

COLD-CLIMATE REMOVAL OF EMERGING CONTAMINANTS IN A TWO-STAGE
VERTICAL FLOW WETLAND AT BRIDGER BOWL SKI AREA

by

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NOMENCLATURE

AOB	Ammonia oxidizing bacteria
CEC	Contaminant of emerging concern
COD	Chemical oxygen demand
DD	Dose depth
DO	Dissolved oxygen
DOC	Dissolved organic carbon
FWS	Free water surface
HFTW	Horizontal flow treatment wetland
HLR	Hydraulic loading rate
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MLR	Mass loading rate
N	Nitrogen
N ₂	Nitrogen gas
NH ₃	Ammonia
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
OC	Organic carbon
OM	Organic matter
PE	Person equivalent
PNEC	Predicted no effect concentration

NOMENCLATURE CONTINUED

PO_4^{3-}	Orthophosphate
POC	Particulate organic carbon
RQ	Risk quotient
SFTW	Surface flow treatment wetland
SNR	Signal to noise
SSFTW	Subsurface flow treatment wetland
TN	Total nitrogen
TNI	Total nitrogen ions
TW	Treatment wetland
U.S.	United States
VFTW	Vertical flow treatment wetland
WW	Wastewater
WWTP	Wastewater treatment plant

ABSTRACT

Contaminants of emerging concern (CECs), including pharmaceuticals, are found in wastewater (WW) at trace levels and can resist removal by conventional wastewater treatment plants (WWTPs). Consequently, CECs are discharged to the environment through WWTP effluent, posing ecological and public health risks. Meanwhile, the U.S. WW sector faces aging infrastructure, tightening effluent standards, and limited budgets, making targeted CEC treatment technologies inaccessible for most municipalities and U.S. households that rely on decentralized treatment. These limitations, combined with rising public awareness and expectations, demonstrate a growing need for low-cost systems capable of both CEC removal and conventional treatment.

Treatment wetlands (TWs), particularly vertical flow treatment wetlands (VFTWs), where water passes vertically through planted sand or gravel media, offer nature-based solutions to municipal WW polishing and decentralized treatment. TWs combine physical, chemical, and biological processes to remove CECs and conventional pollutants. Despite widespread adoption elsewhere, their use in the U.S. is limited by outdated design guidelines and a lack of demonstrated long-term performance.

A knowledge gap in the use of VFTWs for CEC treatment in cold climates was addressed with a pilot-scale study investigating the presence and removal of CECs at the Bridger Bowl two-stage VFTW, which treats ski resort wastewater in winter (Bozeman, MT). The Bridger VFTW has been consistently monitored over its December-April operating season since 2015, providing valuable long-term data. Influent, midpoint, and effluent WW samples were analyzed for chemical oxygen demand (COD), nitrogen species, and target CECs. Solid phase samples were analyzed to identify CECs sorbed to media or biomass.

Despite low WW temperatures (2-4 °C), the VFTW achieved high removal of COD (96%), ammonia (98%), and total nitrogen (71%). Of the 38 CECs detected, half demonstrated removal greater than 80%, primarily in the aerobic zone of the VFTW. Performance equaled or exceeded that of VFTWs and WWTPs in literature studies. By filling the cold-climate data gaps for CEC removal and demonstrating robust treatment of both CECs and conventional pollutants, this work establishes VFTWs as cost-effective, nature-based solutions capable of advanced WW treatment in cold regions.

INTRODUCTION

Background

Wastewater (WW) contains trace levels of contaminants of emerging concern (CECs), including pharmaceuticals, that resist removal by conventional wastewater treatment plants (WWTPs). These compounds enter the environment through WWTP discharge, posing ecological and human health risks. Decentralized wastewater (WW) treatment systems capable of advanced WW treatment are increasingly needed for rural and resource-limited communities, particularly as regulations become more stringent and systems age. In addition to the potential for future regulation, increasing public awareness and expectations for CEC treatment make decentralized solutions capable of removing both conventional contaminants and CECs attractive for replacing or upgrading failing systems.

Among the solutions available, vertical flow treatment wetlands (VFTWs) offer a nature-based, low-cost alternative that combines processes like filtration, sorption, and microbial degradation to remove both conventional contaminants and CECs from WW. Despite broad adoption of these systems in Europe for applications ranging from sludge treatment to water reuse, U.S. guidelines for VFTWs remain outdated, and long term demonstrated performance, especially in cold-climate applications, is scarce. These engineered wetland systems are evaluated in this thesis, with a focus on filling the research gap on their removal of CECs in a cold climate.

System Introduction

The pilot-scale two-stage VFTW evaluated in this thesis is located at the Bridger Bowl Ski Area near Bozeman, Montana (45°49'01.0"N 110°54'22.0"W, elevation 6,430 ft). The system

collects WW from the Saddle Peak and Jim Bridger lodges, which generate WW from lavatories and sinks. The WW at Bridger Bowl is high strength, as it is not influenced by dilution from washing machines, showers, and appliances. Originally designed as a pilot-scale study in 2012, the pilot-scale VFTW demonstrated successful performance in the high-elevation, cold Montana climate, paving the way for construction of a full-scale system to treat the remaining WW from the base area lodges (10,000 gallons per day). Upon construction of the full-scale system in 2018, and adoption of the technology by the Montana Department of Environmental Quality, the pilot-scale VFTW was repurposed for Montana State University research, funded by Bridger Bowl and the Montana Department of Environmental Quality.

The system was designed for carbon and total nitrogen removal through two stages with recirculation and operates with a partially saturated first stage and unsaturated second stage. The surface of the system is planted with native macrophytes, or wetland plants. The Bridger VFTW operates during the ski season from December-April, with an average season snowfall of 300 inches and ambient winter air temperatures well below freezing. Wastewater temperatures during operations are 2-4 °C. The unique configuration and demonstrated long-term performance of the Bridger VFTW provided an opportunity to assess the resilience of cold-climate systems for conventional and emerging contaminant removal.

Thesis Overview

The remainder of this dissertation is as follows:

1. Chapter 2 reviews the need for decentralized WW treatment, treatment wetland configurations, removal mechanisms for conventional contaminants and CECs, and the factors influencing performance of treatment wetlands.

2. Chapter 3 presents a field study at the Bridger Bowl VFTW characterizing CEC occurrence and removal across the system, and the contribution by each stage to the overall removal. It presents the first research on a system with the Bridger VFTW's configuration under cold-climate conditions for CEC removal and presents a novel method for characterizing the role of sorption in treatment of CECs.
3. Chapter 4 provides concluding remarks, including recommended future research.
4. The appendices present supporting information for the chapters of this dissertation. Appendix A provides support for Chapter 3. Appendix B details a field study at the Bridger Bowl VFTW aimed at quantifying the loading limits of the system, utilizing historical data from 2015-2025, and targeted experiments from 2024-2025 to assess the interplay of loading parameters at various operating schemes.

CHAPTER TWO

LITERATURE REVIEW

Abstract

Decentralized wastewater (WW) treatment systems in the United States (U.S.) face increasing challenges from aging infrastructure, more stringent regulations, rising public expectations, and limited funding. Treatment wetlands (TWs), particularly vertical flow treatment wetlands (VFTWs), offer a cost-effective, nature-based solution capable of producing high-quality effluent. Despite extensive research and implementation in Europe, VFTWs remain underutilized in the U.S., due to outdated guidelines and lack of demonstrated long-term performance data, particularly in cold northern climates. These factors also lead to difficulty obtaining permits for VFTWs. This literature review examines the viability of VFTWs as decentralized WW treatment systems to address modern challenges and highlights knowledge gaps in the U.S. The efficacy of VFTWs at treating conventional and emerging contaminants, the success of various intensification strategies, and factors affecting VFTW performance are described in this review. Understanding VFTW performance under realistic and modern conditions is critical for advancing their regulatory acceptance and adoption as a viable decentralized WW treatment option.

Introduction

Wastewater (WW) collection and treatment are essential for maintaining sanitation, protecting public health, and preserving the natural environment (1). In the United States (U.S.), access to WW treatment is widely available, with fewer than one percent of U.S. citizens lacking access to sanitation services (2) compared to 43 percent of the global population in 2022 (3). As

of 2017, approximately 18 percent of U.S. households (roughly 1 in 5) were served by individual decentralized systems or had no WW treatment (4). These decentralized systems collect and treat WW near the source, rather than conveying it to a centralized wastewater treatment plant (WWTP) (5). They are common in small communities (population <10,000) and in areas where centralized systems are geographically or economically impractical, offering a lower cost alternative (2). However, decentralized treatment systems typically have a design life of 40-50 years, and many are near or beyond that limit (6). System failures are worsened by limited funding, difficulty retaining skilled operators, and geographical constraints (7). Simultaneously, more stringent regulations and growing public expectations for advanced WW treatment and removal of emerging contaminants mean that many existing systems do not meet treatment objectives (6). These pressures are felt broadly within the WW industry in centralized and decentralized systems alike and were reflected in a \$69 billion funding gap in 2024 (6). To address these challenges, there is a critical need for systems capable of advanced WW treatment to protect U.S. waters that offer low capital and operational costs.

Treatment wetlands (TWs) offer a sustainable and cost-effective alternative for decentralized WW treatment, particularly in small or rural communities facing aging infrastructure and limited resources. TWs are low-cost natural treatment systems that provide relatively effective treatment of conventional contaminants compared to other decentralized systems of their size and cost (8–11). These highly engineered systems mimic the natural processes of wetlands to remove contaminants through a combination of physical, chemical, and biological mechanisms. TWs achieve high levels of pollutant removal, including nutrients, organic carbon (OC), and emerging contaminants, while operating with low energy and maintenance requirements (9–11). While widely adopted in Europe for municipal WW treatment,

TWs have seen limited use in the U.S. due to slow regulatory adoption and a lack of demonstrated performance in domestic climates. As regulatory standards continue to evolve and funding challenges persist, TWs represent a viable, adaptable solution for achieving advanced WW treatment in a cost-effective and operationally sustainable manner. Thus, there is a need to update outdated research and demonstrate the performance of TWs in the U.S. (12) and provide new guidance on the use of these systems for municipal WW treatment.

Classification of Treatment Wetlands

TWs are classified as surface flow or subsurface flow depending on the water level relative to wetland media. Surface flow treatment wetlands (SFTWs, also known as free water surface wetlands) pass the flow of WW over the soil surface similarly to a natural wetland, creating a relationship between densely rooted vegetation and microorganisms that break down pollutants (9,13). SFTWs are generally used for tertiary WW treatment, due to their risk of exposure to pathogens and relatively low loading capacity (13). Subsurface flow treatment wetlands (SSFTWs) channel WW through a media bed, typically gravel or sand, beneath the organic plant matter, promoting microbial activity in the filter (9,13). SSFTWs are further broken into horizontal flow and vertical flow TWs, which are dependent on the direction of WW flow (13,14). These systems are generally used for secondary treatment of WW following primary treatment, although vertical flow treatment wetlands (VFTW) for the treatment of pre-screened WW and removal of sludge have also been introduced and are known as French VFTWs (13,15). French systems are designed to remove solids from pre-screened WW and are often operated with two stages in series, using the French style VFTW to achieve sludge removal and a second VFTW for nutrient treatment (15).

Both horizontal flow treatment wetlands (HFTWs) and VFTWs consist of a granular media, typically gravel or sand, planted with emergent macrophytes (13). HFTWs pass the flow of WW horizontally through the planted media with the water level maintained just below the media surface, creating a saturated environment that tends to be anaerobic due to oxygen transfer limitations (13). VFTWs direct WW in intermittent doses vertically through the planted media, which allows for enhanced oxygen transfer by pulling air into the unsaturated media behind a dose and facilitating aerobic processes (10,13). Additionally, advantages of HFTWs and SFTWs can be incorporated into VFTW design, as water levels in VFTWs may be controlled to introduce a saturated, anaerobic zone to the TW (13). Because of the increased oxygen transfer, unsaturated VFTWs are typically more compact and can handle higher pollutant loads than other wetland designs, making them a preferred system for most municipal WW treatment applications (9,14). Hybrid systems integrate elements of different TW types to optimize treatment efficiency, similar to combining secondary treatment stages in a WWTP, such as utilizing a two-stage process with saturated and unsaturated zones to promote multiple pathways for nutrient removal (10,14).

The selection of a specific TW type depends on factors such as the composition of the WW, land availability, and treatment goals. Most TWs designed for standalone municipal WW treatment are SSFTWs, usually VFTWs, due to their subsurface treatment and limited risk of exposure to pathogens (13). However, in the U.S., EPA guidelines on the use of TWs for municipal treatment are very limited, and the most recent assessment of the use of SSFTWs for WW was published in 1993 (16), while research and demonstrated performance in Europe has continued to the present. This review will primarily focus on VFTWs due to their high pollutant load capacity, cost efficiency, and diverse removal mechanisms for treating both conventional and emerging contaminants. It will also provide the foundation for assessing the effectiveness of

a VFTW at achieving these treatment objectives in the temperate climate and cold winters of the Northern U.S. in later chapters.

Mechanisms of Treatment

Understanding contaminant removal mechanisms in TWs is essential for optimizing design and predicting treatment performance. TWs utilize a combination of physical, chemical, and biological processes to remove pollutants. TW removal mechanisms occur simultaneously, and may be abiotically, microbially, or plant catalyzed/facilitated making it difficult to isolate specific pathways of removal (13). Consequently, TWs are often treated as "black-box" systems (9), with influent and effluent sampling serving as an efficient method for evaluating performance. While this approach is effective in assessing overall treatment, research into specific mechanisms and operational parameters has provided insight into the internal processes and interactions within TWs, offering the basis for targeted treatment and optimization to enhance treatment. The following sections discuss specific treatment mechanisms, exploring the physical, chemical, and biological processes that drive pollutant removal in TWs. The removal mechanisms and characteristic processes that occur in a typical VFTW design are shown in Figure 1.

Physical removal processes in TWs include sedimentation and filtration. Suspended solids settle out by gravity in pretreatment tanks via sedimentation, and through filtration in the solid media or the bed surface (10,11,13). Filtration occurs as water moves through the granular media, where particles are physically strained and retained within the solid media. The efficacy of physical removal depends on media characteristics and hydraulic loading rate (HLR) (13).

Sorption is primarily a physical process that includes adsorption (binding to surfaces) and absorption (uptake into pores), allowing dissolved substances to adhere to media or biofilm

surfaces. Sorption removes contaminants from the water column and thereby enhances their potential for further transformations due to prolonged retention in the system.

Chemical processes also contribute to contaminant transformation and retention within TWs, working with physical removal to further degrade compounds. Precipitation occurs when dissolved substances form insoluble compounds due to shifts in solubility and pH. These precipitated compounds may then be retained in the system media through physical mechanisms. These processes are influenced by factors such as pH, redox conditions, organic matter content, and media type or surface area (13,17).

Biological processes drive many of the removal mechanisms in TWs, including carbon and nitrogen transformations similar to processes that occur in conventional treatment. Microbial biofilms attach to the surfaces of media and plant roots, where they metabolize contaminants, specifically nutrients and organic matter (9,13). VFTWs use intermittent dosing to maintain aerobic conditions that support processes such as carbon oxidation and nitrification, converting ammonia to nitrate. In multi-stage or modified VFTW systems, saturated, anoxic zones support denitrification and total nitrogen (TN) removal (13). Vegetation contributes by oxygenating the rhizosphere (root zone), creating preferential flow paths by movement of plants and roots, limiting short circuiting by diffusing flow, and facilitating nutrient uptake (13,14). These biological processes are highly sensitive to environmental variables such as temperature, oxygen availability, and loading rates (13).

Removal Mechanisms and Characteristic Processes of a Vertical Flow Treatment Wetland

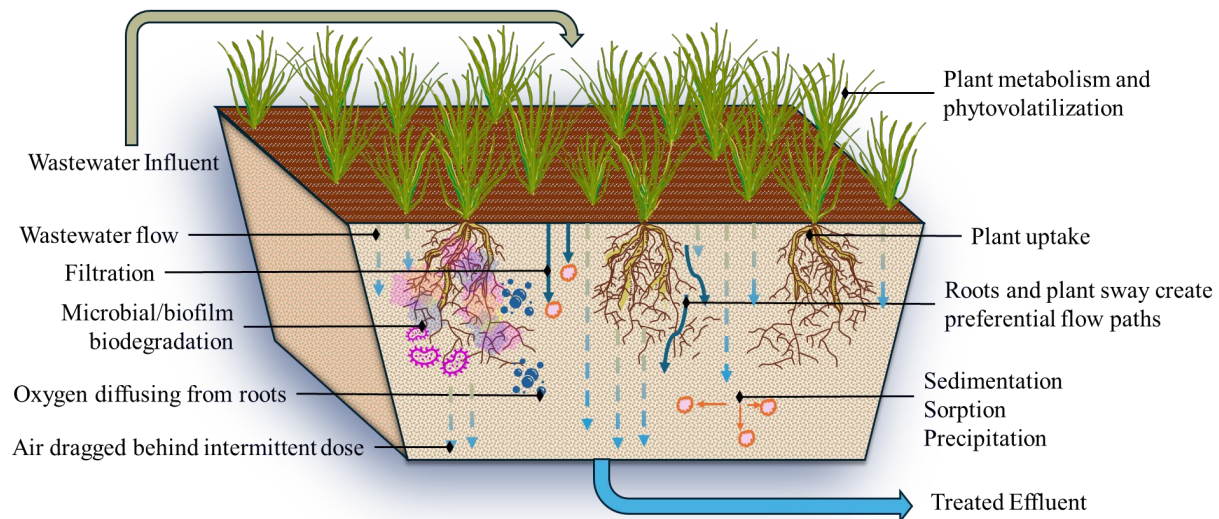


Figure 1: Removal mechanisms and characteristic processes of a typical vertical flow treatment wetland.

Operational and design parameters are critical for promoting specific removal pathways aligned with treatment objectives. These parameters include hydraulic and mass loading rates, dosing frequency and depth, saturation level, media type, and plant species. This review presents the removal mechanisms for conventional and emerging contaminants and demonstrates how operational and design parameters may be adjusted to meet treatment goals.

Contaminants Targeted

This section provides brief summaries on treatment of conventional contaminants in TWs, including organic carbon, nitrogen, phosphorus, and pathogens, while the primary focus of the in-depth review will be on emerging contaminants and their treatment processes within TW systems.

Organic Carbon. Organic carbon in WW is a complex mixture of compounds that can be broadly classified into particulate organic carbon (POC) and dissolved organic carbon (DOC) fractions, each with different implications for removal mechanisms and TW design (13). OC is typically measured as 5-day (carbonaceous) biochemical oxygen demand (BOD_5) or chemical oxygen demand (COD), which represent the biodegradable and total oxidizable (including biodegradable) fractions, respectively. In TWs, POC is primarily removed through sedimentation and filtration, while DOC undergoes biological degradation (13). Typical unmodified SSFTW designs achieve >80% removal of OC in HFTWs, and >90% removal in VFTWs (13).

POC originates from the influent WW and is also generated in the TW via biofilm growth, plant decay, and microbial detritus (13). Plant type and climate may affect the internal contributions of POC, while influent POC levels depend on pretreatment levels and WW composition (13). Retained POC is eventually hydrolyzed, producing DOC that can be further biologically degraded (13).

In SSFTWs, aerobic respiration and denitrification are the dominant DOC removal mechanisms, with unsaturated VFTWs favoring aerobic respiration, and anoxic HFTWs supporting denitrification if nitrate is present (13). Partial saturation and the introduction of an anoxic zone in VFTWs can also enable denitrification (13), which will be discussed relating to nitrogen removal in the following section.

Nitrogen. Nitrogen in WW enters primary and secondary TWs as organic nitrogen and ammonium (NH_4^+), with second, or recycled, stages of 2-stage and hybrid TWs additionally receiving nitrite (NO_2^-) and nitrate (NO_3^-). The cycling of nitrogen involves a series of microbial transformation processes including ammonification, nitrification, and denitrification, with the end goal of WW treatment being the conversion of nitrogen species into nitrogen gas (N_2). In

TWs, there are additional pathways that may contribute transformations to a lesser extent, including the biotic pathways of plant and microbial uptake, nitrogen fixation, anammox, and comammox (18). Abiotic removal processes include ammonia volatilization, adsorption, and precipitation (18). U.S. regulations are shifting towards TN removal rather than focusing solely on transformation to NO_3^- . Typical unmodified SSFTWs achieve 30-50% removal of TN in HFTWs, and <20% removal in VFTWs, indicating that some modifications to promote these processes are necessary if TN removal is a treatment goal (13). Research has shown that intensified and hybrid TWs have achieved higher TN removal, which is discussed in later sections.

Nitrification is the step-wise conversion of NH_4^+ to NO_3^- by autotrophic microbes in an aerobic environment, with NO_2^- as an intermediate product (13). Nitrification is influenced by the presence of NO_3^- , temperature, pH, alkalinity, OC, moisture, microbial populations, and dissolved oxygen (DO) (18). Sufficient OC must be removed prior to the nitrification step, as autotrophic nitrifiers are outcompeted by aerobic heterotrophs in the presence of readily biodegradable OC (13). Typical unmodified SSF TW designs achieve 20-30% removal of NH_4^+ -N in HFTWs, and >90% removal in VFTWs due to higher oxygen transfer capacity (13).

Denitrification is the anoxic step-wise process where facultative organisms reduce NO_3^- to N_2 as the final step in TN removal. In TWs, this process occurs in saturated, anoxic zones, making it effective in HFTWs and saturated zones of VFTWs (13). Denitrification is influenced by DO levels, pH, redox conditions, media type, and OC content (13). Denitrification requires OC as a carbon source, and since nitrification is a prerequisite to denitrification and requires low OC, denitrification is often difficult to achieve. In HFTWs, low nitrification rates result in high NH_4^+ in the effluent, but any NO_3^- produced via nitrification is generally denitrified. Conversely,

VFTWs have high nitrification rates, producing sufficient NO_3^- , but are limited by denitrification (13). Therefore, it may be necessary to combine system types and redox zones to achieve TN removal (8,19). These hybrid systems are discussed in later sections.

