



Research article

Biochar-enhanced bioreactors for agricultural nitrogen mitigation

Jason P. Andras^{a,*}, Rachel L. Rubin^{b,c}, William G. Rodriguez-Reillo^d,
Casey D. Chatelain^{e,f}, Oleander Morrill^b, Kate A. Ballantine^b

^a Mount Holyoke College, Department of Biological Sciences, USA

^b Mount Holyoke College, Department of Environmental Studies, USA

^c Washington State Department of Natural Resources, USA

^d Harvard Medical School, USA

^e Barnstable Clean Water Coalition, USA

^f Horsley Witten Group, USA

ARTICLE INFO

Keywords:

Bioreactor

Woodchip

Biochar

Denitrification

ABSTRACT

Excess nitrogen from agricultural, urban, and wastewater sources is a major contributor to eutrophication and water quality degradation, necessitating effective mitigation strategies. This study evaluates the effectiveness of woodchip-biochar bioreactors in reducing nitrogen loads in agricultural drainage ditches. Biochar, a highly porous and recalcitrant form of charcoal, was incorporated alongside woodchips due to its known capacity to enhance microbial activity and nutrient retention. Three field-scale bioreactors, composed of a 50:50 volumetric mix of woodchips and biochar, were installed in experimental ditches, with three control ditches left untreated. Over an 18-month monitoring period, nitrate concentrations in bioreactor pore water were reduced by an average of 87 % compared to control ditches, suggesting that denitrification - a microbial process converting nitrate to nitrogen gas in low-oxygen conditions - played a dominant role. Biochar amendment enhanced microbial habitat, improved pH buffering, and increased nutrient retention, fostering conditions favorable for denitrification. Prokaryotic amplicon sequencing revealed a distinct microbial community structure in biochar-amended bioreactors, with enrichment of denitrifying taxa and elevated functional potential for nitrogen removal. While transient increases in ammonia and dissolved organic carbon were observed post-installation, these effects did not extend beyond the bioreactor pore water and diminished over time. These findings underscore the denitrifying potential of bioreactors in general, while highlighting the value of biochar as a strategic enhancement to traditional woodchip systems, supporting their adoption as a scalable, cost-effective strategy for reducing nitrogen pollution in agricultural watersheds.

1. Introduction

Nitrogen pollution from agriculture, urban development, and untreated or inadequately treated sewage is a major threat to the ecological health of coastal and near-coastal waterways. Excess nitrogen fuels eutrophication, leading to harmful algal blooms, oxygen depletion, and habitat loss, which disrupt aquatic ecosystems and compromise biodiversity (Anderson et al., 2002; Camargo and Alonso, 2006). Beyond the impacts on environmental health, excess nitrogen can compromise drinking water and harm fisheries and recreation-based economies (Erismann et al., 2013). Agricultural runoff, containing fertilizers rich in nitrogen, is a key driver, while wastewater discharge and stormwater runoff compound the problem by introducing additional nitrogen loads

(Galloway et al., 2008; Howarth, 2008).

Addressing nitrogen pollution in coastal waterways requires innovative and practical approaches that balance effectiveness, cost, and scalability. Conventional strategies to mitigate nitrogen pollution, such as advanced wastewater treatment facilities and expanded sewer infrastructure, are among the most common and effective means of reducing nutrient loads in coastal waterways. These systems can significantly lower nitrogen concentrations in sewage and runoff, thus protecting aquatic ecosystems from the cascading effects of eutrophication. However, their implementation is often constrained by high costs, complex logistical requirements, and lengthy timelines. For many communities, particularly those with limited budgets or urgent ecological challenges, these solutions remain out of reach, underscoring the need for

* Corresponding author. 100 Clapp Labb, Department of Biological Sciences, Mount Holyoke College, 50 College St., South Hadley, MA, 01075, USA.

E-mail address: jandras@mholyoke.edu (J.P. Andras).

<https://doi.org/10.1016/j.jenvman.2025.126260>

Received 28 February 2025; Received in revised form 3 June 2025; Accepted 15 June 2025

Available online 19 June 2025

0301-4797/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

alternative approaches.

Denitrifying bioreactors offer a cost-effective and immediately actionable alternative solution to the nitrogen problem (Cooke et al., 2001; Schipper et al., 2010). These reactors consist of organic waste products, typically woodchips, which provide a carbon source and substrate for microorganisms. When nitrogen-rich water passes through the bioreactor under saturated, low-oxygen conditions, denitrifying microbes convert nitrate into inert dinitrogen gas, effectively removing it from the system. Bioreactors can take the form of a subsurface “wall” to intercept lateral groundwater flow, “layers” to intercept vertical leaching, and “beds” which are typically installed within some containing structure or landscape feature to receive concentrated discharge (Schipper et al., 2010). Denitrifying woodchip bioreactors have been shown to remove up to 100 % of the excess nitrate in field-scale studies of agricultural systems (Addy et al., 2016; Audet et al., 2021; Christianson et al., 2021; Husk et al., 2017; Schipper et al., 2010; vanDriel et al., 2006). Unlike conventional water treatment methods, bioreactors are relatively inexpensive to construct, can be integrated into existing landscapes, and are scalable and adaptable to varying levels of nitrogen pollution. Moreover, their modular design allows them to be deployed in agricultural settings, where they can intercept and treat nitrogen before it enters downstream ecosystems.

In the present study, we explore the efficacy of woodchip bioreactors enhanced with biochar to remove nitrogen from surface and ground water in an agricultural system. Biochar is a carbon-rich, low-nutrient natural product made by pyrolyzing organic material such as wood, leaves, or animal waste in a low oxygen environment. It is highly porous, giving it a large surface area relative to other substrates, and its surface is typically covered with an abundance of functional chemical groups that give it a high sorption capacity for a broad range of pollutants, including nitrogen, phosphorus, heavy metals, and synthetic contaminants such as herbicides and pesticides (Gupta et al., 2016; Tan et al., 2016). This high surface area also provides ample microbial habitat, thereby augmenting microbially driven biogeochemical soil processes such as denitrification (Jaafar et al., 2015). The pH of biochar is typically alkaline, and it can act as a pH buffer, which is critical in acidic environments where optimal denitrification requires near-neutral pH levels. In field-scale applications, biochar has been shown to increase denitrification potential and reduce nitrogen leaching from soil (Cayuela et al., 2024; Clough and Condron, 2010; Nelson et al., 2011; Yao et al., 2017). In light of these properties, biochar filtration has been used as a component process in wastewater treatment, and biochar is commonly applied as a soil conditioner in agricultural systems for the purpose of improving physical and chemical soil characteristics. The properties of biochar suggest that it might also serve as an effective component in bioreactors, potentially enhancing their nitrogen removal capacity.

By combining biochar with woodchips, we aim to capitalize on the complementary benefits of these materials. Woodchips supply the organic carbon necessary to fuel microbial processes, while biochar enhances the microbial habitat, buffers pH, and improves nutrient retention. Additionally, biochar is highly recalcitrant compared to woodchips, which degrade more rapidly. Consequently, incorporating biochar into bioreactors is expected to delay subsidence, maintaining structural integrity and porosity, which are crucial for sustained bioreactor function. Several recent studies have begun to explore the addition of biochar to woodchip bioreactors and have found that it has the potential to substantially improve nitrogen removal rates in small and medium-scale column or mesocosm studies (Ahmadvand and Soltani, 2020; Berger et al., 2019; Bock et al., 2015; Mohanty et al., 2018; Vismontiené and Povilaitis, 2021), as well as larger field-scale pilot bioreactors (Ashoori et al., 2019; Bock et al., 2018; Mohanty et al., 2018). Our research group has also investigated the impact of biochar amendment in the field as well as in laboratory mesocosms of wetland soils. At a 10 % biochar amendment rate by weight, we observed large reductions in the leaching of nitrate (92 %), ammonium (65 %), and phosphate (63 %), along with reductions in emissions of greenhouse

gases – CO₂ (48 %), NO₂ (89 %), and CH₄ (921 %) (Rubin et al., 2020). In the present study, we build upon these prior results with a replicated, field-scale design that aims to explore the efficacy of bioreactors as a tool for nitrogen mitigation in an agricultural setting with a documented history of chronic nitrogen pollution. We hypothesized that installation of bioreactors composed of woodchips and biochar would reduce nitrogen loads in drainage ditches relative to control ditches. In addition to monitoring nutrient concentrations, we aimed to assess how biochar amendment alters the physicochemical environment within the bioreactor substrate and to characterize shifts in microbial community structure and denitrification potential following installation. Findings from this study are intended to inform broader application of this technology across other agricultural systems facing similar environmental challenges.

2. Methods

2.1. Study system

Cranberry farming has historically been a significant and widespread agricultural activity in coastal New England, USA. Cranberry farms are typically located in flow-through or depressional peat bogs near the coast. This placement results in close hydrological connections between cranberry farms and coastal aquatic ecosystems through both surface and groundwater flow. Many of these farms are situated within densely populated suburban and urban landscapes, where they intercept water carrying nutrients and pollutants from stormwater runoff, other agricultural systems, and untreated or inadequately treated sewage from residential septic systems.

