure 4.2) ($p < 0.01$ Mann-Whitney U). Evidence supporting lower plant growth in the RO concentrate-receiving cell was also obtained from visual observation (Figure 4.3). Several bare patches covered in decaying plant litter were observed in the RO concentrate-receiving cell between 0-15 meters of the inlet (Figure 4.3 a & e).

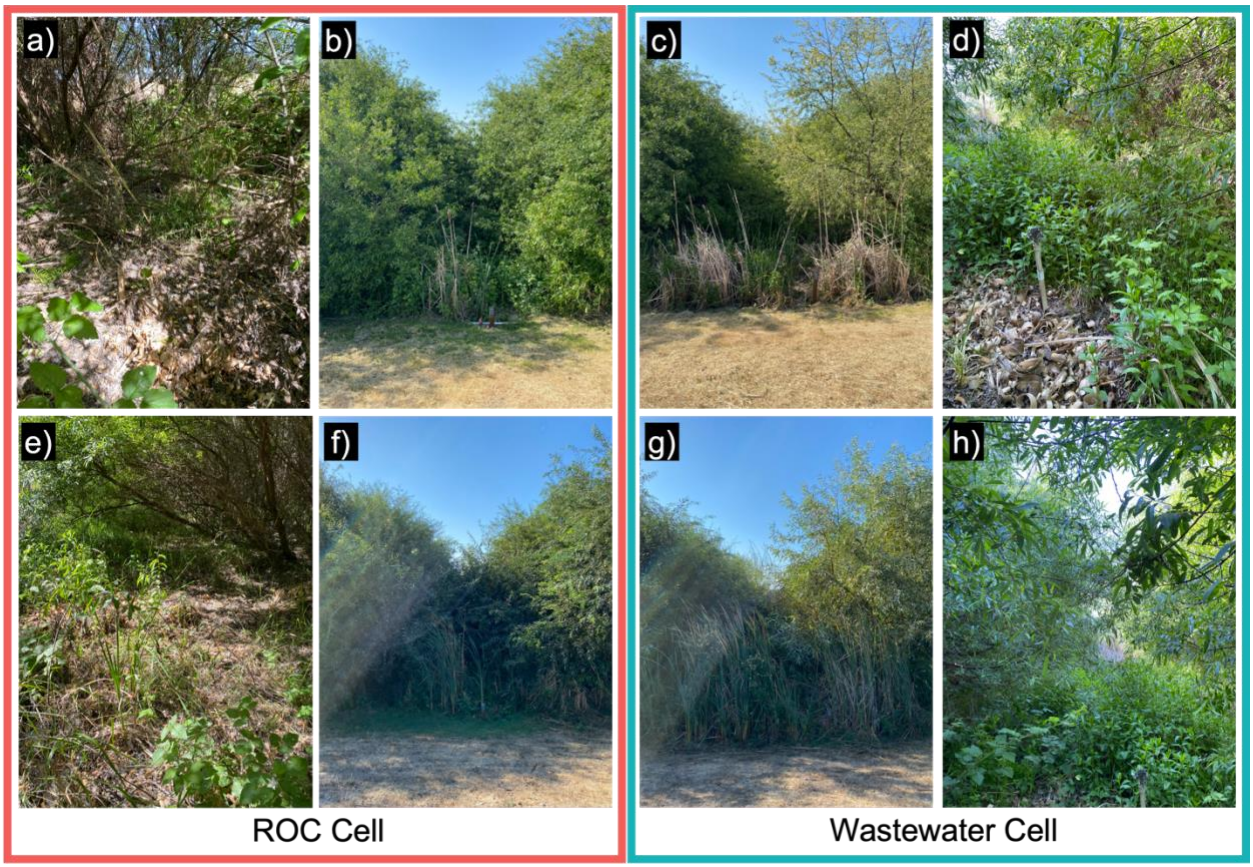


Figure 4.3 Photos of the RO concentrate cell (left) and wastewater cell (right). The RO concentrate exhibited several bare patches in shaded regions (a, e). In contrast, the wastewater effluent-receiving cell exhibited an understory with lush vegetation (d, h). Willow trees in neighboring cells shaded both wetland cells, but willow trees overhanging into the RO concentrate-receiving cell (b, f) shaded the cell to a greater degree than the wastewater effluent-receiving cell (c, g). Photos a-e and h were taken in April 2021, f and g were taken in October 2020.

Lower primary productivity and evapotranspiration rates in the RO concentrate-receiving cell could have been caused by several factors. Shading due to overgrowth of willow trees from neighboring wetland cells likely hindered vegetation growth in the first portion of the RO concentrate-receiving cell (Figure 4.3 b, f). Willow trees planted in the two adjacent wetland cells reached heights of over 10 meters and their branches often extended over the RO concentrate-receiving cell particularly in the first ten meters, where bare sediment patches were visible. Although the wastewater effluent-receiving cell also had willow trees on both of the neighboring cells, the shading was not as extreme (Figure 4.3 c, g). As a result, the understory of the wastewater cell was more densely vegetated (Figure 4.3 d, h).

Differences in the chemical composition of RO concentrate compared to wastewater effluent could have slowed primary productivity or altered the microbial activity in the treatment zone. Although the plants selected for the horizontal levee were well adapted to the salt concentrations typically observed in RO concentrate,[90] increased salinity lowers the osmotic potential around plant roots, slowing transpiration even in well adapted plants.[199] Increased salt concentrations could also alter the species present in the plant community over multiple seasons

because individual plant species have differing tolerances for the salinity of wastewater effluent (i.e., the freshwater range <0.5 ppt) versus RO concentrate (i.e., the oligohaline range 0.5-5 ppt).

It is possible that other solutes present in RO concentrate, such as herbicides, boron, sulfate, and chloride, affected plants. However, visual evidence of plant stress (e.g., chlorosis) was not observed.[200,201] In addition, other researchers have reported that halophytes growing in municipal RO concentrate exhibited high levels of physiological health.[202] Although concentrations of dissolved inorganic species (e.g., boron, sulfate, chloride) in the RO concentrate were above levels recommended for irrigation water,[201] the saturated flow conditions in the horizontal levee prevents salt and contaminant build-up that is typical of irrigated agricultural soils and turfgrass.[203,204] Furthermore, the plant species selected for the horizontal levee were well-adapted to the concentrations of sulfate and chloride found in RO concentrate.[90] Additional research is needed to determine the cause of lowered evapotranspiration rates and plant growth in the RO concentrate-receiving cell and its effects on the redox conditions in the subsurface.

Results from the analysis of wastewater effluent-receiving cells (i.e., Chapter 2) indicated that trace organic contaminants were removed from the subsurface treatment layer of the horizontal levee via biotransformation and that sorption played a minor role in contaminant removal. However, the higher concentrations of trace organic contaminants in RO concentrate necessitated reconsideration of the role of sorption in the RO concentrate-receiving wetland cell, especially during the initial phase of monitoring after the changeover from wastewater effluent because wastewater effluent contained concentrations of trace organic contaminants the were 2-6 times lower than those in RO concentrate.

To estimate the time required for breakthrough of trace organic contaminants in the RO concentrate-receiving wetland cell, we used isotherm-derived K_D values (Table A.5 Summary of K_D values derived from isotherms, microcosms, and field results.) paired with the one-dimensional transport approach described in Chapter 2. If partitioning coefficients (K_D) are consistent in RO concentrate and in municipal wastewater effluent, higher contaminant concentrations in porewater would increase the corresponding equilibrium concentration of trace organic contaminants on surfaces (C_s) (Appendix A2.4). Although the higher ionic strength of RO concentrate would alter the partitioning behavior of trace organic contaminants (e.g., especially those that are ionizable), using K_D's derived from lower concentrations of sorbates would likely yield a conservative estimate of sorption.

Results of the one-dimensional transport models indicated that concentrations of carbamazepine, carboxy-acyclovir and sulfamethoxazole, compounds that are typically persistent in biologically based treatment systems exhibited behavior consistent with predictions made for a scenario in which sorption was coupled with a modest amount of transformation (Figure 4.4). That is, the first detection of the compounds in the outflow were consistent with breakthrough curve predictions, but the maximum concentrations observed after breakthrough were lower than those predicted by assumptions that the compounds did not undergo any transformation. In contrast, more labile contaminants, such as acyclovir, were not observed in the outlet of the wetland, despite predictions that breakthrough should have occurred (Figure 4.4). This implies that these compounds were transformed within the wetland.

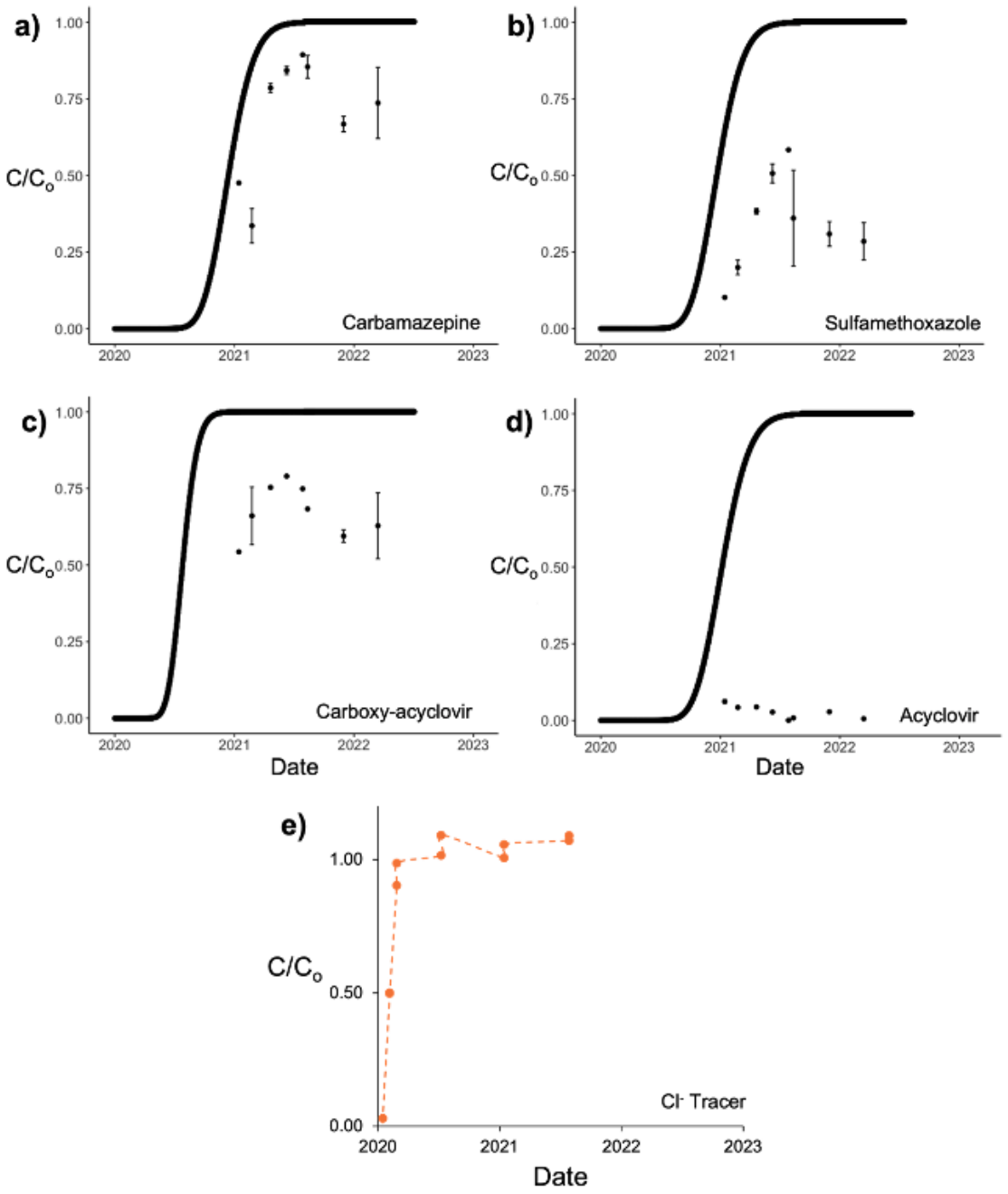


Figure 4.4 a-d: predicted breakthrough curves (Lines) calculated by modeling the horizontal levee as a one-dimensional fixed bed assuming adsorption is the only mechanism resulting in removal of trace organic contaminants. Circles with error bars represent average evapotranspiration-corrected concentrations plus or minus one standard deviation measured in porewater samples collected 42 meters from the inlet relative to the influent concentration collected on the same day (Figure C.4 Mean concentrations of trace organic contaminants in RO concentrate flowing into the horizontal levee). e: monitoring of Cl^- concentrations in the inflow relative to the outflow as a tracer during the system start up and operation. C/C_o calculations for figure e can be found in section A2.2 of the Appendix.

Because sulfate- and Fe(III)-reducing conditions were not prevalent throughout most of the RO concentrate cell (Figure 4.1), we hypothesized that trace organic contaminants that previously required sulfate-reducing conditions would not be completely removed in the RO concentrate-receiving wetland cell after sorption breakthrough occurred. Porewater samples collected in late 2021 and early 2022 supported this hypothesis (Figure 4.5). For example, in the RO concentrate-receiving wetland cell, average concentrations of carboxy-acyclovir, carboxy-metoprolol and carbamazepine were 70% or more of the paired inlet concentrations from the same period during the last four sampling events of the monitoring period (Figure 4.5). In contrast, contaminants for which removal was observed in the wastewater effluent-receiving cells under oxic or nitrate-reducing conditions (e.g., metoprolol, trimethoprim, acyclovir) were removed throughout the RO concentrate-receiving cells. However, trimethoprim and metoprolol removal could not be solely attributed to biotransformation due to their higher affinity for the wetland sediments (i.e., the system had not been run long enough to observe breakthrough of these compounds when these measurements were made).

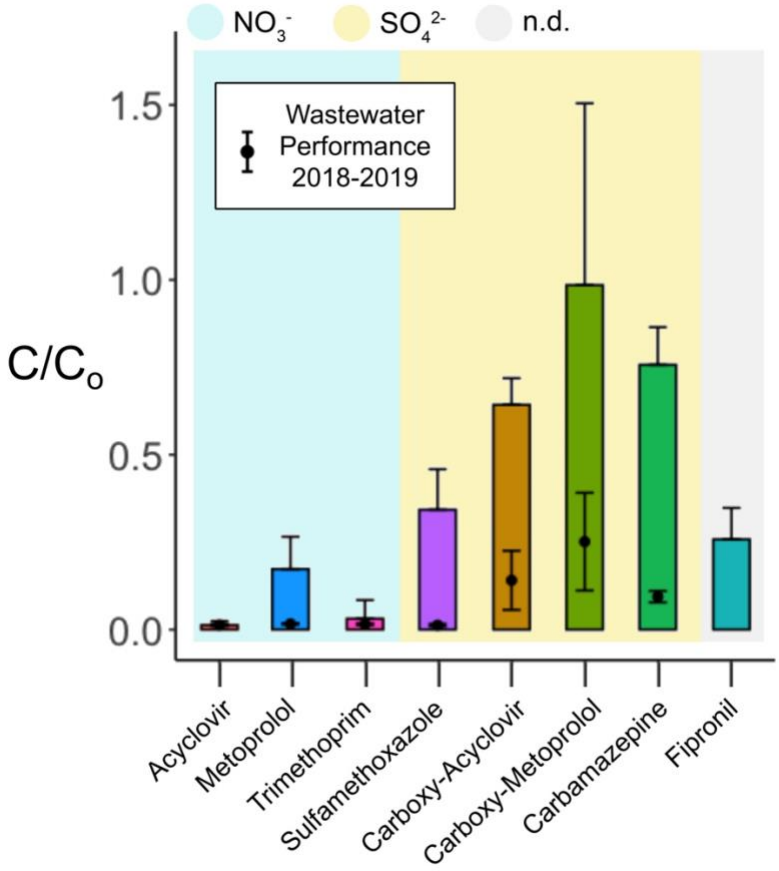


Figure 4.5 Fraction remaining of a suite of trace organic contaminants in porewater samples collected 3 meters from the outlet of the RO concentrate-receiving cell collected between November 2021-June 2022 after sorption breakthrough occurred for all contaminants except metoprolol, fipronil and trimethoprim. Results from monitoring of porewater at similar locations in wastewater effluent-receiving cells between 2018 and 2019 are plotted as dots (2018-2019). Shading represents the redox conditions under which each trace organic contaminant was removed in the wastewater cells (see Chapter 2 for details).

Porewater samples collected 3-12 months after predicted breakthrough times yielded additional insights into the removal processes in the RO concentrate-receiving cells (Figure 4.6). For example, the modest removal of carbamazepine, carboxy-acyclovir and carboxy-metoprolol in the RO concentrate cell only occurred in the last 5-7 meters of the cell, where the first evidence Fe(III)-reducing conditions was detected. Interestingly, nearly all of the removal of sulfamethoxazole took place in this last section of the wetland cell. This is consistent with previous research that indicates sulfamethoxazole can be rapidly removed under Fe(III)-reducing conditions (see Chapter 2 for additional details).[81] These results also suggested that if the horizontal levee was longer or if the sulfate- and Fe(III)-reducing conditions were reached sooner, better removal of these persistent compounds might have been achieved in the horizontal levee.