Phosphorus. Phosphorus in municipal WW exists primarily as orthophosphate (PO_4^{3-}) and in organic forms. In TWs, most organic phosphorus is converted to PO_4^{3-} during organic matter degradation, and this transformation is not typically a limiting factor for phosphorus removal (13). Unlike nitrogen and OC, PO_4^{3-} removal in TWs is primarily through physical and chemical processes, including precipitation, sedimentation, and sorption, with limited long-term microbial pathways and plant uptake (13,18). These mechanisms are relatively slow and provide fewer removal pathways as compared to other contaminants. Additionally, phosphorus removal is difficult to control operationally and long-term treatment of phosphorus is limited, as sorption sites become saturated over time. Typical long-term results for phosphorus show 10-20% removal for both HFTWs and VFTWs (13). Strategies to improve phosphorus removal are discussed in later sections.

Pathogens. Pathogens are removed from WW in TWs through a combination of physical, chemical, and biological processes, including filtration, sedimentation, oxidation, adsorption to organic matter, and aggregation in biofilms (13). In VFTWs, high oxygen availability and dense native microbial communities enhance predation and natural die-off, as well as increasing aerobic stress that some pathogens cannot tolerate. Typical HFTWs designs achieve 2 $\log_{10}$ removal, while VFTWs can achieve 2-4 $\log_{10}$ removal (13). Although TWs facilitate pathogen reduction, they are biologically active systems that may also harbor opportunistic microorganisms. As a result, TWs may require disinfection (e.g., chlorination, UV treatment) to meet stringent discharge or water reuse requirements.

Contaminants of Emerging Concern. Contaminants of emerging concern (CECs), such as pharmaceuticals, personal care products, pesticides, and industrial chemicals, are increasingly detected in WW and surface waters, yet remain unregulated in the U.S. (20,21). These compounds have been listed as priority substances worldwide, and are beginning to be regulated in Europe, highlighting the urgency in finding low-cost treatment options (22). These compounds are persistent and cause ecological damage and threaten human health even at trace concentrations where conventional contaminants would not have an impact (20,23). Conventional treatment systems were not designed to target these compounds, and therefore these compounds generally enter the environment through discharged WWTP effluent (14,24).

TWs are being investigated as a low-cost option for CEC removal due to their various and simultaneous removal mechanisms (25,26). VFTWs particularly are well-suited for removal of aerobically degraded CECs, and studies suggest that aerobic processes are a primary mechanism for removal for many organic CECs at realistic WW concentrations (27). Among wetland types, VFTWs typically show the highest removal rates, followed by HFTWs, with SFTWs performing the least effectively (13). In general, studies on CECs are broken down into the type of contaminant, with many municipal or domestic WW studies focusing on pharmaceuticals, personal care products, and microplastics, as these compounds are found in the WW studied. This review will discuss removal mechanisms of CECs but will focus on pharmaceuticals to complement later chapters.

Research on TWs for CEC treatment has been ongoing for two decades and has demonstrated variable CEC removal in TWs (27–37). Many studies are limited to laboratory-scale setups and/or studies utilizing synthetic WW, reducing the applicability to variable real-world systems (26,38–42). Studies using pilot or full scale VFTWs are less common (14,43–47),

and several are conducted on the same TW at the Langenreichenbach Ecotechnology Research Facility in Germany. Most scaled studies are located in temperate European climates, where freezing is limited to short periods, creating a knowledge gap for cold-climate VFTWs, such as those in the northern U.S., (14,43–45,48,49).

In addition to experimental studies, review papers have investigated the effects of operational strategies, plant and media types, removal processes, and seasonality on CEC removal (9,23–27,50–58). Redox conditions are one of the most important factors, as aerobic zones are required for transformation of many CECs, such as acetaminophen, ibuprofen, and caffeine (14,24,27). In VFTWs, high oxygen availability from intermittent dosing has been shown to support more consistent removal of these compounds (14).

Compound-specific properties such as hydrophobicity, molecular weight, and susceptibility to microbial degradation affect whether a CEC is removed primarily through biodegradation, sorption, or volatilization. For example, carbamazepine, known for high persistence and poor biodegradability in other treatment systems, is also often poorly removed in VFTWs (48), while compounds like ibuprofen and ketoprofen, which are more easily biodegraded, are removed consistently at rates >80% under aerobic conditions (14,27,48).

The presence and type of plants in TWs has also been studied in regard to CEC removal. The leaching of oxygen around the root zone introduces aerobic zones for biodegradation to occur, which may provide treatment of certain compounds (11,48). Plant uptake is also suggested to account for a higher bulk of treatment in CECs than conventional contaminants due to their low concentrations, and the high root biomass is thought to enhance sorption, especially in cold seasons when biodegradation is inhibited (40). In addition to the presence of plants increasing treatment by providing plant uptake and additional biomass for sorption (59), the plant species

itself may also affect treatment, with a large rhizome such as present with *Phragmites australis*, increasing biological activity of associated organisms and providing higher sorption potential (40,52,60).

While microbial community composition has not been extensively studied for CEC removal in full- or pilot-scale TWs, evidence suggests that nitrifiers and other aerobic microbes may play a role in transforming certain CECs (61). Specifically, ammonia-oxidizing bacteria (AOB), which facilitate nitrification, are capable of metabolically transforming a wide range of trace organic contaminants via the ammonia monooxygenase (AMO) enzyme (62). Given their presence in systems with aerobic conditions and ammonia availability, such as a VFTW designed for ammonia removal, AOBs have potential to further enhance the removal of CECs in TWs. Continued study of microbial ecology in full-scale, real-climate systems is needed, but is difficult due to the incredible range of CECs, transformation products, and microbial roles.

Factors Affecting Performance

TW performance is influenced by operational parameters selected based on treatment goals, and environmental parameters that must be considered in the design choice. This section focuses primarily on the operation of VFTWs for municipal WW treatment, as the system described in later chapters is a VFTW. Innovative design, including hybrid and intensified TWs to meet specific treatment goals are also discussed. The selection of TW type depends on the WW origin source, inlet and outlet concentrations, hydraulic and mass loadings, system size, aspect ratio, depth, climate, and ecology (13).

Design Parameters. In VFTWs, the efficiency of removing both conventional and emerging contaminants depends heavily on the filter media selection. Without adequate primary treatment, clogging of the granular media is a common failure mode for VFTWs (11,13). Coarser

media reduces clogging risk but lowers removal efficiency, while finer media enhances removal efficiencies, but slows infiltration, limiting throughput (10,11).

Hydraulic loading rate (HLR) and hydraulic residence time (HRT) are important design parameters influencing the ultimate size of the system, media choice, and overall treatment performance. HLR is defined as the volume of water applied per unit area over time, while HRT reflects the time WW spends in the system. Higher HLRs increase throughput, but reduce HRT and can lower treatment efficiency, as WW has less contact time with microbial communities and the media. Coarse media, which prevents clogging, results in short HRTs and poorer treatment, but if treatment is still sufficient, higher HLR can be applied due to the drainage capacity of coarser media (10,11). Conversely, fine media, which may be clogged with high HLRs, provides longer HRTs due to its smaller pore space, increasing treatment.

Design strategies to balance HLR and HRT include increasing the treatment layer depth or layering media to enhance treatment and reduce the risk of clogging (13). Deeper beds in VFTWs allow for diverse redox conditions created by partial saturation, which can increase possible removal pathways. Dose volume for a given HLR also plays a role in treatment, with smaller dose volumes increasing the HRT, increasing treatment efficiency (13,14). The ratio of the dose volume and retention volume in the TW (dependent on the TW size and media porosity) has been described by one study as an important design parameter capable of predicting performance, with lower dose volumes for a given retention volume increasing performance (63). However, sufficient dose volume is required to distribute flow evenly across the TW surface, sometimes requiring siphons to achieve even application.

Media selection in VFTWs also affects sorptive removal, particularly for PO_4^{3-} , for which sorption is a predominant removal mechanism in TWs. Strategies to increase PO_4^{3-}

removal include the use of highly sorptive media, unplanted beds (to facilitate media replacement), and chemical dosing to precipitate phosphorus into the wetland (13). Media enriched with iron, aluminum, or calcium, such as lightweight expanded clay aggregate (LECA), blast furnace slag, steel slag, zeolite, or alum sludge, has shown higher phosphorus removal capacity than conventional sand or gravel (64–66). For instance, iron slag achieved 60-90% PO_4^{3-} removal over two years in a VFTW treating anaerobic digester effluent, outperforming gravel, marble, and zeolite (67). Another study found that modified steel slag performed well for high phosphorus conditions, while steel slag performed adequately for low concentrations of phosphorus (68). One study found that media selection did not greatly affect phosphorus removal when contact time was limited and removal may have been less affected by sorption (69).

Regardless of the media type, saturation of sorption sites remains a long-term challenge. Incorporating unplanted, replaceable beds of sorptive media in the treatment train to target sorptive contaminants can facilitate regeneration or replacement when capacity is saturated (64–66).

For CECs, finer media enhances higher surface area for sorption and biofilm growth, increasing HRT and filtration. This provides many options for CEC treatment, and although clogging risk increases as media becomes finer, sand media has been shown to outperform gravel in VFTWs for CEC treatment (14,48). Some studies show higher CEC treatment by use of sorptive media, including natural clay, zeolite, biochar or granular activated carbon, particularly for compounds such as benzotriazole and diclofenac which are typically persistent in TWs (24).

Phosphorus retention and CEC treatment are also improved by optimization of HLR and resting periods. Lower HLRs reduce clogging and enhance contact time (69), while resting periods improve oxygen availability, microbial activity, overall performance, and reduce

clogging (14,60). Frequent small doses also achieve similar goals and see improved removal of CECs.

Environmental Parameters. Temperature and climate significantly influence the design, operation, and performance of a TW. In warm climates or summer-only operations, higher HLRs and mass loading rates are feasible due to higher biological activity (11,13,56). In contrast, cold climates typically exhibit reduced treatment efficiency, particularly for nitrogen removal (11,13,14). However, recent findings from a cold-climate two-stage VFTW showed the presence of nitrifiers and high removal of ammonia from WW, indicating that nitrifiers may remain functional at temperatures lower than previously assumed (70). Temperature also influences phosphorus removal, as low temperatures reduce sorptive capacities, while warmer temperatures were found to increase PO_4^{3-} removal by 15% in a small-scale study using synthetic WW (69).

Seasonal variation in CEC removal has been observed in many studies, particularly in biodegradable compounds. Warm season performance tends to be higher due to increased microbial activity and plant growth, although studies from Germany and the Czech Republic have shown that VFTWs maintained removal at winter temperatures near freezing, possibly due to plant uptake (40,43,48). Despite several studies showing seasonal variation, there is a knowledge gap on field-scale VFTWs for CEC removal in cold-climates, where winter temperatures remain below freezing for more than a few days at a time.

If subfreezing temperatures occur, freezing in distribution pipes can be mitigated by preventing water accumulation through drainage design (13). Including a shallow insulating layer can protect against freezing in the treatment layer. However, this may reduce the benefits of plant sway, where plant stalks create preferential flow paths as they move side-to-side during wind, and may increase clogging risk due to slower degradation of plant detritus on the surface

(13). In areas with sufficient snowfall, a snow layer may suffice for insulation. Native emergent macrophytes are typically used for planting VFTWs, and their thick layer can also provide insulation while supplying OC for denitrification (13).

System aging influences performance due to fluctuations in plant growth cycles and maturation, sorption capacity, and microbial establishment and maturation. An initial startup period is typically required in TWs as plants and microbial communities are established, and sorption sites are taken up. Occupation of sorption sites typically leads to a decline in phosphorus treatment following the first few years of operation (11,13). Over time, media porosity may decrease due to clogging, reducing treatment efficiency and requiring maintenance or redesign (13). Plant harvesting, media replacement, weeding, and upkeep of pumps and pipes are necessary to maintain performance over the system lifespan.

Innovative Design. Hybrid and intensified TWs offer additional design options to meet specific treatment goals, particularly TN removal. As regulations shift from denitrification targets to TN limits, interest in optimizing TN removal has grown. While nitrification and denitrification, their limitations, and their role in WW treatment are well understood, achieving both processes and thereby increasing TN removal has proven difficult in single stage TWs due to their opposing redox conditions and OC requirements. A review of TW types showed TN removal efficiencies of 40-50%, with removed loads ranging between 250 and 630 gNm⁻² yr⁻¹ depending on the TW type and influent loading (18). To improve TN removal in VFTWs, strategies such as artificial aeration, internal recycle streams, and hybrid wetlands have been explored, and many of these strategies adopted for TN removal have also improved treatment of other conventional contaminants and CECs.

Hybrid systems combine multiple TW types into one system, such as a VFTW for nitrification and a HFTW for denitrification, providing the contrasting redox conditions necessary for both processes to occur and remove TN (8,19). Hybrid systems also outperform single-stage VFTWs and HFTWs for CEC removal, due to multiple redox zone and longer residence times (27). Hybrid systems are especially useful in treating WW with several types of CECs with various degradation mechanisms or that may be degraded through multiple processes (i.e. both aerobic and anoxic conditions) (27).

Internal recirculation further enhances TN removal by returning nitrified effluent to an upstream, anoxic, OC-rich zone for denitrification, both in hybrid TWs and multi-stage VFTWs (8,19,71). Using an internal recycle stream overcomes limitations on both nitrification (the presence of OC) and denitrification (the presence of nitrate in a high OC zone). For example, 50% effluent recirculation in a multi-stage VFTW achieved TN removal $> 77\%$ (44), while 100% recirculation in a single-stage VFTW yielded 60%, compared to only 25% without recirculation in the same system (72). A long-term cold-climate study (2015-2023) of a two-stage VFTW with a partially saturated first stage (for denitrification) and unsaturated second stage (for nitrification) showed that TN removal was influenced by the portion of effluent recycled. Despite the various operational adjustments and the cold-climate setting, the system achieved an average of 67% TN removal (70). Microbial analysis on the same system identified distinct communities of nitrifying and denitrifying bacteria in their respective zones, supporting the conclusion that NO_3^- and NH_4^+ removal and the reduction of TN was a result of nitrification and denitrification both occurring in the system (70). Recirculation also enhances COD removal by using it further upstream as a carbon source for denitrification. One review reported up to 90% COD removal with effluent recycling in use (73), and the cold-climate long-term study achieved 96% removal

over 8 years (70). The carbon to nitrogen ratio is therefore a key factor in performance of a TW for TN and COD removal (74). While recirculation primarily targets TN, it may benefit CEC removal by providing high redox diversity. One study found that high oxygen with low OC levels was essential for CEC removal, so an internal recycle stream to provide those conditions for nitrification may also enhance CEC removal (48).

Artificial aeration is another intensification strategy implemented in both single-stage and hybrid systems, to increase nitrification in cold climates, at high loadings, or in other cases where nitrification may be limiting to TN removal (73). Aeration also supports removal of other aerobically degraded compounds, including CECs, and may be applied continuously or intermittently through buried perforated pipes, fine bubble diffusers, or air lift systems. Although energy-intensive, aeration generally improves treatment and reduces land requirements (73). In VFTWs, implementation of active aeration increased removal by 74% (74) and 78% (75) for $\text{NH}_4^+\text{-N}$, and 64% and 63% for TN. COD removal also improved by 34% in a VFTW using aeration (75). CEC removal was also improved using aeration in several cases (14,27,60), and it was found that aerated HFTWs and aerated VFTWs outperformed unaerated TW systems, and even a conventional WWTP for CEC removal (48). One study showed that artificially aerating a gravel bed improved the removal of CECs to a level comparable to a free-draining sand bed (14), although another study showed that CEC removal of certain compounds was only improved by aeration in unplanted beds (41). A comparison of intermittent (50%) and continuous aeration yielded similar CEC removal (49).

Conclusions

As decentralized WW systems approach the end of their design life and face increasingly stringent effluent standards, there is a growing need for effective, low-cost replacement systems

to protect water quality and public health. TWs, particularly VFTWs, offer a nature-based solution for decentralized municipal WW treatment, capable of addressing both conventional pollutants and CECs by combining simultaneous physical, chemical, and biological removal mechanisms.

VFTWs have demonstrated high removal efficiencies for conventional contaminants, particularly those subject to aerobic degradation, including OC and TN. Many of the same design and operational strategies that enhance removal of these conventional pollutants, such as optimizing HLR, media selection, redox zone selection, and aeration, also influence removal of CECs. For example, promoting aerobic conditions to support nitrification not only improves TN removal, but also allows for cometabolic degradation of certain CECs by nitrifiers. Similarly, selection of media with a high sorptive capacity for phosphorus removal can increase CEC retention.

While these systems are well characterized in Europe, there is a lack of up-to-date research and long-term studies in the U.S., particularly with regards to shifting public expectations and new regulations. VFTWs also show promise for CECs, but there is a knowledge gap on CEC treatment using VFTWs in cold-climates, such as the northern U.S., with field-scale operations and real municipal WW.

System performance across all contaminant classes is influenced by design and operational parameters, system age, and seasonal variations, making comprehensive and long-term monitoring critical to understanding these solutions. Optimization for targeting specific contaminants is possible, but all treatment objectives and design characteristics must be balanced. The specific gap in field-scale data from cold climates and in the U.S. presents opportunity for further research into these systems. A comprehensive understanding of VFTW

performance for both regulated and emerging contaminants under realistic conditions is essential to support broader application and regulatory acceptance of VFTWs as reliable, high-performance decentralized treatment solutions.

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CHAPTER THREE

CHARACTERIZATION OF CONTAMINANTS OF EMERGING
CONCERN IN A COLD-CLIMATE TWO-STAGE VERTICAL
FLOW TREATMENT WETLANDContribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization.

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Abstract

Contaminants of emerging concern (CECs), particularly pharmaceuticals, are detected in wastewater in low concentrations, but pose environmental and public health risks, and are resistant to conventional treatment. As these compounds are increasingly monitored and studied, treatment methods are required to improve their removal. This study evaluates a well-characterized two-stage vertical flow treatment wetland (VFTW) designed to treat high-strength wastewater in a cold climate.

Conventional wastewater contaminants were removed with high efficiency, including 96% removal of chemical oxygen demand (COD) and 98% removal of ammonia at water temperatures of 2-4°C. Thirty-eight CECs were quantified in the influent wastewater during the winter operating season (December-April), with removal >80% observed for many of these compounds. Most CECs demonstrated higher removal in the unsaturated, aerobic stage of the VFTW. A ranking of the relative abundance of CECs found in liquid samples and solid media samples (porous media and associated biofilm) provided insights into the retention of certain compounds by solid media. It was found that the VFTW significantly reduced the concentrations of most CECs detected in the influent. Compared to literature values for similar systems, the Bridger VFTW demonstrated equal or superior removal for several CECs, despite lower ambient temperatures, than found in the literature, although removal varied by compound.

This study contributes to a broader understanding of VFTWs, adding data that supports the feasibility of VFTWs to remove select CECs, and demonstrating the adaptability of VFTWs to cold climates without compromising their conventional treatment capabilities and their potential for CEC removal.

Introduction

Contaminants of emerging concern (CECs) are chemical compounds used by humans, including pharmaceuticals, personal care products, pesticides, and industrial chemicals, which are increasingly detected in wastewater and surface waters (1,2). Even at low concentrations, CECs may cause ecological damage and threaten human health, and many are resistant to degradation using conventional wastewater treatment processes (1,3). These compounds may be introduced to the environment from wastewater treatment plant (WWTP) effluents (4,5). The investigation of treatment wetlands (TWs) for CEC removal is driven by the interest in low-cost, small-scale systems capable of advanced wastewater treatment under a wide range of environmental conditions (6,7). While studies on TWs have demonstrated effective removal of CECs, investigations under extreme climate conditions are essential to evaluate the robustness of these systems for treating a diverse range of contaminants under various conditions.

TWs can function as low-cost, decentralized wastewater treatment systems (8) and achieve reduction of contaminants through simultaneous processes including sorption, microbial degradation, photodegradation, volatilization, and plant accumulation (4), (9–15). The interplay of these diverse treatment processes in TWs and other nature-based systems have proven effective at reducing CECs (16,17). Vertical flow treatment wetlands (VFTWs) have compact designs that direct wastewater vertically by gravity through planted gravel or sand media containing native wetland plants, resulting in treatment of high pollutant loads (5,8). VFTWs can be operated unsaturated, to facilitate oxygen transfer in the subsurface, or saturated to promote anoxic conditions (7). VFTWs are well-suited for removing aerobically degradable compounds, and studies suggest that aerobic biodegradation is favored for many CECs at realistic wastewater concentrations (14).

Investigation of CEC removal in TWs has been ongoing for nearly 20 years (14,18–27). Much of this research has been restricted to laboratory scale studies and/or studies utilizing synthetic wastewater (16,28–32). Studies using pilot- or full-scale VFTWs are less common (5,33–37), and several are conducted on the same TW system at the Langenreichenbach Ecotechnology Research Facility in Germany. In addition to these experimental studies, review papers have investigated the effects of operational strategies, plant and media types, removal processes, and seasonality on CEC removal (3,4,8,10,13–17,38–43). Most extensively characterized pilot- or full-scale TWs for raw wastewater treatment in Europe do not experience prolonged subfreezing temperatures. Systems in eastern Ukraine (35), Germany (33–36) and Prague (37) experience freezing for no more than a few weeks at a time, and those in Spain (5,34) and Belgium (29) rarely freeze. This presents a knowledge gap regarding CEC removal capability in cold-climate VFTWs, especially in regions with sustained subfreezing temperatures, such as the northern United States.