The design and management of cranberry farms further influence their hydrology and potential for nutrient export. To make the bogs suitable for cranberry cultivation, the substrate surface is elevated by regular addition of sand, and hydrological infrastructure such as ditches, flumes, dams, and dykes is installed to control water levels. These features allow the bog surface to remain drained during the growing season and facilitate flooding during harvest. However, this design also reduces the natural interaction between water and the bog soil and its associated microbial communities, limiting the soil's capacity to process nutrients. The application of fertilizers and pesticides in cranberry farming further increases the nutrient and pollutant loads in water flowing through the bogs, contributing to nitrogen and other pollution in downstream aquatic ecosystems.

Because cranberry farms are designed with ditches that collect surface and groundwater flow, they are well-suited for the installation of denitrifying bioreactors. By loading bioreactor substrates, such as woodchips and biochar, directly into these ditches – a type of bed bioreactor – it is possible to create in-line treatment systems that intercept nitrogen-laden water before it enters downstream ecosystems.

This study was conducted at Hamblin Bogs, a 150-acre cranberry farm located at the headwaters of the Marston Mills River in the Three Bays Watershed, Cape Cod, Massachusetts, USA (Fig. 1A). These bogs are well-positioned to intercept substantial nitrogen flows, including those originating from residential septic systems and agricultural runoff within the surrounding watershed. Nitrogen concentrations in the Three Bays Estuary, just downstream of the bogs, consistently exceed total maximum daily loads (TMDLs) by nearly two-fold, and water quality monitoring has estimated that approximately 40 % of this excess N load originates or flows through Hamblin Bogs (Mass D.E.P. 2004). These features make the Hamblin Bogs cranberry farm an ideal location for testing the ability of woodchip-biochar bioreactors to reduce nitrogen loads in surface and ground water.

2.2. Study design and bioreactor installation

The study was conducted in a region of a large cranberry farm selected based on previous ground-penetrating radar studies that

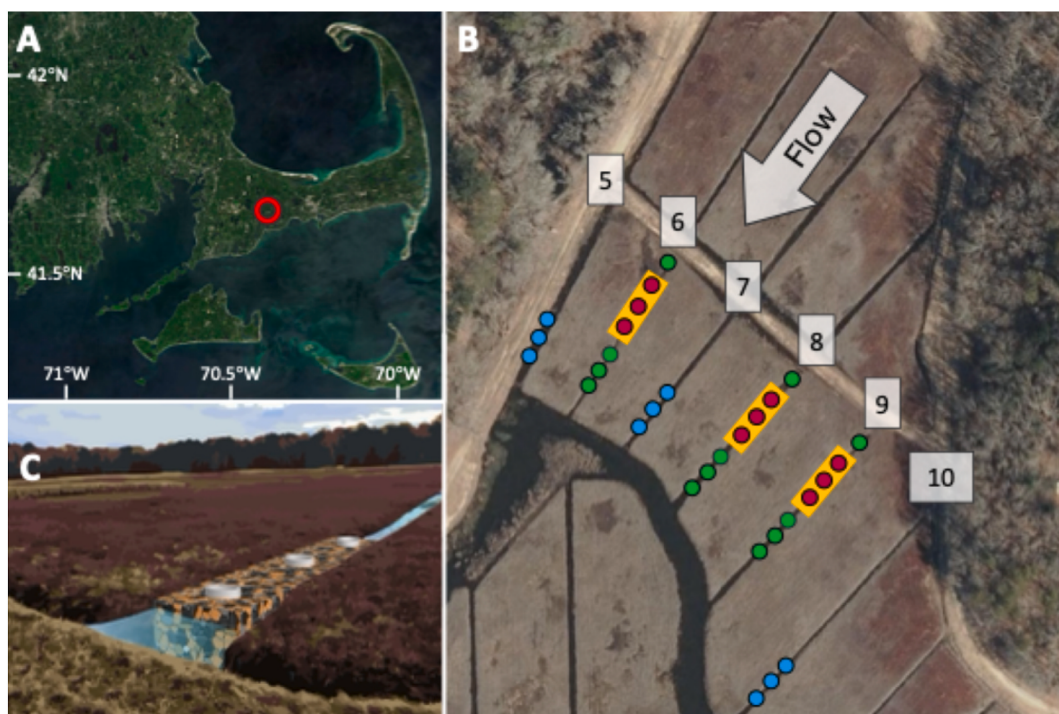


Fig. 1. Study site and experimental design. **A**, The study site (red circle) was located at Hamblin Bogs cranberry farm in Marston Mills, Cape Cod, Massachusetts, USA. **B**, The three replicate woodchip-biochar bioreactors, represented by yellow rectangles, were located in ditches 6, 8, and 9. “Within Bioreactor” water samples, represented by red circles, were drawn from PVC sampling wells installed in the bioreactor substrate. “Treated Ditch” water samples, represented by green circles, were drawn at ~4-m intervals from surface water in the same ditch as the bioreactors. “Control Ditch” water samples, represented by blue circles, were drawn at ~4-m intervals from ditches 5, 7, and 10, where no bioreactors were located. All study ditches intercept water flowing into the Marston Mills River, the main artery of the bogs and major tributary of the Three Bays Estuary (image from [MassGIS Data: 2019 Aerial Imagery \[Mass.gov\]](#)). **C**, The woodchip-biochar bioreactors were installed in existing drainage ditches within the cranberry farm and measured ~12m long x 1.5m wide x 1.2m high. Each reactor had three PVC sampling wells installed along its length. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

indicated the presence of a potential nitrogen-rich groundwater upwelling in this area (BCWC, unpublished data). Within this region, six drainage ditches with similar nutrient concentrations and flow regimes were identified based on preliminary analysis (Fig. 1B). The ditches available at the site tended to exhibit either high flow or very low flow conditions. To minimize the risk of bioreactor clogging and flooding—issues encountered during preliminary studies with woodchip-only pilot bioreactors—six ditches with consistent surface water and low flow were selected for this study.

Three replicate bioreactors were installed in separate ditches, with three adjacent ditches serving as control sites where no bioreactors were installed (Fig. 1B). Each bioreactor was designed to incorporate a woodchip-biochar substrate mixture at a 50:50 volumetric ratio. The amendment rate was based on application rates from previous studies on denitrifying biochar-woodchip bioreactors where successful denitrification was observed (Ashoori et al., 2019). This rate was also roughly comparable to our prior mesocosm experiments, where a 10 % biochar amendment by weight significantly reduced the leaching of nitrate, ammonium, and phosphate from cranberry farm soils (Rubin et al., 2020). A volumetric ratio was used for this study to facilitate ease of large-scale field implementation. Given the low bulk density of biochar, a 50 % volumetric amendment ratio was approximately equivalent to the 10 % weight-based ratio used in the previous mesocosm study.

The bioreactors were installed over four workdays in April and May of 2021. Target ditches were cleared of vegetative matter and excess sediment using hand tools and a backhoe. Wire mesh barriers were constructed to contain the substrate, and the woodchip and biochar substrate mixture was manually loaded into the bioreactors. Virgin white pine (*Pinus strobus*) woodchips, a readily available and inexpensive local forestry waste product, were sourced from Govoni Forest Products (Mashpee, MA). The biochar used in this study was produced

from the same white pine woodchip feedstock, heated to 500 °C over 8 h, and sieved to 1–2 inches (pH 8.6; CEC 6.7 meq/100 g; SOM 48.6 %, NO₃-N 7 ppm, total P 29.9 ppm, 88–92 % C, 0.48 % N, New England Biochar LLC, Eastham, MA)

Each bioreactor measured 12 m in length and filled the ditch to the bog surface – a depth of approximately 1.2 m and width of approximately 1.5 m – for a total reactor volume of approximately 21.6 m³ (Fig. 1C, Supplementary Fig. 1). To secure the substrate and prevent loss during flooding, 2 × 2-inch mesh black polypropylene deer fencing was installed as a cover and secured with 16-inch galvanized rebar J-hook stakes.

2.3. Water sample collection and analysis

Three monitoring wells were installed in each bioreactor to sample water from the upper, middle, and lower thirds of the reactor. The wells were constructed from 6-inch slotted PVC pipes extending to the full depth of the reactor. This design allowed for detailed spatial sampling within the bioreactors. A total of 30 locations for repeat water sampling were established across the study site, distributed into three treatment categories: 1) ‘Within Bioreactor’ samples were drawn from pore water in the wells installed directly in the bioreactor substrate; 2) ‘Treated Ditch’ samples were collected from surface water in ditches upstream and downstream of a bioreactor. 3) ‘Control Ditch’ samples were collected from surface water in control ditches without a bioreactor. Our study is distinguished from traditional bioreactor studies that compare influent concentrations with effluent concentrations. Due to the unique characteristics of cranberry farms (minimal gradient and fluctuating water levels), we compared water in treated ditches to water from adjacent control ditches. While we did collect one influent water sample from each bioreactor for quality control purposes, we decided to

ultimately pool these samples with the downstream samples because surface flow in the ditches was very low (generally not detectable via flow meter), and statistical analysis showed no significant difference in nutrient concentrations above and below the reactors (data not shown).