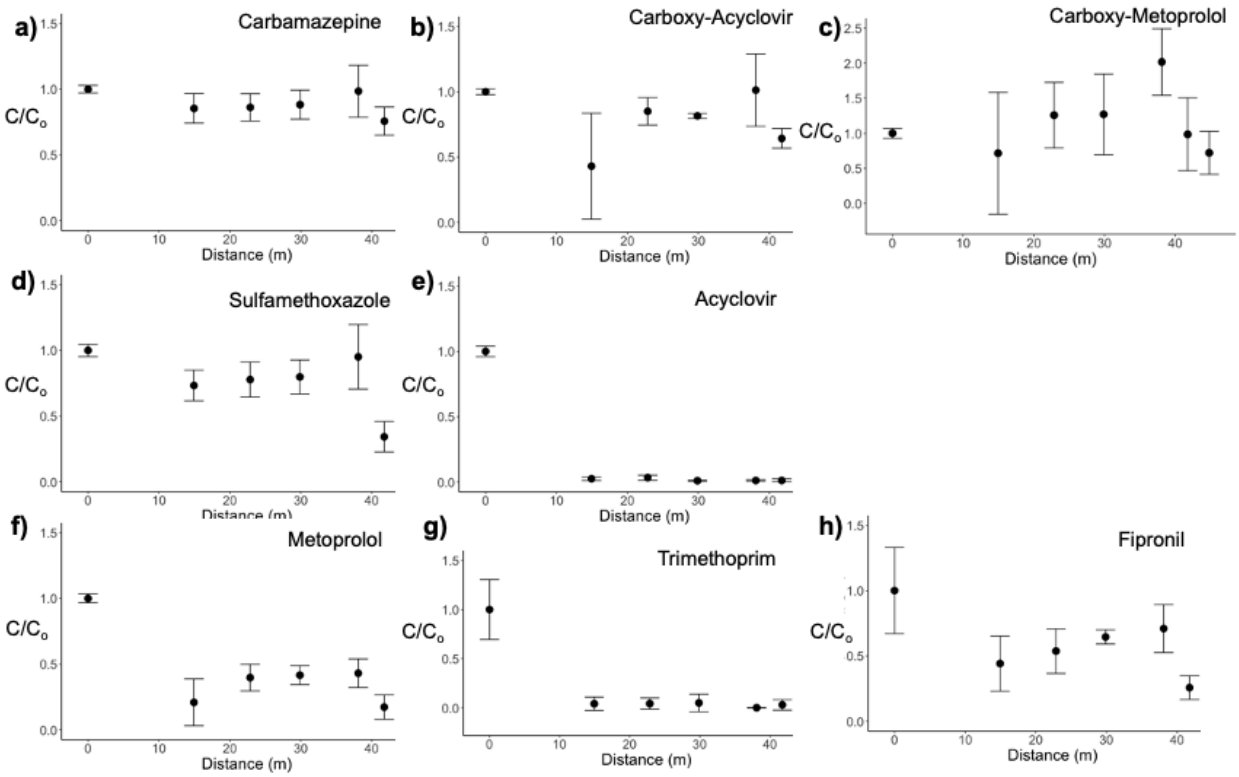


Figure 4.6 Average fraction remaining of trace organic contaminants in porewater and monitoring wells (15m, 30m) collected from the RO concentrate-receiving cell collected over four sampling dates between November 2021-June 2022. Error bars represent +/- 1 standard deviation. (n = 12 at each location)

To compare the removal of nitrate and trace organic contaminants from RO concentrate in horizontal levees to other RO concentrate treatment wetlands, we calculated the annual mass of contaminants removed per unit area. This is an important metric because land availability is often a significant cost and design constraint for constructed wetlands, especially in urban areas where wastewater-derived RO treatment systems are likely to be built.[205] Results from this analysis indicated that the horizontal levee removed 5-7 times more acyclovir and trimethoprim from RO concentrate per unit area year-round than open-water wetlands that only achieved contaminant removal during summer (Figure 4.7). The horizontal levee also removed sulfamethoxazole during all seasons— a phenomenon that was not observed in open-water wetlands. These results

combined with observations reported in Chapter 2 (section A4.2 of the Appendix) suggest that horizontal levees treating RO concentrate may not be subject to seasonal variability issues that are common in open-water wetlands due to lower temperatures and less available sunlight for photolysis of contaminants during winter.[39] However, because the experimental horizontal levee was built in the San Francisco Bay Area where temperatures are mild year-round, its suitability for colder climates is uncertain.

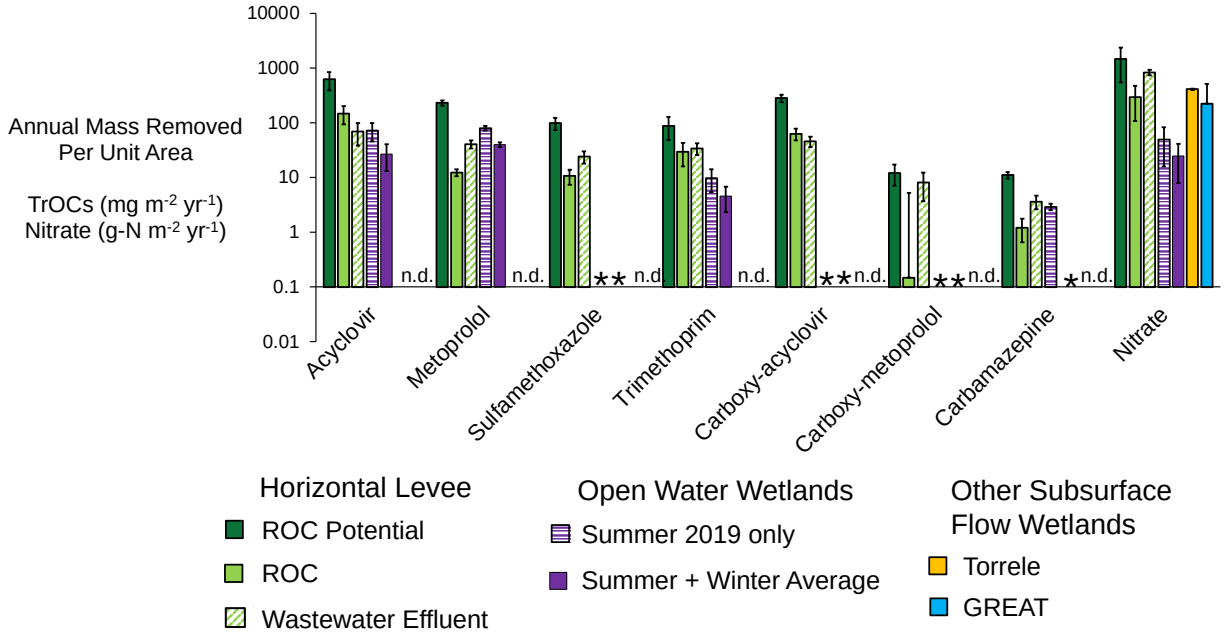


Figure 4.7 Annual mass of contaminants removed per unit area for horizontal levees treating RO concentrate and wastewater effluent plotted on a log scale. The potential removal that could be achieved in RO concentrate wetland cells that are designed to reach the same redox conditions as those treating wastewater effluent is also plotted (Dark green, bar 1). Horizontal levees are compared to open-water wetlands[39,191] in the summer with peak performance (2019) and those that were averaged over the winter and summer seasons. Horizontal levees are also compared to subsurface flow treatment wetlands treating RO concentrate.[206,207] (* is zero removal, n.d. is no data reported for the study)

The horizontal levee also removed 11 ± 7 times more nitrate on average annually per unit area than open-water wetlands, which only removed nitrate during summer.[191] It also exhibited similar performance to two subsurface flow wetlands treating RO concentrate (GREAT and Torrele systems outlined in Table 4.1).[206–209] Similar to the results for trace organic contaminants, the horizontal levee consistently removed nitrate in all seasons whereas open-water wetlands did not remove nitrate during winter months.

The mechanisms through which nitrate is removed in subsurface flow constructed wetlands merits additional consideration because nitrate that is not lost from the system through denitrification could be released during plant decomposition. The nutrient removal mechanisms in the other two subsurface flow treatment systems (e.g., GREAT and Torrele) are unknown. It is possible that plant uptake contributed to nitrogen removal because these systems did not integrate a supplemental carbon source in the sediment layers. In contrast, plant uptake played a minor role

in nitrate removal in the horizontal levee[60] because nitrate was primarily removed via denitrification.[59]

Although the horizontal levee outperformed other nature-based RO concentrate treatment systems, it did not remove as high of a mass of recalcitrant trace organic contaminants as the wastewater effluent-receiving cells when expressed on an annual mass removed per unit area basis Figure 4.7). This lower performance is likely due to slower rates of denitrification in the RO concentrate cell and the lack of sulfate- and Fe(III)-reducing conditions within significant sections of the wetland because these conditions are important for the removal of persistent trace organic contaminants such as carboxy-metoprolol, carbamazepine, and sulfamethoxazole (Chapter 2). However, because RO generates a smaller volume of water that needs to be treated relative to wastewater effluent, horizontal levees would require the acquisition of significantly less shoreline to process the entire volume of wastewater produced by the adjacent community.

If RO concentrate-receiving horizontal levees were designed to exhibit the same redox conditions as those observed in wastewater effluent-receiving cells, it is likely that the areal removal of contaminants in horizontal levees would be improved (i.e., the "ROC in horizontal levee potential" bar in Figure 4.7). These conditions could increase the mass removed per unit area for wastewater-derived contaminants in horizontal levees that treat RO concentrate by factors ranging from 3-82 times depending on the contaminant. This would make horizontal levees several orders of magnitude more efficient than the other wetland designs that have been tested for RO concentrate treatment thusfar.

Several design improvements to the horizontal levee might enhance their performance during RO concentrate treatment. For example, to accelerate denitrification, additional carbon could be added to the RO concentrate cell prior to its construction. Additional labile organic carbon has been shown to increase denitrification rates in open-water wetlands that treat RO concentrate[191] and in subsurface and surface flow wetlands (e.g., denitrification walls).[210] After nitrate is removed from the subsurface, this additional carbon could stimulate sulfate- and Fe(III)-reducing conditions which could provide a pathway for removal of persistent trace organic contaminants (e.g., carbamazepine) from RO concentrate. However, experiments to determine the amount of carbon needed for this purpose would be necessary because the slower rates of denitrification observed in the RO concentrate-receiving wetland cell could have been due to something other than a lack of labile organic carbon.

As an alternative approach, contaminants could be retained in the subsurface of horizontal levees if activated carbon or another sorbent material is incorporated into the subsurface treatment layer. Addition of a sorbent media may also allow for the removal of trace organic contaminants that are not amenable to biotransformation (e.g., per- and polyfluoro alkyl substances). However, addition of sorbent media that requires frequent regeneration to reliably remove trace organic contaminants over time is impractical. Therefore, such design alterations require further research and validation prior to implementation at scale.

Design improvements to increase the removal of trace organic contaminants via biotransformation may also limit risks to terrestrial wildlife posed by trace organic contaminants found in the plants growing in horizontal levees that treat RO concentrate. According to research

conducted in Chapter 3, carbamazepine is the only monitored trace organic contaminant that is expected to be readily taken up by plants. If the average concentration of carbamazepine in plants increased proportionally to the concentrations in wetland porewater and if plant biomass is proportional to evapotranspiration,[198] we estimate that after sorption breakthrough, up to approximately 8% of the mass of carbamazepine that entered the horizontal levee would be taken up by plants. This uptake mechanism could be more significant for the exposure of terrestrial organisms in the RO concentrate-receiving cell than it was in wastewater effluent-receiving cells because after sorption breakthrough occurred only a small amount of carbamazepine (i.e., <25%) was removed from RO concentrate. In addition, the concentrations found in some plants (*Juncus spp.* and *B. maritimus*) growing in the first 0-7.5 meters of the more dilute wastewater cells exceeded those that caused behavioral alterations in the insect *N. cinerea.*[153] However, design improvements that increase the removal of trace organic contaminants via biotransformation or other mechanisms in the subsurface could limit the concentrations found in plants by lowering porewater concentrations. In addition, wetland plants exhibit considerable inter-species variability in carbamazepine uptake and translocation (Figure 3.1). Therefore, plants grown in horizontal levees intended for the treatment of RO concentrate could be selected strategically to lower the exposure of carbamazepine and other trace organic contaminants to terrestrial wildlife.

4.4 Conclusions and Outlook on Nature-based Treatment of RO Concentrate

Our research demonstrated that the horizontal levee can remove multiple contaminants from the concentrate produced during RO treatment of municipal wastewater effluent. This may reduce the number of steps needed in a treatment train because physical, chemical, and biological removal mechanisms take place simultaneously in horizontal levees (Figure 4.8), whereas other treatment systems make use of only one set of processes at a time. Although only a few early-stage experiments of RO concentrate treatment in constructed wetlands have been reported, the results presented here indicate that horizontal levees have the potential to be more efficient on a surface area basis than nature-based systems employed for the treatment of wastewater effluent, provided the proper redox conditions are established in the subsurface. In addition, horizontal levees and other constructed wetlands may provide a means of removing trace metals, another class of priority contaminants, from RO concentrate. For example, although metals have not been the focus of research on constructed wetlands treating municipal wastewater effluent, copper and zinc concentrations have been reduced by up to 60-80% in wetlands treating industrial process water via plant uptake, sorption, and precipitation.[50,56,57] Presumably, these removal mechanisms for metals could be achieved in horizontal levees that are also designed to remove nutrients and trace organic contaminants from RO concentrate. However, the removal of trace metals requires more research because they are often present as metal-EDTA complexes in RO concentrate and may therefore be more difficult to remove in subsurface flow treatment wetlands.[211]

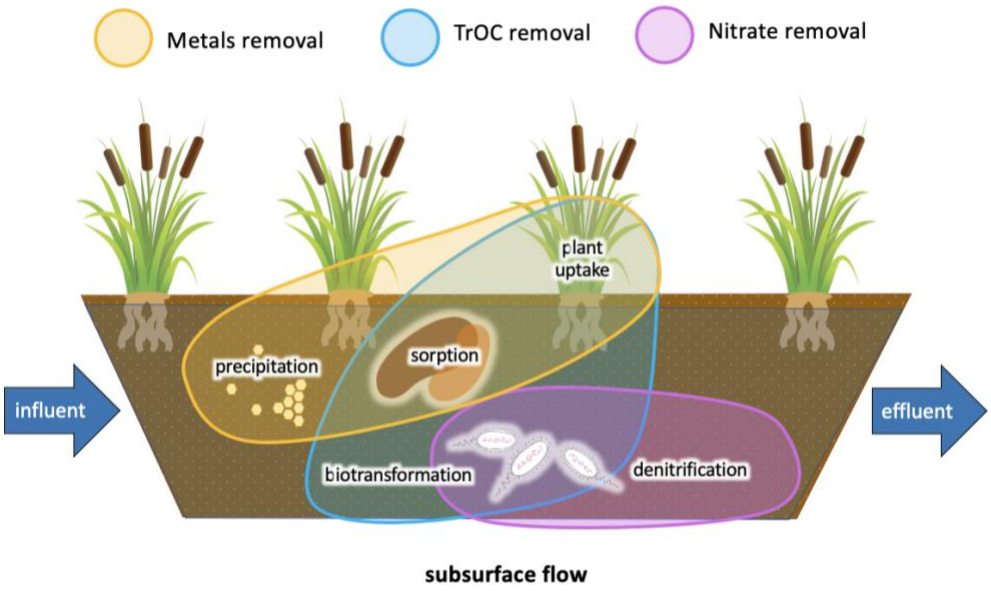


Figure 4.8 Main metals, trace organic contaminants, and nitrate removal mechanisms occurring simultaneously in subsurface flow wetlands.

Beyond their attraction as cost-effective treatment systems, horizontal levees and other nature-based systems are also appealing to decision-makers because members of the community appreciate the additional benefits that wetlands can provide. For example, constructed wetlands can be designed to create wildlife habitat,[63,64] improve biodiversity,[65] and provide recreation and educational opportunities.[60] Results of a mixed-methods stakeholder analysis that assessed the importance of these ancillary factors in the selection of nutrient reduction strategies indicated that ancillary benefits (e.g., sea level rise protection, habitat creation) combined with the water quality benefits of constructed wetlands made them more favorable than other approaches like installation of advanced treatment technologies at existing wastewater treatment plants for nutrient removal.[46] RO concentrate treatment wetlands that provide a public interaction component may be able to capitalize on the positive association that decision-makers have with wetlands, especially because they could enhance overall public support for the entire potable water reuse project.

Our research indicates that horizontal levees and other constructed wetland systems may offer a means of managing RO concentrate and that constructed wetlands designed to treat RO concentrate may take up less land than those used to treat municipal wastewater effluent. Using constructed wetlands for RO concentrate treatment could therefore provide synergistic benefits – enabling water reuse while improving the efficiency of constructed wetlands and providing additional benefits such as habitat creation. Although more research is needed to realize the full potential of horizontal levees for RO concentrate treatment while minimizing unintended consequences, these multiple benefits could make them an attractive nature-based solution for cities facing diverse challenges such as sea level rise, wetland habitat loss, impaired surface water quality and water scarcity.

CHAPTER 5. Conclusions

5.1 Summary

Horizontal levees are a recently developed, subsurface flow constructed wetland designed to improve water quality while creating habitat and protecting communities and infrastructure from the effects of sea level rise. To understand the mechanisms controlling the removal of trace organic contaminants in horizontal levees, we monitored an experimental horizontal levee that received municipal wastewater effluent over a period of 2.5 years by multi-media sampling (i.e., plants, water and soil) at various distances along the flow path. Efforts to determine the mechanisms controlling contaminant removal included: isotherm experiments and microcosms using soil collected from the field site; spatial, temporal and species-specific sampling of plants; porewater sampling before and after upgrades to the wastewater treatment plant eliminated nitrate from the water flowing into the horizontal levee; and linking contaminant removal to the redox conditions in different zones of the treatment system. Findings were translated to environmental systems that experience similar conditions, such riparian zones and wetlands located adjacent to effluent-dominated streams where municipal wastewater effluent flows through the subsurface. We also tested the ability of horizontal levees to remove contaminants from reverse osmosis concentrate, an application that could improve the efficiency of constructed wetlands from a land-use perspective. This chapter highlights the key findings from this dissertation and identifies future research needs.

5.2 Research Findings

5.2.1 Mechanisms of Trace Organic Contaminant Removal in Horizontal Levees

Results from Chapter 2 indicated that horizontal levees remove trace organic contaminants primarily through transformation reactions that take place in the subsurface treatment zone. Contaminants that are typically persistent in aerobic biologically-based treatment systems such as carbamazepine, carboxy-acyclovir, and sulfamethoxazole were rapidly removed once sulfate- and Fe(III)-reducing conditions were achieved. In horizontal levee wetland cells that did not exhibit appreciable sulfate reduction, removal of these contaminants was limited (Chapter 4). In the absence or presence of nitrate, relatively biodegradable trace organic contaminants, such as acyclovir and metoprolol, were also rapidly transformed. These results suggested that providing enough supplemental carbon to remove nitrate and establish sulfate and Fe(III)-reducing conditions in horizontal levees and other subsurface treatment systems could enable them to remove a wide range of trace organic contaminants that are persistent in aerobic treatment systems.