Characterization of CEC removal in TWs has revealed several factors influencing treatment efficiency. Studies have found that porous media size (5), the presence of plants (44), and plant species may play a role in removal of CECs (45). Predominantly aerobic VFTWs demonstrated higher removal for most CECs than anaerobic systems, and frequent dosing helped maintain an aerobic environment, benefiting CEC removal, nitrification, and chemical oxygen demand (COD) removal (5,45). Seasonal performances were observed in some systems, with higher removal in warmer months. Microbial communities have not been extensively studied for CEC removal in full- or pilot-scale TWs, but evidence suggests that nitrifying bacteria in TWs can oxidize organic pollutants, including some CECs (46).

This study addresses the current knowledge gaps by investigating CEC removal in a pilot-scale, cold-climate VFTW receiving high-strength raw wastewater from ski area lodges. The pilot-scale TW was designed for total nitrogen (TN) and carbon removal, and is operated with two VFTW stages, one partially saturated (anoxic) and one unsaturated (aerobic), allowing for a comparison of contaminant removal between the stages (7,8,47). The study characterizes the presence and removal of CECs across the system and compares performance to other TWs and conventional systems. To the authors' knowledge, this is the first study to present data on CEC removal at low water temperatures (2–4°C) in a two-stage VFTW with a comparison of removal performance between the anoxic and aerobic stages.

Materials and Methods

Site Description and System Operations

This study was conducted during the 2022-23 operational season (December to April) at the Bridger Bowl Ski Area TW (45°49'01.0"N 110°54'22.0"W, elevation 1,960 m (6,430 ft)). The VFTW system has been operational since 2013 and removes TN and COD from clarified, high-strength wastewater using two stages with partial recycling (Figure 2).

The first stage of the TW was partially saturated to enhance denitrification of nitrate in the recycle stream and to remove COD. It was saturated to a depth of 0.71 m with a total media layer depth of 0.91 m to prevent freezing, composed of crushed gravel ($d_{50} = 5.3$ mm). The second stage was fully unsaturated for nitrification and consisted of a 0.91 m deep media layer of washed concrete-sand ($d_{50} = 0.53$ mm). Both stages consisted of two identical treatment cells fed in parallel for a total treatment area of 48 m² per stage. The TW was planted with *Carex utriculata* and *Schoenoplectus acutus* in equally sized, separate, checkerboard quadrants.

Volunteer species have appeared and are removed each summer. During the study, wastewater

temperature was 2-4 °C, and influent total suspended solids were 52-87 mg/L, measured using EPA Method 160.2 (48).

Influent raw wastewater was collected through a series of pretreatment tanks before application to the pilot-scale system. The high strength raw wastewater was collected from the ski area base lodge lavatories and kitchens. Settled wastewater was dosed onto the first stage at 1,500 gpd (3.6 m³/d), alternating with doses from the recycle stream and resulting in a 2:1 recycle-to-influent ratio and a total system hydraulic loading of 4,500 gpd.

The first-stage effluent, containing ammonia, was collected in a transfer tank and applied to the fully unsaturated second stage, facilitating nitrification of ammonia to nitrate. The second stage was dosed 9 times a day, with each cell receiving 250 gal per dose at a depth of 3.98 cm/dose. The second stage effluent was collected in a recirculation tank, where a portion (2:1) was recycled to the first stage. The final effluent of 1,500 gpd was discharged to the full-scale VFTW treating the ski area's wastewater.

Wastewater Sampling

Grab samples and 3-day average composite samples were collected from the septic tank (raw unmixed influent to system), transfer tank (mixed first-stage effluent and second-stage influent), and recirculation tank (final system effluent and partial influent to first stage) (Figure 1). Wastewater samples were analyzed at least weekly over the season for COD, ammonia-nitrogen (NH₃-N), nitrate (NO₃⁻), nitrite (NO₂⁻), and orthophosphate (PO₄³⁻). Samples were transported on snow/ice and analyzed within one day of collection. COD was measured using HACH® digestion vials (HACH® Co., Loveland, CO, USA). NH₃-N was measured using a modified Berthelot reaction approach for 96 well plates, with absorbance readings taken at a wavelength of 660 nm on a BioTek Synergy HTX Multimode Reader (Agilent Technologies,

Inc., Santa Clara, CA, USA) (49). NO_3^- , NO_2^- , and PO_4^{3-} were measured using a Metrohm Eco ion chromatograph with a Metrosep A Supp 5150/4.0 column, Metrohm Suppressor Module and 3.20/1.00 mM sodium bicarbonate/sodium carbonate eluent at 0.7 mL/min (Metrohm USA, Riverview, FL, USA).

BRIDGER BOWL PILOT SCALE VERTICAL FLOW WETLAND SYSTEM

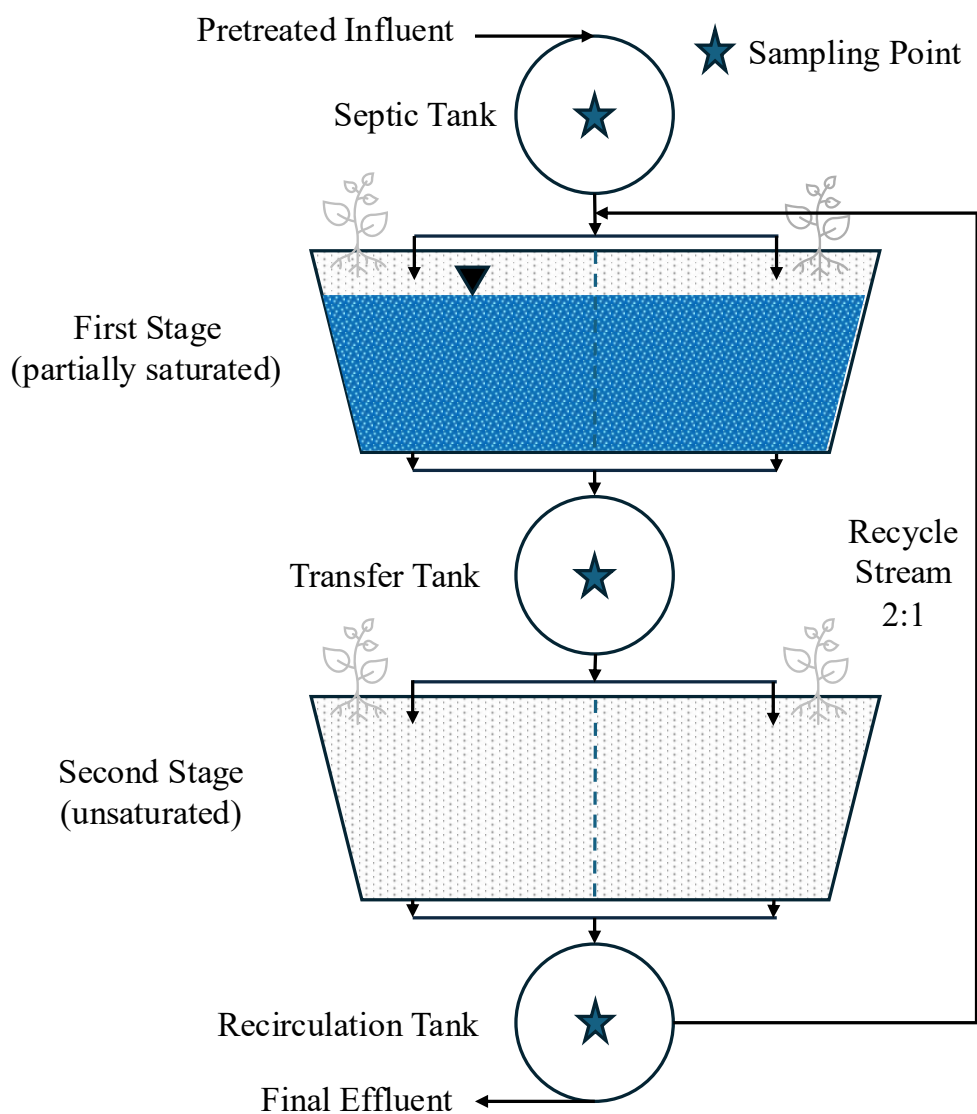


Figure 2: Bridger Bowl pilot scale vertical flow treatment wetland flow schematic. Circles represent intermediate collection tanks. Grab sampling points are denoted by stars.

Contaminants of Emerging Concern Sampling

Liquid wastewater grab samples for CEC analysis were collected from the septic tank, transfer tank, and recirculation tank (Figure 2) three times per week (Monday, Wednesday, Friday) once per month across the operational season (sampling dates shown in Table S7). A total of 30 unique samples were collected in duplicate or triplicate from the 3 tanks over 10 sampling days. Samples were pipetted at 2 mL from a triple-rinsed HDPE dipstick directly from the sampling tank into 4 mL amber vials. Lab blanks were prepared using DI water at the same volume with a pipette and the same amber vials. All samples were transported in coolers with snow/ice and immediately frozen in the amber vials, where they were stored frozen until shipping on dry ice for analysis.

Solid samples were taken on two sampling days from both the gravel media ($n = 6$) and a pure biofilm ($n = 3$) in the first stage and the sand media ($n = 3$) in the second stage to investigate the presence of CECs associated with the wetland media and biofilm. An in-situ sampling method was used (50), where microbial samplers were constructed from stainless steel mesh rolled into 5 cm long tubes and fitted with PVC endcaps. Stainless steel eyebolts (15 cm) were threaded through the samplers and connected to 1.8 m paracord. The samplers were sterilized with 10% bleach and 2.5 g/L sodium thiosulfate solutions before being filled with media from their respective stages using a sterilized shovel. Samplers were placed into 5 cm by 1.5 m PVC pipes with 0.95 cm bores spaced every 5 cm along a 60 cm length, allowing wastewater to flow into the sampling ports. During sampling, the samplers were removed from the wetland, transported in sterile bags, and frozen. Samples were lyophilized for several days under vacuum (~ 0.015 mTorr, -50 °F) until all water was removed. Samplers were installed in November 2021 and five were collected in March 2022 during the operating season and four were collected in September 2022, while the system was not receiving wastewater. Additionally, three solid

samples of a biofilm that formed on the outside of the samplers were collected from the first stage and placed into a sterile sampling bag (Whirl-pak®). These biofilm samples were transported identically to the granular media samples.

Both liquid and solid samples were shipped to Aarhus University, Denmark for analysis, where they were analyzed for CECs using high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS).

Analytical Methods for Contaminants of Emerging Concern

The wastewater samples were prepared by pipetting 1,800 μ L of each sample into an HPLC vial and centrifuging at 4,000 g (Hermle Labortechnik GmbH Z 206 A) for 7 minutes to settle the particles. Subsequently, 900 μ L of each supernatant was transferred into a new HPLC vial and mixed with 100 μ L of a methanolic internal standard solution.

The lyophilized media and biofilm samples were prepared by weighing 0.3 g of each sample into a 10 mL vial, followed by a sequential 3-step extraction using an ultrasonic bath for 15 minutes with each of the following solvents: 1) 10 mL of methanol (0.2% formic acid), 2) 10 mL of a 50:50 methanol and water mixture (0.2% formic acid), and 3) water (0.2% formic acid). After each extraction step, the media and biofilm sample was centrifuged, and the supernatant was collected. The three supernatants were combined in a single vial, vortexed, and 1,800 μ L of the combined supernatant was pipetted into an HPLC vial and centrifuged under the same conditions as the wastewater samples. 900 μ L of each supernatant was transferred into a new HPLC vial and mixed with 100 μ L of a methanolic internal standard solution.

The wastewater and media and biofilm supernatant samples were analyzed by injecting 20 μ L of the mixtures in duplicates, into a high-performance liquid chromatography (HPLC) system (Agilent 1290, Waldbronn, Germany) coupled with tandem mass spectrometry (Sciex Qtrap

5500, LC-MS/MS, Framingham, MA, USA). The chromatographic separation was conducted with a solvent flow rate of 250 $\mu\text{L}/\text{min}$ using a Synergi Polar-RP column (150 $\times$ 2 mm, 4 μm particle size; Phenomenex, Torrance, CA, USA) thermostated at 20°C. The data was treated with software SCIEX OS 3.3. Detailed information, including chromatographic retention times, precursor and product ions, compound-specific optimized MS parameters, and details about analytical and internal standards, can be found in (51) together with the detailed figures of the merit of the method. The full list of screened compounds can be found in Table S4. The limits of quantification (LOQ) and limits of detection (LOD) for aqueous samples can be found in Figure S8 and Figure S9.

Data Analysis and Calculations

System Flow Definitions and Dilutions. The influent contaminant concentrations to the first stage were calculated using the dosed 2:1 recycle-to-septic flow ratio for the 2022-23 season. Thus, two system influent concentrations are defined: “raw influent”, which refers to the septic tank concentration, and “first stage influent”, which refers to the weighted average of recycle and septic applied to the first stage. The measured concentration in the transfer tank, also called midpoint, was defined as both the first stage effluent and the second stage influent. The measured concentration in the recirculation tank was defined as the second stage effluent and was used in calculating the first stage influent.

In this study, grab samples from the septic, transfer, and recirculation tanks on one day were assumed to be paired. However, due to low concentrations of CECs, this assumption may affect the analysis of specific compounds that appear intermittently or were found to be highly variable throughout the season.

Contaminant Quantification and Removal. Percent and mass removal were calculated for each contaminant at each stage and for the overall system using paired samples from the same date. Stage percent removal was normalized to the influent of the stage, with first-stage removal based on the mixed influent concentration to determine removal across the cell, and system removal based on the raw (septic) influent vs. effluent.

To ensure conservative estimates of removal percentages, sample concentrations below the LOD were replaced with the LOD value, and sample concentrations between the LOD and LOQ values were replaced with the LOQ value. Estimated removal rates were indicated as achieving at least the estimated value using " $\geq$ ". In quantification of the number of detections, values less than the LOQ but greater than the LOD were considered detections, while values less than the LOD were considered non-detects.

A signal to noise ratio (SNR) was used to filter compounds for analysis of removal by stage. The SNR was defined as the concentration of the compound divided by the LOD. To compare removal between the first and second stages, compounds were filtered to include only those with influent concentrations in both stages that were at least three times the compound's LOD ($\text{SNR} > 3$). Compounds that were fully removed in the first stage were discarded from the comparison based on SNR. The screening ensured that only compounds with a sufficiently high influent load were compared across stages and provided confidence that decreasing concentrations across the system were due to quantifiable TW removal.

The ecotoxicity of each compound was assessed using freshwater predicted no effect concentrations (PNECs). PNECs provide a ranking of the relative ecotoxicity of each compound, based on each compound's effect on three freshwater trophic levels of aquatic organisms (52,53). PNECs for compounds previously detected in wastewater treatment applications were taken from

a compiled list (52). Additional values were found in the NORMAN ecotoxicology database from experimental data when possible, and from quantitative structure-activity relationship predictions in cases of unavailable or insufficient data (54). PNECs were compared to the influent and effluent concentration of each compound as a risk quotient (RQ), with RQs greater than 1 indicating potential toxicity to aquatic life based on the freshwater PNEC. A dilution factor of 10 to the receiving waters is assumed for most PNEC applications (53), and since the Bridger VFTW is discharged to groundwater through a drain field, the cutoff of $RQ < 1$ is arbitrary, and used for comparing risk between compounds and sampling locations in this analysis. This approach using RQs relative to PNECs for wastewater is used in a similar study for pharmaceuticals (53), and is described by the EPA for ecological risk assessment (55) and by a recent publication on treatment of CECs in wastewater (56).

Results and Discussion

Conventional Contaminants

Data for conventional wastewater contaminants for the 2022-23 season (Table 1) show concentrations and removal consistent with previous operational seasons (6,57). Mean influent concentrations show high values of COD (829 mg/L) and $\text{NH}_3\text{-N}$ (193 mg/L) typical of the high-strength wastewater at Bridger Bowl. Average seasonal removal of COD (97%), $\text{NH}_3\text{-N}$ (98%), TN (70%) demonstrate high performance consistent with previous seasons and the design parameters. Concentrations measured in the septic tanks are diluted with recycled water before application to the first stage, which primarily removes COD, suspended solids, and TN, while the second stage primarily removes $\text{NH}_3\text{-N}$. Decreasing concentrations between the measured septic tank concentrations and transfer tank concentrations are due to these dilution effects in addition to any removal through the first stage.

Table 1: Average concentrations and removal of conventional contaminants at Bridger Bowl during the study period. TN is calculated as the sum of $\text{NH}_3\text{-N}$, NO_3^- , and NO_2^- . Concentrations represent season average $\pm$ standard error. Percent removal represented average.

Parameter	COD	$\text{NH}_3\text{-N}$	TN	$\text{PO}_4\text{-P}$
Septic Tank (mg/L)	829 ± 10.5	193 ± 1.9	193 ± 1.9	14.1 ± 1.5
Transfer Tank (mg/L)	66.0 ± 3.0	47.8 ± 1.6	59 ± 1.9	9.9 ± 1.1
Recirculation Tank (mg/L)	27.8 ± 1.7	3.7 ± 1.0	57 ± 1.5	9.1 ± 1.0
Removal (%)	96.6 ± 0.2	98.1 ± 0.5	70.5 ± 0.8	32.9 ± 3.4
Number of Samples	26	26	26	18

The total hydraulic retention time of the system was estimated to be just over one day. Most of this time occurred in the saturated zone of the first stage. The first stage was estimated to have a 14-hour retention time, based on of the assumption of total volume of the saturated zone to be 34 m^3 with 30% porosity, meaning that each cell contained about 1,300 gallons (about 5 m^3) of water. Flow through the unsaturated second stage was estimated to take approximately thirty minutes based on observation of the effluent weir. Retention in the collection tanks between each stage means that the effluent of several doses was mixed.

Contaminants of Emerging Concern

Classification and Quantification. Of the 74 compounds that were screened, 38 were identified above their LOD in at least one sample. Apart from N,N-diethyl-meta-toluamide (DEET), an active ingredient in insect repellent products (58), all detected compounds were pharmaceuticals. The detected compounds, their pharmaceutical use classes, LODs, LOQs, and the number of detections across the ten sampling days can be found in Table S5.

The anti-infective agents use class had the greatest number of detected compounds (7 detected compounds out of 38 total), followed by antiepileptics and psychoanaleptics (6), stimulants (5), and beta-blockers (4). Other detected compound classes included antihistamines

(3), contrast agents (3), hypertensives (2), lipid-lowering agents (2), local anaesthetic (2), anti-inflammatory agents (1), diuretics (1), and other (1, DEET).

Of the 38 compounds detected, 18 were detected in all three sampling points on all ten sampling days. Six compounds (citalopram, azithromycin, trimethoprim, codeine, THC-COOH, and amphetamine) were detected in the influent each day but were never detected in the effluent. Azithromycin was also never detected in the mid-stage. Clarithromycin was never detected in the effluent, but was only detected 7 times in the influent. Clindamycin and oxcarbazepine were not detected in the influent, but were detected at least once downstream (mid stage and/or effluent).

Season averages and ranges for the three sampling points are shown in Table 2. Many compounds demonstrated substantial reductions in concentration from the raw influent to the final effluent, particularly those with high influent concentrations. Several compounds were reduced to below the LOD or LOQ in the final effluent. The wide range in influent concentrations for some compounds may also reflect variability in wastewater composition. When assessing reductions, it is noted that in addition to mass removal occurring in the system, there is a difference in concentration between the septic and mixed first-stage influent due to dilution of 2:1 by recycled water.

Caffeine (211 µg/L) and gabapentin (111 µg/L) had the highest average influent concentrations for the operational season, at least one order of magnitude higher than any other compound. Lamotrigine (21 µg/L), amoxicillin (7 µg/L), fexofenadine (7 µg/L), THC-COOH (5 µg/L), and iopamidol (5 µg/L) also had relatively high concentrations in the raw influent. On average, 11 compounds were removed to levels below their LOQ in the final effluent, with 6 of

these compounds removed to levels below their LOD. Clindamycin and oxcarbazepine were not detected in the raw influent but were detected further in the system at levels near their LOQ.

Table 2: Season average (minimum, maximum) measured concentrations and average system removal of detected compounds at grab sample points. Compounds <LOD or <LOQ in the influent were not reported for removal due to distortion by these limits. Compounds <LOD or <LOQ in the effluent were estimated conservatively using the upper limit and are indicated by “ $\geq$ ”.

Pharmaceutical Class	Compound	Septic Tank Average (Min, Max) ($\mu\text{g/L}$)	Transfer Tank Average (Min, Max) ($\mu\text{g/L}$)	Recirculation Tank Average (Min, Max) ($\mu\text{g/L}$)	System Removal Average
Antiepileptics and psycho-analeptics	Gabapentin	111 (100, 136)	102 (87.7, 115)	102 (88, 117)	7%
	Carbamazepine	0.16 (0.03, 0.29)	0.14 (0.07, 0.21)	0.13 (0.07, 0.22)	1%
	Venlafaxine	0.88 (0.68, 1.26)	0.19 (0.13, 0.21)	0.07 (0.06, 0.08)	92%
	Citalopram	1.08 (0.91, 1.21)	0.06 (0.06, 0.07)	<0.01 (LOD)	$\geq 99\%$
	Oxcarbazepine	<0.1 (LOD)	<0.1 (LOD)	0.22 (0.10, 0.42)	-
	Lamotrigine	20.9 (13.3, 25.3)	14.2 (10.3, 16.3)	11.2 (7.58, 13.4)	46%
Antihistamines	Cetirizine	2.74 (1.92, 3.72)	1.27 (1.04, 1.62)	0.70 (0.58, 0.88)	74%
	Fexofenadine	7.26 (4.19, 9.17)	2.25 (1.29, 2.78)	0.33 (0.26, 0.42)	95%
	Ranitidine	0.27 (0.05, 0.46)	<0.05 (LOD)	<0.1 (LOQ)	$\geq 59\%$
Antihypertensives	Losartan	2.83 (2.22, 3.37)	1.08 (0.88, 1.23)	0.35 (0.23, 0.43)	88%
	Valsartan	3.66 (2.72, 5.89)	1.14 (0.83, 1.85)	0.25 (0.13, 0.39)	93%
Anti-infective agents	Sulfadiazine	0.04 (0.03, 0.06)	0.04 (0.03, 0.05)	<0.02 (LOQ)	55%
	Sulfamethoxazole	1.60 (0.51, 2.31)	0.93 (0.44, 1.37)	0.41 (0.06, 0.69)	77%
	Trimethoprim	0.39 (0.18, 0.63)	0.05 (0.02, 0.09)	<0.01 (LOD)	$\geq 97\%$
	Clindamycin	<0.05 (LOD)	<0.1 (LOQ)	0.15 (0.10, 0.21)	-
	Clarithromycin	0.24 (0.05, 1.13)	0.11 (0.05, 0.29)	<0.05 (LOD)	$\geq 49\%$
	Azithromycin	4.47 (3.54, 6.81)	<1 (LOD)	<1 (LOD)	$\geq 77\%$
	Amoxicillin	7.32 (5.11, 8.49)	3.61 (3.25, 4.13)	2.47 (2.11, 2.92)	66%
Anti-inflammatories	Diclofenac	0.15 (0.11, 0.19)	0.11 (0.09, 0.13)	0.10 (0.06, 0.12)	34%

Table 2 Continued.