Water samples were collected monthly from June 2021 through December 2022, with exceptions in October 2021 and October 2022, when the bogs were flooded due to nearby cranberry harvests, and February 2022, when the bogs were frozen and snow-covered. This sampling schedule captured two full growing seasons. Samples were analyzed for pH, conductivity, dissolved oxygen (DO), salinity, nitrate, and ammonia. A subset of 12 samples—four from each experimental category—was also analyzed for dissolved organic carbon (DOC), a more costly analysis. Nutrient analyses were conducted following standard EPA methods (Ammonia: SM-4500NH₃-BH, Nitrate/Nitrite: SM-4500NO₃-F, DOC: SW-846 9060A). Data quality was evaluated based on a minimum detection limit (MDL) of 0.0228 mg/L for nitrate and nitrite, which was a sufficiently low threshold to give us confidence that we would be able to detect changes in nitrogen levels attributable to the bioreactors.

Water quality data (nitrate, ammonia, DOC) were statistically analyzed with a univariate split-plot repeated-measures ANOVA with sample date, treatment category, and the interaction term date*treatment as fixed effects and ditch ID (nested within treatment) as a random effect. Post-hoc comparisons of water quality variables among treatment categories on given dates were performed via mixed-model ANOVA with treatment category as a main effect and ditch ID as a random effect. This experimental design allowed for a robust comparison of nutrient concentrations among the three sampling categories to assess whether the bioreactors effectively reduced nitrogen levels in treated ditches relative to control ditches.

2.4. Soil/substrate sample collection and analysis

In July 2023 after water quality sampling for the project was complete, we collected soil/substrate samples (~5000 cm³) from each of the 30 water sampling locations (Fig. 1B) as well as three additional samples from the cranberry bog surface (hereafter referred to as 'Bog Surface' samples). Samples were collected using either a 1.5-cm diameter soil corer (for soil samples) or a hand trowel (for bioreactor substrate samples) to a depth of 10 cm. Collection tools were flame-sterilized between each sample. All vegetative matter was removed from the surface of each sample, and samples were homogenized in their own bags and subsampled in the field: half of each sample was stored at 5 °C for immediate physicochemical analysis, and the other half was flash frozen in liquid nitrogen and stored at -80 °C for subsequent genetic analysis of the soil microbial community.

We measured six key soil variables to characterize the physicochemical environment [bulk density, pH, cation exchange capacity (CEC), soil organic matter (SOM), soil nitrate, and soil phosphorus]. Nutrient concentrations were determined using the modified Morgan extraction procedure. Cation exchange capacity was determined using hydrochloric acid cation displacement method (Hendershot et al., 2007). Soil organic matter was determined through loss on ignition at 360 °C. Soil variables were statistically analyzed via mixed-model ANOVA with treatment category as a main effect and ditch ID as a random effect. Post-hoc comparisons among treatment categories were performed with pairwise Tukey tests.

2.5. Microbial community profiling

We used 16S amplicon sequencing to characterize the resident prokaryotic microbial communities of all 33 soil/substrate samples from the present experiment, as well as three substrate samples collected from a woodchip-only pilot bioreactor (hereafter referred to as 'Woodchip Bioreactor' samples) that was constructed by another team of researchers in the same region of the cranberry farm. Though this

bioreactor was not considered in any other aspect of our study, we felt it would provide an interesting point of comparison for the microbial community of our woodchip-biochar bioreactors. Total DNA was extracted from a 0.2 g portion of each soil or substrate sample using a DNeasy Powersoil Kit (QIAGEN Inc, Hilden DE). Amplicon sequencing of the 16S rRNA gene was performed targeting the V4 region using primers 515F and 806R, following established protocols (Caporaso et al., 2012; Walters et al., 2016). Sequencing was performed at Argonne National Lab on an Illumina MiSeq platform using paired-end 151x151 cycles. Raw sequencing data was demultiplexed and imported into QIIME2 (v.2024.2) (Bolyen et al., 2019) for downstream processing. Sequences were processed using the DADA2 plugin in QIIME2 to denoise, remove chimeras, and generate exact sequence variants (ASVs). Feature tables, representative sequences, and phylogenetic trees were generated for downstream analyses. Taxonomic classifications were assigned using a Naive Bayes classifier trained on the Greengenes 13.8 99 % OTU database, targeting the 515–806 hypervariable region (McDonald et al., 2011). Metadata linking samples to experimental treatments were used throughout the pipeline to enable group comparisons.

Differences in community composition among treatment groups were evaluated at the phylum level. Taxonomic composition was visualized using bar plots displaying the relative abundances of microbial taxa, with taxonomic summaries aggregated by treatment groups. These plots were generated in QIIME2 and refined in R. Alpha and beta diversity metrics were calculated with QIIME2's core-metrics-phylogenetic pipeline, using a sampling depth of 500 sequences per sample. Alpha diversity was assessed at the phylum level using richness, Shannon diversity, Simpson's diversity, and Pielou's evenness, while beta diversity was evaluated using unweighted UniFrac distances.

To ensure that observed differences in community composition were not confounded by variations in dispersion, a multivariate analysis of homogeneity of group dispersions was conducted using the betadisper function with Bray-Curtis dissimilarity distances (Anderson, 2005). Permutational tests for homogeneity of dispersion confirmed that variance among groups was sufficiently homogeneous to proceed with further analyses. Differences in microbial community composition among treatment groups were assessed using permutational multivariate analysis of variance (PERMANOVA), implemented in the adonis2 function of the vegan package in R, with 999 permutations (Dixon, 2003; Oksanen et al., 2024). Pairwise comparisons between treatment groups were conducted using the pairwise.adonis2 function from the pairwiseAdonis package, enabling identification of specific group differences. To visualize patterns in microbial community composition and diversity, principal coordinate analysis (PCoA) plots were generated for Unweighted UniFrac distances with point size scaled to Shannon diversity values using ggplot2 package in R.

To assess relationships between microbial community composition and environmental factors, we performed a canonical correspondence analysis (CCA) using the cca function in the vegan package in R (Dixon, 2003; Oksanen et al., 2024). The model included soil pH, cation exchange capacity (CEC), bulk density, and soil organic matter (SOM) as predictor variables. The significance of CCA axes was evaluated using an ANOVA-like permutation test (anova.cca), and model selection was refined using stepwise variable selection via the ordstep function. Bray-Curtis dissimilarity was used as the distance metric for microbial community composition. The resulting ordination plot was visualized with ggplot2, incorporating environmental vectors to illustrate relationships between microbial assemblages and soil characteristics. Samples were color coded by treatment group and point sizes in the plot were scaled to Shannon diversity, allowing visualization of diversity gradients across sites. These analyses enabled identification of key environmental gradients structuring microbial communities and provided insight into how bioreactor substrate composition influences microbial functional potential.

Additional functional inferences were drawn using PICRUSt2, a tool that predicts the functional potential of microbial communities by

inferring metagenomic content from 16S rRNA gene sequences and reference genomes (Douglas et al., 2020). Predicted pathway abundances for each sample were derived from KEGG Orthology (KO)-based metabolic pathways and aggregated by treatment group. We focused specifically on the denitrification pathway, and used ANOVA and pairwise Tukey tests to statistically compare the inferred abundance of denitrifying bacteria among treatment groups.

3. Results

3.1. Water quality results

Our 18-month water-quality monitoring revealed statistically significant differences in nutrient concentrations among the bioreactors, the treated ditches, and the untreated control ditches (Table 1). The most notable finding was a substantial and consistent reduction in nitrate concentrations within the bioreactors, confirming their effectiveness in reducing nitrogen loads. Mean nitrate levels in bioreactor pore water were approximately five-fold (87 %) lower than those in control ditches (0.19 mg/L vs. 1.45 mg/L), with similar reductions observed in the treated ditch water (0.27 mg/L; Fig. 2A). These analyses indicated that date, treatment category, and their interaction were all statistically significant factors influencing nitrate concentrations (Table 1).

Ammonia concentrations, in contrast, showed a different temporal pattern. Levels were moderately elevated in pore water sampled from within the bioreactor over the first several months post-installation (~months 3–7) but later stabilized to levels indistinguishable from the treated and control ditches (Fig. 2B). Both date and treatment category were significant factors influencing ammonia concentrations (Table 1), with a notable interaction effect between date and treatment.