5.2.2 Plant Uptake of Trace Organic Contaminants: Implications for Effluent-Dominated Streams

Plant uptake and translocation of trace organic contaminants were evaluated in the horizontal levee to provide insights into the mass balance for compounds amenable to removal through this mechanism (Chapter 2). Among the monitored compounds, carbamazepine was the only

contaminant that underwent significant plant uptake and translocation. Concentrations of carbamazepine in plants exhibited significant spatial, temporal, and species-specific variability (Chapter 3). Using in-plant concentrations informed by data on spatial, species-specific and temporal variability, we estimated that plant uptake removed no more than 12% of the mass of carbamazepine that entered in horizontal levee during the monitoring period.

The results from this objective also provided insight into the importance of plant uptake to the fate of wastewater-derived contaminants in important ecosystems that exhibit similar conditions as the horizontal levee (Chapter 3). For example, effluent-dominated riparian zones in the American west are important to a variety of terrestrial wildlife. Using results from the experimental horizontal levee, we predict that plant uptake of trace organic contaminants could be an important overlooked terrestrial exposure pathway in effluent-dominated riparian zones. This research highlights that terrestrial exposures and effects should be considered when assessing the ecotoxicological risks associated with discharges of municipal wastewater effluent.

The approach used in this research objective highlights that nature-based treatment systems may be an adequate model to represent other environmental systems, such as riparian zones that obtain water from effluent-dominated rivers. The experimental set-up of the horizontal levee and other experimental nature-based treatment systems also allows researchers to test a variety of hypotheses at a spatial scale that can be applied to natural ecosystems. Another example of this is the use of open-water wetlands for wastewater effluent treatment because studies of open-water wetlands allowed researchers to gain new insights into the overlooked role of reactive nitrogen species for contaminant transformation in natural sunlit waters.[212] Therefore, mechanistic studies of nature-based treatment systems may further our understanding of the fate of chemical contaminants in the environment while providing design insights intended to enhance their performance.

5.2.3 Horizontal levee treatment of RO Concentrate

RO concentrate management is a significant barrier to RO-based water reuse in water-stressed cities worldwide because traditional engineered approaches (e.g., chemical oxidation, sorption) are not economically feasible for the treatment of RO concentrate derived from municipal wastewater effluent.[171] Horizontal levees were evaluated to determine their suitability for the treatment of RO concentrate from municipal wastewater reuse projects. Results showed that nitrate was removed from RO concentrate, but that nitrate reduction occurred over larger distances than in the wetland cells treating municipal wastewater effluent. Similar trends were observed for some trace organic contaminants that were previously removed under nitrate-reducing conditions (e.g., metoprolol). Relatively biodegradable compounds, such as acyclovir were rapidly removed from both RO concentrate and municipal wastewater effluent. Because the wetland cell that received RO concentrate did not exhibit sulfate-reducing conditions, some especially persistent contaminants were not removed to an appreciable degree (e.g., carbamazepine, carboxy-metoprolol, carboxy-acyclovir).

Our results suggested that the performance of horizontal levees that treat RO concentrate could be enhanced by employing anaerobic redox conditions (i.e., sulfate- and Fe(III)-reducing

conditions). Insight into the mechanisms of nitrate reduction inhibition as well as methods to overcome this issue (e.g., increasing the supply of labile organic carbon), could improve the performance of horizontal levees significantly. The ability of horizontal levees to remove multiple contaminants from RO concentrate and their potential for more removal suggested that horizontal levees may be an attractive approach for managing RO concentrate.

5.3 Future Research

5.3.1 Additional Mechanistic Research Areas

More research is needed to gain a better understating of the interplay between removal mechanisms that take place simultaneously to design subsurface flow treatment systems that perform consistently. For example, treatment systems intended to remove trace organic contaminants via biotransformation could be designed to retain contaminants via sorption during the initial phases of operation when microbial communities have yet to mature or plant roots have yet to be established in the subsurface. Additionally, the presence of plant roots could indirectly impact the performance of subsurface flow treatment systems via stimulation of rhizosphere microbial activity. Properly designed subsurface flow treatment systems would need to account for uncertainty associated with these factors in their sizing and design. Although our approach quantitatively delineated the roles of each removal mechanism, it did not explicitly consider potential synergies between removal mechanisms.

Additional research into the pathways of contaminant biotransformation under anaerobic conditions is also needed. This is critical to ensuring that wetland treatment systems do not form persistent transformation products that are more toxic than the chemicals entering subsurface treatment systems. These efforts could leverage new tools for understanding the microbiology of complex systems (i.e., metagenomics and metatranscriptomics), chemical transformation products (e.g., non-target or suspect screening high resolution mass spectrometry), and their toxicity (e.g., high throughput toxicity screening) paired with experiments designed to unravel the microbial communities, their enzymatic activity, and the chemical transformation pathways involved in the transformation of trace organic contaminants under anaerobic conditions. These insights may allow for the design of treatment systems that generate minimal persistent toxic transformation products.

Improvements to the hydraulic performance of horizontal levees and other subsurface treatment systems should also be assessed with attention to the tradeoffs that occur due to design decisions. For example, research into the role of wetland slope (i.e., hydraulic head), subsurface porosity (e.g., as a function of sediment grain size), and treatment layer thickness is needed to determine if more water could be passed through the subsurface. However, these changes could compromise treatment performance (e.g., through lowered contact with microbial biomass, altering biofilms through increased shear stress, alterations to the chemical composition of the subsurface media). Research into how these hydraulic performance alterations impact removal rates is critical prior to their implementation.

5.3.2 Avoiding Unintended Consequences in Constructed Wetlands

Unintended consequences could result from treatment systems that employ sulfate-reducing conditions. For example, although mercury concentrations in wastewater effluent tend to be low,[213] sulfate-reduction is associated with the production of methylmercury in wetlands,[214] a chemical with high ecotoxicity. Also, sulfides produced during sulfate reduction are toxic to plants and some microbial communities. Sulfate reduction could result in the release of $H_2S_{(g)}$ which can cause significant odor issues for the communities adjacent to the wetlands. However, the horizontal levee may have been less susceptible to these issues because the surface sediments likely limit $H_2S_{(g)}$ release from the underground treatment layer and because excess Fe(II) can substantially lower concentrations of dissolved S(-II) species. However, research into methods to prevent these issues in horizontal levees that receive higher concentrations of sulfate (e.g., in horizontal levees designed to reach sulfate-reducing conditions in RO concentrate) and in other contexts should be considered.

Another set of unintended consequences that should be considered is related to the potential for constructed wetlands to expose wildlife and insects living inside them to contaminants present in the edible parts of plants. Plant uptake is an is an overlooked wildlife exposure pathway in effluent-dominated waterways, but it may also be relevant in constructed wetlands that treat wastewater effluent, agricultural runoff laden with pesticides[215] or in wetlands growing in regions without industrial discharge restrictions.[158] Because constructed wetlands and effluent-dominated riparian zones can attract wildlife, methods to minimize unintended exposures of wildlife to contaminants present in plants are needed.

5.3.3 The Provision of Additional Benefits

The horizontal levee is designed to improve water quality and provide habitat. However, the quality of the horizontal levee habitat has not been quantified. In addition, in full-scale applications, the relationship between horizontal levee habitat size and value is unclear. For this reason, horizontal levees should be designed through consultation with wildlife ecologists who can assess the quality and value of habitat that is produced.

Another benefit of the horizontal levee is its ability to mitigate against sea level rise. The experimental system was not designed in a manner that provides direct insight into the ability of the system to protect shorelines from storm surges. The connection between the treatment system and the receiving waterbody is critical because the horizontal levee must be structurally stable while also providing water quality improvement. The intrusion of salty water near the bottom section of the horizontal levee could affect the microbial community of the upstream treatment zone through tidal action. In addition, the integration of horizontal levees with shallow regions on the tidal plane could provide additional unquantified benefits to wildlife (e.g., fish breeding territory). Therefore, horizontal levee design and implementation should be considered as part of a larger system of shoreline management and habitat restoration.[216]

5.4 Final Thoughts

Natural ecosystems provide human society with irreplaceable supporting, regulating, provisioning, and cultural services that result in an estimated 125 trillion 2007 USD per year in value.[217] In particular, coastal wetlands can perform multiple ecosystem functions such as dissipation of storm surges, hydrology modulation, building up land elevation through accretion and settling of sediments, improving water quality, providing valuable wildlife habitat, as well as providing social and cultural functions.

Recognition of these important ecosystem functions has inspired natural approaches to environmental and societal issues (i.e., nature-based solutions). Nature-based solutions that provide the functions of mature ecosystems when integrated into urban or human-influenced environments are especially promising because they may be capable of benefiting wildlife and a large portion of human society. However, the performance of nature-based solutions as compared to native or restored ecosystems or engineered alternatives must be validated to ensure that they provide the prospective benefits. In particular, nature-based solutions within the context of water infrastructure must provide reliable water quality and/or quantity benefits while interfacing with existing water technologies, infrastructure, and policies. Research into the performance of nature-based solutions, such as that conducted in this dissertation, could support decision makers in their efforts to assess the benefits that these approaches provide as they aim to tackle diverse socio-environmental issues simultaneously. However, due to the complexity and dynamism of the water landscape, successful implementation of nature-based solutions for water quality improvement requires a shift towards collaboration across disciplinary, societal, and institutional boundaries.[218]

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APPENDIX A: Supporting Information for Chapter 2

A1.1 Cross-section of the Horizontal Levee Subsurface

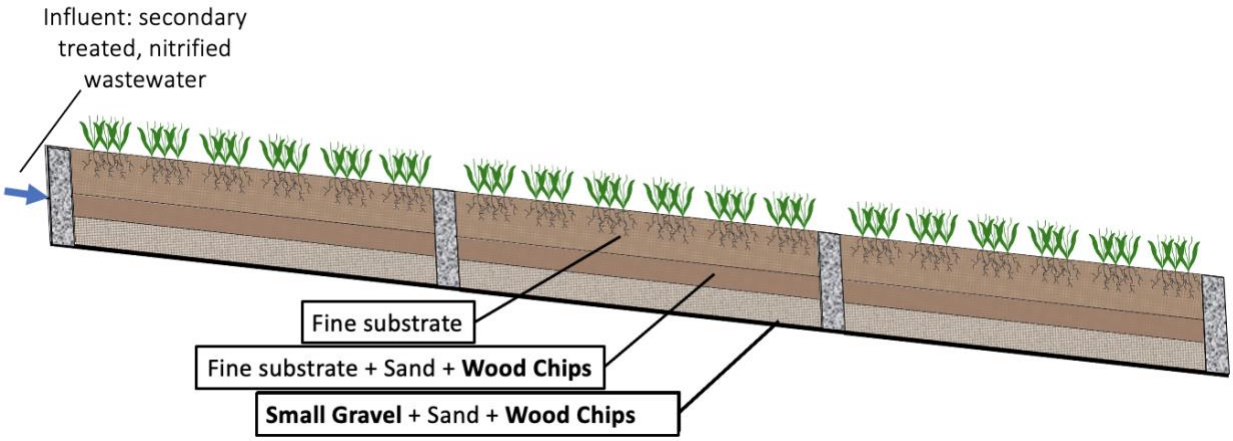


Figure A.1 The subsurface design of horizontal levees.

A1.2 Microcosm Set-up

Table A.1 Experimental treatments and corresponding shorthand used for labels in Table A.2

Treatments	Shorthand	#of time points	Total Vials needed
Nitrate + nitrate reducing soil + spiked porewater	N#	6	18
Sulfate + sulfate reducing soil + spiked porewater	S#	12	36
Control - no soil + nitrate + spiked porewater	NS-N#	3	9
Control - no soil + sulfate + spiked porewater	NS-S#	3	9
Control - nitrate reducing soil + spiked porewater	NN#	3	9
Control - sulfate reducing soil + spiked porewater	NS#	3	9

Table A.2 Sampling plan for the microcosm experiments including plans for periodic extraction and porewater sampling

Time point	Hour #	Days	Weeks	Day of week/time target	Description	Samples taken (description)	Porewater	Soil Extract	N	NS-N	NN	S	NS-S	NS
t0	0	0.0	0.0	Wednesday March 17	sample before introducing anything into the microcosms (soil alone, porewater alone)	unamended porewater, porewater + nitrate, porewater + sulfate, porewater + sulfate+CBZ, triplicate unamended soil extracts, triplicate unamended soil microbial	4	3						
t1	1	0.0	0.0	Wednesday March 17	sorption test	porewater, soil	6	6	pw+s			pw+s		
t2	3	0.1	0.0	wednesday March 17		poerwater	6		pw			pw		
t3	17	0.7	0.1	Thursday, March 18 AM	peak nitrate reducing	porewater, soil	14	11	pw+s	pw	pw+s	pw+s		
t4	24	1.0	0.1	Thursday, March 18 PM	peak nitrate reducing	poerwater	9		pw	pw	pw			
t5	41	1.7	0.2	Friday March 19, AM	near end nitrate reducing	porewater, soil	14	11	pw+s			pw+s	pw	pw+s
t6	47	2.0	0.3	Friday March 19, PM	end nitrate reducing	poerwater	9		pw	pw	pw			
t7	113	4.7	0.7	Monday March 22 AM	s reducing	poerwater	5					pw		
t8	185	7.7	1.1	Thursday March 25 AM	s reducing	poerwater	3					pw		
t9	281	11.7	1.7	Monday March 29 AM	s reducing	poerwater	3					pw		
t10	353	14.7	2.1	Thursday April 1 AM	peak S reducing	porewater, soil	11	8				pw+s	pw	pw+s
t11	449	18.7	2.7	Monday april 5 AM	s reducing	poerwater	3					pw		
t12	521	21.7	3.1	Thursday april 8 am	s reducing	poerwater	3					pw		
t13	617	25.7	3.7	Monday april 12 AM	s reducing	poerwater	3					pw		
t14	689	28.7	4.1	Thursday april 15 AM	end S reducing	porewater, soil	11	8				pw+s	pw	pw+s
						sum -->	104	47						

A1.3 Additional Analytical Details

An analyte list and spike-recoveries for soil extractions can be found in this section. For specific details on the LC-MS/MS methods, see Appendix B1.2.

Table A.3 Analyte list

Analyte type	**Analyte**	**Method**
Anions	Cl^-	Ion Chromatography
	NO_3^-	
	NO_2^-	
	SO_4^{2-}	
	Br^-	
Cations	NH_4^+	
	Na^+	
	Mg^{2+}	
	Ca^{2+}	
	K^+	
	Li^+	

Organic Carbon	TOC	Total Organic Carbon Analyzer
Nitrogen	TN	Total Nitrogen Analyzer
Metals	Fe	ICP-MS
	Mn	
Trace Organic Contaminants	Acyclovir	LC-MS/MS
Metabolite	Carboxy-Acyclovir	
	Metoprolol	
Metabolite	Carboxy-Metoprolol	
	Trimethoprim	
	Sulfamethoxazole	
	Carbamazepine	
Metabolite	10-OH-Carbamazepine*	
Metabolite	DiOH-Carbamazepine*	
Metabolite	9-Acridine-COOH*	
General Water Quality Parameters	pH	Field Meters
	Dissolved Oxygen	
	Electrical Conductivity	
	Temperature	

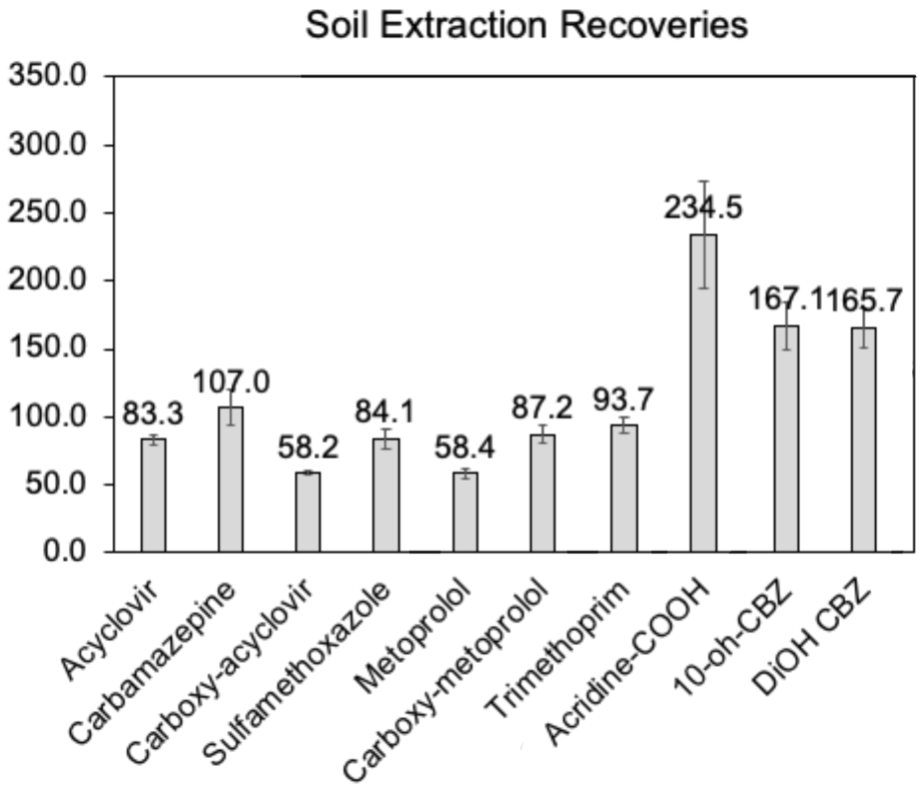


Figure A.2 Recoveries for monitored trace organic contaminants in spike-recovery experiments.