Pharmaceutical Class	Compound	Septic Tank Average (Min, Max) (µg/L)	Transfer Tank Average (Min, Max) (µg/L)	Recirculation Tank Average (Min, Max) (µg/L)	System Removal Average
Beta-blockers	Propranolol	0.24 (0.20, 0.28)	0.05 (0.04, 0.06)	<0.02 (LOQ)	≥ 92%
	Atenolol	0.27 (0.18, 0.37)	0.05 (0.04, 0.06)	0.03 (0.03, 0.03)	89%
	Metoprolol	0.62 (0.39, 0.80)	0.29 (0.22, 0.34)	0.19 (0.16, 0.23)	68%
	Sotalol	0.10 (0.02, 0.28)	0.07 (0.05, 0.10)	0.06 (0.05, 0.09)	49%
Contrast agents	Diatrizoic acid	0.25 (0.10, 0.55)	0.34 (0.10, 0.84)	0.37 (0.10, 0.96)	36%
	Iopamidol	5.33 (0.31, 10.6)	11.7 (0.30, 43.6)	12.9 (0.28, 51.2)	70%
	IoHexol	0.87 (0.25, 2.87)	<0.5 (LOQ)	<0.5 (LOQ)	≥ 31%
Diuretics	Hydrochlorothiazide	<2 (LOQ)	<2 (LOQ)	<2 (LOQ)	-
Immunosuppressant	Mycophenolic Acid	3.16 (1.75, 4.40)	0.63 (0.20, 1.22)	0.08 (0.03, 0.17)	97%
Lipid-lowering agents	Gemfibrozil	0.87 (0.10, 2.41)	0.57 (0.20, 1.33)	0.44 (0.10, 1.13)	8%
	Rosuvastatin	1.25 (0.85, 1.52)	0.49 (0.41, 0.59)	<0.2 (LOQ)	≥ 83%
Local anesthetic	Lidocaine	0.16 (0.08, 0.55)	0.14 (0.10, 0.24)	0.13 (0.10, 0.18)	6%
	Codeine	0.10 (0.08, 0.12)	0.04 (0.03, 0.04)	<0.02 (LOD)	≥ 79%
Stimulants	Caffeine	211 (179, 237)	42.3 (34.6, 53.1)	1.50 (0.79, 2.23)	99%
	Benzoylcegonine	3.23 (1.93, 5.35)	1.16 (0.50, 2.15)	<1 (LOQ)	≥ 77%
	THC-COOH	5.27 (4.12, 6.58)	1.01 (0.56, 1.30)	<1 (LOQ)	≥ 94%
	Amphetamine	2.04 (1.66, 2.34)	2.49 (2.08, 3.23)	<0.05 (LOD)	≥ 98%
	Methamphetamine	0.59 (0.27, 1.01)	0.07 (0.05, 0.11)	<0.02 (LOD)	≥ 95%
Other	DEET	0.07 (0.05, 0.10)	0.06 (0.05, 0.08)	<0.05 (LOQ)	≥ 19%

Concentrations for some compounds, especially those at higher values such as caffeine and gabapentin, remained relatively consistent across sampling events, while others with lower concentrations such as atenolol and diatrizoic acid, exhibited high variability. Variability in the raw influent may reflect daily changes in influent wastewater composition, particularly for low-concentration compounds influenced by sporadic use from a small number of visitors to the ski

area. Variability in effluent concentrations may be due to changes in operations or environmental conditions, such as temperature. While no clear trends in removal efficiency by compound class are apparent, 20 of the 38 compounds were removed on average 70% or more across the system.

Samples were collected during several days of the week and across the ski season to capture potential short- and long-term temporal trends in wastewater composition and its treatment conditions. No clear temporal trends in CEC concentrations were observed, either seasonally or by day of the week. While wastewater flow and overall composition likely fluctuated during these periods, the holding tanks throughout the system and the low concentrations of these compounds likely helped buffer short-term flow variability, stabilizing influent concentrations over time.

The freshwater PNEC value for each compound, along with RQs of the PNEC to the raw influent and the effluent concentrations, were used to assess ecotoxicity across the system. PNECs are not suitable as design treatment parameters (52) but can determine each compound's relative ecotoxicity to assess the freshwater impact by compound. These values are presented in Table S6. There is a reduction in the number of compounds with RQs > 1 from the raw influent to the final effluent, indicating a decrease in potential ecological risk following treatment in the TW.

Citalopram had the highest RQ in the influent, where it was >2 orders of magnitude higher than all other compounds. It also had the highest effluent RQ but was greatly reduced. Of 20 compounds with raw influent RQs > 1 , 7 compounds (clarithromycin, codeine, diclofenac, mycophenolic acid, propranolol, THC-COOH and venlafaxine) were reduced to final effluent RQs < 1 . Atenolol, DEET, diatrizoic acid, iohexol and iopamidol had RQs < 0.00 in both the influent and effluent, indicating negligible risk. Some compounds still exhibited high effluent

RQs, including amoxicillin (32), azithromycin (11), citalopram (133), clindamycin (10), lidocaine (51), and ranitidine (39). Clindamycin was the only compound to show an increase in risk following treatment. In total, 13 compounds had effluent RQs >1. For compounds with potential ecological risk in the influent, all but clindamycin demonstrated a reduction in relative risk following treatment by the system.

Removal by Stage. CEC removal across each stage of the TW was compared using influent and effluent concentrations. To do this accurately, dilution caused by mixing of the recycle and septic influent streams in the first stage was first accounted for. The calculated diluted first-stage influent was then compared with measured midpoint concentrations to determine removal across the first stage. Removal across the second stage was determined using measured midpoint and effluent concentrations. However, using this approach, the sum of removal in the two stages does not equal the total system removal, as accounting for dilution in the first stage leads to an underestimation when compared to removal calculated using the raw influent concentrations without the recycle stream.

Only compounds present at concentrations three times or greater than their LOD (SNR > 3) in the applied influent to both stages were considered, as removal could be calculated in both stages. Of the 38 compounds found in the system, 27 passed this screening. Compounds excluded were present at negligible or low concentration entering the second stage, and included oxcarbazepine, ranitidine, sulfadiazine, clindamycin, clarithromycin, azithromycin, iohexol, hydrochlorothiazide, codeine, benzoylecgonine, and DEET. Percent removal by stage normalized to the respective influent for compounds that passed screening are shown in Figure 3.

Percent Removal Normalized by Stage

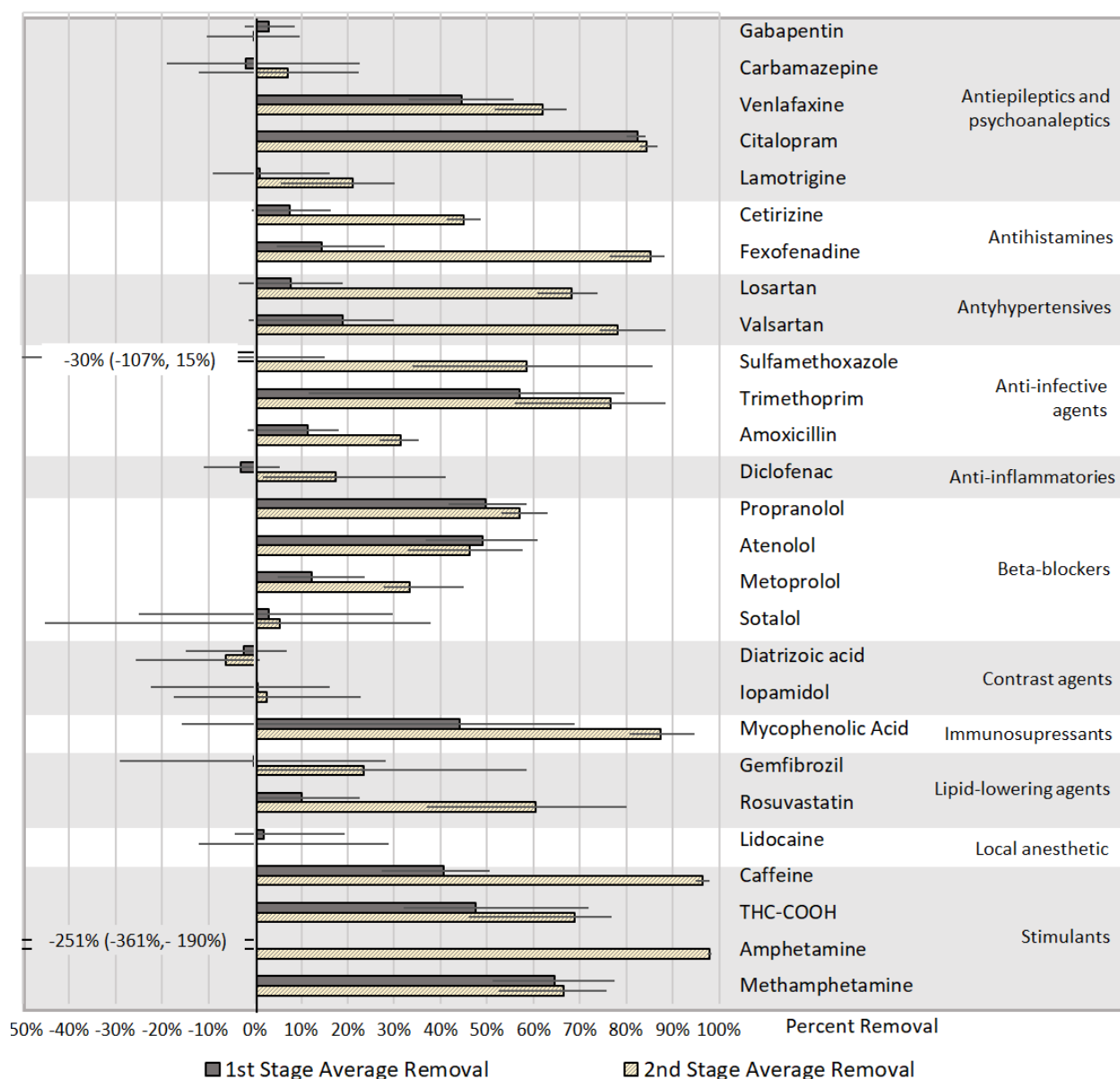


Figure 3: Percent removal by stage of compounds detected in the applied influent (SNR >3) of both stages at the Bridger Bowl VFTW. Positive bars indicate removal, while negative bars represent an increase across a stage. Error bars represent the range of minimum and maximum removal across the season. For sulfamethoxazole and amphetamine, values exceed the chart bounds and are given as text.

Most compounds in this analysis showed higher removal in the second stage, suggesting the unsaturated environment played a larger role in reducing CEC concentrations. A few compounds showed higher removal in the first stage than the second, including atenolol,

diatrazoic acid, gabapentin, and lidocaine. Of these, atenolol is the only compound showing high removal through the system. Benzoylgonine, which did not pass screening for this comparative analysis as described previously was also primarily reduced in the first stage. Removal in the first stage could occur in either the saturated zone, which is thought to be anoxic, or the unsaturated zone above it, which is likely aerobic. Removal in the second stage was assumed to be fully aerobic. Given the first stage's longer residence time and different influent concentrations to each stage, no direct comparisons between anoxic or aerobic removal efficiency can be made. However, the compounds with higher removal in the first stage may be responsive to the extended residence time and anoxic degradation. In contrast, the second stage may remove compounds that are responsive to aerobic degradation, but its lower residence time provided less interaction between compounds and various removal processes.

Increase in Concentration of Contaminants in the System. Some CECs showed an increase in concentration across individual stages or the system. Many of these compounds were present at low concentrations and demonstrated very low mass increases. Compounds that showed an increase in mass greater than the compounds' LOD and a percent increase greater than 5% are presented.

Compounds increasing across the first stage included sulfadiazine (51%, +0.01 µg/L increase; LOD of 0.01 µg/L), sulfamethoxazole (30%, +0.13 µg/L; 0.02 µg/L), and amphetamine (251%, +1.8 µg; 0.05 µg/L). The small concentration increase for sulfonated compounds suggests that they are recalcitrant and are likely not produced, as poorly removed compounds may be recirculated several times. However, long residence times in intermediate tanks resulted in well-mixed doses, and the assumption for paired samples is valid at concentrations high enough to be undistorted by detection and quantification limitations. Deconjugation is common

for antibiotics, including sulfadiazine and sulfamethoxazole. In wastewater, especially under anaerobic conditions, antibiotics may deconjugate from metabolites formed in the body, reforming the parent compound (59–61). Since sulfadiazine and sulfamethoxazole increase slightly in the first stage, deconjugation may occur. The second stage removed sulfadiazine and sulfamethoxazole (0.02 µg/L, 0.52 µg/L), leading to overall treatment in the system.

In the second stage, clindamycin (68%, +0.06 µg increase; LOD of 0.05 µg/L), and oxcarbamazepine (118%, +0.12 µg; 0.10 µg/L) increased in concentration. These again indicate recalcitrant properties with very small mass increases. Compounds increasing across the system were clindamycin (191%, +0.10 µg/L; 0.05 µg/L), diatrizoic acid (36%, +0.12 µg; 0.1 µg/L), iopamidol (70%, +7.6 µg; 0.15 µg/L), and oxcarbamazepine (118%, +0.12 µg; 0.1 µg/L).

The relatively high concentration increases of amphetamine in the first stage and iopamidol across the system were investigated in literature. In wastewater treatment, biodegradation of methamphetamine may form amphetamine (62–65). Amphetamine is also known to be a metabolite of other drugs and medications, and these precursors were mostly not screened in this study (66). Methamphetamine in the first stage decreased on average by 0.14 (seasonal range 0.05-0.27) µg/L, while amphetamine increased by an average of 1.78 (1.36-2.53) µg/L, more than ten times higher than the methamphetamine being removed. In the second stage, amphetamine decreased by 2.44 (2.03-3.18) µg/L and methamphetamine decreased by 0.05 (0.03-0.06) µg/L, showing effective removal of both compounds, even if amphetamine is produced in the system due to degradation of other compounds.

Iopamidol, an iodinated contrast agent, shows more pronounced temporal variability than other compounds, and on average increased in mass across the system. Iohexol, another iodinated contrast agent present in the system, is more structurally complex than iopamidol, and

degrades through several distinct pathways (67–72). These compounds are directly applied to the skin in hospital settings, and therefore may be present in the system with very few users, increasing their variability and making their trends difficult to capture without a targeted sampling campaign.

Contaminants of Emerging Concern in Solid Media Samples. A comparison between the relative abundance of CECs in aqueous and solids (media and biofilm) samples is presented here. Due to the limited quantity of solid samples collected, a direct comparison between solid and aqueous samples was not feasible. However, a ranking of relative contents of CECs in solid media compared to aqueous samples offers insight into the accumulation of these compounds in different media types, including pure biofilm, mixed gravel media, and mixed sand media samples. By examining the relative concentrations of compounds within these samples, their retention, potential for biodegradation, and overall behavior in the system may be better understood.

Rankings were developed by averaging solid and aqueous samples by category and assigning a value of 1 to the most concentrated compound in the category, 2 to the next, and so forth in descending order of concentration. Categories included: first-stage aqueous, second-stage aqueous, pure biofilm (taken from the saturated zone in the first stage), mixed gravel media (from the saturated and unsaturated first-stage zones) and mixed media sand (from the unsaturated second stage). Sampling dates and locations within a stage for the solids categories were averaged. Aqueous samples were ranked as the average influent concentration for the respective stage, to best represent the water encountered by the media. Overall average results for aqueous and solid samples by category can be seen in Table 3.

Table 3: Solid and aqueous sampling locations and media categories ranked by relative CEC contents. A low ranking indicates high concentrations (dry weight) of a compound as compared to other compounds within that category. Aqueous samples were ranked by influent wastewater to the respective stage. ND indicates no numerical values, either <LOQ or <LOD on average.

Compound	1 st Stage Liquid	2 nd Stage Liquid	Pure Biofilm	Mixed Gravel Media	Mixed Sand Media
4-Methyl-1H-benzotriazole	ND	ND	ND	12	2
Amoxicillin	5	5	ND	ND	ND
Amphetamine	16	6	ND	ND	ND
Atenolol	33	33	ND	ND	20
Azithromycin	7	14	8	5	6
Benzotriazole	ND	ND	6	9	10
Benzoylcegonine	10	10	11	ND	ND
Caffeine	2	2	2	2	4
Carbamazepine	28	23	ND	17	18
Cetirizine	12	9	ND	ND	ND
Ciprofloxacin	ND	ND	1	ND	ND
Citalopram	20	31	9	3	12
Clarithromycin	31	26	ND	16	15
Clindamycin	30	28	ND	ND	ND
Codeine	37	38	ND	ND	ND
DEET	36	32	ND	ND	ND
Diatrizoic acid	23	20	ND	ND	ND
Diclofenac	32	25	ND	ND	ND
Fexofenadine	6	7	5	4	1
Gabapentin	1	1	3	8	5
Gemfibrozil	17	17	ND	ND	ND
Hydrochlorothiazide	9	8	ND	ND	ND
Iohexol	19	19	ND	ND	ND
Iopamidol	4	4	18	ND	17

Table 3 Continued.

Compound	1st Stage Liquid	2nd Stage Liquid	Pure Biofilm	Mixed Gravel Media	Mixed Sand Media
Irbesartan	ND	ND	10	13	9
Lamotrigine	3	3	ND	ND	ND
Lidocaine	27	24	ND	19	19
Losartan	13	12	12	14	11
Methamphetamine	24	29	16	18	ND
Metoprolol	22	21	ND	20	21
Miconazole	ND	ND	ND	11	ND
Mycophenolic Acid	14	16	14	ND	ND
Oxcarbazepine	25	27	ND	ND	16
Propranolol	34	35	15	7	7
Ranitidine	26	36	ND	ND	ND
Rosuvastatin	18	18	ND	ND	ND
Sertraline	ND	ND	4	1	3
Sotalol	35	30	ND	ND	ND
Sulfadiazine	38	37	ND	ND	ND
Sulfamethoxazole	15	15	13	ND	13
THC-COOH	8	13	7	6	ND
Trimethoprim	29	34	17	15	14
Valsartan	11	11	ND	ND	ND
Venlafaxine	21	22	ND	10	8

Gabapentin, caffeine, and fexofenadine ranked highly in both aqueous and solid samples, suggesting continuous input of these compounds into the system. The relative abundance of CECs in the first and second stage aqueous samples was similar for most compounds, with gabapentin, caffeine, and lamotrigine ranking the highest in both stages. When using the applied

first stage influent concentrations for ranking, gabapentin ranked higher than caffeine due to its recirculated concentrations.

Some compounds, including amoxicillin, iopamidol, and lamotrigine ranked highly in aqueous samples but ranked low or were not detected in the solid-phase samples. In contrast, sertraline, propranolol, and benzotriazole were among the highest-ranked compounds in solids despite low aqueous-phase presence. Ciprofloxacin was the highest ranked compound in biofilm samples and is not found elsewhere in the system, demonstrating high sorptive behavior corroborated by literature (61). The mixed sand media had 21 detected compounds, mixed gravel had 20, and pure biofilm had 18. While many compounds were consistent in the solid samples, some were variably detected between sand, gravel, and biofilm. Additional samples might determine variability due to temporal or location (i.e. TW stage) trends.

These results suggest that sorption may play a role in controlling transformation processes, although reported partitioning coefficients did not always correspond with observed behavior. Compounds exhibiting strong partitioning to biofilm or media were retained within the system, aiding removal from the aqueous phase. Because of the seasonal operations at Bridger, there is an opportunity for further degradation of compounds retained in the solid phase during the rest of the period. This demonstrates the importance of collecting solid-phase samples in intermittently loaded systems to fully consider the fate of compounds within the system. Concentrations measured in the solid samples (porous media and biofilm) may be referenced in Appendix A.

Comparison to Other Systems. Making direct comparisons to other systems for all detected compounds is challenging due to the wide range of operational parameters and system designs. However, comparisons are made for compounds that are commonly reported in reviews

and were also detected in the Bridger system. Eleven compounds screened in this study have reported typical concentrations for surface water and municipal wastewater (see Table S10) (56).

Every compound in the Bridger TW raw influent fell below the reported surface water and municipal wastewater concentrations, except for azithromycin and caffeine. Azithromycin fell between the surface water and municipal wastewater values, while caffeine exceeded the high range of reported values. Ciprofloxacin, which was reported at very high values in surface waters, was not detected in the Bridger raw influent but was found in pure biofilm samples. Because the Bridger system serves a unique population and does not collect wastewater from the typical population range or hospitals, lower concentrations of most pharmaceuticals and high concentrations of caffeine were expected.