Dissolved organic carbon (DOC) exhibited an initial surge in bioreactor pore water, with significantly higher levels compared to treated and control ditches during the first three months after installation. However, this effect was transient, and DOC levels declined quickly, becoming statistically indistinguishable from the surrounding ditch water after this initial period (Fig. 2C). Date and the interaction of date and treatment were significant predictors of DOC variation, while treatment alone was not (Table 1), indicating that DOC differences were largely driven by temporal changes rather than persistent treatment effects.

Patterns for temperature, dissolved oxygen (DO), conductivity, and pH were more complex. Temperature fluctuated seasonally as expected, with all treatments showing similar mean summer highs of

approximately 20 °C and winter lows of approximately 5 °C (Supplementary Fig. 2A). Although treatment category was not a significant factor, the interaction of date and treatment was (Table 1), suggesting potential seasonal differences in temperature buffering effects of the bioreactors. DO levels were generally lower within the bioreactors compared to control ditches for most of the study period, aligning with conditions favorable for denitrification (Supplementary Fig. 2B). Treatment category was a significant predictor of DO concentrations, with bioreactors consistently showing lower oxygen levels (Table 1). Conductivity and pH were slightly higher in control ditches compared to treated ditches and bioreactors throughout the study (Supplementary Fig. 2C and 2D). Both variables were significantly affected by date, treatment category, and their interaction (Table 1), suggesting that bioreactor installation influenced water chemistry over time.

Taken together, these results demonstrate that woodchip-biochar bioreactors were highly effective at reducing nitrate levels while temporarily affecting ammonia and DOC concentrations in the early months post-installation. The lower DO levels within the bioreactors further suggest favorable conditions for microbial denitrification.

3.2. Substrate/soil physicochemical characteristics

Analysis of soil and substrate samples revealed significant differences in physicochemical properties among the bioreactors, treated and control ditch sediments, and cranberry bog surface soils, reflecting the distinct composition of each substrate. The bioreactor substrate had the lowest bulk density (Table 2; Fig. 3A), suggesting that the porosity of the reactor is greater than the surrounding soil, and that the preferential flow path of ground and surface water is likely through the reactor. Organic matter content was also highest in the bioreactor substrate, consistent with its 50 % woodchip composition (Table 2; Fig. 3B). This abundant organic material likely provided a sustained carbon source to support microbial denitrification.

The bioreactor substrate had the highest pH, aligning with the known alkalinity-buffering capacity of biochar (Table 2; Fig. 3C). This could enhance microbial denitrification, as optimal rates typically occur in near-neutral to slightly alkaline conditions. Similarly, cation exchange capacity (CEC) was highest in the bioreactor substrate, though not all pairwise comparisons were statistically significant (Table 2; Fig. 3D). Elevated CEC may have facilitated nutrient retention and stabilization of positively charged ions such as ammonium (NH_4^+).

Nutrient concentrations also varied significantly among substrates, with the bioreactor substrate containing considerably higher nitrate and phosphorus levels compared to the surrounding soils (Table 2; Fig. 3E and F). Biochar's high surface area and functional groups are known to bind nitrate and phosphate, which may account for both the elevated nutrient levels in the substrate and the reduction in nitrate concentrations in bioreactor pore water.

Overall, these findings confirm that the bioreactor substrate created a distinct physicochemical environment that promoted water flow, increased organic matter availability, buffered pH, and retained nutrients. These conditions likely contributed to the observed reductions in nitrogen concentrations in the treated ditches, supporting the bioreactor's function as a nitrogen removal system.

3.3. Substrate/soil microbial communities

Microbial communities within the bioreactor were significantly different from those in the soil of the ditches or from the bog surface. An overall PERMANOVA analysis of microbial community structure identified substrate type as the primary significant effect (Fig. 4, Supplementary Figs. 3 and 4), and pairwise comparisons revealed that the microbial composition of the woodchip-biochar bioreactor was significantly distinct from all other soil and substrate types (Supplementary Table 1). Interestingly, the microbial community of the woodchip-only

Table 1
Water quality statistics.

Response Variable	Fixed Effect	F-value	p-value
Nitrate-N	Date	2.42	0.0023
	Treatment Category	14.95	<0.0001
	Date*Treatment	2.84	<0.0001
Ammonia	Date	5.32	<0.0001
	Treatment Category	4.82	0.0162
	Date*Treatment	1.72	0.0121
DOC	Date	3.54	<0.0001
	Treatment Category	2.43	0.1435
	Date*Treatment	3.26	<0.0001
Temperature	Date	348.11	<0.0001
	Treatment Category	2.48	0.102
	Date*Treatment	4.9	<0.0001
DO	Date	35.96	<0.0001
	Treatment Category	9.82	0.0006
	Date*Treatment	400.2	<0.0001
Conductivity	Date	15.48	<0.0001
	Treatment Category	8.54	0.0013
	Date*Treatment	2.18	0.0004
pH	Date	15.3	<0.0001
	Treatment Category	9.1	0.0009
	Date*Treatment	1.41	0.0776

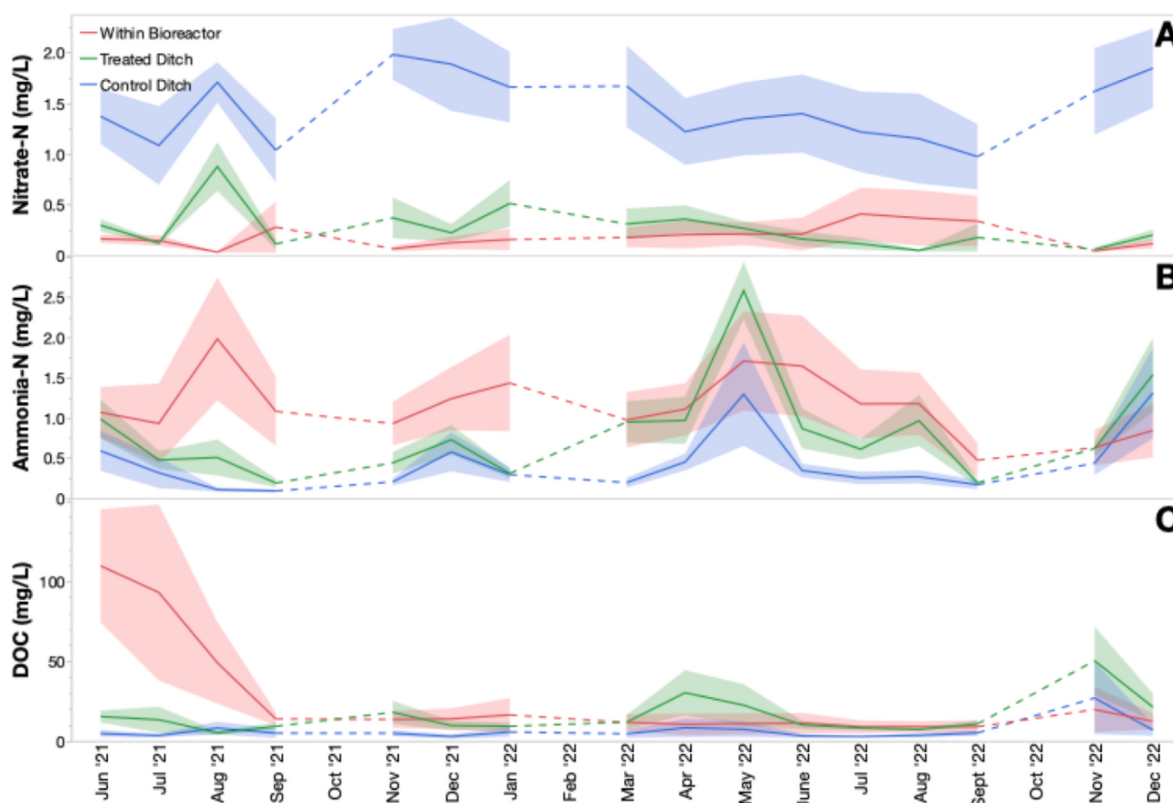


Fig. 2. Key water quality variables in the three treatment categories (Within Bioreactor, Treated Ditch, and Control Ditch) through the 18-month course of monitoring. Shaded bands depict standard error. Dashed lines signify the three months when samples could not be collected and no data was available.

Table 2
Substrate/soil physicochemical statistics.

Response Variable	F-Value	P-Value
Bulk Density	11.3657	0.0001
Organic Matter	10.0447	0.0001
pH	5.4582	0.0048
CEC	2.2413	0.1073
Nitrate	3.5184	0.029
Phosphorus	6.5283	0.0019

bioreactor, which was sampled from a separate pilot experiment for comparison, was more similar on average to the sediment of the treated and control ditches than it was to the woodchip/biochar bioreactor (Fig. 4), suggesting that the addition of biochar substantially altered microbial composition within the reactor substrate, fostering a unique assemblage of microbial taxa.

Canonical Correspondence Analysis (CCA) revealed that while none of the tested environmental variables (pH, CEC, bulk density, SOM) were statistically significant predictors of microbial community composition ($p > 0.05$ for all terms), the CCA ordination nonetheless provided insight into how trends in these factors aligned with community structure (Supplementary Fig. 4). Specifically, the microbial assemblages within our experimental woodchip-biochar bioreactors were associated with higher pH, SOM, and CEC, and lower bulk density.