A2.1 Mass balance calculations

The following equation was used to describe the mass balance in the wetland over the course of the monitoring period.

$$m_{in} = m_{out} + m_{porewater} + m_{sorbed} + m_{roots} + m_{plants} + m_{transformed} \quad A.1$$

Where *m* is the mass in each wetland compartment over a defined time interval. The mass that entered the wetland was quantified using flowrates and inflow concentrations averaged over the monitoring period:

$$m_{in} = \int C_{in}(t)Q_{in}(t)dt = \overline{C_{in}}\ \overline{Q_{in}}\ \Delta t \quad A.2$$

Where m_{in} is the mass of a trace organic contaminant entering the wetland over the time interval Δt at an average inflow concentration, $\overline{C_{in}}$, and inflow flowrate $\overline{Q_{in}}$. The mass that exited the wetland was quantified similarly.

$$m_{out} = \int C_{out}(t)Q_{out}(t)dt = \overline{C_{out}}\ \overline{Q_{out}}\ \Delta t \quad A.3$$

Where m_{out} is the mass of a trace organic contaminants exiting the wetland over the time interval Δt at an average outflow concentration, $\overline{C_{out}}$, and outflow flowrate $\overline{Q_{out}}$. $\overline{Q_{out}}$ was approximated by accounting for evapotranspiration through increases in the concentration of the conservative ion, Cl^-.

$$Q_{out} = Q_{in}\frac{[Cl]_{in}}{[Cl]_{out}} \quad A.4$$

In other words, if Q_{out} is assumed to be equal to Q_{in}, $\overline{C_{out}}$ must be multiplied by the factor $\frac{[Cl]_{in}}{[Cl]_{out}}$ to account for loss of flow due to evapotranspiration. Therefore:

$$m_{out} = \overline{C_{out_et-adj}}\ \overline{Q_{in}}\ \Delta t = \overline{C_{out_measured}}\ \overline{\frac{[Cl]_{in}}{[Cl]_{out}}}\ \overline{Q_{in}}\ \Delta t \quad A.5$$

The mass of contaminants sorbed to soil particles, m_{sorbed}, was calculated using isotherm K_D values and porewater concentrations, $C_{pw}(x)$. Because porewater concentrations varied spatially across the wetland, concentrations were integrated over the length of the wetland:

$$m_{sorbed} = K_D \rho_b A \int C_{pw}(x)dx = K_D \rho_b A \sum \overline{C_{pw}} \Delta x$$

A.6

A uniform bulk density, ρ_b, of 1.5 g cm^{-3}, and cross sectional area, *A*, equal to the width of each wetland cell (10^4 cm) times the total depth (10^2 cm), was used to provide a maximum estimate of sorption in the subsurface.

The mass of contaminants in porewater, $m_{porewater}$, was approximated similarly, assuming a porosity, ϕ, of 0.4.

$$m_{porewater} = \phi A \int C_{pw}(x)dx = \phi A \sum \overline{C_{pw}} \Delta x$$

A.7

The mass of contaminants in plants, m_{plants}, taken up during the monitoring period, Δt, was approximated using the following equation because plant biomass and concentrations in plants varied over time and space.

$$m_{plants} = w \iint C_{plants}(x) B_{shoots}(t) dt dx = B_{shoots} w \Delta t \sum \overline{C_{plants}} \Delta x$$

A.8

Where w, is the width of each wetland cell (10 m), $C_{plants}(x)$, is the total concentration of contaminants and their plant-derived metabolites in plants across the wetland per dry mass of plants, B_{shoots} is the annual above-ground biomass produced in the wetland per unit area after the system had been operating for approximately 2 years and plant communities had matured. Biomass produced during the monitoring period was assumed to be equal to that produced during one year.

$$C_{plants}(x) = C_{measured}(x) + C_{metabolized}(x)$$

A.9

$$C_{plants}(x) = \frac{C_{measured}(x)}{(1 - f_{metabolized})}$$

A.10

In order to generate a range of possible plant uptake scenarios (Table A.4), low and high B_{shoots}, $f_{metabolized}$, and C_{plants} scenarios published in the literature or reported in Chapter 3 were used and the average of these scenarios formed the basis of the estimates reported herein. Concentrations measured in meadow plants in the summer of 2019 were estimated to represent

half of the annual uptake of carbamazepine because samples were taken at approximately the midpoint of the season and concentrations increase in plants over the growing season (Chapter 3).

The contribution of plant roots to sorption was likely small because the mass of plant roots added to the subsurface per year (<700 kg)[60] is much lower than the mass of sediment in the subsurface (>6 x 10^5 kg). For example:

$$m_{roots} = \mathrm{BCF}_{root} B_{roots}\ \mathrm{K_D} w \sum \overline{C_{pw}}\ \Delta x \qquad A.11$$

$$m_{sorbed} + m_{roots} = (BCF_{root} B_{roots} + \rho_b A)\ \mathrm{K_D} \sum \overline{C_{pw}}\ \Delta x \qquad A.12$$

$$BCF_{root} B_{roots} \ll \rho_b A \qquad A.13$$

$$m_{sorbed} + m_{roots} \approx m_{sorbed} \qquad A.14$$

Where BCF_{root} is the bioconcentration factor in roots given by the ratio of the concentration of a trace organic contaminant in roots to the concentration in soil per gram of dry soil and B_{roots} is the annual below-ground biomass produced in the wetland per unit area. BCF_{root} values are much less than 10^2 for hydrophilic compounds such as those monitored in this study.[219]

A2.1.1 Plant Uptake Scenarios

Table A.4 Plant uptake scenarios used to estimate the contribution of plant uptake and translocation to the removal of trace organic contaminants in the horizontal levee.

Variable	Values tested
$\overline{C_{measured}}$	Average of plant species with highest concentrations were representative of concentrations across the entire slope (e.g. concentration were uniformly high) Average of plant species with lowest concentrations were representative across the entire slope (e.g. concentrations were uniformly low) Average of concentations at each location was representative of concentrations at that location. Concentrations were integrated across the slope.

B_{shoots}[60]	Mean - 1 standard deviation estimate of biomass production Mean estimate of biomass production Mean + 1 standard deviation estimate of biomass production
$f_{metabolized}$[70,71]	33% of the carbamazepine taken up into plants is metabolized 75% of the carbamazepine taken up into plants is metabolized
Plant species	B_{shoots} and $\overline{C_{measured}}$ estimates were made on the basis of concentrations measured in willow and meadow vegetation.

A2.2 Estimates of HRT

In January of 2020, one of the wetland cells was converted to operation with RO concentrate (Chapter 4). During the transition to RO concentrate, Cl^- concentrations were monitored in the effluent of the RO concentrate cell periodically (Figure A.3) . Monitoring of the increase in Cl^- concentrations in the effluent of the RO concentrate cell allowed us to validate the hydraulic retention time estimates made using Lithium Bromide tracer test injections into smaller portions of the wetland previously.[44] Using the following set of equations, we estimated that the hydraulic retention time is approximately 19 days in the RO concentrate cell.

$$\frac{[Cl]}{[Cl]_{o_{relative}}} = \frac{[Cl]}{[Cl]_o} - \overline{\frac{[Cl]}{[Cl]_{o_{final}}}} \qquad A.15$$

$$HRT = \sum \Delta \frac{[Cl]}{[Cl]_{o_{relative}}} * t_{avg} \qquad A.16$$

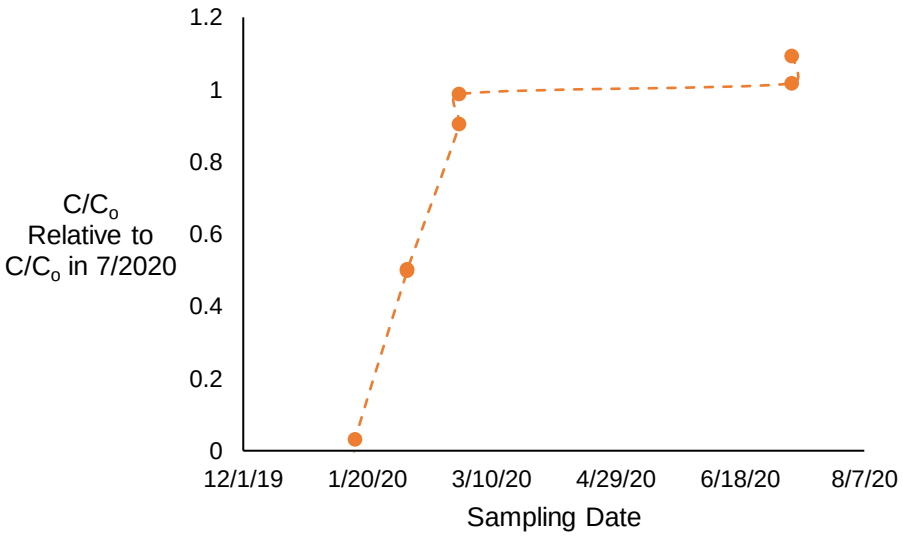


Figure A.3 Increases in fraction remaining for Cl^- relative to the final fraction remaining of Cl^- during the transition from wastewater effluent to RO concentrate.

A2.3 Contaminant Half-Lives

To calculate contaminant half-lives, we fit first-order removal rate models to porewater data. Examples of rate fitting can be found in Figure A.10 & Figure A.11. Implicit in this analysis is that the system is operated after contaminant breakthrough and that contaminants loaded into each section of the wetland are removed via biotransformation (e.g., the contaminant removal profiles are no longer migrating across the wetland as they would if sorption was the primary mechanism of removal). A lack of seasonal removal and the low contribution of sorption to the mass balance suggests that the contaminant removal profiles were relatively stable in 2019 when rate constants were derived (Section A4.2 of the Appendix).

A2.4 Methods Used to Calculate Distribution Coefficients

All isotherms were linear apart from sulfamethoxazole (Figure A.4 Isotherm results for the monitored trace organic contaminants. Therefore distribution coefficients were used to estimate sorption given as the ratio between the concentration of contaminants on soil (C_s µg/g-dry soil) to the concentration in the aqueous phase at equilibrium (C_{aq} µg/L).

$$K_D = \frac{C_s}{C_{aq}} \qquad A.17$$

Isotherm K_D's were derived by measuring the mass added into each microcosm, m_{added}, and the mass measured in the aqueous phase, m_{aq}, after equilibration. The amount sorbed to the solid, m_{sorb}, was calculated as the difference between the mass added and the mass measured after equilibration.

$$m_{added} = m_{sorb} + m_{aq}$$

A.18

$$m_{added} = C_{aq}V_{aq} + C_s m_s$$

A.19

$$C_s = \frac{m_{added} - C_{aq}V_{aq}}{m_s}$$

A.20

Field and microcosm derived K_D's were derived by measuring concentrations in porewater and on sediments through solid-liquid extractions. The mass of contaminants in the porewater of wet sediments was accounted for by assuming porewater concentrations were uniform in sediments using the following equation

$$C_s = C_{extract}V_{extract} - C_{aq}f_w m_{wet\ soil}\rho_w$$

A.21

Where f_w is the moisture content of the wet soil.

A2.5 One-Dimensional Fixed Bed Sorption Modeling and Breakthrough Times

The following equation[1] describes the fate and transport of contaminants undergoing sorption in a fixed-bed column assuming empty bed conditions. Using a numerical solution to this equation, we modeled the anticipated breakthrough curve in the horizontal levee if sorption alone controlled the fate of trace organic contaminants and assumed that sorption is instantaneous (e.g., ignoring sorption kinetics). This assumption provides a conservative estimate of breakthrough time because if sorption kinetics are slower than anticipated, we would expect trace organic contaminants to exit the wetland prior to their sorption breakthrough times.

$$\frac{\partial c}{\partial t} = -\frac{v}{\phi + \rho_b\left(\frac{\partial C_s}{\partial C_{aq}}\right)_{eq}}\frac{\partial c}{\partial z}$$

A.22[1]

$$v = \frac{L_{wetland}}{HRT_{av}}$$

A.23

$$\left(\frac{\partial C_s}{\partial C_{aq}}\right)_{eq} = \mathrm{K_D}$$

A.24

Where $L_{wetland}$ and HRT_{av} are the wetland length and hydraulic retention time respectively.

The accuracy of the results of the numerical solution were validated by comparing the time at which 50% of the influent concentration was expected to appear at the outlet (e.g. breakthrough times, $t_{50\%\,breakthrough}$) to those generated by calculating retardation factors, *R*.

$$t_{50\%\,breakthrough} = R * HRT_{avg}$$

A.25

$$R = 1 + \left(\frac{\rho_b}{\phi}\right)\mathrm{K_D}$$

A.26

A3.1 Redox Zonation

Redox zones were delineated in individual wetland cells to determine the fraction of contaminant removal occurring in each zone. The nitrate and oxygen reducing zone was defined as the distance from the start of the wetland to the first porewater sample where concentrations of nitrate declined to below 1mg-N/L. We defined the start of the sulfate reducing zone as the first porewater sample that had sulfate concentrations more than 10% less than the influent value and continued until concentrations declined to below 1mg/L. Contaminant removal in each zone relative to the total amount removed was calculated. Wetland cells with overlapping nitrate and sulfate reducing zones were excluded from the analysis because we could not isolate the redox communities in these sections of the wetland.

A4.1 Sorption Isotherm Results

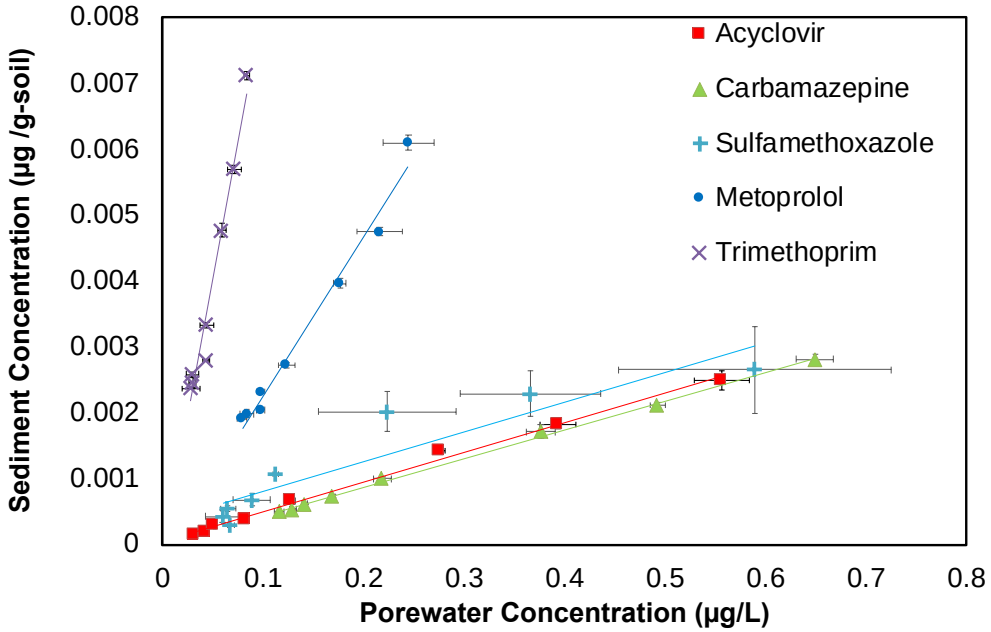


Figure A.4 Isotherm results for the monitored trace organic contaminants

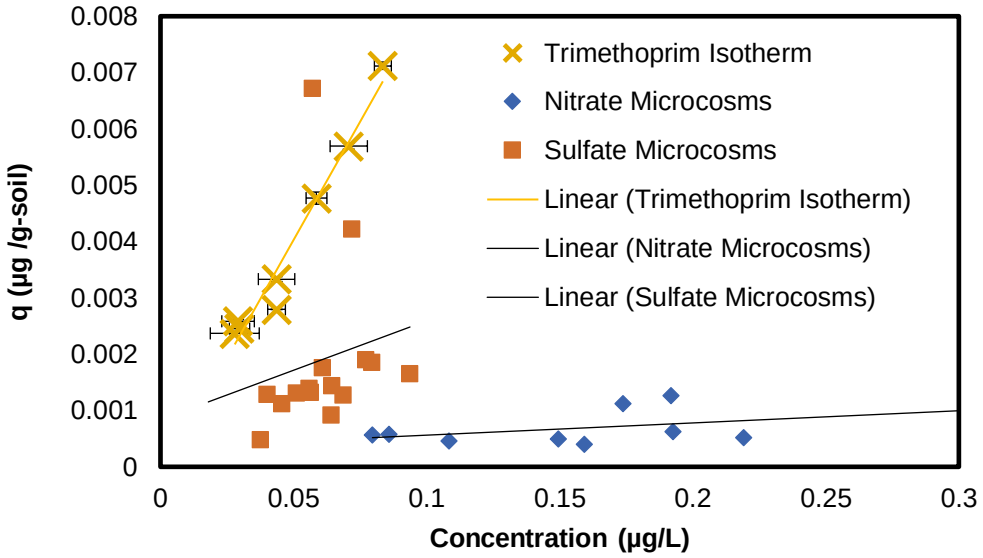


Figure A.5 Trimethoprim isotherm results compared to the distribution in microcosm experiments. C_s (q) was determined by sediment extraction in microcosm experiments.

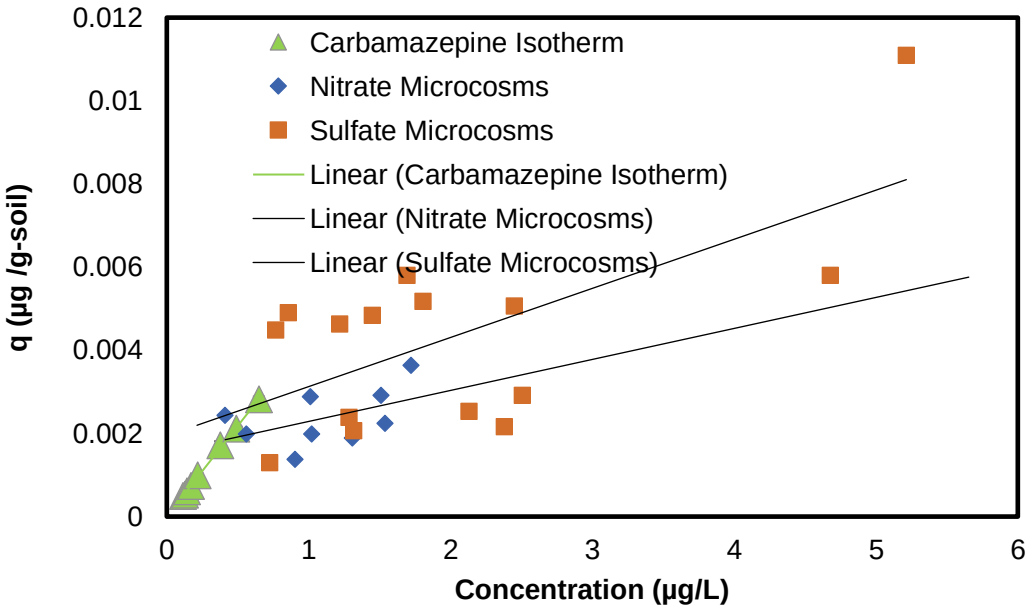


Figure A.6 Carbamazepine isotherm results compared to the distribution in microcosm experiments. C_s (q) was determined by sediment extraction in microcosm experiments.