Based on referenced removal for compounds in TWs (56), sulfamethoxazole, trimethoprim, amoxicillin, and caffeine exhibited higher removal than reported values, while diclofenac, metoprolol, and carbamazepine showed lower removal. One study with a similar two-stage vertical flow TW and a WWTP alongside the TW (33) reported three of the same compounds detected at Bridger Bowl. Diclofenac exhibited removal of 53%, 77%, and 34% removal and carbamazepine had -12%, -1%, and 1% removal for the WWTP, VFTW, and Bridger systems, respectively, while caffeine was removed 99% or greater in all three systems. This system reached a minimum water temperature of 6 °C, with average monthly wastewater temperatures hovering between 10-20 °C, significantly warmer than the Bridger TW wastewater. A review reported average removal values for VFTWs for diclofenac (58% average, compared to 34% Bridger), caffeine (94%, 99%), sulfamethoxazole (65%, 77%), and carbamazepine (-8.5%, 1%) (45).

These comparisons demonstrate the variability in pharmaceutical concentrations and removal across wastewater treatment systems. The Bridger TW had high removal of caffeine and sulfamethoxazole. Lower removal of diclofenac and metoprolol indicate limitations in treatment of more recalcitrant compounds, consistent with findings in other VFTWs. Overall, these results suggest that the Bridger system performs comparably or superior to similar systems for certain compounds, even at its extremely low water temperatures of 2-4 °C, while highlighting the need for more comprehensive reporting of compounds to allow for broader comparisons.

Conclusions

This study investigated the removal of CECs in a two-stage VFTW, offering a first look at performance in a cold-climate. The study assessed overall system performance and compared the two stages in the system and the aqueous and solid phases of CECs in each stage. Key findings include:

- The system achieved significant reduction of most detected compounds, including conventional contaminants and CECs, even under extremely cold conditions (wastewater 2-4 °C). The system also reduced the risk quotients of measured compounds in the system.
- The second, unsaturated stage of the TW demonstrated higher removal for most compounds present at concentrations high enough to analyze, despite its shorter hydraulic retention time, indicating that these compounds were more effectively treated in aerobic conditions.
- The ranking of compounds by relative abundance in aqueous and solid samples indicated that certain compounds were more susceptible to retention by solid materials. This

ranking approach is a valuable first step in understanding how CECs are retained within the system, especially for intermittently loaded TWs.

- The Bridger system demonstrated removal comparable to or exceeding that of other VFTWs and conventional systems for many congruently reported compounds. Lower removal of recalcitrant compounds such as diclofenac and metoprolol highlights system limitations consistent with broader trends in TW performance. These findings support the potential for wider adoption of VFTWs as decentralized treatment options in cold-climates, particularly where conventional treatment is not feasible.

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CHAPTER FIVE

CONCLUSION

Overview

Vertical flow treatment wetlands (VFTWs) for municipal wastewater (WW) polishing and decentralized treatment are capable of treating both conventional contaminants and contaminants of emerging concern (CECs). Thus, these systems offer a solution to tightening effluent standards, budget constraints, aging systems, and rising public expectations. VFTWs combine physical filtration, sorption to porous media and biofilms, and microbial degradation in a compact footprint, resulting in high treatment of organic carbon, total nitrogen, and a growing list of reported CECs. Design parameters such as hydraulic loading rate, media selection, redox zoning, recirculation, and aeration drive both conventional pollutant and CEC removal. For example, engineering diverse redox conditions and recirculation to optimize nitrification simultaneously promotes cometabolic breakdown of aerobically biodegraded pharmaceuticals, while choosing media with strong phosphorus sorption capabilities may also boost sorptive removal of organic CECs.

Despite decades of European experience, U.S. guidelines and long-term system monitoring, especially regarding CECs, have lagged. Additionally, there is a global knowledge gap on the performance of established VFTWs for removing CECs in cold climates. A pilot-scale experimental study, located at the Bridger Bowl two-stage VFTW, directly addresses this gap. During the study period, the Bridger VFTW demonstrated its advanced WW treatment abilities, achieving removal of 96% COD, 98% ammonia-nitrogen, and 71% total nitrogen, at water temperatures of 2–4 °C. Of the 38 CECs detected in the Bridger VFTW WW, half were removed

over 80%, with the aerobic stage of the VFTW, which was designed for nitrification, showing higher removal for most compounds. Additionally, a media retention study revealed compound-specific sorption trends and demonstrated a novel approach to assessing sorption of CECs that are difficult to quantify and compare at trace levels.

Together, these outcomes confirm that well-configured VFTWs can perform advanced treatment of conventional contaminants and CECs in cold climates. The performance of the Bridger VFTW shows the resilience and reliability of these systems and provides valuable long-term performance data to support the broader use of VFTWs for cold-climate wastewater treatment, and the adoption of these systems in the U.S. Future research should explore the combination of anoxic and aerobic zones for CEC removal in cold climates, particularly across a wide range of contaminants. Some CECs may benefit from recirculating flows, multiple redox zones, or a longer residence time, especially those that may undergo stepwise treatment similar to nitrogen species. Investigating these variables could provide guidelines and targeted optimization of two-stage systems. Comparative studies are also recommended between two-stage VFTWs and other decentralized treatment options for CEC removal. Given the demonstrated performance of the Bridger VFTW over conventional WWTPs, and existing literature comparing WWTPs with other decentralized systems for conventional treatment, it is likely that TWs outperform many decentralized alternatives. With further validation in cold climates, design guidelines should be updated to include two-stage VFTWs for both nutrient and CEC removal.

Future Research

Although this thesis demonstrates that two-stage VFTWs can effectively remove CECs from high-strength WW in cold-climate conditions, several research gaps must be closed to

prove reliable performance and move toward adoption of these systems. First, inconsistent reporting of indicator compounds across CEC studies makes it difficult to compare performance. These indicator compounds vary from study to study and often are a result of local interests or regulations but make comparisons to different WW compositions and other regulated compounds difficult. Comprehensive screening and reporting may add workload to these studies but would enable meaningful comparisons despite varying local guidelines and regulations. Additionally, CEC transformation products are not well described, and removal of any given compound may result in an undesirable transformation product. Further investigation into CEC degradation and transformation pathways will strengthen screening lists and in turn ensure treatment goals are met. Also, little is known about the cold-adapted microbial communities in VFTWs, and their degradation of CECs. Across the treatment wetland field, more research into both microbial degradation of CECs and cold-climate-specific communities is needed.

More field trials are also recommended across various WW compositions and treatment scales, ranging from pilot- to full-scale systems, to validate cold-climate removal efficiencies. These expanded field trials should also systematically optimize hydraulic loading rates, mass loading rates, dose depths, redox zones, and media selection for CEC treatment in cold climates. Direct comparisons of CEC treatment in VFTWs to conventional wastewater treatment plants in cold climates are also missing from the literature. These side-by-side evaluations of different VFTW configurations and alternative systems could further clarify the advantages and trade-offs of each with regards to both conventional contaminants and CECs for cold-climate application.

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APPENDICES

APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 3

Supporting Information

This appendix contains supporting information referenced in Chapter 3 of this thesis.

Refer to Chapter 3 for clarification and methods regarding this data.

Table S4: List of screened compounds and their Chemical Abstract Service (CAS) numbers.

Compound	CAS #	Compound	CAS #
4-Methyl-1H-benzotriazole	29878-31-7	Gabapetin	60142-96-3
Amisulpride	71675-85-9	Gemfibrozil	25812-30-0
Amoxicillin	26787-78-0	Hydrochlorothiazide	58-93-5
Amphetamine	300-62-9	Ibuprofen	15687-27-1
Ampicillin	69-53-4	Iohecol	66108-95-0
Atenolol	29122-68-7	Iomeprol	78649-41-9
Azithromycin	83905-01-5	Iopamidol	60166-93-0
Azoxystrobin	131860-33-8	Iopromide	73334-07-3
Benzophenone	119-61-9	Irbesartan	138402-11-6
Benzotriazole	95-14-7	Isoproturon	34123-59-6
Benzoylcegonine	519-09-5	Lamotrigine	84057-84-1
Bicalutamide	90357-06-5	Lidocaine	137-58-6
Caffeine	58-08-2	Losartan	114798-26-4
Candesartan	139481-59-7	MDMA	64057-70-1
Carbamazepine	98-46-4	Mefenamic acid	61-68-7
Carbendazim	10605-21-7	Methamphetamine	537-46-2
Cetirizine	83881-51-0	Metoprolol	51384-51-1
Ciprofloxacin	85721-33-1	Miconazole	22916-47-8
Citalopram	59729-33-8	Mycophenolic acid	24280-93-1
Clarithromycin	81103-11-9	Olmesartan	144689-63-4
Clindamycin	18323-44-9	Oxazepam	604-75-1
Codeine	76-57-3	Oxcarbazepine	28721-07-5
DDAC-C10	7173-51-5	Phenazone	60-80-0
Diatrizoic acid	117-96-4	Propranolol	525-66-6
Diclofenac	15307-86-5	Propyphenazone	479-92-5
Diethyltoluamide (DEET)	134-62-3	Ranitidine	82530-72-1
Doxycycline	564-25-0	Rosuvastatin	287714-41-4
Eprosartan	133040-01-4	Roxithromycin	80214-83-1
Erythromycin	114-07-8	Sertraline	79617-96-2
Estrone	53-16-7	Simvastatin	79902-63-9
Fexofenadine	83799-24-0	Sotalol	3930-20-9
Fluopyram	658066-35-4	Sulfadiazine	68-35-9
Furosemide	54-31-9	Sulfamethizole	144-82-1

Table S4 Continued

Compound	CAS #	Compound	CAS #
Sulfamethoxazole	723-46-6	Trimethoprim	738-70-5
Sulfapyridine	144-83-2	Valsartan	137862-53-4
THC-COOH	56354-06-4	Venlafaxine	93413-69-5
Tramadol	27203-92-5		

Table S5: Detected compounds at the Bridger Bowl VFTW. Each number of detects is out of the 10 total sampling days.

Pharmaceutical Use Class	Compound Name	LOD (µg/L)	LOQ (µg/L)	Septic Tank Detects	Transfer Tank Detects	Recirculation Tank Detects
Antiepileptics and psychoanaleptics	Gabapentin	0.02	0.03	10	10	10
	Carbamazepine	0.01	0.02	10	10	10
	Venlafaxine	0.02	0.03	10	10	10
	Citalopram	0.01	0.02	10	10	0
	Oxcarbazepine	0.1	0.2	0	0	5
	Lamotrigine	2	3	10	10	10
Antihistamines	Cetirizine	0.03	0.1	10	10	10
	Fexofenadine	0.02	0.03	10	10	10
	Ranitidine	0.05	0.1	8	0	1
Antihypertensives	Losartan	0.02	0.03	10	10	10
	Valsartan	0.1	0.2	10	10	10
Anti-infective agents	Sulfadiazine	0.01	0.02	10	10	6
	Sulfamethoxazole	0.02	0.03	10	10	10
	Trimethoprim	0.01	0.01	10	10	0
	Clindamycin	0.05	0.1	0	8	10
	Clarithromycin	0.05	0.1	7	8	0
	Azithromycin	1	3	10	0	0
	Amoxicillin	1	2	10	10	10
Anti-inflammatories	Diclofenac	0.03	0.05	10	10	10
Beta-blockers	Propranolol	0.01	0.02	10	10	10
	Atenolol	0.01	0.01	10	10	10
	Metoprolol	0.05	0.1	10	10	10
	Sotalol	0.02	0.05	6	10	10
Contrast agents	Diatrizoic acid	0.1	0.2	6	6	6
	Iopamidol	0.15	0.25	10	10	10
	Iohexol	0.25	0.5	4	4	2
Diuretics	Hydrochlorothiazide	1	2	7	7	7
Immunosuppressants	Mycophenolic acid	0.03	0.05	10	10	5
Lipid-lowering agents	Gemfibrozil	0.1	0.2	7	10	8
	Rosuvastatin	0.1	0.2	10	10	7
Local anesthetic	Lidocaine	0.01	0.02	10	10	10
	Codeine	0.02	0.03	10	10	0
Stimulants	Caffeine	0.01	0.02	10	10	10
	Benzoylcegonine	0.5	1	10	7	4
	THC-COOH	0.3	1	10	10	0
	Amphetamine	0.05	0.1	10	10	0
	Methamphetamine	0.02	0.05	10	10	1
Not a pharmaceutical	N,N-Diethyl-meta-toluamide (DEET)	0.02	0.05	10	10	10

Table S6: Freshwater Predicted No Effect Concentrations (PNEC) and Risk Quotients (RQ) using raw influent and effluent contaminant concentrations. Bold cells indicate a RQ >1, suggesting potential ecotoxicity.

* Ecotoxicity of compound reduced from raw influent RQ > 1 to final effluent PNEC ratio < 1.

† Value not found in referenced literature.

Compound Name	PNEC (µg/L)	Raw Influent Risk Quotient	Final Effluent Risk Quotient
Amoxicillin	0.078 (1)	94	32
Amphetamine	25 (2)	0.08	0.00
Atenolol	128 (1)	0.00	0.00
Azithromycin	0.09 (1)	49	11
<i>Benzoyllecgonine</i> [†]	Not Found	NA	NA
Caffeine	1.2 (2)	176	1.25
Carbamazepine	0.5 (1)	0.32	0.26
Cetirizine	0.41 (2)	6.68	1.70
Citalopram	0.000075 (1)	14385	133
Clarithromycin*	0.06 (1)	3.97	0.83
Clindamycin	0.014 (1)	3.57	10
Codeine*	0.06 (1)	1.64	0.33
DEET	88 (2)	0.00	0.00
Diatrizoic acid	10000 (1)	0.00	0.00
Diclofenac*	0.1 (1)	1.45	0.95
Fexofenadine	200 (2)	0.04	0.00
Gabapentin	100 (1)	1.11	1.02
Gemfibrozil	0.15 (1)	5.77	2.95
Hydrochlorothiazide	100 (2)	0.02	0.02
Iohexol	1000000 (1)	0.00	0.00
Iopamidol	100000 0 (1)	0.00	0.00
Lamotrigine	8 (2)	2.62	1.40
Lidocaine	0.0026 1 (1)	61	51
Losartan	331 (1)	0.01	0.00
Methamphetamine	9.74 (2)	0.06	0.00
Metoprolol	0.1 (1)	6.18	1.94
Mycophenolic acid*	0.1 (1)	32	0.82
Oxcarbazepine	2.95 (2)	0.03	0.07
Propranolol*	0.1 (1)	2.44	0.20
Ranitidine	0.002 (1)	137	39
Rosuvastatin	1.8 (1)	0.69	0.11
Sotalol	13 (1)	0.01	0.00
Sulfadiazine	4.6 (1)	0.01	0.00
Sulfamethoxazole	0.118 (1)	14	3.44
THC-COOH*	0.62 (2)	8.51	0.48

Table S6 Continued.

Compound Name	PNEC (µg/L)	Raw Influent Risk Quotient	Final Effluent Risk Quotient
Trimethoprim	<i>10 (1)</i>	<i>0.04</i>	<i>0.00</i>
Valsartan	<i>85 (1)</i>	<i>0.04</i>	<i>0.00</i>
Venlafaxine*	<i>0.1 (1)</i>	8.79	<i>0.70</i>

Table S7: Sampling dates for micropollutants throughout the operational season. On each date, grab samples were collected in duplicate or triplicate from the septic, transfer, and recirculation tanks.

Dates Sampled

18 January, 2023
2 February, 2023
3 February, 2023
6 February, 2023
6 March, 2023
8 March, 2023
10 March, 2023
29 March, 2023
31 March, 2023
3 April, 2023

Table S8: Raw data for solid media samples. This list reflects all screened compounds for solid media samples. Values in compounds are as measured in 30 mL of solvent from extracted from their original total dry sample.

Sample ID	1	2	3	4	5	6	7	8	9	10	11	12		
Date	3/17/2022								9/21/2022					
Stage	2	2	1	1	1	1	1	1	2	1	1	1		
Cell	1	2	2	1	1	1	1	1	1	1	2	1		
Location	5	5	3	5	3	6	2	3	2	6	3	3		
Depth (inches)	6	6	6	24	24	24	24	24	6	6	24	24		
Sample Type	sand	sand	gravel	gravel	gravel	biofilm	biofilm	biofilm	sand	gravel	gravel	gravel		
Weight (g)	8.4	10.28	8.96	7.94	10.77	0.06	0.06	0.06	5.41	7.51	5.84	9.2		
Sample ID	LOD	LOQ	1	2	3	4	5	6	7	8	9	10	11	12
Compound	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
4-Methyl-1H-benzotriazole	0.3	1	1.35	1.66	0.86	<LOD	<LOD	<LOD	<LOD	<LOD	0.67	0.85	<LOD	<LOD
Amoxicillin	1.00	1.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Amphetamine	0.05	0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Atenolol	0.01	0.02	0.03	0.05	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Azithromycin	0.3	0.3	0.49	0.69	0.45	0.77	0.45	0.34	0.30	0.41	0.38	1.67	1.20	0.59
Azoxystrobin	0.05	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzotriazole	0.1	0.3	0.28	0.30	0.22	0.37	0.20	0.47	0.37	0.56	0.26	0.72	0.45	0.26
Caffeine	0.01	0.01	1.33	0.88	0.57	5.58	4.99	7.67	5.25	5.79	0.59	0.75	1.27	0.69
Candesartan	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	0.02	0.05	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.04	0.07	<LOD	<LOD
Carbendazim	30.00	30.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ciprofloxacin	10	10	<LOD	<LOD	<LOD	<LOD	<LOD	17.7	13.5	15.5	<LOD	<LOD	<LOD	<LOD
Citalopram	0.02	0.02	0.16	0.30	0.20	1.06	0.31	0.24	0.14	0.16	0.13	2.83	1.00	0.49
Clarithromycin	0.05	0.05	0.04	0.05	0.04	<LOD	<LOD	<LOD	<LOD	<LOD	0.08	0.06	0.03	<LOD
Clindamycin	0.05	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Diatrizoic Acid	0.05	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Diclofenac	0.02	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Doxycycline	0.05	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Eprosartan	0.02	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erythromycin	0.1	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Estrone	0.3	1.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fexofenadine	0.02	0.05	2.43	4.18	0.49	1.05	0.97	0.51	0.59	0.50	0.96	3.69	<LOD	<LOD
Fluopyram	0.02	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Furosemide	0.3	1.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Gabapentin	0.01	0.02	1.14	1.60	<LOQ	1.32	0.60	2.57	4.26	2.16	0.31	0.43	0.18	<LOQ
Gemfibrozil	0.1	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Hydrochlorothiazide	10.00	30.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Iohexol	0.25	0.25	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Iomeprol	0.05	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Iopamidol	0.1	0.1	0.14	<LOD	<LOD	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD

Table S8 Continued.

Sample ID	LOD	LOQ	1	2	3	4	5	6	7	8	9	10	11	12
Compound	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Iopromide	0.1	0.25	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Irbesartan	0.01	0.01	0.33	0.66	0.05	0.15	0.21	0.18	0.19	0.15	0.17	0.46	0.06	0.03
Isoproturon	0.1	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lidocaine	0.01	0.01	<LOD	0.06	0.03	<LOD	<LOD	<LOD	<LOD	<LOD	0.04	<LOD	<LOD	<LOD
Losartan	0.05	0.1	0.33	0.61	<LOQ	0.12	0.13	0.17	0.18	0.15	<LOQ	0.29	<LOQ	<LOQ
Mefenamic acid	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Metoprolol	0.02	0.02	0.01	0.03	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.01	<LOQ	<LOQ
Miconazole	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.66	<LOD	<LOD
Mycophenolic acid	0.1	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	0.16	<LOD	0.17	<LOD	<LOD	<LOD	<LOD
Olmesartan	0.01	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oxazepam	0.01	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Phenazone	0.1	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
propranolol	0.01	0.01	0.40	0.83	0.17	0.29	0.35	0.05	0.05	0.06	0.28	1.20	0.35	0.14
Propyphenazone	0.02	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ranitidine	10.00	10.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Rosuvastatin	0.05	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Roxithromycin	0.3	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sertraline	0.05	0.05	1.37	2.12	0.72	1.77	1.10	1.29	1.27	1.41	0.37	8.57	1.48	0.70
Simvastatin	0.3	1.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sotalol	0.02	0.05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfadiazine	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfamethizole	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	0.02	0.02	0.13	0.27	<LOD	<LOD	<LOD	0.14	0.12	0.09	<LOD	<LOD	<LOD	<LOD
Sulfapyridine	0.3	1.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Thiacloprid	1.00	3.00	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tramadol	1.00	3.00												
Trimethoprim	0.02	0.02	0.15	0.20	0.03	0.11	0.15	0.05	0.04	0.06	0.02	0.04	0.03	<LOQ
Valsartan	0.05	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Venlafaxine	0.05	0.05	0.40	0.51	0.12	0.22	0.23	<LOQ	<LOQ	<LOQ	0.28	0.30	0.57	0.34

Table S9: Concentrations of compound per gram of dry media measured in the solids samples. Not Quantified (NQ) represents values <LOD in raw sample since LOD values are for total raw sample, and do not apply to compound contents per gram of dry media. Values here were used to rank compound contributions. Each category represents averages across stage location and depth for each media type.