Functional inference based on PICRUST2 pathway analysis showed that the abundance of denitrifying bacteria was much higher within the woodchip-biochar bioreactor than the bog surface or ditch substrate samples and was also significantly higher than the woodchip-only reactor (Fig. 5), suggesting that biochar amendment enhanced conditions favorable for denitrification. Despite this functional enrichment, the resident microbial communities of the woodchip-biochar substrate exhibited the lowest taxonomic diversity among all samples, as

indicated by reduced Shannon diversity, Simpson diversity, Pielou's evenness, and richness metrics (Supplementary Fig. 5), suggesting a microbial community strongly shaped by environmental filtering, with a relatively small number of dominant taxa.

4. Discussion

4.1. Efficacy of woodchip-biochar bioreactors for nitrogen removal

This study evaluated a cost-effective, field-scalable strategy for reducing nitrogen loads in a coastal agricultural setting by installing woodchip-biochar bioreactors directly into the drainage ditches of an active cranberry farm. We hypothesized that the combination of woodchips and biochar would facilitate enhanced microbial denitrification relative to untreated ditches, driven by biochar's capacity to buffer pH, adsorb nutrients, and support diverse microbial communities. In line with this hypothesis, our data show that nitrate concentrations were consistently and substantially lower - by approximately fivefold or 87 % - in the bioreactor pore water and the treated ditch water compared to control ditches (Fig. 2A). These findings reinforce prior research demonstrating that woodchip-based bioreactors can intercept and remove nitrate, particularly when they are integrated into existing hydrological infrastructure (Addy et al., 2016; Schipper et al., 2010).

Evidence from both water chemistry and substrate analysis points to microbial denitrification as the dominant pathway of nitrate attenuation. The lower bulk density and higher hydraulic conductivity of the bioreactor substrate provided a preferential flow path, prolonging contact time and allowing nitrate-rich water to traverse the reactor rather than bypass it. Within the bioreactors, elevated levels of dissolved organic carbon (DOC) coupled with lower levels of dissolved oxygen (DO), created conditions favorable to denitrifiers. The elevated pH of the bioreactor substrate provides another layer of support, as denitrification generally peaks at near-neutral to slightly alkaline conditions.

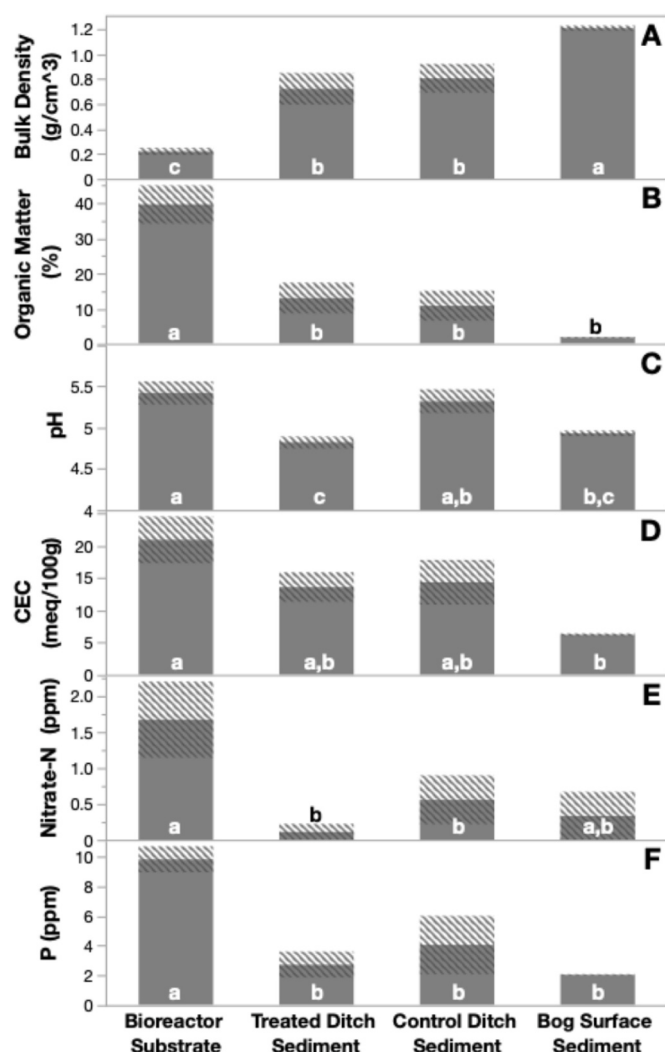


Fig. 3. Key soil/substrate variables within the bioreactors, adjacent to the bioreactors in the treated ditches, in the untreated control ditches, and from the cranberry bog surface. Hash bands depict standard error. Lowercase letters set in bars indicate significant differences among categories based on pairwise Tukey tests – categories not connected by the same letter are significantly different.

Importantly, these characteristics were measured either in the pore water of the bioreactor or in a standard slurry of homogenized substrate prepared for experimental measurement, and it is likely that the observed physicochemical trends favoring denitrification were considerably more pronounced in situ, at the surface of the bioreactor substrate. Taken together, these factors indicate that woodchip–biochar bioreactors can foster environments favorable to denitrification.

In contrast to the patterns observed in water chemistry, the bioreactor substrate exhibited significantly higher levels of nitrate and phosphorus compared to the surrounding soils (Fig. 3E and F). Since wood decomposition is not typically an appreciable source of nitrate or phosphorus, these elevated levels may reflect nutrient adsorption, a characteristic of biochar that has been reported in agricultural soils amended with biochar (Lehmann and Joseph, 2024). While the high CEC of the substrate primarily facilitates the retention of positively charged ions such as ammonium, biochar is also known to interact with anions like nitrate and phosphate via electrostatic interactions with positively charged functional groups, physical trapping within its porous structure, and chemical binding to surface oxides (Lehmann and Joseph, 2024). These properties suggest that biochar amendment may also enhance microbial denitrification by the prolonged retention of

nutrients in bioavailable form.

4.2. Transient increases in ammonia and DOC

Beyond the reductions in nitrate, we observed elevated ammonia levels in the pore water of the bioreactors for several months after installation (approximately months 3 through 7), after which they became indistinguishable from the concentrations in both treated and control ditch water (Fig. 2B). A parallel trend was seen in DOC, which was significantly higher within the bioreactors during the first three months post-installation but declined rapidly thereafter, eventually matching the background levels in the surrounding ditch water (Fig. 2C).

It is unclear whether the carbon and nitrogen were mobilized from the disturbed bog soils during bioreactor installation, or from the added substrate itself. Nonetheless, transient peaks in ammonia and DOC are consistent with previous reports of initial leaching from newly established woodchip bioreactors (Christianson et al., 2021; Healy et al., 2012; Lepine et al., 2021; Sharrer et al., 2016). In addition, the pyrolysis process used to create biochar can transform organic compounds, making them more bioavailable and thus prone to a period of initial leaching (Cairns et al., 2022). However, such leaching tends to be temporary, especially for nutrient-poor substrates like wood, as the limited pool of readily bioavailable carbon and nitrogen is quickly metabolized or leached away, leaving behind a more recalcitrant matrix. This pattern was evident in our study, where DOC concentrations returned to baseline by month three and ammonia by month six.

Several other mechanisms may have also helped to produce these transient ammonia peaks. The high DOC initially observed in the bioreactor pore water could have supported dissimilatory nitrate reduction to ammonium (DNRA), while the elevated cation exchange capacity (CEC) of the biochar–woodchip mixture may have stabilized ammonia within the substrate. As DOC levels diminished, DNRA likely declined, and denitrification became the predominant nitrogen transformation pathway. Importantly, elevated ammonia and DOC were only detected in pore water sampled directly from within the bioreactors, with no evidence of increased nutrient levels in the surrounding ditch water. This finding indicates that, regardless of their source, these initial pulses did not negatively impact water quality beyond the reactor itself, highlighting the localized and self-limiting nature of any early nutrient release.