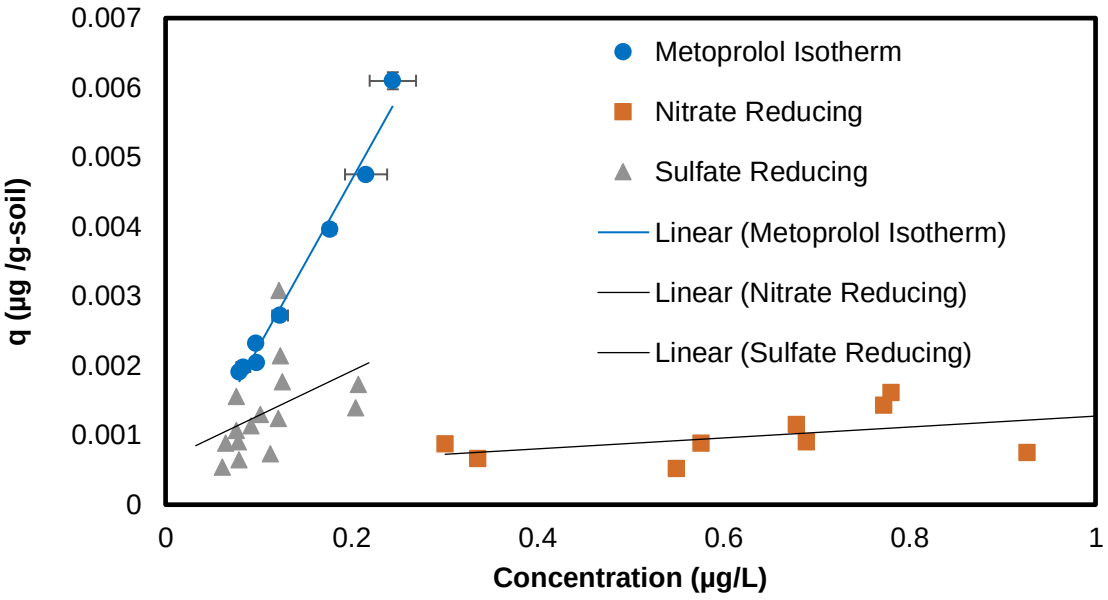


Figure A.7 Metoprolol isotherm results compared to the distribution in microcosm experiments. Cs (q) was determined by sediment extraction in microcosm experiments.

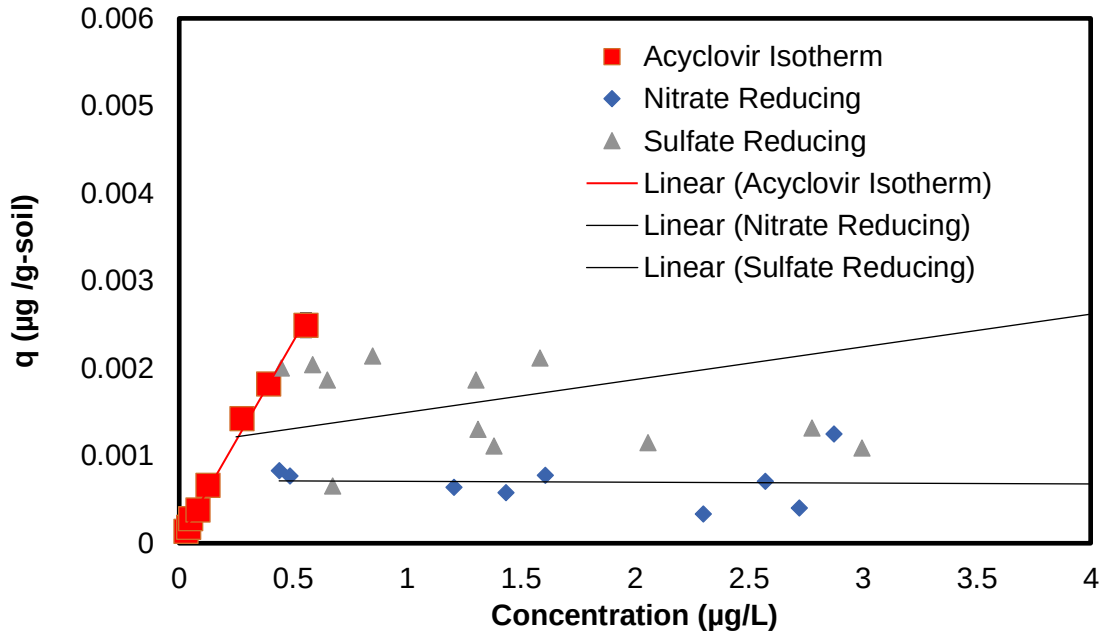


Figure A.8 Acyclovir isotherm results compared to the distribution in microcosm experiments. Cs (q) was determined by sediment extraction in microcosm experiments.

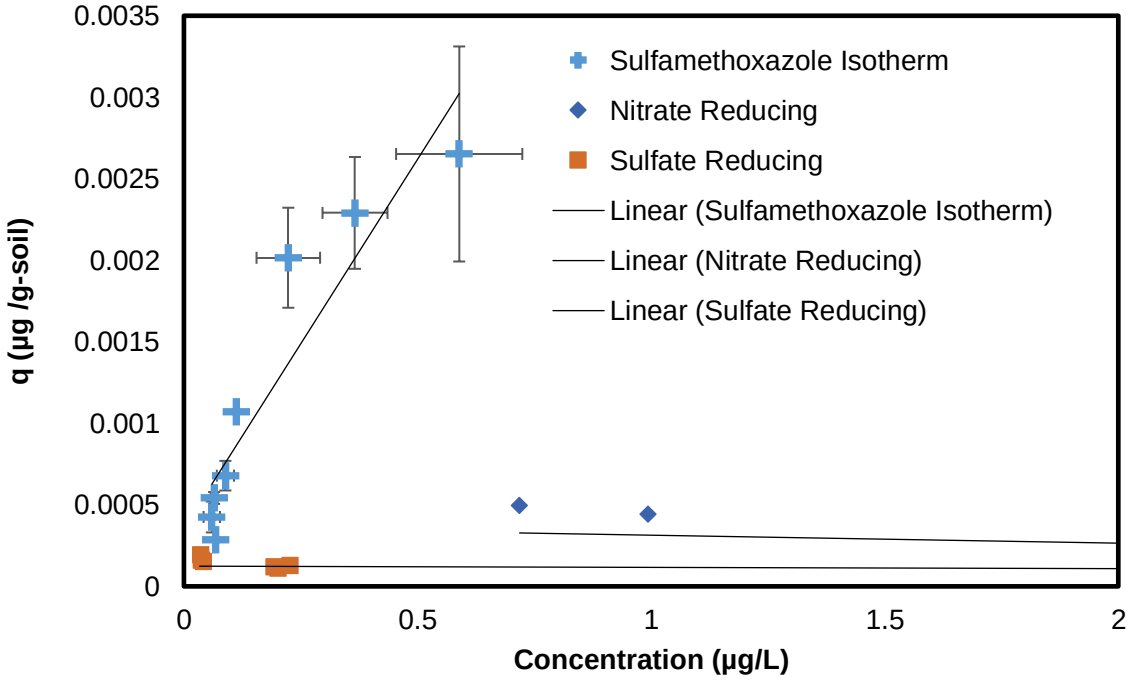


Figure A.9 Sulfamethoxazole isotherm results compared to the distribution in microcosm experiments. Cs (q) was determined by sediment extraction in microcosm experiments

Table A.5 Summary of K_D values derived from isotherms, microcosms, and field results.

Compound	Field K_D	Nitrate-reducing Microcosm K_D	Sulfate-reducing Microcosm K_D	Surface Layer Isotherm-derived K_D	Treatment Layer Isotherm- derived K_D
Acyclovir	0.0046 +/- 0.0005	0.0007 +/- 0.0006	0.0015 +/- 0.0012	0.0048 +/- 0.0010	0.0040 +/- 0.0016
Carbamazepine	0.0093 +/- 0.0028	0.0025 +/- 0.0014	0.0026 +/- 0.0016	0.0032 +/- 0.0005	0.0052 +/- 0.0050
Carboxy-acyclovir	0.0011 +/- 0.0012	0.0005 +/- 0.0003	0.0004 +/- 0.0001	0.0027 +/- 0.0003	0.0018 +/- 0.0234
Sulfamethoxazole	0.0026 +/- 0.0020	0.0002 +/- 0.0003	0.0017 +/- 0.0021	0.0012 +/- 0.0002	0.0043 +/- 0.0035
Metoprolol	0.0034 +/- 0.0013	0.0017 +/- 0.0006	0.0127 +/- 0.0052	0.0150 +/- 0.0034	0.0212 +/- 0.0042
Carboxy-metoprolol	0.0012 +/- 0.0005	0.0006 +/- 0.0002	0.0005 +/- 0.0002	Negligible sorption	Negligible sorption
Trimethoprim	0.0051 +/- 0.0035	0.0047 +/- 0.0020	0.0315 +/- 0.0262	0.0376 +/- 0.019	0.0259 +/- 0.0152
10-OH Carbamazepine	n.d.	0.0003 +/- 0.0004	0.0007 +/- 0.0004	n.d.	n.d.
DiOH-Carbamzepine	n.d.	0.0006 +/- 0.0005	0.0006 +/- 0.0004	n.d.	n.d.

A4.2 Impact of Wetland Cell Type and Temperature on Performance

Because the horizontal levee removed trace organic contaminants via biotransformation, water temperature and wetland cell design (e.g., hydraulic residence time) could be important to system performance. For example, the temperature of the wetland porewater varies by about 7 degrees between winter and summer. Microbial activity typically decreases by about 40% over this temperature range,[220] though this estimate may vary significantly between sediment microbial communities.[221]

Porewater concentrations from the summer of 2019 and winter of 2019 show that all trace organic contaminants were removed at similar rates in the summer and winter (Figure A.10). Air temperature changes between seasons are modest in northern California, USA, and subsurface treatment systems exhibit even lower temperature fluctuations than those in the air due to thermal insulation provided by sediment and relatively constant temperatures of wastewater effluent. For example, wastewater effluent flowing into the horizontal levee only varied from 14.7 – 21.7 ºC between seasons. The lack of an effect of temperature on the removal of trace organic contaminants could be related to seasonal shifts in biogeochemistry. For example, during winter, the zone in which nitrate is reduced was not significantly different than the zone in the summer.[59] As the activity of heterotrophic organisms declines, autotrophic nitrate-reducing organisms that employ sulfide minerals as an energy source contribute more to nitrate reduction in winter. Our results suggest that despite seasonal shifts in microbial activity, trace organic contaminant removal continues at similar rates. In addition, our results suggest that other seasonal factors such as plant senescence or changes in rhizosphere-associated microbial communities were not important for the removal of trace organic contaminants. However, there is a need to study subsurface systems like horizontal levees under conditions that exhibit greater seasonal variations in temperature and microbial activity.

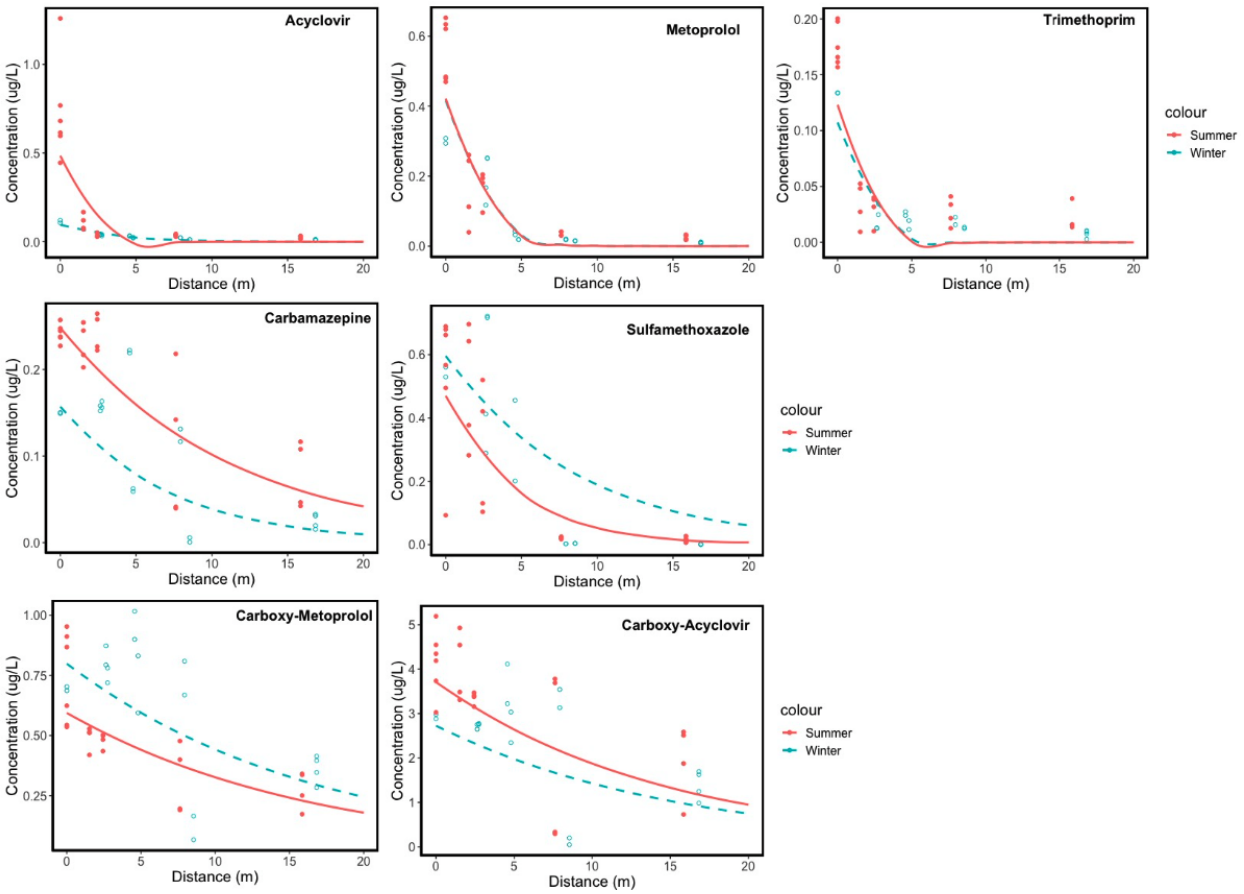


Figure A.10 Concentrations of trace organic contaminants versus distance in horizontal levee inflow and wetland cells. Seasonal data were collected from fine cells in winter and summer of 2019. Rate constants were not statistically significantly different in winter and summer, except for acyclovir, but this is due to low concentrations in the inflow during the winter of 2019.

Different design parameters in each wetland cell type may influence horizontal levee performance by changing subsurface mass loading and hydraulic retention times. For example, despite the application of a similar head, willow cells with coarse sediments exhibited higher subsurface flows (3.6 m^3/d) than coarse meadow (2.3 m^3/d) or fine meadow (1.4 m^3/d) cells during the period when flows were calibrated so that overland flow was eliminated from the wetland cells[44] Monitoring of concentrations of trace organic contaminants at the outlet of the wetland cells from the second year of operation show that the percentage removal of trace organic contaminants in each wetland cell type is similar (Figure 2-1). However, because loading into each cell was different, the willow and coarse cells removed significantly more mass of contaminants than fine cells. In addition, porewater samples reveal modest differences among cell types. For example, compounds that were removed under sulfate reducing conditions (e.g., carbamazepine) required longer distances for removal in wetland cell types that had sulfate reduction over larger areas (e.g., willow cells) due to higher flow rates and presumably shorter hydraulic retention times in those cells. In contrast, compounds that were removed under nitrate reducing conditions (e.g. metoprolol) were removed over the same distance in all wetland cell types (Figure A.11).

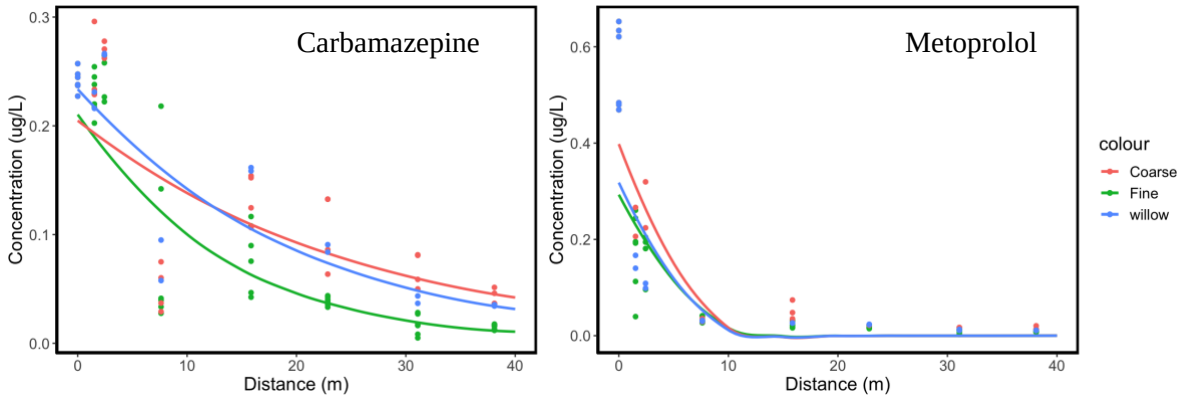


Figure A.11 Concentrations of carbamazepine and metoprolol versus distance in horizontal levee influent and wetland cells. Data from all three wetland cell types collected in the Summer of 2019 were plotted along with pseudo first order rate fits. Carbamazepine removal rate constants were statistically significantly different, however the magnitude was modest. Metoprolol removal rate constants were not significantly different.