*Reference values from (3)

Compound	Average Biofilm Only (µg/g)	Average Gravel (µg/g)	Average Sand (µg/g)
4-Methyl-1H-benzotriazole	NQ	1.04E-03	4.47E-03
Amoxicillin	NQ	NQ	NQ
Amphetamine	NQ	NQ	NQ
Atenolol	NQ	NQ	7.52E-05
Azithromycin	0.18	3.41E-03	1.95E-03
Benzotriazole	0.23	1.45E-03	1.11E-03
Benzoyllecgonine	0.08	NQ	NQ
Caffeine	3.12	8.11E-03	3.54E-03
Carbamazepine	NQ	4.53E-05	1.30E-04
Cetirizine	NQ	NQ	NQ
Ciprofloxacin	7.79	NQ	NQ
Citalopram	0.09	3.92E-03	7.21E-04
Clarithromycin	NQ	8.95E-05	2.42E-04
Clindamycin	NQ	NQ	NQ
Codeine T2	NQ	NQ	NQ
DEET	NQ	NQ	NQ
Diatrizoic acid	NQ	NQ	NQ
Diclofenac	NQ	NQ	NQ
Fexofenadine	0.27	3.84E-03	8.74E-03
Gabapentin	1.50	1.55E-03	3.48E-03
Gemfibrozil	NQ	NQ	NQ
Hydrochlorothiazide	NQ	NQ	NQ
Ioexol	NQ	NQ	NQ
Iopamidol	0.02	NQ	1.64E-04
Irbesartan	0.09	5.97E-04	1.36E-03
Lamotrigine	NQ	NQ	NQ
Lidocaine	NQ	1.41E-05	1.29E-04
Losartan	0.08	3.29E-04	9.89E-04
Methamphetamine	0.03	3.36E-05	NQ
Metoprolol	NQ	8.10E-06	4.03E-05
Miconazole	NQ	1.11E-03	NQ
Mycophenolic Acid	0.05	NQ	NQ
Oxcarbazepine	NQ	NQ	1.98E-04
Propranolol	0.03	1.61E-03	1.81E-03
Ranitidine	NQ	NQ	NQ
Rosuvastatin	NQ	NQ	NQ

Table S9 Continued.

Compound	Average Biofilm Only (µg/g)	Average Gravel (µg/g)	Average Sand (µg/g)
Sertraline	0.66	9.38E-03	4.38E-03
Sotalol	NQ	NQ	NQ
Sulfadiazine	NQ	NQ	NQ
Sulfamethoxazole	0.06	NQ	4.23E-04
THC-COOH	0.23	2.17E-03	NQ
Trimethoprim	0.02	2.04E-04	4.09E-04
Valsartan	NQ	NQ	NQ
Venlafaxine	NQ	1.18E-03	1.49E-03

Table S10: Concentrations of pharmaceuticals in surface water and municipal wastewater as compared to Bridger Bowl raw influent.

Compound	Surface Water* (µg/L)	Municipal Wastewater* (µg/L)	Bridger Raw Influent (µg/L)
Codeine	0.31 ± 0.41	8.11 ± 13.70	0.10 (0.08, 0.12)
Diclofenac	1.22 ± 2.64	3.52 ± 6.59	0.15 (0.11, 0.19)
Ibuprofen	9.65 ± 24.12	21.19 ± 18.41	<LOD
Ciprofloxacin	1302.78 ± 2905.34	7.30 ± 9.89	<LOD
Sulfamethoxazole	4.45 ± 11.65	13.42 ± 27.59	1.60 (0.51, 2.31)
Trimethoprim	1.78 ± 2.38	9.96 ± 23.68	0.39 (0.18, 0.63)
Erythromycin	1.15 ± 2.38	3.75 ± 4.38	<LOD
Azithromycin	0.02	300.00 ±	4.47 (3.54, 6.81)
Atenolol	6.56 ± 11.04	38.99 ± 98.43	0.27 (0.18, 0.37)
Metoprolol	3.11 ± 4.02	190.29 ± 424.69	0.62 (0.39, 0.80)
Carbamazepine	0.54 ± 0.96	2.52 ± 5.39	0.16 (0.03, 0.29)
Caffeine	35.57 ± 62.38	61.68 ± 60.72	211 (179, 237)

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APPENDIX B

QUANTIFYING LOADING LIMITS OF A COLD-CLIMATE
TWO-STAGE VERTICAL FLOW TREATMENT WETLAND

Abstract

This study evaluates the effects of hydraulic loading rate (HLR), mass loading rate (MLR), and dose depth (DD) on chemical oxygen demand (COD) and ammonia-nitrogen ($\text{NH}_3\text{-N}$) removal in a two-stage vertical flow treatment wetland. Specifically, it investigates the second stage, designed for nitrification, for loading limits described by HLR, MLR, and DD. A comprehensive 2015–2025 dataset spanning first-stage HLRs of 5.5–62 cm/d (median MLRs of 109 g/m²/d COD, 22 g/m²/d $\text{NH}_3\text{-N}$) and second-stage HLRs of 5.5–60 cm/d (median MLRs of 27 g/m²/d COD, 16 g/m²/d $\text{NH}_3\text{-N}$) revealed that second-stage $\text{NH}_3\text{-N}$ removal declined sharply past HLRs ~40 cm/d regardless of mass load. Targeted 2024–2025 experiments spanned typical, low-HLR, and high-MLR/moderate-HLR schemes to quantify loading limits. These experiments showed that low-HLR schemes yielded high percent removal, but high MLRs were not achieved for these schemes, and therefore little mass was removed. Under typical HLRs of 24–41 cm/d, all observed MLRs for $\text{NH}_3\text{-N}$ in the second stage achieved consistently high percent and mass removal with no upper MLR limit quantified in this study. These findings underscore HLR as the primary control on second-stage performance, and highlight the importance of evaluating HLR, MLR, and DD across their combined ranges, since each parameter's influence can control performance at different crossover points.

Introduction

Small-footprint treatment wetlands (TWs) offer nature-based, low-energy wastewater (WW) treatment solutions for decentralized communities lacking the capital or population density to support conventional wastewater treatment plants (WWTPs) (1–5). These highly engineered systems mimic natural wetland processes to effectively remove nutrients, organic carbon (OC), and emerging contaminants (2–4). Among TW configurations, vertical flow

treatment wetlands (VFTWs) are preferred for municipal WW treatment due to their high oxygen transfer capacity, which support complete nitrification even under high hydraulic loads (3,5).

Total nitrogen (TN) removal in VFTWs requires both nitrification and denitrification, which may be achieved using a two-stage VFTW with an internal recycle stream to return nitrified effluent WW from an aerobic zone to an upstream carbon-rich anoxic zone for denitrification (1,6–8).

The design and performance of VFTWs are governed by three interrelated hydraulic parameters: hydraulic loading rate (HLR), mass loading rate (MLR) and dose depth (DD) (4,5). HLR defines the daily volumetric flow per unit surface area, while MLR quantifies the mass of contaminant applied over that area per day. DD refers to the volume of WW applied per dose over the entire surface area applied. The treatment efficacy for a given MLR and HRT may be predicted through the hydraulic retention time (HRT), which depends on media porosity, TW configuration, and dose volume (5,9). HRT defines the amount of time water spends in the TW. While HRT correlates positively with treatment efficiency, its estimation in unsaturated systems is complicated by pore volume changes due to plant growth and organic matter accumulation (5).

Longer HRTs increase contact time with porous media and microbial communities, allowing for sorption and biodegradation. Selection of media and TW configuration relies on determining the HRT required to meet treatment goals. Influent WW concentrations and treatment area influence the MLR, with higher MLRs requiring longer retention, and increased HRT. The selected HRT in turn constrains the HLR and DD, both of which can be adjusted via media selection and configuration of the wetland. To achieve adequate HRT for treatment, HLR must be limited to reduce throughput of WW, yet high throughput results in more WW treated in a smaller area (3,4). As such, optimization of a TW requires holistic consideration of all three

parameters, especially at their performance extremes, where one factor may have more influence than another.

The hydraulic parameters guide the design and operation of VFTWs through a variety of approaches. Design manuals often still treat them independently, relying on generalized “rules-of-thumb” that do not represent a wide range of influent WW characteristics, design configurations, and climates. These rules-of-thumb give specific surface area requirements per person equivalent (PE), maximum organic loading rates (MLRs), filter materials and diameters, and treatment layer depths (5). They also give maximum HLRs and loading intervals, which control DDs and ensure treatment performance (5). Another design approach, called the first-order plug-flow kC^* method, uses influent concentrations, a background concentration (C^*), HLR, and an areal reaction rate coefficient (k) to predict performance of VFTWs or size the design to meet a treatment goal (5). Alternatively, mass loading charts based on over 1,500 small-scale TWs plot influent and effluent concentrations and may be used to predict performance (4,5).

Infiltration capacity, clogging risk, oxygen transfer and renewal, and short-circuiting risk scale with HLR (3–5). The viable MLR is governed by the microbial demand for electron donors and acceptors, meaning that in tropical climates or other conditions amenable to high microbial activity, higher MLRs may be feasible (5). Finally, DD for a given HLR controls the redox conditions and oxygen transfer of each dose, with smaller, more frequent doses increasing oxygen transfer.

While single stage VFTW hydraulics are well documented, comparatively few field studies have examined the interplay of HLR, MLR, and DD in multi-stage TWs. Long-term datasets in cold climates and in the U.S are also lacking, leaving knowledge gaps such as

maximum nitrogen removal rates in cold climates, and the influence of HLR, MLR, and DD on overall performance in a range of operating conditions.

The Bridger Bowl pilot-scale two-stage VFTW has been operational since 2013, with ten years of water quality data collected. Throughout its lifespan, numerous experimental studies have been conducted to optimize operational parameters for its primary design goal of removing organic carbon and total nitrogen from high-strength WW(10–12). Water quality samples from the Bridger Bowl VFTW have shown consistently high removal efficiencies for ammonia and nitrate over the ten years of study. Recent metagenomics analyses further corroborate these findings, demonstrating nitrification and denitrification occurring at temperatures much lower than previously thought to be the limit (12).

Long-term monitoring and system optimization have confirmed the Bridger VFTW's ability to achieve its design objectives while also demonstrating a more robust treatment capacity than expected. However, despite various experiments examining dosing and loading effects, little has been done to quantify the system's operational limits. This is particularly true in the second stage where influent WW composition is difficult to control due to initial treatment in the first stage. Hydraulic loading to the second stage is limited by flow rates through the first stage, and mass loading is limited by the level of treatment achieved in the first stage. This study analyzes long-term monitoring data (from 2015 to the present) for the system with a focus on second stage loading dynamics, while also providing additional insights into the system's performance at the outer limits of each loading parameter. During the 2024-2025 operating seasons, the facility was intentionally pushed beyond its historical loading limits, adding data points to the upper bounds of HLR to both stages, COD MLR to the first stage, and ammonia MLR to the second stage. By combining these new loading regimes with historical data, this study provides an assessment of

how HLR, MLR, and DD interact to govern ammonia nitrification and TN removal in a two-stage VFTW with a second stage nitrification bed.

Methods

Site Description

The study was conducted at the Bridger Bowl Ski Area pilot-scale VFTW (45°49'01.0"N 110°54'22.0"W, elevation 6430 ft (1960 m)) during its winter operating season from December-April. The VFTW has been operational since 2013 and was designed to treat clarified, high-strength WW generated from base area lodges' lavatories and kitchens. By 2015, the system was planted and operated as a VFTW. The system is a two-stage, subsurface vertical flow configuration with partial internal recycling to remove OC and TN from WW. This study uses data collected from the 2015-2025 operating seasons, with an emphasis on the 2024-2025 period. Over the course of the study, a series of experimental operational schemes were implemented to evaluate treatment under varying loading conditions, with the 2024-2025 seasons aimed at quantifying the limits of these conditions.

The first stage of the TW was designed for carbon removal and denitrification. It consisted of two identical, parallel cells (called A1 and A2) filled with crushed gravel ($d_{50} = 5.3$ mm) to a depth of 0.91 m, and operated under partial saturation to create anoxic conditions favorable for denitrification. Saturation depth was controlled via adjustable Agri-Drain™ plates in the TW cell outlets, with a typical water level maintained at 0.71 m, leaving 0.20 m of unsaturated media to prevent freezing. Alternative saturation depths of 0.53 m and 0 m were also implemented under the various experimental schemes throughout the study.

The second stage (also containing two cells, B1 and B2) was configured for aerobic removal of ammonia via nitrification and was operated fully unsaturated over the course of the

study. It was filled to a depth of 0.91 m with washed concrete-sand ($d_{50} = 0.53$ mm). Both stages included two parallel treatment cells, each independently operated, allowing for either mirrored or differential dosing. Each stage had a total area of 48 m^2 and were dosed intermittently via a perforated pipe network, with gravity drainage to intermediate collection tanks. The retention of WW therefore varied by stage due to media differences, saturation depth, and dosing strategy.

The TW was planted with *Carex utriculata* and *Schoenoplectus acutus*, with opportunistic vegetation and weed growth removed during summer months prior to the operational season. Winter WW temperatures were 2-4 °C, and influent total suspended solids (TSS) were 52-87 mg/L, measured using EPA Method 160.2.

Influent to the TW consisted of primary-treated WW following sedimentation in a series of pretreatment tanks. The system influent was dosed intermittently onto the first-stage cells, alternating with doses of nitrate-rich recycle water returned from the second stage. The volumetric ratio of recycled water to influent WW applied was described using the recycle ratio, which ranged from 0-3 across the study period, with influent WW flow rates ranging from 350-2500 gallons per day. Dose volumes and frequencies varied with each scheme and are detailed in later sections.

The first-stage effluent, containing ammonia, was collected in a transfer tank and dosed onto the fully unsaturated second stage, matching the combined volume applied to the first cells per day. The second stage effluent was collected in the recirculation tank, where a portion was recycled to the first stage, and the remainder was discharged to the ski area's full-scale VFTW.

The MLR varied depending on the influent flow rate and the composition of the source water. For the first stage, the MLR depended on the system influent WW concentrations (septic stream), the recycle ratio, and the system influent flow rate. For the second stage, the MLR

depended on treatment achieved in the first stage, or composition of the first stage effluent, and the system flow rate.

BRIDGER BOWL VERTICAL FLOW TREATMENT WETLAND

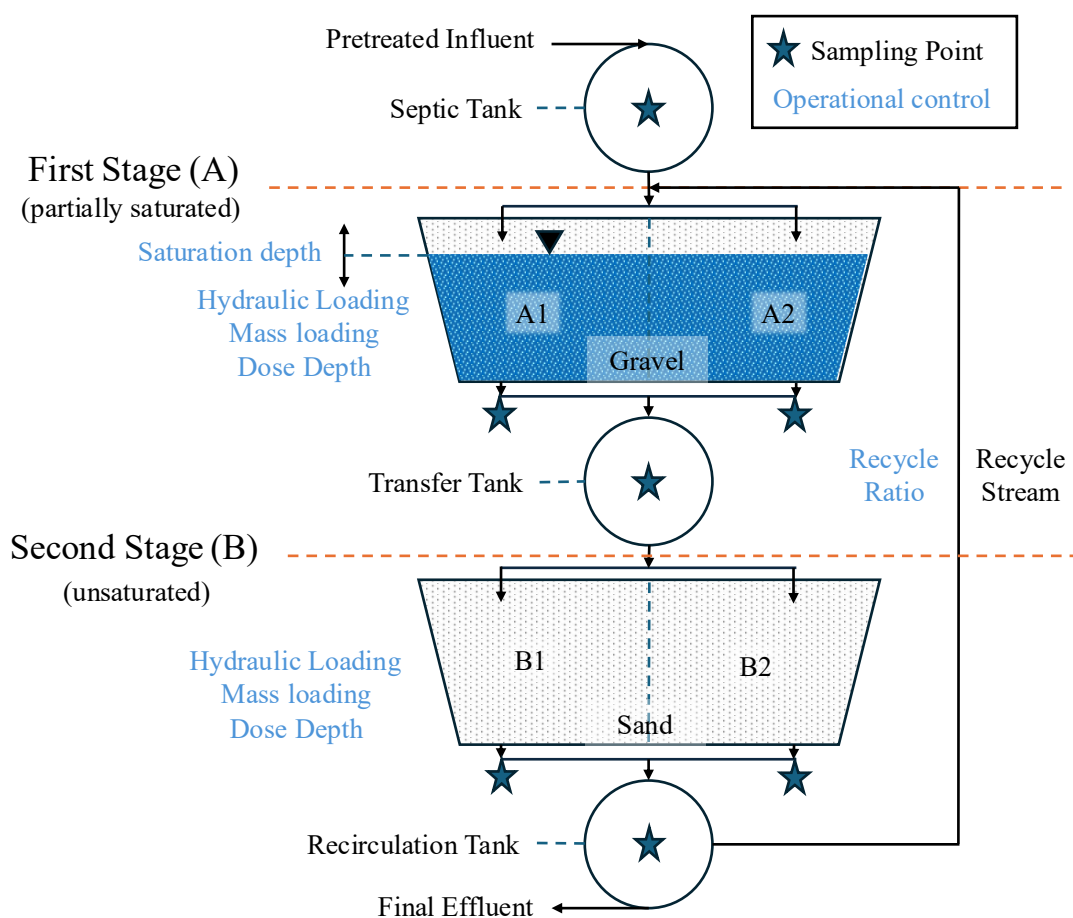


Figure S4: Bridger Bowl treatment wetland flow schematic with sampling points and operational controls.

Sampling and Analysis

Over the course of the historical dataset, both grab samples and 3-day composite WW samples were collected from three sampling tanks within the system. The 2024-2025 experiments used grab samples only, collected three days per week on average over the operating

season. The septic tank samples, or influent, represented the raw, unmixed influent WW composition. Samples from the transfer tank were collected from the mixed tank, representing the influent WW to the second stage, and from each weir at the outfall of the first stage. These weirs, separately representing the outfall of each first-stage cell (A1 and A2), contained treated water from the first stage. Similarly, samples collected from the mixed recirculation tank represented the final effluent WW, as well as the recycle stream applied to the first stage, dosed alternating with the septic stream. Samples collected from each weir within the recirculation tank represented treated water from each individual second-stage cell (B1 and B2). The seven sampling points may be seen in Figure S4.

Sampling across the entire dataset occurred at least weekly and included analyses for chemical oxygen demand (COD), a measurement of the organic carbon in the system, ammonia-nitrogen ($\text{NH}_3\text{-N}$), nitrate (NO_3^-), nitrite (NO_2^-), and orthophosphate (PO_4^{3-}). Total nitrogen ions (TNI) were also recorded as a sum of ammonia, nitrate, and nitrite as nitrogen. All samples were acidified or analyzed within 24 hours of collection and were transported on ice or snow for preservation.

COD was determined using HACH® digestion vials (HACH® Co., Loveland, CO, USA). Ammonia-nitrogen was measured via a modified Berthelot reaction adapted for 96-well microplates, with absorbance recorded at 660 nm using a BioTek Synergy HTX Multimode Reader (Agilent Technologies, Inc., Santa Clara, CA, USA)(13). Nitrate, nitrite, and orthophosphate concentrations were quantified using a Metrohm Eco ion chromatograph equipped with a Metrosep A Supp 5150/4.0 column, a Metrohm Suppressor Module, and a 3.20/1.00 mM sodium bicarbonate/sodium carbonate eluent at a flow rate of 0.7 mL/min (Metrohm USA, Riverview, FL, USA).

Calculations

Hydraulic loading rates (HLRs) (cm/d) were calculated for each treatment stage by dividing the respective daily WW flows (m^3/d) by the wetland surface area (m^2). In the first stage, septic HLRs were calculated by dividing the septic flow applied per day to each cell (A1 and A2) by each cell's surface area (16x16ft, $\sim 24 \text{ m}^2$), while recycle HLRs were computed the same way using the daily recirculated flow applied to each cell. The total HLR to the first stage thus equaled as the sum of the septic and recycle HLRs, or the total daily WW volume applied to each cell divided by its surface area. For the second stage, HLR was calculated by dividing the daily transfer flow applied to each cell of the second stage (B1 and B2) by its surface area ($\sim 24 \text{ m}^2$).

Mass loading rates (MLRs) ($\text{g}/\text{m}^2/\text{d}$) were calculated for COD, ammonia-nitrogen, and TNI based on the respective influent concentrations and corresponding HLRs. Each HLR was converted to meters per day and multiplied by the corresponding concentration at the location of interest (g/m^3). In the first stage, the MLR reflected a weighted contribution from both the septic and recycle streams, as each stream's HLR was multiplied by its respective contaminant concentration, and the two products were summed. The second stage MLR was simply the product of the second stage HLR and the transfer tank concentrations.

To quantify mass removal, mass removal rates ($\text{g}/\text{m}^2/\text{d}$) were determined for each cell by first calculating effluent mass flux by multiplying the cell's HLR by the measured effluent concentration and converting to grams. This effluent flux was then subtracted from the corresponding influent MLR. The effluent concentration used to calculate effluent flux was taken from cell weirs for individual cells (i.e. A1, A2, B1, B2) to obtain cell-specific performance, rather than using the mixed effluent concentrations in the tanks. Thus, effluent flux was

calculated from two measured points (each cell) and influent MLR was calculated from one measured point (the mixed influent) but resulted in two data points, one for each cell influent, when multiplied by respective HLRs.

Dose depth (DD) (cm/dose) was defined as the depth of water delivered in each dosing event, disregarding porosity or filter volume. For every loading stream this was calculated by dividing the stream-specific HLR by its daily dosing frequency. Thus, the first stage had DDs calculated separately for septic and recycle flows, and the second stage's DD was the transfer HLR divided by the number of transfer doses applied per day.

Removal efficiencies (%) were calculated for both individual stages and for the system by dividing the mass removed by the mass loaded for each location. First-stage efficiencies (A1 and A2) used the combined mass load from the septic and recycle inputs and the weir-specific effluent mass fluxes leaving the stage. Second stage efficiencies (B1 and B2) used mass loading and removal based on those calculated from the transfer and second-stage effluent weirs. Finally, the overall system efficiency used mass removed from the system influent (septic tank measurements) to the system effluent (recirculation tank measurements), using only the influent flow rate (septic) without the recirculating flow rate inside the system (sum of septic and recycle).

Statistical descriptors were selected based on the distributional characteristics and interpretive relevance of each variable. Median and interquartile range (IQR) were used to summarize mass loading rates (MLRs), percent removal, and concentration data, as these metrics are robust to outliers and better reflect central tendency and variability in environmental datasets. Due to the long study period of the historical dataset, outliers were present due to changes in operations or seasonal system startup periods, which were not always recorded. Consequently, it

was difficult to hand-select outliers for exclusion from the dataset, so metrics resistant to outliers were used. For more discrete or operationally controlled variables, such as hydraulic loading rate (HLR) and dose depth (DD), the mode, minimum, maximum, and the number of observations at the modal value were reported. This approach provided insight into typical system operation and dosing behavior, particularly where values were fixed or clustered due to design or control protocols. With the exception of the targeted 2024-2025 experiments, most of the historical dataset was focused on optimizing the system's performance. The mode and number of samples at the modal value thus summarized the optimized conditions frequented by the system, while the minimum and maximum values provided bounds, especially relevant to the 2024-2025 experiments.