4.3. Microbial community Structure and functional potential

Genetic analysis of resident microbial communities revealed distinct compositions within the substrate of the woodchip–biochar bioreactors compared to surrounding ditch sediments and cranberry bog surface soils (Fig. 4, Supplementary Figs. 3 and 4). Canonical Correspondence Analysis (CCA) suggested that microbial community structure in the bioreactors aligned with gradients of higher pH, soil organic matter (SOM), and cation exchange capacity (CEC), and lower bulk density (Supplementary Fig. 4). However, statistical tests did not identify these relationships as significant, indicating that while trends in microbial community composition appeared to correspond with these environmental variables, additional factors are likely also influencing community structure. Functional profiling based on taxonomic community composition further supported these findings, revealing a higher abundance of taxa associated with denitrification pathways in biochar-enhanced bioreactors. Notably, abundance of denitrifiers was significantly higher in the biochar-amended bioreactor than in a comparable bioreactor comprised of woodchips alone (Fig. 5), suggesting that the addition of biochar enhanced conditions favorable for denitrifying microbes. Despite this functional enrichment, the bioreactor substrate exhibited the lowest taxonomic diversity among all samples (Supplementary Fig. 5). This pattern suggests a microbial community strongly shaped by environmental filtering, with a relatively small

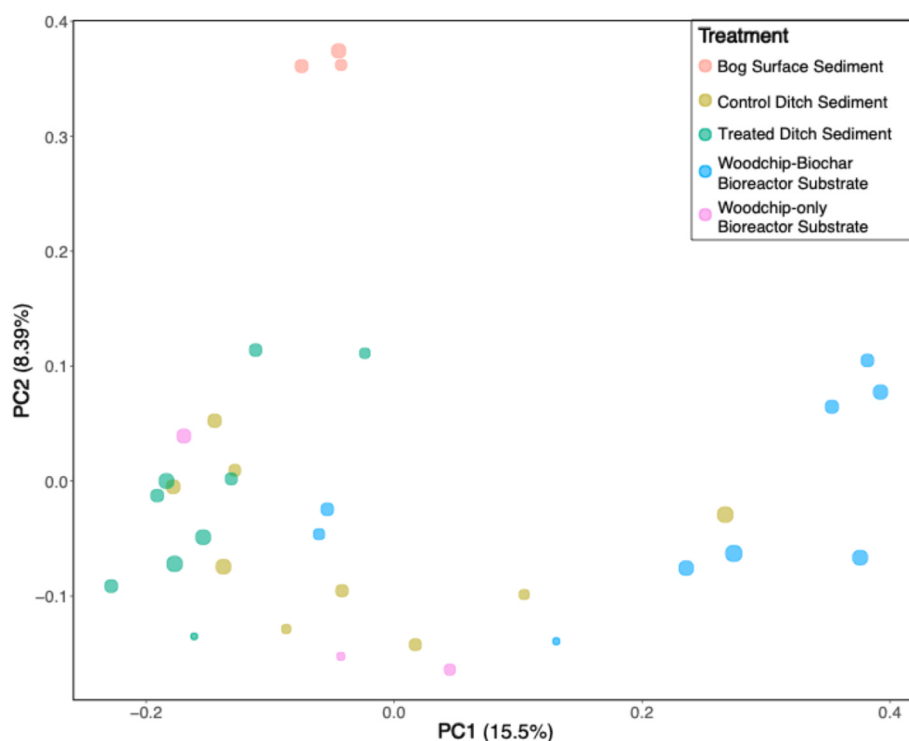


Fig. 4. Principal Coordinate Analysis (PCoA) of Microbial Community Composition – PCoA plot of microbial beta diversity based on Unweighted UniFrac distances, illustrating differences in microbial community composition among bioreactor substrate, ditch sediments, and cranberry bog surface soils. Points are color-coded by treatment group, and point size is scaled to Shannon diversity to highlight trends in microbial diversity across samples. Axis labels indicate the percentage of variation explained by each principal coordinate.

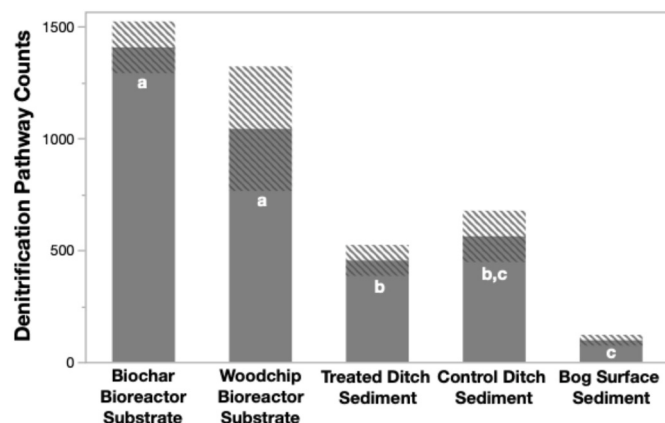


Fig. 5. Functional Inference of Denitrification Pathways via PICRUST2 Bar plot showing the relative abundance of predicted denitrification-related genes in microbial communities across different substrate types. The woodchip-biochar bioreactor exhibited significantly higher denitrifier abundance than both the control ditch and bog surface soils, as well as the woodchip-only bioreactor sampled from a separate study. Hash bands depict standard error. Lowercase letters set in bars indicate significant differences among categories based on pairwise Tukey tests – categories not connected by the same letter are significantly different.

number of dominant taxa. The selective pressures within the bioreactor, such as low oxygen and high nitrate availability, likely favored specialized denitrifiers while limiting overall community diversity. These findings highlight the trade-off between taxonomic richness and functional efficiency, demonstrating that bioreactors can support targeted microbial processes critical for nitrate removal even in the context of reduced microbial diversity. The strong influence of substrate

composition on microbial function underscores the importance of biochar amendments in optimizing bioreactor performance for enhanced nitrogen removal.

4.4. Study Limitations and future research directions

While this study provides strong evidence for the effectiveness of biochar-amended bioreactors, several limitations warrant further investigation. Our decision to incorporate biochar into the bioreactor substrate was informed by our prior research demonstrating that biochar amendment substantially reduced nutrient leaching from wetland soils (Rubin et al., 2020). However, due to site constraints, we were unable to include a side-by-side comparison of bioreactors with and without biochar. Although our results demonstrate significant nitrate removal, future studies should employ a factorial experimental design to explicitly test the impact of biochar amendment rate on bioreactor performance.

Our study differs from other agricultural bioreactor studies due to low flow rates and episodic flooding that is characteristic of coastal Massachusetts cranberry farms. Our results and field observations suggest that treated water may have moved bidirectionally throughout the monitoring period, with bioreactor pore water mixing with water upstream and downstream of the bioreactor. This was supported by the lack of statistically significant differences in nitrate, ammonia, and DOC concentrations between the upstream and downstream water samples. Therefore, we compared water from ditches that did not have bioreactors to water from ditches that contained bioreactors, combining the upstream and downstream samples in the treated water category. This unique study design should be considered when making comparisons to other bioreactor studies.

Another avenue of future investigation relates to the nitrogen concentrations in our study system. The nitrate inputs we observed (range of 0.02–3.1 mg/L; mean of 1.5 mg/L in control ditches) exceed total

maximum daily loads in the watershed by nearly two-fold and have caused significant impairment and degradation in the receiving waterways and coastal ecosystems, including a total loss of eelgrass beds in the estuary downstream ([Massachusetts Department of Environmental Protection. Massachusetts Estuaries Project Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Popponesset Bay, Mashpee & Barnstable, MA., 2004](#)). For reference, it is recommended that thresholds for nitrate-N should be on the order of 0.5 mg/L to avoid eutrophication in freshwater and coastal systems ([Zeng et al., 2016](#)). Nonetheless, many prior field-scale studies of bioreactors have focused on agricultural tile drainage systems with nitrate concentrations substantially higher than those observed in our system, often well in excess of 10 mg/L ([Christianson et al., 2021](#)), and the concentrations observed in our study may be below the saturation point for denitrifying bacteria ([Kouanda and Hua, 2021](#); [Schipper et al., 2010](#); [Wrightwood et al., 2021](#)). Given that our bioreactors successfully reduced nitrate loads under local conditions, it would be valuable to assess their efficacy in settings with even higher nitrogen concentrations to determine whether performance scales proportionally with nitrate loads.

The hydraulic properties of woodchip-biochar bioreactors represent another potential area for optimization. Our bioreactor design incorporating biochar provided higher porosity than the surrounding substrate, successfully creating a preferential flow path for water to move through the bioreactor material. However, this highlights an inherent trade-off in bioreactor design: higher porosity facilitates water movement through rather than around the bioreactor but may potentially reduce hydraulic retention time needed for complete denitrification. In our system, the slow flow conditions characteristic of cranberry farm ditches likely provided sufficient retention time despite the relatively high porosity of our substrate mixture. We observed that the woodchip-biochar bioreactors maintained better hydraulic performance compared to woodchip-only bioreactors previously installed at the site, which experienced clogging and flooding issues. Future research should systematically evaluate how different ratios of biochar to woodchips affect porosity, hydraulic conductivity, and retention time to identify optimal substrate compositions for different flow regimes and nitrogen loading conditions. The ability to 'tune' hydraulic properties by adjusting biochar proportion represents a promising avenue for optimizing bioreactor performance, though such adjustments may also influence the biogeochemical environment and microbial community structure within the reactor.