A4.3 Microcosm Results

Additional evidence for the importance of microbial communities that utilize different terminal electron acceptors was obtained from microcosm experiments. These experiments were insightful because some compounds (e.g., acyclovir, 10-OH carbamazepine and trimethoprim) were fully removed before they reached the sulfate-reducing zone in the wetland (Figure 2.4), which made it impossible to observe their fate in the sulfate-reducing zone. Analysis of the total mass of trace organic contaminants in microcosm porewater and soil extracts allowed us to attribute removal in the microcosms to biotransformation. Acyclovir, trimethoprim, and 10-OH carbamazepine, three compounds that were depleted within the first five meters of the inlet in the wetland, were removed in microcosms that contained sediment from both the nitrate-reducing zone and the sulfate-reducing zone (Figure A.13a, Figure A.14a, Figure A.15). These results are consistent with previous reports because acyclovir is removed under anaerobic conditions in membrane bioreactors[87] and trimethoprim is removed under anoxic and anaerobic conditions in lab-scale experiments.[116] However, 9-acridine carboxylic acid, a metabolite of 10-OH carbamazepine only increased in the microcosms containing sediment from the nitrate-reducing zone (Figure A.12) suggesting that the 10-OH carbamazepine degradation pathway differed under nitrate-reducing and sulfate-reducing conditions in the microcosms.

The removal of trace organic contaminants in the microcosms containing sediments collected from the nitrate-reducing zone was more modest than the removal observed in the field likely because only 2.5mg-N/L of nitrate was available to nitrate-reducing microbial communities in the microcosms. This concentration of nitrate was rapidly removed within 20 hours (i.e.. the first trace organic contaminant sampling point in Figure A.13a) indicating that nitrate-reducing conditions were not predominant for the period between 20 and 48 hours.

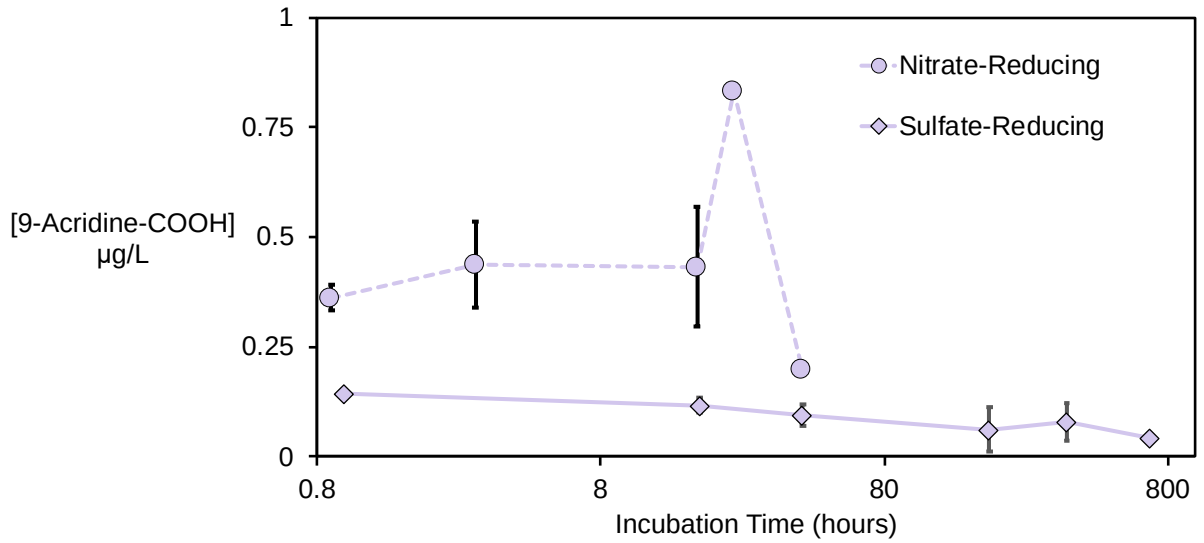


Figure A.12 9-Acridine-COOH was formed in the microcosms that contained sediment from the nitrate-reducing zone, but not in the microcosms that contained sediment from the sulfate-reducing zone.

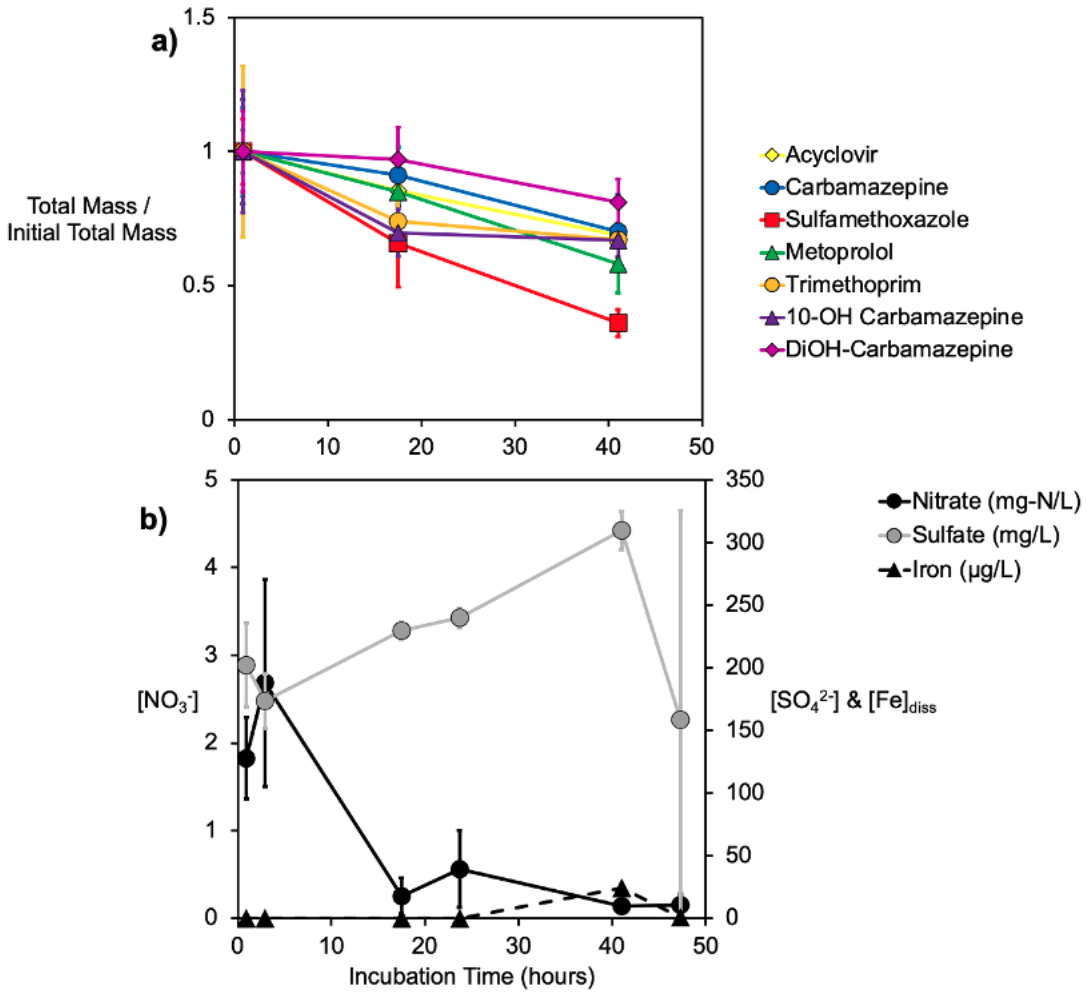


Figure A.13 Total mass of trace organic contaminants remaining relative to initial total mass after the first sampling point in microcosms containing sediment from the nitrate-reducing zone (a) and concentrations of redox-active inorganic species (b). Iron concentrations are represented by the dashed symbol.

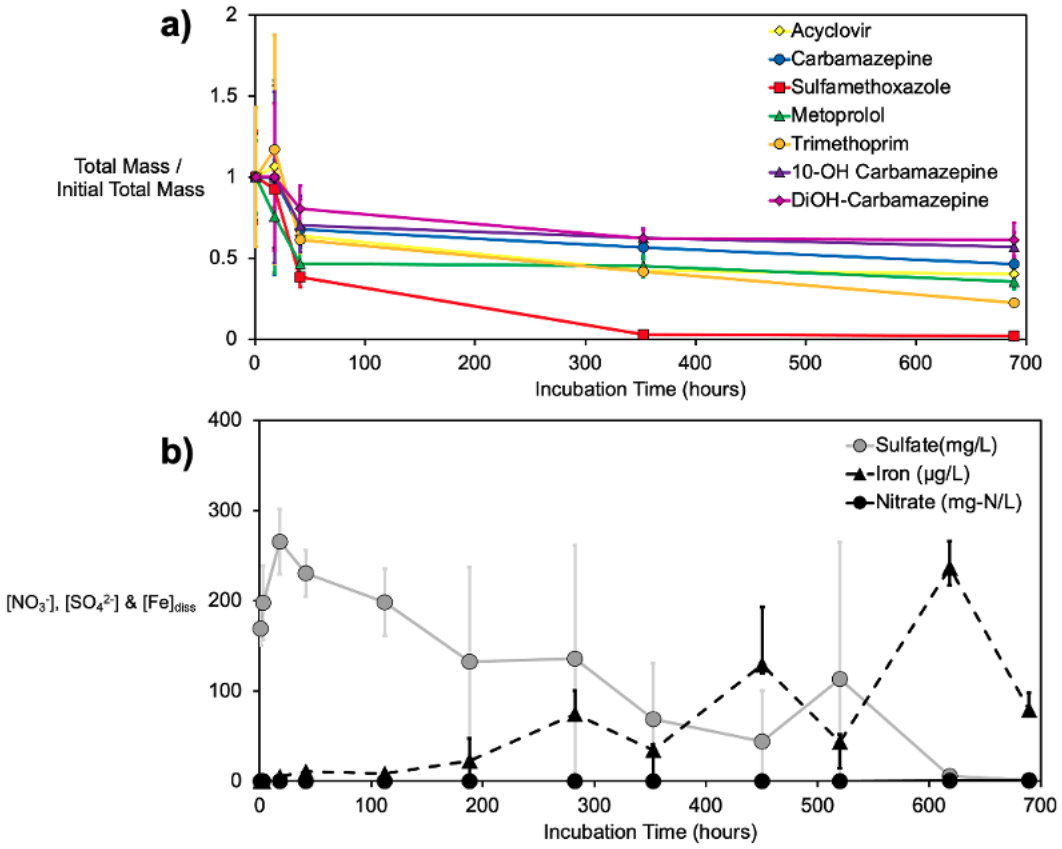


Figure A.14 Total mass of trace organic contaminants relative to initial total mass after the first sampling point in microcosms containing sediment from the sulfate-reducing zone (a). Concentrations of redox indicator species are also plotted over time (b). Iron concentrations are represented by the dashed symbol.

Persistent trace organic contaminants (e.g., carbamazepine and sulfamethoxazole) were also removed in microcosms that contained sediments collected from the nitrate-reducing and the sulfate-reducing zones. For sulfamethoxazole, this finding is consistent with prior research that indicates it is removed under nitrate, iron, and sulfate-reducing conditions via biotransformation and through reactions with microbially-produced reactive species (e.g., adsorbed Fe(II)).[82,89,93] Carbamazepine was only removed in these microcosms after nitrate decreased to less than 0.5mg-N/L in the microcosms (e.g., after 20 hours). After this period, sulfate and iron reduction could not be entirely ruled out in these microcosms (Figure A.13b).

Although microcosm results suggest that trace organic contaminants could be removed with or without added nitrate, removal rates derived from microcosm experiments cannot be directly extrapolated to field conditions.[222] Substantial variability in the removal of trace organic contaminants under laboratory and field conditions have been reported[108] and are likely due to disturbances to the microbial communities during the set-up of microcosm experiments, insufficient maturation[37] or adaptation time[30] and an inability to replicate the conditions encountered in the field (e.g., spatial gradients of solutes in porous media). For example, although various diverse consortia of soil microorganisms can be resilient to changing redox conditions in the environment,[223] disturbances to the subsurface microbial community that occurred during the

set-up of our microcosm experiments such as physical disruption of biofilms or exposure to oxygen could have altered the metabolic capabilities of the community. The removal of persistent trace organic contaminants that require sulfate-reducing conditions could be disproportionately impacted by these disturbances because oxygen is more toxic to strict anaerobes (i.e., many sulfate-reducers) than it is to facultative aerobes (i.e., most nitrate-reducers).[224] We observed evidence for the importance of this limitation in our microcosm experiments because, despite similar solids-to-water ratios (e.g., approximately 3 g-dry soil/mL water) in the field and microcosms, sulfate reduction was much faster in the field (i.e., 70 mg/L of sulfate was removed within about 9 days in the field versus over the last 14 days in the microcosm experiments; Figure A.14b) whereas nitrate-reduction occurred over similar timescales in our microcosm and field experiments (e.g., < 2 days Figure A.13b). However, future microcosm experiments could be used to provide insight into trace organic contaminant transformation pathways through the detection of transformation products.

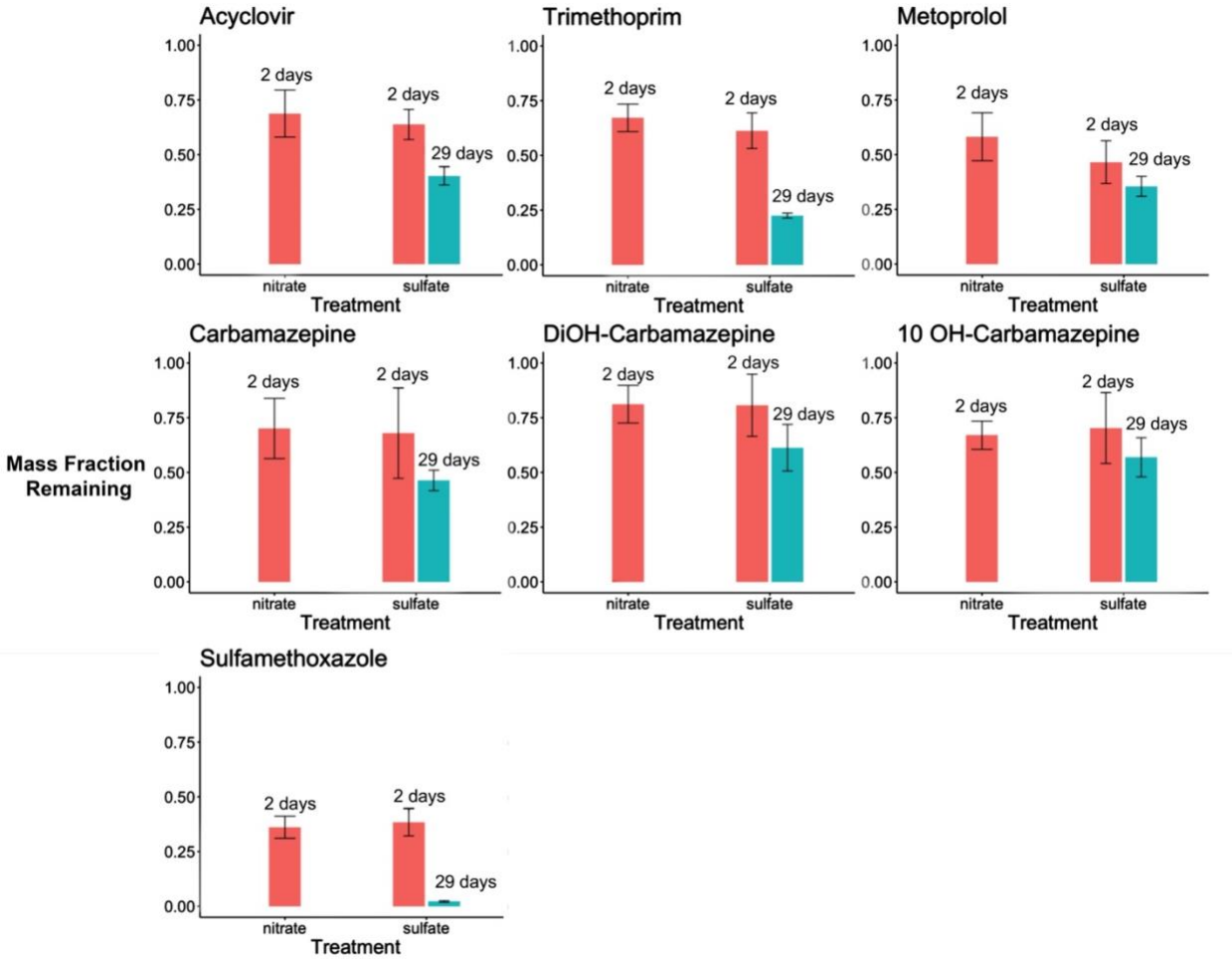
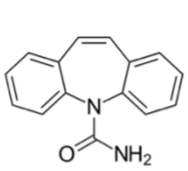
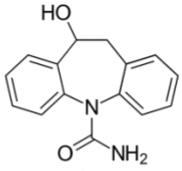


Figure A.15 Total mass fraction remaining (aqueous and adsorbed) of trace organic contaminants in microcosms containing sediment from the nitrate-reducing and sulfate-reducing zones. Incubation times are indicated above each bar. (For each bar, n=3)

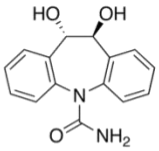
A4.4 Other Supplemental Results



Carbamazepine



10-OH Carbamazepine



Dihydroxy Carbamazepine

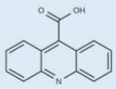
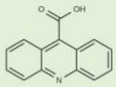
Aerobic Conditions (0-1.5m in horizontal levee)	Persistent	Removed	Formed  9-Acridine-COOH	Slowly Removed
Nitrate-reducing conditions (0-10m in horizontal levee)	Persistent	Removed	Formed  9-Acridine-COOH	Slowly Removed
Sulfate-reducing & iron-reducing conditions (5-30m in horizontal levee)	Removed	Removed*	Removed	Slowly Removed

Figure A.16 Redox dependent removal of carbamazepine and related hydroxylated structures. Hydroxylation of carbamazepine alters the rates and pathways of carbamazepine transformation under diverse redox conditions. 9-Acridine-COOH was only formed under aerobic and nitrate-reducing conditions. *only measured in microcosm experiments.