Operational Data

Hydraulic dosing was controlled using pump runtimes and schedules. For each operating scheme, a runtime was set for the septic and recycle pumps to the first TW stage, and for the transfer pump to the second TW stage. Pump rating curves were developed to determine flow rates, and upon installation of a flow meter in the first stage in 2024, the septic and recycle pump flows were validated. Weep valves were used to allow backflow of water left within the distribution system after a dose to ensure extra water was not applied to the system. Pump schedules were set for each operating scheme to dictate the frequency and duration of doses within a 24-hour cycle. Thus, using the area of the TW and the pump runtimes and schedules, the HLR (volume per unit area per day), DD (dose volume per unit area) and dose frequency were set. The MLR was more difficult to control, as it was a result of the influent WW composition and the septic HLR. For the second stage, MLR was dependent on first stage treatment and loading, meaning efforts to increase MLR to the second stage necessitated increased loading to

the first stage. The operating parameters contributing to each operating scheme for the 2024-2025 experiments, conducted over the respective ski seasons, are shown in Table S11. Operating schemes across the entire historical dataset are summarized in Results along with their resulting treatment performance and MLRs.

Table S11: 2024-2025 Bridger Bowl VFTW operating schemes for maximizing second-stage loading.

Operating Scheme	1	2	3	4	5	6	7	8	9	10
Recycle Ratio	3	2	2	0	2	2	1	0	0	0
A Cell Saturation Depth (cm)	71	71	71	71	71	0	0	0	0	0
A1: Septic HLR (cm/d)	12	12	12	12	12	12	18	18	18.	-
A1: Recycle HLR (cm/d)	38	25	25	-	25	25	19	-	-	-
A1: Total HLR (cm/d)	50	38	38	12	38	38	37	18	18	-
A2: Septic HLR (cm/d)	12	12	12	12	12	12	18	18	18	24
A2: Recycle HLR (cm/d)	38	25	25	-	25	25	19	-	-	-
A2: Total HLR (cm/d)	50	38	38	12	38	38	37	18	18	24
B1: Total HLR (cm/d)	50	38	55	24	40	40	40	-	36	24
B2: Total HLR (cm/d)	50	38	20	-	35	35	35	36	-	-
A1: Septic Dose Depth (cm)	1.5	1.5	1.5	0.8	1.5	1.5	0.8	0.8	0.8	-
A1: Recycle Dose Depth (cm)	1.6	1.1	1.1	-	1.1	1.1	0.8	-	-	-
A2: Septic Dose Depth (cm)	1.5	1.5	1.5	0.8	1.5	1.5	0.8	0.8	0.8	1.0
A2: Recycle Dose Depth (cm)	1.6	1.1	1.1	-	1.1	1.1	0.8	-	-	-
B1: Dose Depth (cm)	2.1	1.6	1.7	1.5	5.0	5.0	4.9	-	1.5	1.0
B2: Dose Depth (cm)	2.1	1.6	1.7	-	4.4	4.4	4.4	4.5	-	-

During the 2024-2025 experiments, upper bounds on HLR, MLR, and DD were investigated both separately and simultaneously. These experiments specifically focused on achieving upper loading datapoints in the second stage, which is focused on removing ammonia-nitrogen, as fewer investigations on the second stage have been completed. MLRs are presented in the Results, as they required concentration data results to calculate, but since MLR scales with septic HLR, each operating scheme's effect on MLR may be approximated. Operating Scheme 1 was intended to provide an upper HLR with more typical MLR and DD. Operating Scheme 2 was the typical operating scheme, optimized for organic carbon and TN removal. Schemes 3 and

4 aimed to increase both HLR and MLR to the second stage, while maintaining a typical DD. To increase only ammonia MLR to the second stage, it was necessary to allow ammonia to pass through the first stage, while removing organic carbon to avoid clogging in the second stage. This was also necessary to prevent autotrophic nitrifiers being outcompeted by aerobic heterotrophs in the presence of readily biodegradable COD in the second stage. To achieve this, schemes 6-10 desaturated the first stage to allow for aerobic biodegradation of COD. Since nitrifiers were not as active in the first stage, as it had been saturated for several years, this allowed for ammonia to pass through while removing organic carbon from the system. Thus, schemes 6-10 increased ammonia MLRs to the second stage under typical HLRs, and various DDs. They also investigated high COD MLRs to the first stage in both saturated and unsaturated conditions. Schemes 5 and 6 were identical at typical operating conditions to compare saturated and unsaturated conditions of the first stage.

Results and Discussion

Historical Dataset

Overall Performance. Data for conventional WW contaminants for the entire historical study period, regardless of individual experiments, is presented in Table S12. Median concentrations show high values of COD (781 mg/L) and NH₃-N (171 mg/L) typical of the high-strength WW at Bridger Bowl. Average removal of COD (95%), NH₃-N (99%), TNI (69%) demonstrate high performance regardless of ongoing experiments. Concentrations measured in the system influent were diluted with recycle water before application to the first stage, which primarily removes COD, suspended solids, and TN, while the second stage primarily removes NH₃-N.

Table S12: Median concentrations and removal of contaminants across entire historical dataset at Bridger Bowl (2015-2025). Parentheses represent an interquartile range (IQR).

Parameter	COD	NH₃-N	TNI	PO₄-P
System Influent Median (IQR) (mg/L)	781 (178)	171 (50)	169 (48)	14 (2.9)
Mid-Point Median (IQR) (mg/L)	69 (48)	45 (22)	55 (24)	11 (4.0)
System Effluent Median (IQR) (mg/L)	35 (22)	02 (04)	50 (24)	11 (4.1)
Removal Median (IQR) (%)	95 (2.4)	99 (2.7)	69 (16)	19 (23)
Number of Samples	257	277	255	114

Samples and measured concentrations for COD, NH₃-N, and TNI are presented for the historical dataset and for the 2024-2025 experiments in Figure S5, Figure S6, and Figure S7, respectively. 2024-2025 operating schemes presented in each figure may be referenced in Table S11.

The dataset concentrations presented in Figure S5, Figure S6, and Figure S7 all show rising concentrations in the transfer tank during the 2024-2025 experiments, due to the changes in operating schemes to increase throughput to the second stage. Additionally, 2018-2019 data show more variable results than other years with 2020-2025 showing similar trends to each other, and 2016-2017 showing similar values. For the 2024-2025 period, it can be seen that Scheme 4, which had no recycle stream and 0.71 m of saturation, increased COD in the transfer tank the most. For NH₃-N, high values were seen in the transfer tank for Schemes 4, and Schemes 8-10. TNI showed decreasing performance as the first stage was desaturated and loading was increased in Schemes 6-10. Discussion on each loading variable and their effects on performance is included in later sections.

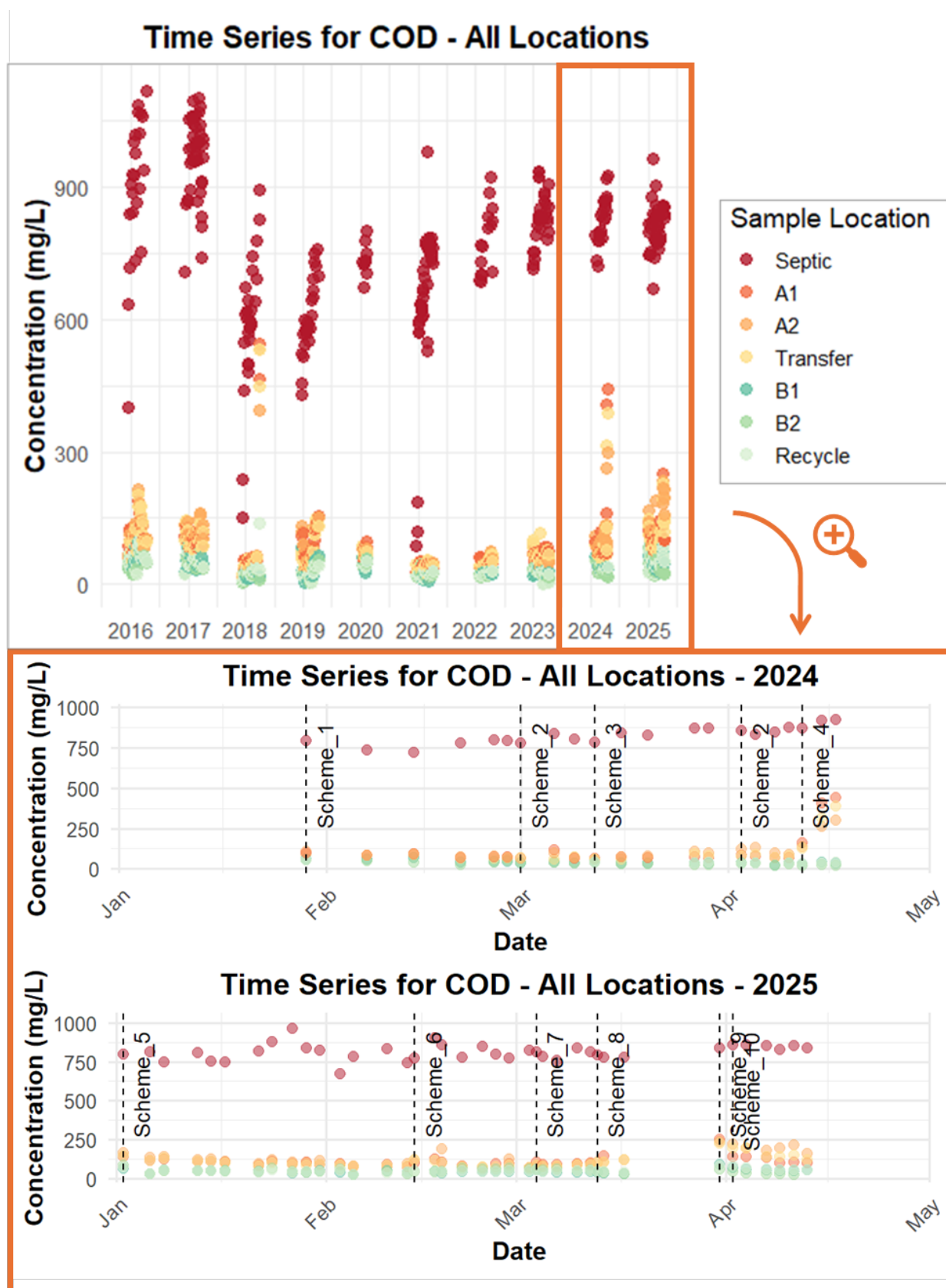


Figure S5: Concentration data collected for COD at the Bridger VFTW.

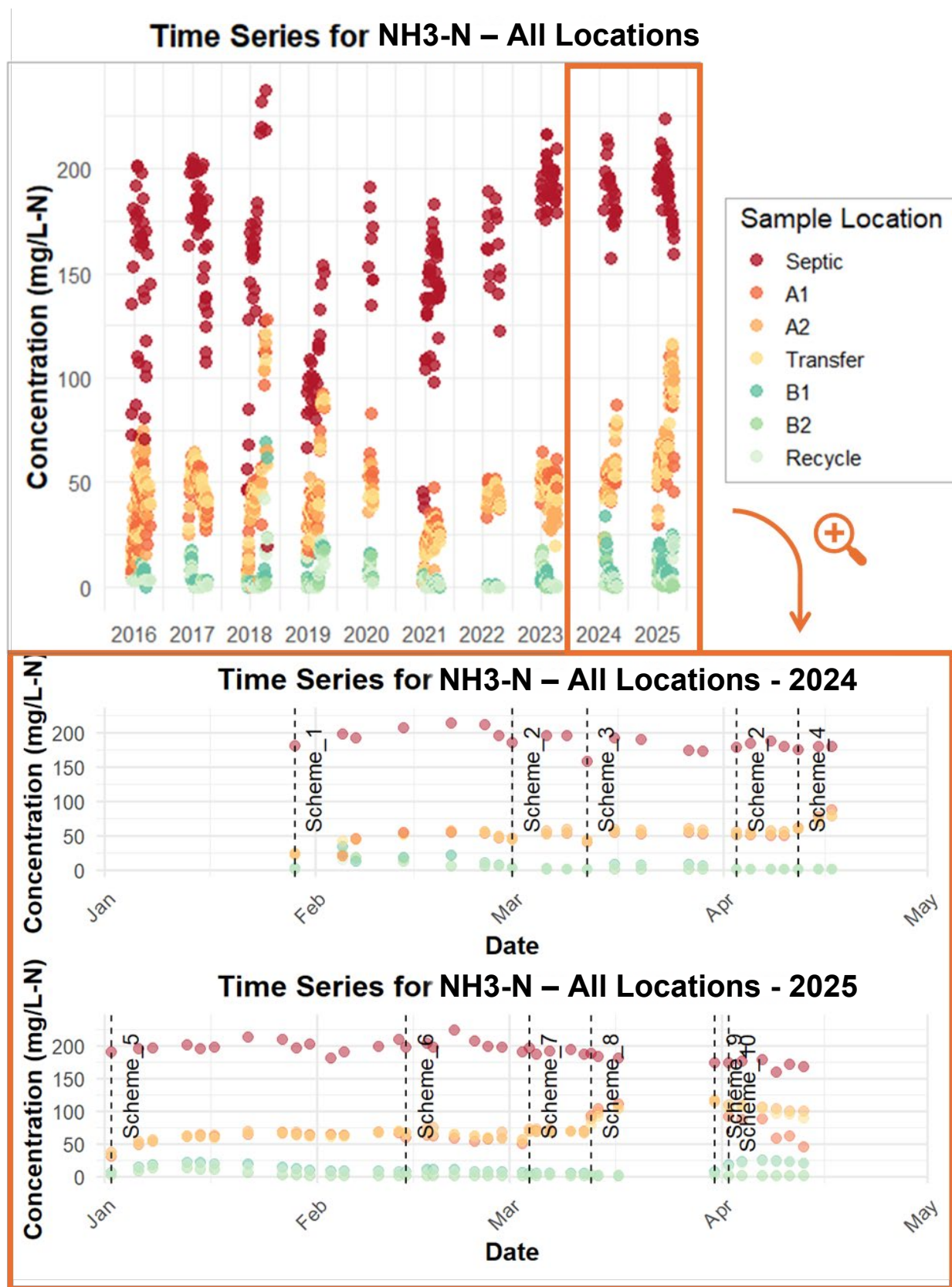


Figure S6: Concentration data collected for NH₃-N at the Bridger VFTW.

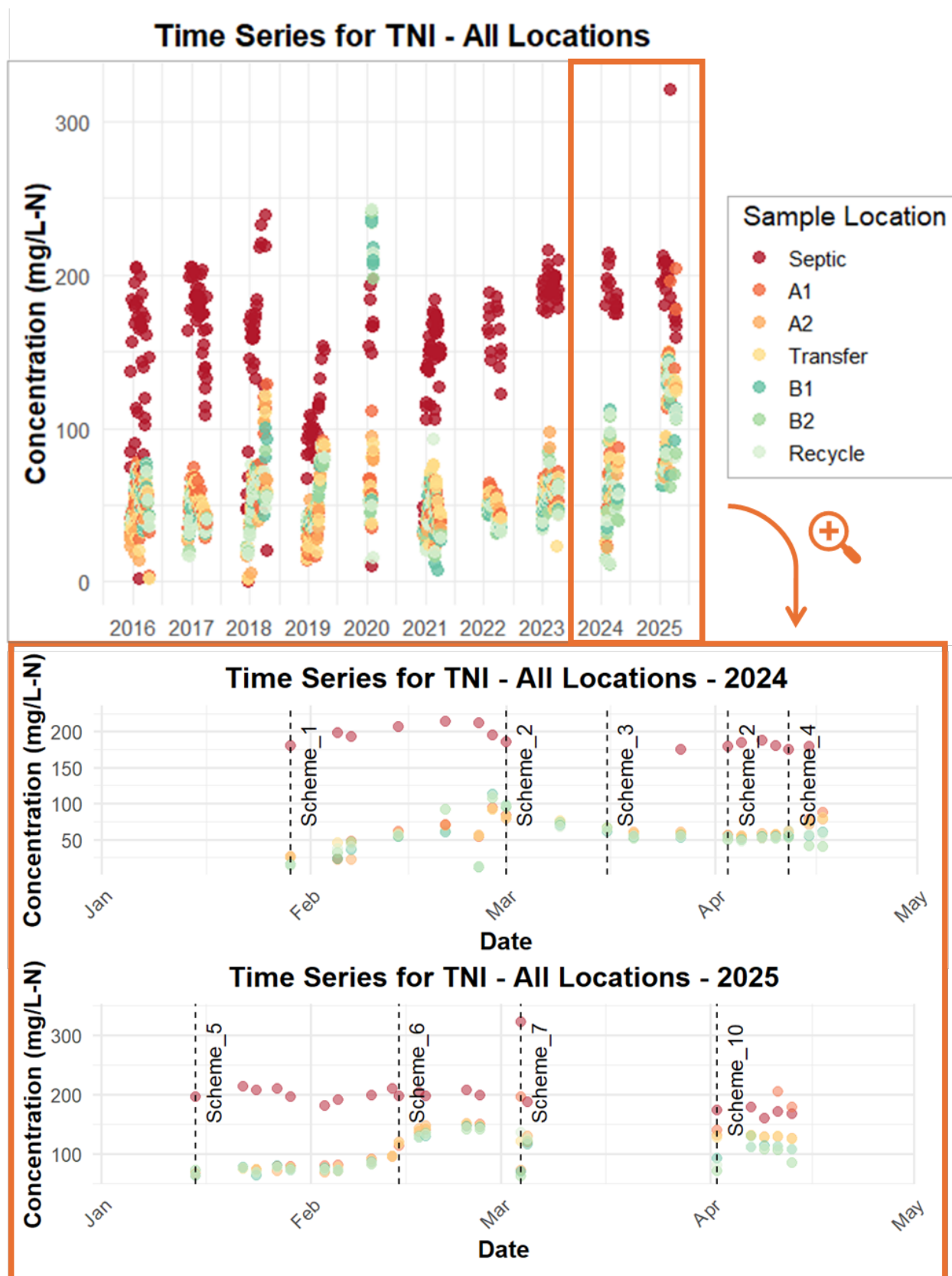


Figure S7: Concentration data collected for TNI at the Bridger VFTW.

Loading Conditions. A summary of the system loading conditions achieved across the 2015-2025 dataset is laid out for each constituent of interest to this analysis in Table S13, and Table S15. These contaminants include COD, ammonia-nitrogen, and TNI, due to their effects on performance in the second TW stage. Differences in operationally controlled parameters for each contaminant in each scheme were a result of certain compounds being analyzed more frequently than others. The table represents sample numbers and modal values with minimums and maximums for datasets containing only the contaminant of interest, so if the contaminant was not assessed at a given loading scheme, the values reflect that. The first stage combines A1 and A2 datasets, while the second stage combines B1 and B2 datasets, as some operating schemes used only one cell. This means that for sampling days with both cells operating, one sample was collected from the tank influent to the stage, while two samples were collected from the effluent, one from each cell. The numbers represent the latter, or the total number of values calculated.

For the total dataset, hydraulic loading to the first stage ranged from 5.5-62 cm/d, although COD measurements were only taken up to 56 cm/d. Samples were collected most frequently (n=68 to n=77) in the 36-40 cm/d range. This resulted in a median MLR of 109 g/m²/d COD, 22 g/m²/d NH₃-N, and 32 g/m²/d TNI (including influence from the recycle stream) loaded onto the first stage. Dose depths ranged from 0.65-4.0 cm/dose for septic doses, with a modal value of 1.5 (or 1.1 for COD) cm/dose, and no loading to 2.0 cm/dose for recycle, with a modal value of 1.1 (2.0 for TNI) cm/dose. The first stage removed a median of 77 g/m²/d COD, 3.9 g/m²/d NH₃-N, and 11 g/m²/d TNI.

In the second stage, hydraulic loading ranged from 5.5-60 cm/day. Measurements were taken most frequently from 24-41 cm/day. The median mass loads were 27 g/m²/d COD, 16

$\text{g/m}^2/\text{d}$ $\text{NH}_3\text{-N}$, and $17 \text{ g/m}^2/\text{d}$ TNI. Dose depths ranged from 1.0-5.0 cm/dose, with most measurements taken at 4.0 cm/dose. The second stage removed a median of $12 \text{ g/m}^2/\text{d}$ COD, $15 \text{ g/m}^2/\text{d}$ $\text{NH}_3\text{-N}$, and $1.2 \text{ g/m}^2/\text{d}$ TNI.

Table S13: Hydraulic loading conditions achieved over 2015-2025 dataset by contaminant. First stage includes samples taken from A1 and A2, while the second stage includes samples taken from B1 and B2, with some operating schemes used only one cell.

COD		First Stage	Second Stage
	Total Number of Samples	404	399
Hydraulic Load	Mode (min, max) (cm/d)	40 (5.5, 56)	41 (5.5, 60)
	Number of Samples at Mode	77	78
	Number of Unique Values	21	23
Septic Dose Depth	Mode (min, max) (cm)	1.1 (0.7, 4.0)	-
	Number of Samples at Mode	77	-
	Number of Unique Values	19	-
Recycle Dose Depth	Mode (min, max) (cm)	1.1 (0.0, 2.0)	-
	Number of Samples at Mode	64	-
	Number of Unique Values	19	-
Transfer Dose Depth	Mode (min, max) (cm)	-	4.0 (1.0, 5.0)
	Number of Samples at Mode	-	84
	Number of Unique Values	-	23
	Mass Load Median (IQR) ($\text{g/m}^2/\text{d}$)	109 (35)	27 (20)
	Mass Removal Median (IQR) ($\text{g/m}^2/\text{d}$)	77 (26)	12 (11)
	Percent Removal Median (IQR)	75% (12)	51% (19)

Table S14: Hydraulic loading conditions achieved over 2015-2025 dataset for NH₃-N. First stage includes samples taken from A1 and A2, while the second stage includes samples taken from B1 and B2, with some operating schemes used only one cell.