Temperature effects on nitrogen removal also merit further study. Multiple field studies have documented seasonal reductions in bioreactor performance during winter, as denitrification is a temperature-sensitive microbial process ([Robertson and Merkley, 2009](#)). In contrast, nitrate removal in our bioreactors remained consistent throughout the winter months. This may be partly explained by the slow flow and long hydraulic residence time of our reactors, as previous studies have found that longer residence time can improve nitrate removal efficiency at low temperatures ([Cooke et al., 2001](#)). Our findings also align with previous research indicating that biochar amendments may enhance cold-weather nitrogen removal by providing a stable source of labile carbon for microbial metabolism. In a study by [Vismontiené and Povilaitis \(2021\)](#), amendment with 20 % biochar improved denitrification in woodchip bioreactors at temperatures below 10 °C, which the author attributed to biochar's capacity to adsorb and slowly release DOC and nutrients for microbial use. Our biochar amendment rate was considerably higher at 50 %, which may have further amplified this effect. The potential for biochar to buffer seasonal declines in nitrogen removal warrants additional investigation, particularly in temperate and boreal regions where climate change is expected to shift precipitation patterns toward more frequent winter rain events.

Design refinements could further improve bioreactor efficacy. The early pulse of ammonium observed in our study suggests that initial substrate composition and hydraulic conditions could be optimized to

minimize potential DNRA or ammonification effects. Additionally, long-term monitoring is needed to assess substrate stability and microbial activity over time. While biochar is highly recalcitrant, woodchips degrade more rapidly, and maintaining structural integrity and adequate porosity is crucial for sustained bioreactor function. Future work should explore strategies for extending substrate lifespan, such as periodic replenishment or incorporation of different recalcitrant substrates.

Beyond optimizing individual bioreactor performance, future research should continue to explore broader applications of this technology, particularly as it incorporates biochar. Integrating bioreactors into urban stormwater treatment networks, restored wetlands, or wastewater treatment infrastructure could significantly expand their role in nutrient management. Additionally, developing cost-benefit models for different deployment scenarios would help municipalities and policymakers assess the feasibility of large-scale bioreactor implementation.

4.5. Broader implications

Although this study was conducted in a relatively specialized agricultural system in southeastern Massachusetts, the findings have broad relevance for agricultural and nutrient-impaired systems worldwide. Bioreactors offer a scalable and adaptable solution for mitigating nitrogen pollution in diverse settings, including agricultural drainage systems, urban stormwater management, and wastewater treatment. Their cost-effectiveness, driven by the ease of installation and the use of low-cost substrates such as forestry and agricultural waste products, further enhances their viability, particularly in resource-limited regions. Unlike large-scale infrastructure improvements, which often require significant capital investments and long implementation timelines, bioreactors provide an immediate and relatively inexpensive intervention to reduce nitrogen loads in waterways. Moreover, their modular design enables integration into existing hydrological systems without disrupting land use, making them an attractive solution for agricultural landscapes where nutrient runoff is a persistent concern.

The inclusion of biochar in bioreactors further enhances their effectiveness by promoting microbial denitrification, buffering pH, and adsorbing nutrients. These properties create an optimal environment for nitrogen removal, likely improving bioreactor function beyond what woodchips alone can achieve. Additionally, biochar contributes to carbon sequestration by stabilizing plant-derived carbon in a recalcitrant form that resists decomposition. This process effectively transfers atmospheric CO₂ into long-term storage in the geosphere, providing an added climate benefit alongside water quality improvements.

In addition to their utility in active agricultural systems, bioreactors may also serve as a valuable management tool in the context of retired or transitioning farmland. In New England, more than 13,500 acres of historic cranberry bogs remain in cultivation, but socio-economic pressures are expected to drive the retirement of approximately 40 % of these farms over the next 10–15 years ([Hoekstra et al., 2020](#)). Many of these farms are likely to undergo full-scale wetland restoration, involving hydrological modifications such as removing irrigation structures, refilling drainage ditches, and reconstructing stream sinuosity. During this transition, bioreactors could serve as a temporary yet effective means of intercepting nitrogen-laden runoff before it enters sensitive downstream ecosystems. Additionally, bioreactors could be incorporated into the restoration design itself, with irrigation ditches being refilled not only with sand or soil but also with a mixture of woodchips and biochar to provide ongoing nitrogen removal capacity, working in concert with the newly restored ecological functions of the larger wetland landscape. Our findings suggest that bioreactors could play a pivotal role not only in active agricultural settings but also in ecological restoration efforts aimed at mitigating legacy nutrient pollution.

4.6. Conclusions

Taken together, our results demonstrate that woodchip–biochar bioreactors provide an effective, scalable, and cost-efficient approach to reducing nitrogen pollution in agricultural drainage systems and beyond. Their adaptability across different landscapes and management scenarios makes them a valuable tool in the broader effort to mitigate nutrient pollution. Integrating bioreactors into land and water management strategies presents an opportunity to enhance nutrient removal efficiency, protect and restore aquatic ecosystems, and improve environmental resilience on a broad scale.

CRediT authorship contribution statement

Jason P. Andras: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rachel L. Rubin:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **William G. Rodriguez-Reillo:** Visualization, Software, Formal analysis. **Casey D. Chatelain:** Methodology, Investigation, Funding acquisition. **Oleander Morrill:** Investigation. **Kate A. Ballantine:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding sources

This work was funded by a grant from the U.S. Environmental Protection Agency Southeast New England Program (SNEP Grant#: SNEPWG20-9-MTHO).

Declaration of competing interest

This manuscript represents original work that has not been published or submitted elsewhere. All authors have approved the submission, and we have no conflicts of interest to disclose.

Acknowledgements

The authors would like to express their gratitude to the many individuals and organizations who contributed to this study. We are especially grateful to the team at the Barnstable Clean Water Coalition (BCWC)—Zee Crocker and Livia Graham—for their support with site access, grant development, bioreactor installation, and sample collection. Additionally, we appreciate the contributions of the bioreactor working group, including scientists from EPA Region 1, EPA Office of Research and Development, The Nature Conservancy, and the Horsley Witten Group, for their collaborative exchange of ideas that enriched this project. We also extend our thanks to Bob Wells at New England Biochar for his guidance on biochar production and for supplying the biochar used in this study. Finally, we thank the team of volunteers from BCWC and the local community whose dedication and hard work made the bioreactor installation possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.126260>.

Data availability

Data will be made available on request.

References

- Addy, K., Gold, A.J., Christianson, L.E., David, M.B., Schipper, L.A., Ratigan, N.A., 2016. Denitrifying bioreactors for nitrate removal: a meta-analysis. *J. Environ. Qual.* 45, 873–881. <https://doi.org/10.2134/jeq2015.07.0399>.
- Ahmadvand, M., Soltani, J., 2020. Effect of wheat-straw biochar on nitrate removal in laboratory denitrifying bioreactors. *Int. J. Environ. Res.* 14, 205–213. <https://doi.org/10.1007/s41742-020-00248-3>.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 704–726. <https://doi.org/10.1007/bf02804901>.
- Anderson, M.J., 2005. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62, 245–253. <https://doi.org/10.1111/j.1541-0420.2005.00440.x>.
- Ashoori, N., Teixedo, M., Spahr, S., LeFevre, G.H., Sedlak, D.L., Luthy, R.G., 2019. Evaluation of pilot-scale biochar-amended woodchip bioreactors to remove nitrate, metals, and trace organic contaminants from urban stormwater runoff. *Water Res.* 154, 1–11. <https://doi.org/10.1016/j.watres.2019.01.040>.
- Audet, J., Jégliot, A., Elsgaard, L., Maagaard, A.L., Sørensen, S.R., Zak, D., Hoffmann, C. C., 2021. Nitrogen removal and nitrous oxide emissions from woodchip bioreactors treating agricultural drainage waters. *Ecol. Eng.* 169, 106328. <https://doi.org/10.1016/j.ecoleng.2021.106328>.
- Berger, A.W., Valencia, R., Miao, Y., Ravi, S., Mahendra, S., Mohanty, S.K., 2019. Biochar increases nitrate removal capacity of woodchip biofilters during high-intensity rainfall. *Water Res.* 165, 115008. <https://doi.org/10.1016/j.watres.2019.115008>.
- Bock, E., Smith, N., Rogers, M., Coleman, B., Reiter, M., Benham, B., Easton, Z.M., 2015. Enhanced nitrate and phosphate removal in a denitrifying bioreactor with biochar. *J. Environ. Qual.* 44, 605–613. <https://doi.org/10.2134/jeq2014.03.0111>.
- Bock, E.M., Coleman, B.S.L., Easton, Z.M., 2018. Performance of an under-loaded denitrifying bioreactor with biochar amendment. *J. Environ. Manag.* 217, 447–455. <https://doi.org/10.1016/j.jenvman.2018.03.111>.
- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Silva, R.D., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwards, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., Hooft, J.J.J. van der, Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Pippel, M. von, Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Cairns, S., Robertson, I., Holliman, P., Street-Perrott, A., 2022. Treatments of wood ash amended biochar to reduce nutrient leaching and immobilise lead, copper, zinc and cadmium in aqueous solution: column experiments. *Environ. Sci.: Water Res. Technol.* 8, 1277–1286. <https://doi.org/10.1039/d1ew00962a>.
- Camargo, J.A., Alonso, Á., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environ. Int.* 32, 831–849. <https://doi.org/10.1016/j.envint.2006.05.002>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624. <https://doi.org/10.1038/ismej.2012.8>.
- Cayuela, M.L., Spott, O., Pascual, M.B., Sánchez-García, M., Sánchez-Monedero, M.A., 2024. Key biochar properties linked to denitrification products in a calcareous soil. *Biochar* 6, 90. <https://doi.org/10.1007/s42773-024-00386-3>.
- Christianson, L.E., Cooke, R.A., Hay, C.H., Helmers, M.J., Feyereisen, G.W., Ranaivoson, A.Z., McMaine, J.T., McDaniel, R., Rosen, T.R., Pluer, W.T., Schipper, L. A., Dougherty, H., Robinson, R.J., Layden, I.A., Irvine-Brown, S.M., Manca, F., Dhaese, K., Nelissen, V., Ahnen, M. von, 2021. Effectiveness of denitrifying bioreactors on water pollutant reduction from agricultural areas. *Trans. ASABE (Am. Soc. Agric. Biol. Eng.)* 64, 641–658. <https://doi.org/10.13031/trans.14011>.
- Clough, T.J., Condron, L.M., 2010. Biochar and the nitrogen cycle: introduction. *J. Environ. Qual.* 39, 1218–1223. <https://doi.org/10.2134/jeq2010.0204>.
- Cooke, R.A., Doheny, A.M., Hirschi, M.C., 2001. Bio-reactors for edge-of-field treatment of tile outflow. 2001 Sacram. <https://doi.org/10.13031/2013.7373>. CA July 29–August 1, 2001.
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14, 927. [https://doi.org/10.1658/1100-9233\(2003\)014\[0927:vaporf\]2.0.co;2](https://doi.org/10.1658/1100-9233(2003)014[0927:vaporf]2.0.co;2).
- Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUST2 for prediction of metagenome functions. *Nat. Biotechnol.* 38, 685–688. <https://doi.org/10.1038/s41587-020-0548-6>.
- Erismann, J.W., Galloway, J.N., Seitzinger, S., Bleeker, A., Dise, N.B., Petrescu, A.M.R., Leach, A.M., Vries, W. de, 2013. Consequences of human modification of the global