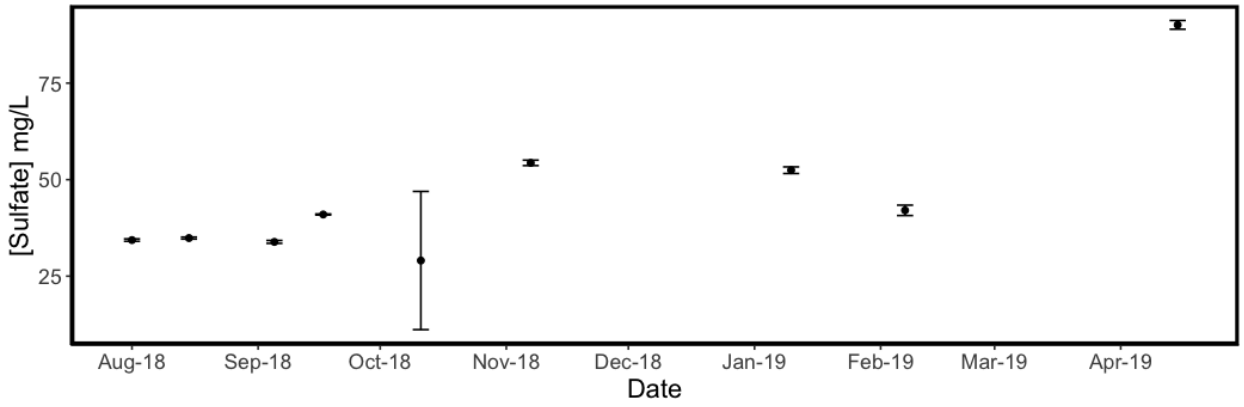


Figure A.17 Sulfate concentrations over the monitoring period.

APPENDIX B: Supporting Information for Chapter 3

B1. Materials and Methods

B1.1 Winter Plant Sampling

Plant samples were collected in February 2019 from two fine meadow cells by collecting all above-ground plant biomass (shoots) rooted within a representative 0.25m^2 quadrat. Plant samples were stored in plastic bags on ice prior to returning to the lab where samples were separated into live and dead biomass, manually cut into 5-10 cm pieces, and homogenized prior to subsampling. Triplicate subsamples were collected from bulk samples and freeze-dried in glass vials. Subsamples weighed before and after freeze-drying to determine moisture content. Analytes in samples collected in February 2019 were quantified without matrix-matching and results can be found in section S7 of the supplementary information. Samples collected from this period were used to screen for the presence of a suite of trace organic contaminants as well as carbamazepine metabolites.

B1.2 LC/MS Methods

B1.2.1 High Performance Liquid Chromatography

Trace organic contaminants were separated using a reversed-phase C18 Hydro-RP column with a flow rate of 0.6 mL/min. Sample injection volume was set to 25µL with 3 needle wash cycles in a solution containing 50% LCMS-grade methanol and 50% Milli-Q water. Two chromatography methods were employed as indicated below.

Table B.1 List of trace organic contaminants included in preliminary screening experiments in winter 2019 (only winter 2019 indicated with*) and final plant analyses.

Compound	Chromatography method
Metoprolol*	A
Sulfamethoxazole*	A
Propranolol*	A
Atenolol*	A
Carboxy-metoprolol*	A
Carboxy-abacavir*	A
Carboxy-acyclovir*	A
Acyclovir*	A
Carbamazepine	B
Acridine	B
Acridine-COOH	B
Carbamazepine epoxide	B
10-OH-Carbamazepine	B
DiOH-Carbamazepine	B
Fipronil	B
Fipronil sulfide	B
Fipronil sulfone	B

Table B.2 HPLC gradient used for a suite of pharmaceutical compounds. Column temperature was set to 25 ºC.

Chromatography method A

Time	0.1% Acetic Acid	Methanol
2 min	100 %	0 %
8 min	40 %	60 %
11 min	5 %	95 %
15 min	5 %	95 %
15.1 min	100 %	0 %
20 min	100 %	0 %

Table B.3 HPLC gradient used for a carbamazepine, fipronil and a suite of their metabolites. Column temperature was set to 30 ºC.

Chromatography method B		
Time	**0.1% Acetic Acid**	**Methanol**
2 min	55 %	45 %
5 min	47%	53 %
7 min	5 %	95 %
13 min	5 %	95 %
13.1 min	85 %	15 %
18 min	85 %	15 %

B1.2.2 MS/MS Parameters

Table B.4 Electrospray ionization source settings and parameters

Parameter	**Units**	**Value (+)**	**Value (-)**
Gas Temperature	ºC	350	350
Gas Flow	l/min	9	9
Nebulizer	psi	25	25
Capillary Voltage	V	3600	3500

Table B.5 MS/MS parameters used for the detection and/or quantification of trace organic contaminants.

Compound	Precursor to product ion transitions (amu)	Ionization Mode	Collision energy (V)	Fragmentor (V)	Internal standard
Metoprolol	268 → 159 268 → 116	ESI +	17 14	130 130	Metoprolol d7
Metoprolol d7	275→ 123	ESI +	14	130	-
Sulfamethoxazole	254→156 254→92	ESI +	10 25	110 110	Sulfamethoxazole d4
Sulfamethoxazole d4	258 → 96	ESI +	25	110	-
Propranolol	260→183 260→116	ESI +	12 13	98 98	Propranolol d7
Propranolol d7	267→116	ESI +	13	98	-
Atenolol	267→190 267→ 145	ESI +	16 24	130 130	Atenolol d7
Atenolol d7	274→ 145	ESI +	24	130	-
Carboxy-metoprolol	268.1→190.9 268.1→144.9	ESI +	17 25	130 130	Carboxy-metoprolol d5
Carboxy-metoprolol d5	273.1→196	ESI +	17	130	-
Carboxy-abacavir	301→191 301→150	ESI +	20 35	100 100	Abacavir d4
Abacavir d4	291→195	ESI +	15	100	-
Carboxy-acyclovir	240→152 240→135	ESI +	15 30	80 80	Acyclovir d4
Acyclovir d4	230→152	ESI +	10	70	-
Acyclovir	226→152 226→135	ESI +	10 45	75 75	Acyclovir d4
Carbamazepine	237→ 194 237→179	ESI +	20 35	120 120	Carbamazepine d10
Carbamazepine d10	247→204	ESI +	20	120	-
Acridine	180→152	ESI +	35	70	Carbamazepine d10
Acridine-COOH	224→196 224→167	ESI +	37 50	120 120	Carbamazepine d10
Carbamazepine epoxide	253→ 256 253→180	ESI +	10 25	80 80	Carbamazepine d10
10-OH-Carbamazepine	255→237 255→194	ESI +	5 17	92 92	Carbamazepine d10
DiOH-Carbamazepine	271→210 271→180 253→180	ESI +	15 33 35	80 80 120	Carbamazepine d10
Fipronil	434.9→ 330 434.9→ 250	ESI -	15 30	90 90	Fipronil 13C 15N
Fipronil 13C 15N	439→334 439→252	ESI -	10 25	85 85	-
Fipronil sulfide	419→382.9 419→261.9	ESI-	10 30	90 90	Fipronil 13C 15N
Fipronil sulfone	451→414.9 451→281.9	ESI -	25 25	90 90	Fipronil 13C 15N

B1.2.3 Limits of Quantification and Detection

The limit of quantification (LOQ) and limit of detection (LOD) for each analyte was determined as 10 or 3.3 times the standard deviation of the response over the slope of the calibration curve respectively[225]. When concentrations were less than the limit of quantitation, but greater than the limit of detection, a value equal to half of the limit of quantification was used for the purpose of summary statistics and data visualization. When concentrations were less than the limit of detection, zero was used for the purpose of summary statistics and data visualization.

Table B.6 Limits of detection and quantification for carbamazepine and a suite of its metabolites in matrix-matched calibration curve.

Compound	**LOD** (µg/L, ng/g-d.w.)	**LOQ** (µg/L, ng/g-d.w.)
Carbamazepine	0.0069, 0.90	0.023, 3.0
Acridine	0.015, 2.0	0.051, 6.6
Acridine-COOH	0.070, 9.1	0.23, 30
Carbamazepine epoxide	0.011, 1.4	0.037, 4.8
10-OH-Carbamazepine	0.021, 2.7	0.071, 9.2
DiOH-Carbamazepine	0.029, 3.8	0.097, 13
Fipronil	0.0089, 1.2	0.030, 3.9
Fipronil Sulfide	0.011, 1.4	0.038, 4.9
Fipronil Sulfone	0.023, 3.0	0.075, 9.8

B1.3 Extraction Method Validation

B1.3.1 Spike-recovery Tests

To validate recoveries from the plant residues, triplicate ground plant residues were spiked with 32.5 ng of each trace organic contaminant and allowed to dry for several hours. After drying, the solid-liquid extraction method was performed on the spiked plant residues to determine the recovery efficiencies reported below.

Table B.7 Recoveries reported for carbamazepine and its metabolites in plant residues.

Compound	**Recovery (mean ± one standard deviation)**
Acridine	96.0 ± 7 %
9-Acridine-COOH	104 ± 3 %
Carbamazepine	105 ± 4 %
Carbamazepine - epoxide	97.6 ± 5 %
10-OH Carbamazepine	104 ± 3 %
DiOH Carbamazepine	117 ± 2 %

B1.3.2 Additional Extraction Step

An additional extraction step was conducted by repeating the solid-liquid extraction method another time using the final methanol:Milli-Q water ratio (50:50). Extract from this final step was reserved and processed separately to determine if trace organic contaminants were detectable in the final extract. No trace organic contaminants were detected in the final extract indicating that the extraction procedure retrieved all extractable trace organic contaminants from the plant residues.

B1.4 Methods to Estimate Doses to Animals

We used the following allometric relationships from the EPA's Wildlife Exposure Factors Handbook to calculate the relative food ingestion rate (RFIR, dry mass of food consumed in g per day per g of body weight) for rodents, herbivorous mammals, birds, and reptiles/amphibians:

$$\text{Birds: } RFIR = 0.648(m_{b.w}^{0.651})/m_{b.w.}$$

$$\text{Rodents: } RFIR = 0.621(m_{b.w}^{0.564}) /m_{b.w.}$$

$$\text{Herbivorous mammals} = RFIR = 0.577(m_{b.w}^{0.727}) /m_{b.w.}$$

$$\text{Herbivorous reptiles and amphibians} = RFIR = 0.019(m_{b.w}^{0.841}) /m_{b.w.}$$

Where $m_{b.w.}$ is the body weight mass of the organism of interest.

The estimated ingestion of carbamazepine relative to the human acceptable daily intake (ADI) was calculated as:

$$CBZ_{injested}/ADI = RFIR*[CBZ]_{in\ plants}/ADI$$

$$[CBZ]_{in\ plants} = \text{ng CBZ/g dry mass}$$

$$ADI = \text{ng CBZ / g body weight / d}$$

To provide a worst-case estimate of dietary ingestion, the value equal to the mean concentration of carbamazepine in species with the highest affinity for carbamazepine uptake in the first 0-7.5m of the wetland (i.e., *Juncus spp.* and *B. maritimus*) was used in dietary estimate calculation (i.e., $[CBZ]_{in\ plants}$).

B2. Results

B2.1 Fipronil Detection and Background Contamination

Fipronil, fipronil sulfone, and fipronil sulfide were detected in 91%, 77% and 74% of the plant samples respectively. However, attempts to quantify these compounds were hindered by high levels of background contamination in the willow leaves used for matrix matching. The presence of fipronil in the matrix-matched willow leaves was confirmed by a consistent qualifier to quantifier ratio and retention time in spiked and unspiked samples. Background levels of fipronil were estimated using standard addition (i.e., the lower range of the calibration curve). Fipronil levels were approximately 3.1 ng/g-dry mass in the willow leaves used for matrix matching.

B2.2 Supplementary Figures

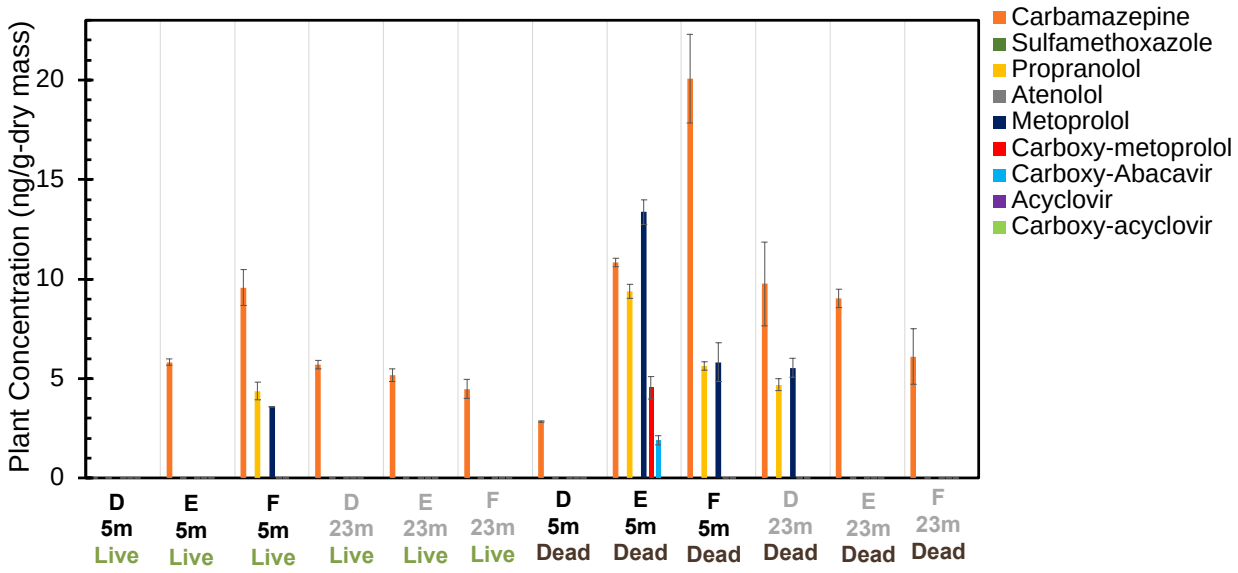


Figure B.1 Semi-quantitative detection of carbamazepine and other pharmaceuticals in live plants and dead plants collected in winter of 2019. Carbamazepine metabolites can be found in figure S4. **Note: These samples were quantified without matrix matching**. (At each sampling location, n = 3)

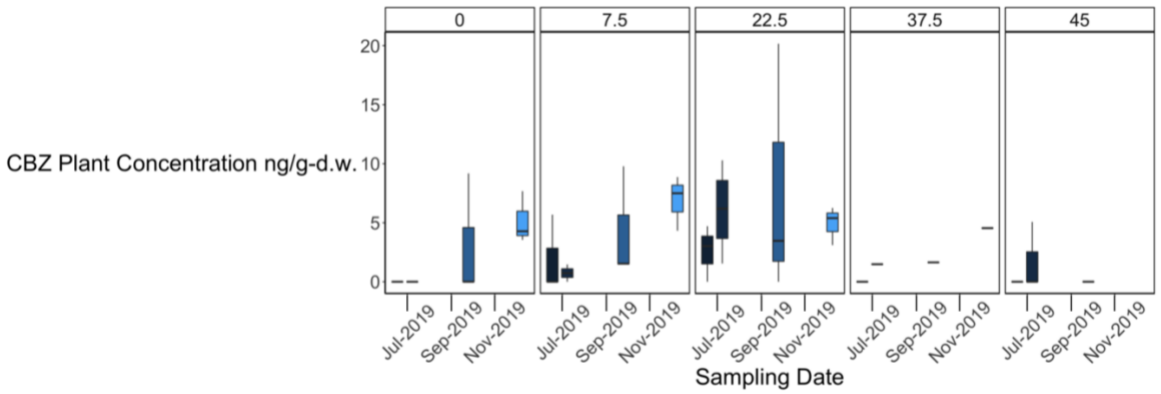


Figure B.2 Concentrations of carbamazepine found in *S. lasiolepis* leaves collected at different distances and sampling times along the horizontal levee slope. The distance (m) along the slope is indicated at the top of each panel.

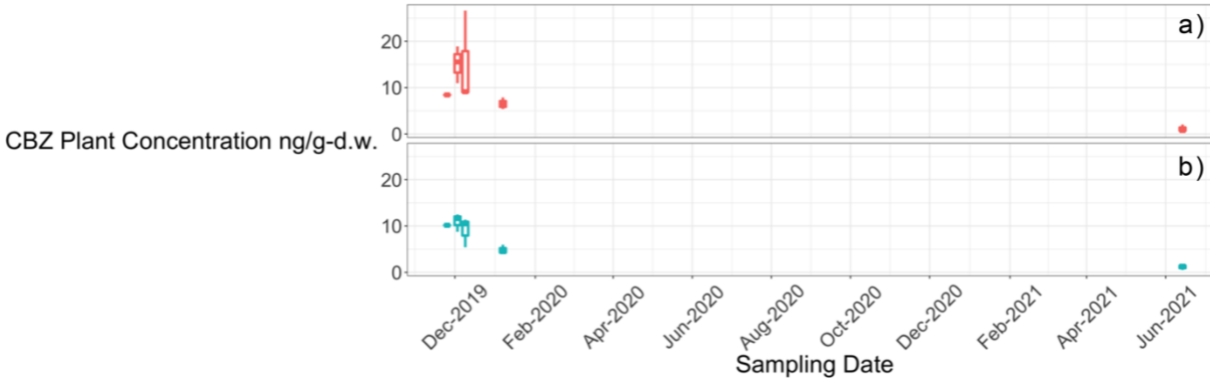


Figure B.3 Concentrations of carbamazepine found in decomposing a) Juncus ssp. and b) B. maritumus in litter-bag experiments.