NH ₃ -N		First Stage	Second Stage
	Total Number of Samples	455	438
Hydraulic Load	Mode (min, max) (cm/d)	38 (5.5, 62)	24 (5.5, 60)
	Number of Samples at Mode	68	74
	Number of Unique Values	24	24
Septic Dose Depth	Mode (min, max) (cm)	1.5 (0.7, 4.0)	-
	Number of Samples at Mode	82	-
	Number of Unique Values	21	-
Recycle Dose Depth	Mode (min, max) (cm)	1.1 (0.0, 2.0)	-
	Number of Samples at Mode	68	-
	Number of Unique Values	20	-
Transfer Dose Depth	Mode (min, max) (cm)	-	4.0 (1.0, 5.0)
	Number of Samples at Mode	-	84
	Number of Unique Values	-	24
	Mass Load Median (IQR) (g/m²/d)	22 (12)	16 (10)
	Mass Removal Median (IQR) (g/m²/d)	3.9 (4.3)	15 (9.1)
	Percent Removal Median (IQR)	21% (23)	95% (11)

Table S15: Hydraulic loading conditions achieved over 2015-2025 dataset for TNI. First stage includes samples taken from A1 and A2, while the second stage includes samples taken from B1 and B2, with some operating schemes used only one cell.

TNI		First Stage	Second Stage
	Total Number of Samples	426	436
Hydraulic Load	Mode (min, max) (cm/d)	36 (5.5, 62)	24 (5.5, 60)
	Number of Samples at Mode	54	84
	Number of Unique Values	23	21
Septic Dose Depth	Dose Depth Mode (min, max) (cm)	1.5 (0.7, 4.0)	-
	Number of Samples at Mode	50	-
	Number of Unique Values	20	-
Recycle Dose Depth	Dose Depth Mode (min, max) (cm)	2.0 (0.0, 2.0)	-
	Number of Samples at Mode	54	-
	Number of Unique Values	23	-
Transfer Dose Depth	Dose Depth Mode (min, max) (cm)	-	4.0 (1.0, 5.0)
	Number of Samples at Mode	-	84
	Number of Unique Values	-	22
	Mass Load Median (IQR) (g/m²/d)	32 (18)	17 (10)
	Mass Removal Median (IQR) (g/m²/d)	11 (7.3)	1.2 (3.1)
	Percent Removal Median (IQR)	40% (14)	6.7% (19)

Loading Parameter Effect on Treatment Performance. Overall interactions between HLR, MLR, and mass removal were investigated across the entire historical dataset with specific interest in COD performance in the first stage and ammonia-nitrogen removal in the second stage. Typical plots showing mass removal and mass loading with 1:1 lines were created, with the additional HLR variable indicated by color. These plots are shown in Figure S8 and Figure S9. The 1:1 line in each figure represents 100% removal. Figure S8 showed a decrease in mass removal of COD with an increasing mass load. Mass removal became more variable at MLRs exceeding about 60 g/m²/d, with points from a range of high HLRs showing poorer treatment than lower MLRs. Above about 125 g/m²/d, high HLRs resulted in mass removal lower than low HLRs for the same MLR, although data points were more scarce above 125 g/m²/d. Figure S9, showing the ammonia-nitrogen plot, showed a similar trend, but indicated a potential HLR limit for treatment above 40 cm/day, with low HLRs still demonstrating high performance even at high MLRs.

While Figure S8 and Figure S9 provide insight into the removal by MLR, the addition of HLR as a more significant variable and the influence of DD is not fully clear for second-stage ammonia-nitrogen and is presented further in Figure S10. Two HLRs were excluded from Figure S10 as they were outliers on the low end that were used before the system was fully established. Dose depths were binned into those greater or equal to 2 cm/dose, and those less than 2 cm/dose. Once again, HLRs greater than about 40 cm/day resulted in poorer mass and percent removal, even across several MLRs and DDs. There did not appear to be a clear MLR limit to this dataset, as high MLRs at low HLRs still exhibited high mass and percent removal. Limited data at the high end prevents conclusive limit points.

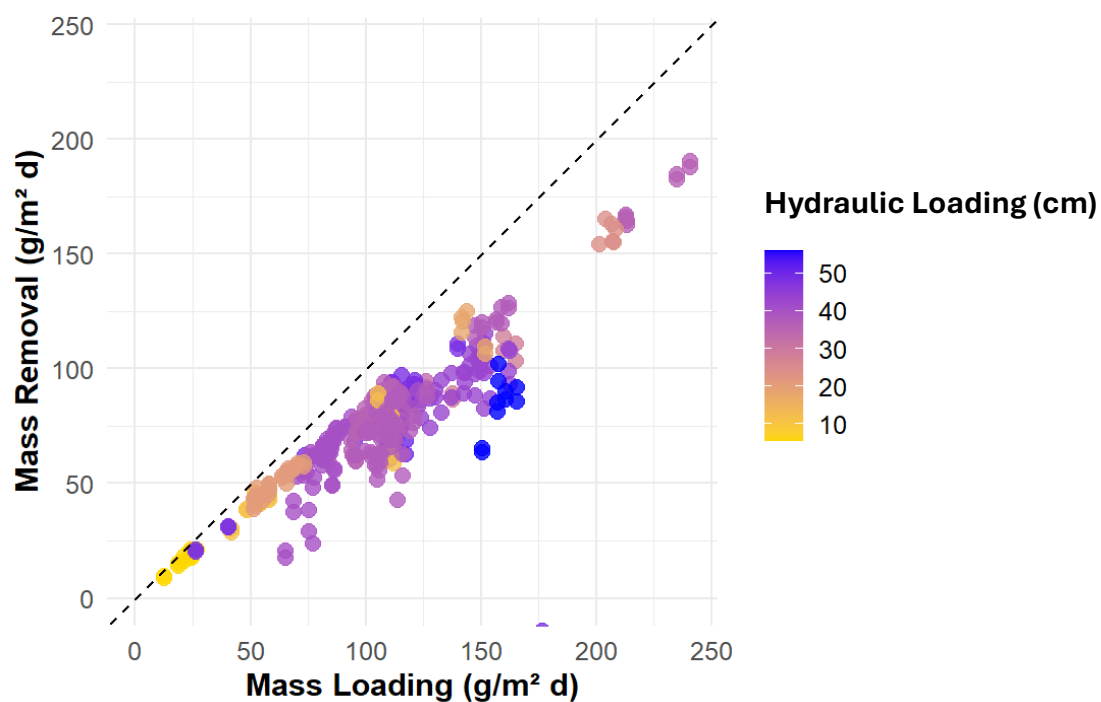
Mass Loading vs Removal in First Stage - COD

Figure S8: Mass loading and removal by hydraulic loading for COD in the first stage.

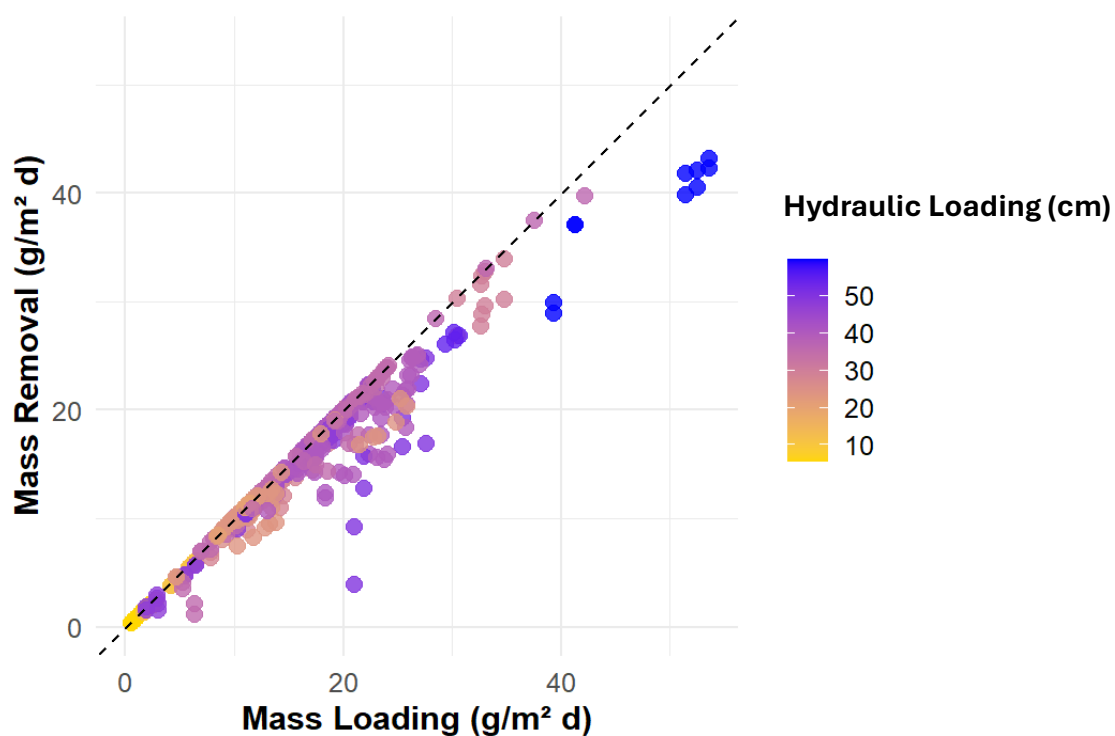
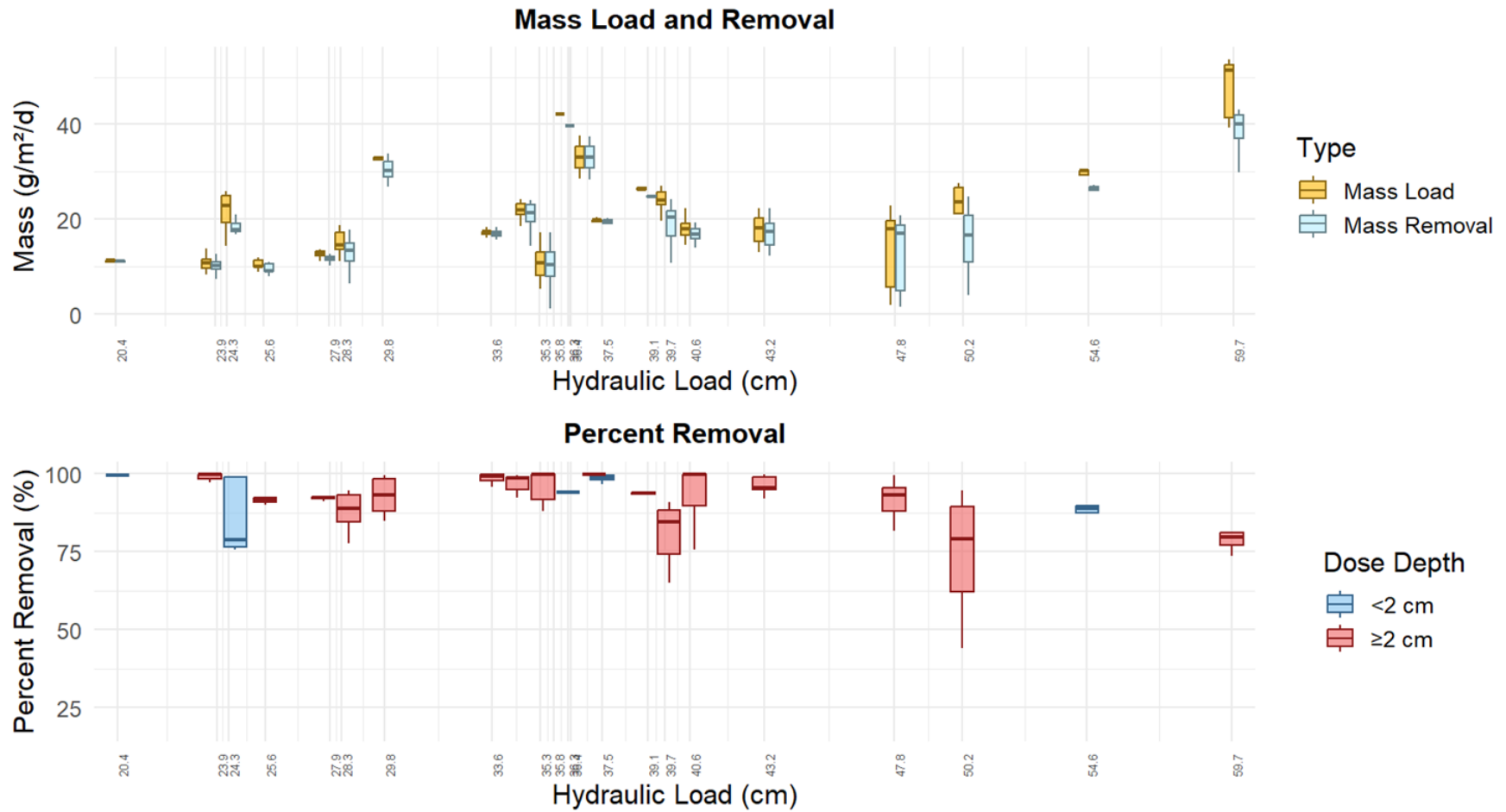
Mass Loading vs Removal in Second Stage – NH₃-N

Figure S9: Mass loading and removal by hydraulic loading for NH₃-N in the second stage.

NH3-N Mass Load, Removal, and Percent Removal by Hydraulic Load in Second Stage



1

Figure S10: Effects of hydraulic load, mass load, and dose depth on percent and mass removal of NH3-N from the second stage.

2024-2025 Experiments

To better understand the potential limits of each loading parameter and the influence of each at various intersections between parameters, a deeper investigation was done on the 2024-2025 experiments. These experiments aimed to increase the bounds of the HLR and MLR, especially with regards to the second stage loading of ammonia-nitrogen. However, because everything loaded to the second stage must first undergo treatment in the first stage, new operating parameters were introduced to the first stage, including desaturating it to promote COD removal and enhance ammonia-nitrogen throughput. Reduction and elimination of the recycle ratio, and increased septic loads were also introduced to increase concentrations passing through the first stage. This successfully increased ammonia-nitrogen loading to the second stage, as seen in Figure S6. The experiments used the operating schemes detailed in Table S11 and were previously described in Methods. The results are presented in Figure S11 for ammonia loading to the second stage for each operating scheme.

Scheme 1 and Scheme 5 shown in Figure S11 show high variability in percent removal, MLR, and mass removal. These schemes were both utilized during the startup periods of the 2024 and 2025 season, respectively, before the system was fully equilibrated and performing with little variation.

NH₃-N Second Stage (B1 and B2 Cells)

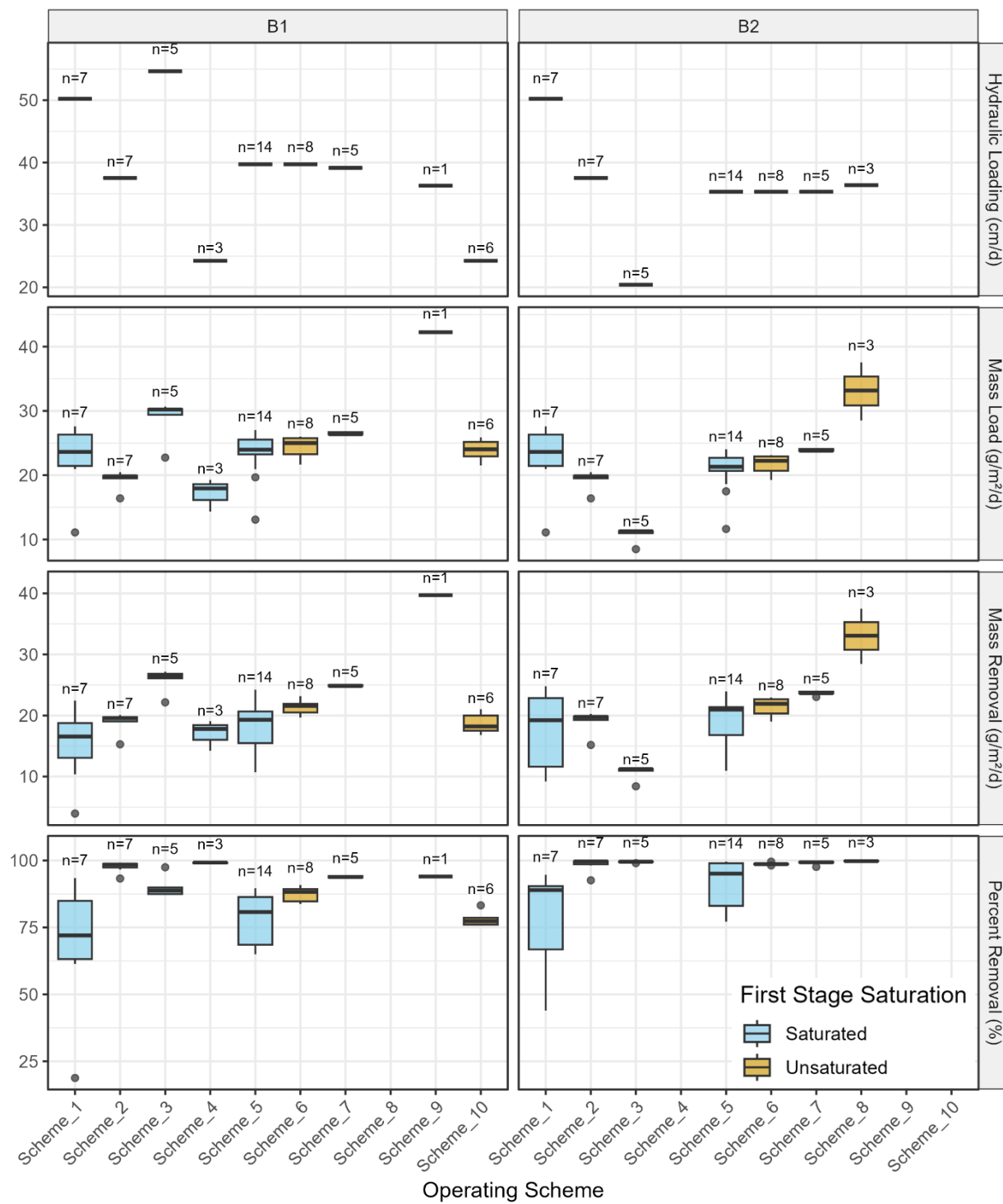


Figure S11: 2024-2024 NH₃-N loading parameters and removal across operating schemes.

At low HLRs (Scheme 4 and Scheme 10 in B1, Scheme 3 in B2), removal percentages appeared to scale with MLRs, with Scheme 3 (B2) having the lowest MLR and highest percent removal, though with little mass loaded to the system, little mass was removed. Scheme 4 (B1) had a slightly higher MLR, and demonstrated higher mass removal, and high percent removal. Scheme 10 (B1) showed mass removal not much higher than Scheme 4, although it had a higher MLR and similar low HLR. This was also reflected in the percent removal, which was lower.

At high HLRs greater than or equal to 50 cm/day (Scheme 1 and 3 in B1 and Scheme 1 in B2), MLRs in the dataset were all within a similar range, but percent removal was consistently poorer than schemes with lower MLRs, apart from Scheme 5, which demonstrated variable removal. High MLRs exceeding 30 g/m²/day with typical HLRs in Scheme 9 of B1 and Scheme 8 of B2 showed high percent removal and high mass removal. While there were less data points at high MLRs, there did not appear to be a limit reached for mass loading of ammonia-nitrogen to the second stage, while an HLR limit of about 50 cm/day showed decreased removal, despite typical MLRs.

Overall, the startup schemes (1 and 5) exhibited high variability in removal metrics due to system equilibration, while low-HLR schemes showed percent removal scaling inversely with MLR but achieved limited mass removal with small loads. At HLRs above 50 cm/day, percent removal declined despite typical MLRs, whereas sustained high MLRs (> 30 g/m²/d) under typical HLRs delivered consistently high percent and mass removal, with no apparent upper limit to ammonia-nitrogen loading in the second stage.

Conclusions

The comprehensive dataset demonstrates that HLR is the dominant control on treatment performance in the second VFTW stage in the Bridger VFTW, where the ability to increase MLR is dependent on HLR, the first stage, and source water characteristics. Additionally, it was found that the first stage was the limiting factor on MLR to the second stage, rather than the treatment capacity of the second stage. In the first stage, median COD and nitrogen loadings (109 g/m²/d COD, 22 g/m²/d NH₃-N) yielded an overall removal of 77 g/m²/d COD and 3.9 g/m²/d NH₃-N. In the second stage, ammonia-nitrogen removal remained robust at moderate HLRs (24-41 cm/d) and high MLRs (>30 g/m²/d) but declined sharply when HLR exceeded ~40 cm/d, regardless of load, indicating an operational upper bound for HLR. This research highlights the importance of evaluating how HLR, MLR, and DD interact across their combined ranges, since each parameter's influence can dominate at different crossover points. However, these findings require verification in other two-stage VFTWs, as the Bridger VFTW is a unique system.

The targeted 2024-2025 experiments improved the historical dataset, especially for the second stage with relatively few optimization experiments by providing data points at the extreme upper ends of loading to the Bridger VFTW. However, due to operational constraints and system malfunctions, fewer loading schemes were achieved than originally proposed. An upper limit of ~40 cm/d HLR to the second stage was suggested, but no upper limit for mass loading was found. Recommended future research includes increasing trials at HLRs above 40 cm/d and MLRs beyond 40 g/m²/d if possible. Additional investigation into dose depth effects, especially at the higher end of MLRs, may also improve second-stage treatment. Finally, comparisons to single-stage VFTW performance and limits should be made, especially regarding

the carbon to nitrogen ratio in the second stage, as the presence of OC may reduce nitrification efficiency.

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