- nitrogen cycle. *Philos. Trans. R. Soc. B: Biol. Sci.* 368, 20130116. <https://doi.org/10.1098/rstb.2013.0116>.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892. <https://doi.org/10.1126/science.1136674>.
- Gupta, P., Ann, T., Lee, S.-M., 2016. Use of biochar to enhance constructed wetland performance in wastewater reclamation. *Environ. Eng. Res.* 21, 36–44. <https://doi.org/10.4491/eer.2015.067>.
- Healy, M.G., Ibrahim, T.G., Lanigan, G.J., Serrenho, A.J., Fenton, O., 2012. Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. *Ecol. Eng.* 40, 198–209. <https://doi.org/10.1016/j.ecoleng.2011.12.010>.
- Hendershot, W., Lalonde, H., Duquette, M., 2007. *Soil Sampling and Methods of Analysis*, second ed. <https://doi.org/10.1201/9781420005271.ch18>.
- Hoekstra, B.R., Neill, C., Kennedy, C.D., 2020. Trends in the Massachusetts cranberry industry create opportunities for the restoration of cultivated riparian wetlands. *Restor. Ecol.* 28, 185–195. <https://doi.org/10.1111/rec.13037>.
- Howarth, R.W., 2008. Coastal nitrogen pollution: a review of sources and trends globally and regionally. *Harmful Algae* 8, 14–20. <https://doi.org/10.1016/j.hal.2008.08.015>.
- Husk, B.R., Anderson, B.C., Whalen, J.K., Sanchez, J.S., 2017. Reducing nitrogen contamination from agricultural subsurface drainage with denitrification bioreactors and controlled drainage. *Biosyst. Eng.* 153, 52–62. <https://doi.org/10.1016/j.biosystemseng.2016.10.021>.
- Jaafar, N.M., Clode, P.L., Abbott, L.K., 2015. Soil microbial responses to biochars varying in particle size, surface and pore properties. *Pedosphere* 25, 770–780. [https://doi.org/10.1016/s1002-0160\(15\)30058-8](https://doi.org/10.1016/s1002-0160(15)30058-8).
- Kouanda, A., Hua, G., 2021. Determination of nitrate removal kinetics model parameters in woodchip bioreactors. *Water Res.* 195, 116974. <https://doi.org/10.1016/j.watres.2021.116974>.
- Lehmann, J., Joseph, S., 2024. Biochar for environmental management, science, technology and implementation 1–14. <https://doi.org/10.4324/9781003297673-1>.
- Lepine, C., Christianson, L., Soucek, D., McIsaac, G., Summerfelt, S., 2021. Metal leaching and toxicity of denitrifying woodchip bioreactor outflow—Potential reuse application. *Aquac. Eng.* 93, 102129. <https://doi.org/10.1016/j.aquaeng.2020.102129>.
- Massachusetts Department of Environmental Protection, 2004. *Massachusetts Estuaries Project Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Popponesset Bay, Mashpee & Barnstable, MA*. Massachusetts Department of Environmental Protection.
- McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2011. An improved green genes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6, 610–618. <https://doi.org/10.1038/ismej.2011.139>.
- Mohanty, S.K., Valencia, R., Berger, A.W., Yu, I.K.M., Xiong, X., Saunders, T.M., Tsang, D. C.W., 2018. Plenty of room for carbon on the ground: potential applications of biochar for stormwater treatment. *Sci. Total Environ.* 625, 1644–1658. <https://doi.org/10.1016/j.scitotenv.2018.01.037>.
- Nelson, N.O., Agudelo, S.C., Yuan, W., Gan, J., 2011. Nitrogen and phosphorus availability in biochar-amended soils. *Soil Sci.* 176, 218–226. <https://doi.org/10.1097/ss.0b013e3182171eac>.
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2024. *Vegan: community ecology package*. <https://doi.org/10.32614/cran.package.vegan>.
- Robertson, W.D., Merkley, L.C., 2009. In-stream bioreactor for agricultural nitrate treatment. *J. Environ. Qual.* 38, 230–237. <https://doi.org/10.2134/jeq2008.0100>.
- Rubin, R.L., Anderson, T.R., Ballantine, K.A., 2020. Biochar simultaneously reduces nutrient leaching and greenhouse gas emissions in restored wetland soils. *Wetlands* 40, 1981–1991. <https://doi.org/10.1007/s13157-020-01380-8>.
- Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C., 2010. Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. *Ecol. Eng.* 36, 1532–1543. <https://doi.org/10.1016/j.ecoleng.2010.04.008>.
- Sharrer, K.L., Christianson, L.E., Lepine, C., Summerfelt, S.T., 2016. Modeling and mitigation of denitrification ‘woodchip’ bioreactor phosphorus releases during treatment of aquaculture wastewater. *Ecol. Eng.* 93, 135–143. <https://doi.org/10.1016/j.ecoleng.2016.05.019>.
- Tan, X., Liu, Y., Gu, Y., Xu, Y., Zeng, G., Hu, X., Liu, Shao-bo, Wang, X., Liu, Si-mian, Li, J., 2016. Biochar-based nano-composites for the decontamination of wastewater: a review. *Bioresour. Technol.* 212, 318–333. <https://doi.org/10.1016/j.biortech.2016.04.093>.
- vanDriel, P.W., Robertson, W.D., Merkley, L.C., 2006. Denitrification of agricultural drainage using wood-based reactors. *Trans. ASABE (Am. Soc. Agric. Biol. Eng.)* 49, 565–573. <https://doi.org/10.13031/2013.20391>.
- Vismontiené, R., Povilaitis, A., 2021. Effect of biochar amendment in woodchip denitrifying bioreactors for nitrate and phosphate removal in tile drainage flow. *Water* 13, 2883. <https://doi.org/10.3390/w13202883>.
- Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 1. <https://doi.org/10.1128/msystems.00009-15>, 10.1128/msystems.00009-15.
- Wrightwood, O.M., Hattaway, M.E., Young, T.M., Bischel, H.N., 2021. Assessment of woodchip bioreactor characteristics and their influences on joint nitrate and pesticide removal. *ACS EST Water* 2, 106–116. <https://doi.org/10.1021/acsestwater.1c00277>.
- Yao, S.Q., Groffman, P.M., Alewell, C., Ballantine, K., 2017. Soil amendments promote denitrification in restored wetlands. *Restor. Ecol.* 26, 294–302. <https://doi.org/10.1111/rec.12573>.
- Zeng, Q., Qin, L., Bao, L., Li, Y., Li, X., 2016. Critical nutrient thresholds needed to control eutrophication and synergistic interactions between phosphorus and different nitrogen sources. *Environ. Sci. Pollut. Res.* 23, 21008–21019. <https://doi.org/10.1007/s11356-016-7321-x>.