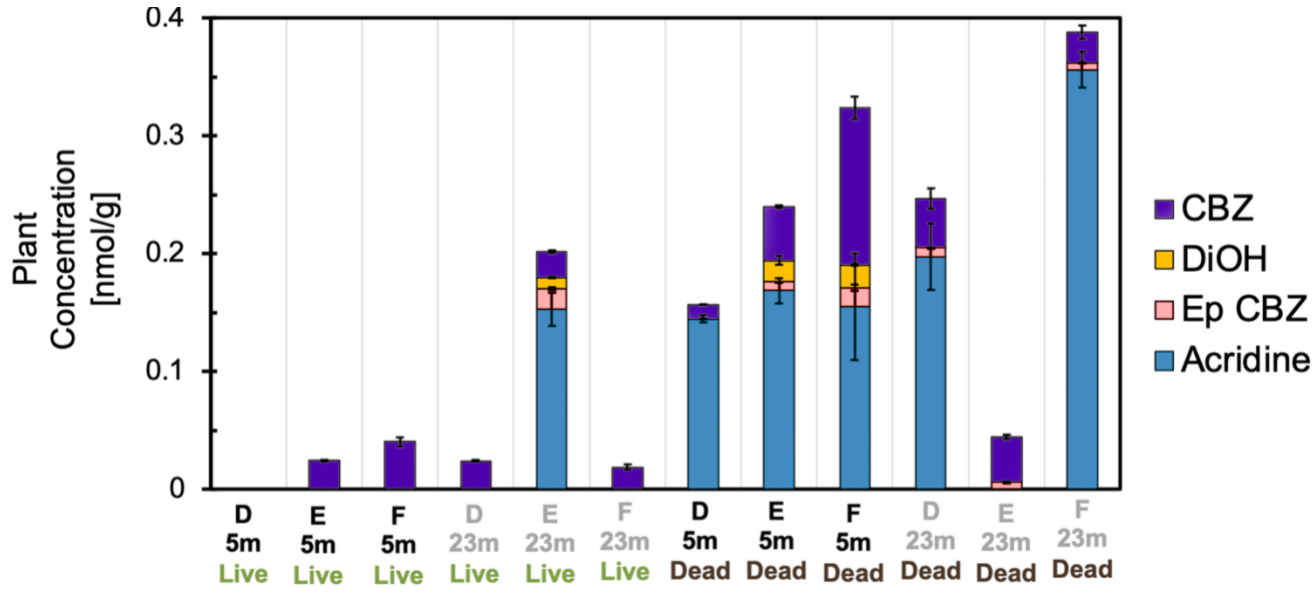


Figure B.4 Semi-quantitative detection of carbamazepine and metabolites in live plants and dead plants collected in winter of 2019. **Note: These plant samples were quantified without matrix matching.**

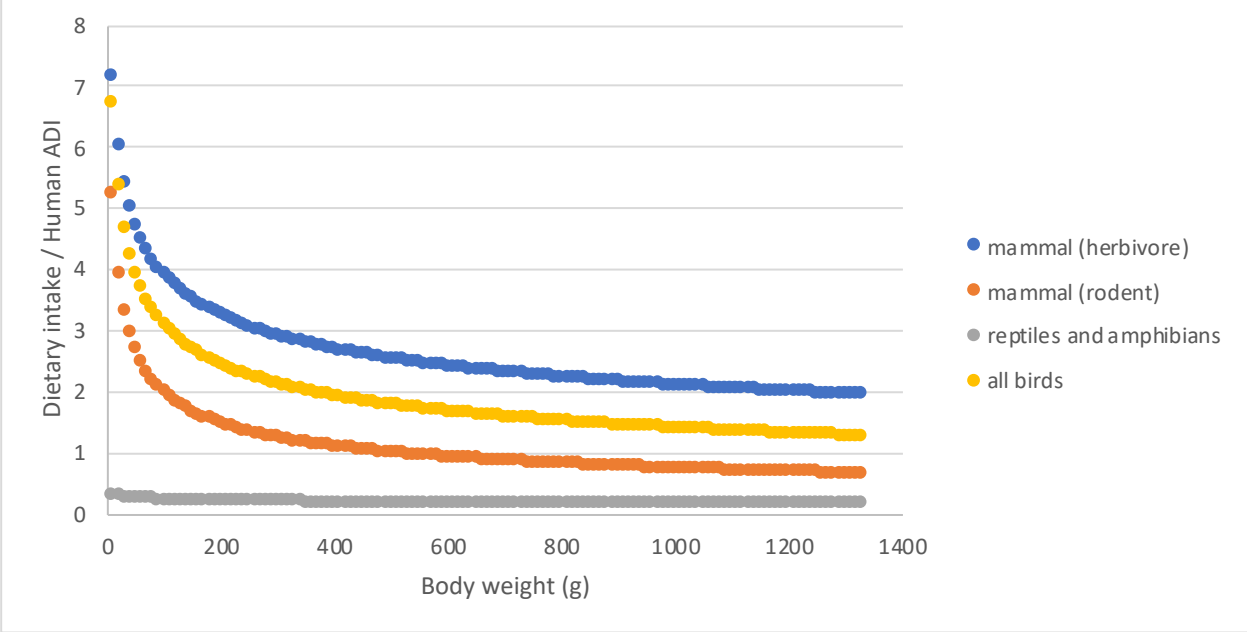


Figure B.5 Intake of carbamazepine relative to the human ADI for various animals of different body weights assuming they are feeding exclusively on vegetation containing concentrations similar to those found in plants within the 0-7.5 meter zone of the meadow wetland cells.

APPENDIX C. Supporting Information for Chapter 4

C1.1 "The Blob"

A significant, temporary drop in nitrate concentrations was observed between 3-12 meters, indicating a possible zone of limited flux in the subsurface. Other dissolved species such as sulfate exhibited similar trends (Figure C.1). Additionally, elevated nitrate and sulfate concentrations on the edges of the wetland cell in this zone provided evidence that water flowed around this zone (Figure C.2). This observation could be explained by heterogeneities introduced during the construction of the field site, by compaction that occurred during sampling (e.g. due to walking or using an auger for soil sampling), or by clogging over time due to the migration of fine particles, precipitation of minerals in that zone, or biofilm growth. Sampling from other lateral positions further downstream in the wetland and sampling from intermediate and effluent monitoring wells suggested that other low permeability areas were not present in the subsurface.

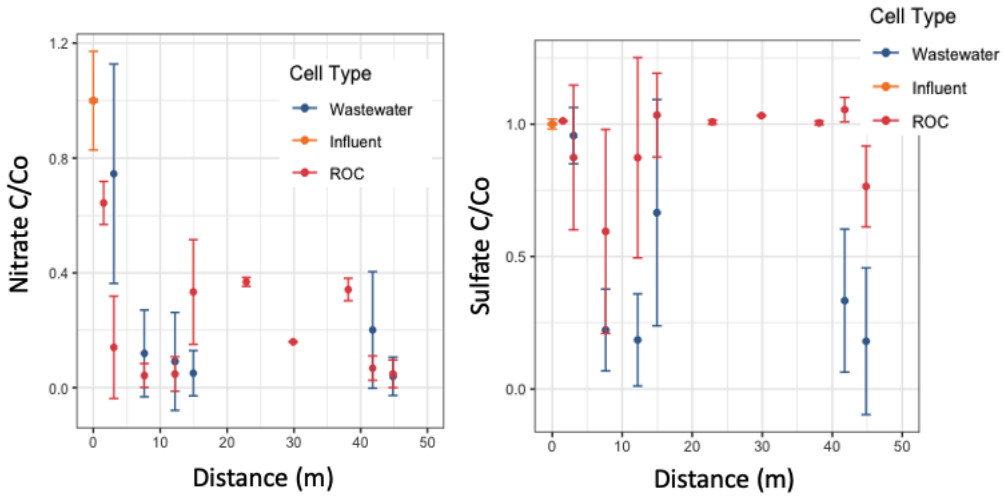


Figure C.1 Porewater sampling of nitrate and sulfate including the zone of low permeability "the blob" between 3-12 meters.

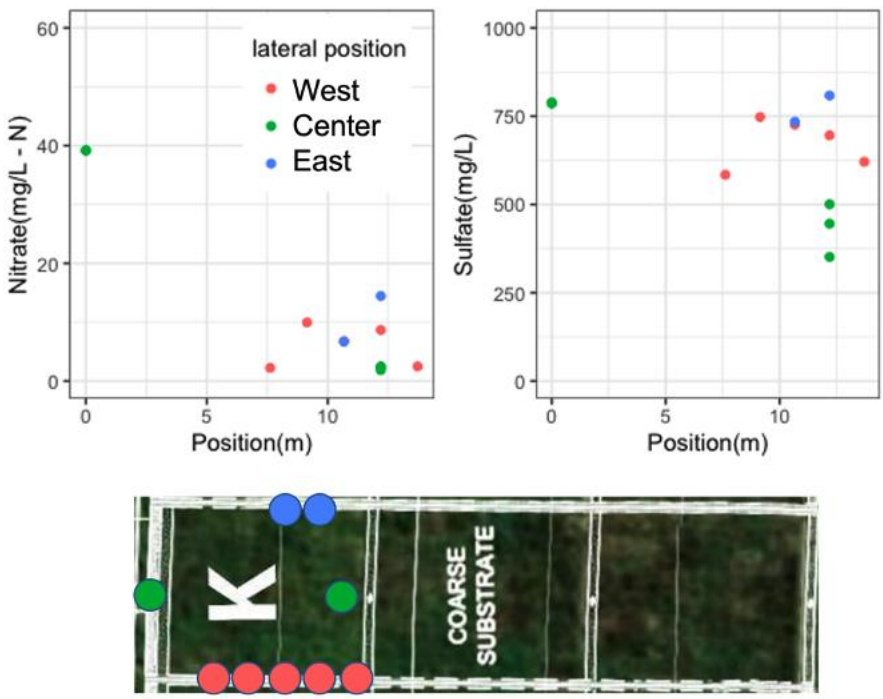


Figure C.2 (top) concentrations of nitrate and sulfate in porewater samples collected in the center (green), and along the east and west edges (blue, red respectively). Approximate porewater sampling locations are shown on the aerial map (bottom, water flows from left to right).

C1.2 Plant Uptake of Carbamazepine in the RO Concentrate Cell

The mass of carbamazepine, m_{plants_roc}, that entered plants in the RO concentrate wetland is calculated below. To provide a conservative estimate of uptake into plants in the RO concentrate, the highest estimate for the mass in plants in the wastewater cell, m_{plants_ww} was used in the following equation:

$$m_{plants_roc} = m_{plants_ww} * \frac{\overline{C_{o_{ROC}}}}{\overline{C_{o_{ww}}}} * \frac{\overline{ET_{ROC}}}{\overline{ET_{ww}}} \qquad C.1$$

See equation *A.8* for the calculation of m_{plants_ww}.

The ratio of the mean influent concentrations, $\frac{\overline{C_{o_{ROC}}}}{\overline{C_{o_{ww}}}}$, and amount of evapotranspiration, $\frac{\overline{ET_{ROC}}}{\overline{ET_{ww}}}$, in the in the RO concentrate cell relative to the wastewater cell was used to convert the estimate to the conditions expected in the RO concentrate cell.

The amount of evapotranspiration in the wetland cells was estimated using the following equation:

$$\overline{Q_{ET}} = \left(1 - \frac{\overline{[Cl^-]_{in}}}{[Cl^-]_{out}}\right) * \overline{Q_{in}} \qquad C.2$$

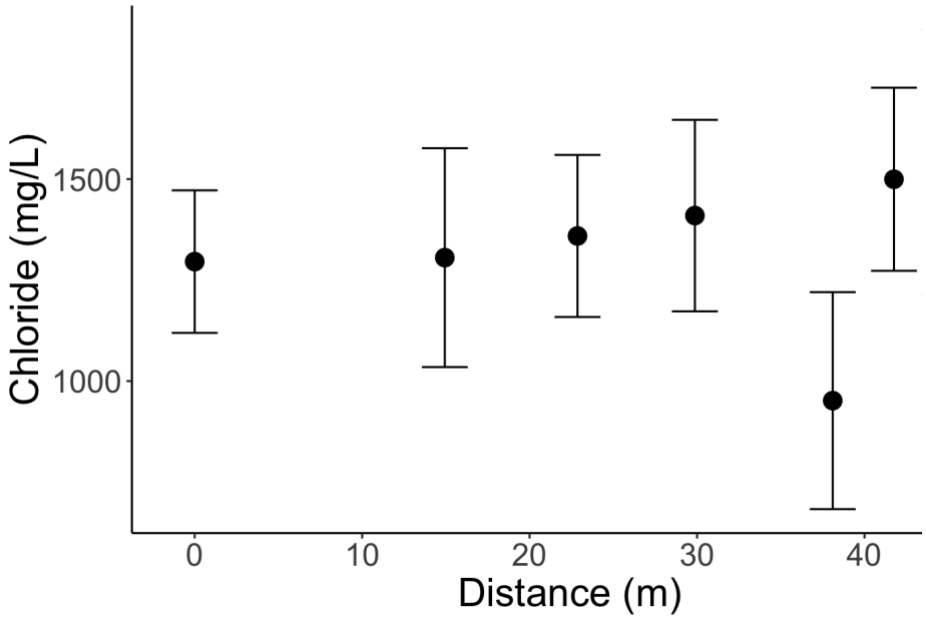


Figure C.3 Chloride concentrations along the horizontal levee flow-path.

C1.3 Annual Mass Removed per Unit Area

To calculate the annual mass of contaminants removed per unit area (M_{RA}), we use measured concentration declines over defined areas in wetland porewater in the following equation:

$$M_{RA} = \overline{C_o} * \overline{Q_{in}} * \frac{\left(1 - \frac{\overline{C}}{\overline{C_o}}\right)}{l * w} \qquad C.3$$

Where *l* is the distance required to achieve removal and *w* is the wetland cell width. In the case of RO concentrate, the zone between 3-15m where low permeability was observed was subtracted from distances longer than 15 meters (section C1.1). In the case of wastewater effluent, the distance was calculated on the basis of porewater concentration profiles presented in Chapter 2. In open-water wetlands, we assumed $\overline{C_o}$ was approximately equal to that measured in the horizontal levee because the RO concentrate was sourced from the same treatment plant. $\frac{\overline{C}}{\overline{C_o}}$ values for the open-water wetlands were extracted from published data[39] using WebPlotDigitizer. In the RO concentrate potential bar, contaminant removal profiles (i.e., $\frac{\overline{C}}{\overline{C_o}}$) from the wastewater-receiving cells were applied to the concentrations measured in the RO concentrate flowing into the wetland.

C1.4 Supplemental Figures

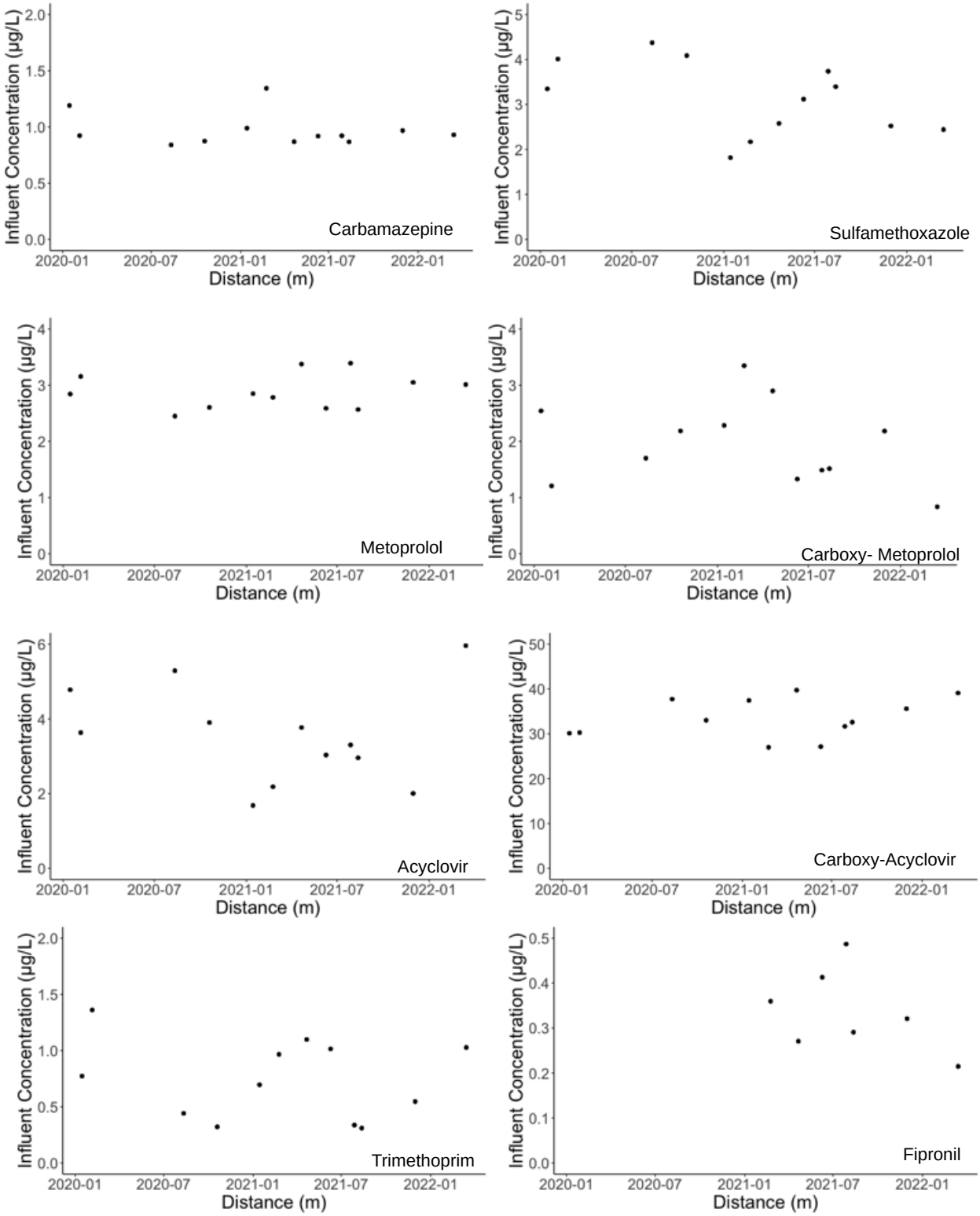


Figure C.4 Mean concentrations of trace organic contaminants in RO concentrate flowing into the horizontal levee

APPENDIX D. Photos of the Field Site

D1.1 Field Site Photos



Figure D.1 Horizontal levee in January 2017 view from the effluent sampling wells



Figure D.2 Horizontal levee in October 2017 - view from the effluent sampling wells



Figure D.3 Willow Sampling in November 2017



Figure D.4 Horizontal levee in winter of 2022 (left) and summer of 2021 (right) at approximately the same location near the inlet



Figure D.5 Wildlife use of the horizontal levee field site. A variety of other birds, insects, and mammals (e.g. jackrabbits) were also seen onsite but are not pictured.