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Resilience in Function, Microbial Community Structure, and Nitrifier Composition of Bench-Scale Biofilm Reactors during Wet Weather Disturbances

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Cite This: ACS EST Water 2025, 5, 575−582
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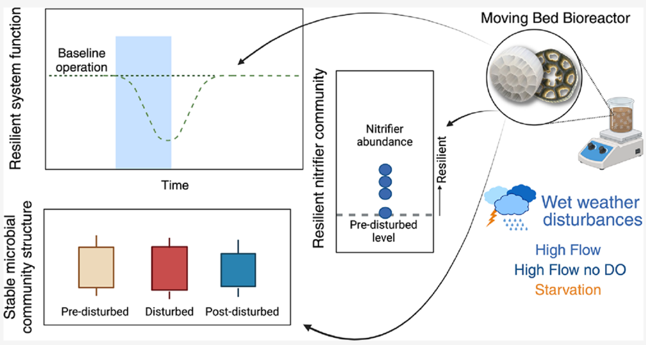

ABSTRACT: Wet weather events, such as hurricanes and tropical storms, are on the rise globally due to climate change. Activated sludge systems are vulnerable to wet weather, as hydraulic overloading can cause a washout of biomass. Biofilm-based treatment technologies, such as moving bed biofilm reactors (MBBR), can improve resiliency by preventing biomass washout and protecting slow-growing nitrifiers. In this study, we investigated the resilience of a biofilm system challenged by wet weather events and examined the impact of different disturbances on the system's microbial community. We performed three simulated wet weather stressor experiments on replicate bench-scale MBBR bench reactors: (1) high flow and high load (representative of flooding and a first flush); (2) high flow, high load, and no dissolved oxygen (DO) (representative of flooding with power outage); and (3) starvation and no DO (temporary plant shut down). The biofilm system's function, in terms of ammonia-N and soluble organic carbon removal, was resilient to the wet weather disturbances as the function recovered to the baseline performance after disturbance within hours. The biofilm microbial community structure was resistant (not impacted by the disturbance), and the nitrifier community was resilient (the ability to recover to baseline conditions after the disturbance).

KEYWORDS: *resilience, wastewater, biofilm, MBBR, wet weather, disturbance, nitrifier*

■ INTRODUCTION

Wet weather events such as hurricanes, tropical storms, and heavy precipitation are increasing in frequency and duration as a result of climate change.[1] In conventional activated sludge water resource recovery facilities (WRRFs), frequent storms and extreme flooding can cause increased flow coming into the facilities by up to 70% of the base influent flow.[2] Increases in flow pose a risk to the secondary treatment of a conventional WRRF that harnesses active biomass to aerobically transform ammonia-N and organic carbon into nitrate-N and CO_2, respectively.[3] Increased flow also decreases the hydraulic retention time (HRT) of the system. This can result in the washout of suspended activated sludge from the clarifiers due to hydraulic overloading, producing diluted return activated sludge (RAS) that is sent back to the secondary aeration basins. Loss of active biomass threatens the wastewater system's ability to comply with effluent discharge permits. In addition to biomass washout, increased flow can also flush solids that have accumulated in sewer lines during dry weather conditions, resulting in an increase in the solids load to the WRRF, referred to as the "first flush".[4] Beyond flow impacts, wet weather events frequently cause widespread power outages, interrupting wastewater treatment unit processes.[5] Power outages can shut down critical equipment such as blowers and pumps, which can result in a cessation of aeration in the bioreactors. Because aerobic biological treatment processes rely on external oxygenation, the absence or reduction of aeration in bioreactors can considerably hinder the aerobic biological oxidation of pollutants such as carbon and ammonia. As WRRFs are vulnerable to wet weather events, there is a need to advance the resiliency of biological wastewater treatment systems to these events.

In the context of critical infrastructure like WRRFs, resilience refers to the ability to reduce the magnitude and/or duration of the impact of disruptive events.[6] Resilience

Received: June 6, 2024
Revised: January 2, 2025
Accepted: January 6, 2025
Published: January 15, 2025

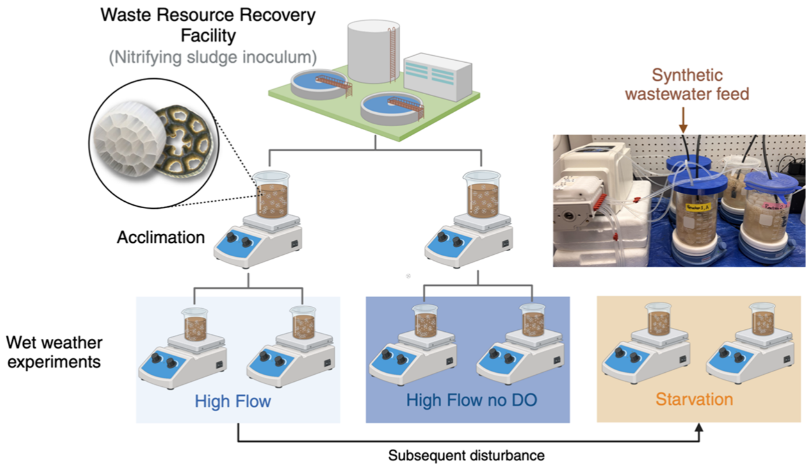

Figure 1. Experimental design of the wet weather disturbances on bench scale MBBR reactors.

includes factors such as the ability to maintain treatment performance and effluent water quality during hazards, as well as the ability to recover from process interruptions or failures.[7] Additionally, the microbial community in activated sludge plays a critical role in determining how a WRRF's treatment process resists and/or recovers after a disturbance. Prior studies suggest that taxonomically and metabolically diverse activated sludge microbial communities contribute to the system's functional stability.[8]

In ecology, ecosystem stability is a microbial community's response to disturbance[9] and a disturbance is an event that triggers discrete changes in the physical or chemical environment that may affect the microbial community composition and structure.[10] Disturbances can be classified as pulses or presses. A pulse disturbance is characterized as a short, discrete event (e.g., toxic shock load), whereas a press disturbance is a long-term or continuous event (e.g., long-term temperature shift).[10] In some cases, the stability of a microbial community can vary, depending on the duration of a disruption. Some instances of wet weather events, such as increased hydraulic loading due to rainfall and increased substrate loading during the first flush, are examples of pulse disturbances, as their durations are typically only hours. In contrast, a press disturbance occurs for a prolonged period, for example, plant shutdown due to equipment damage for weeks or months following a wet weather event. After a disturbance, microbial community composition may shift in structure and/or function, and individual taxa may go extinct while others increase in abundance. This may disrupt or destabilize ecosystem function. Stability can be expressed in terms of resistance (insensitivity to disturbance) or resilience (capacity to regain structure, function, and processes post-disturbance). If the microbial community structure and composition of biomass are resilient to wet weather disturbances, the WRRF can rapidly recover after the disturbance has subsided.[10] A limited number of prior studies have investigated the impact of pulse and press disturbances on activated sludge system function and microbial community structure.[3,11−13] However, very little attention has been devoted to the combined effect of pulse and press disturbances.[10,14,15] This lack of knowledge limits the accuracy of predictions of the response of WRRF systems to complex, and realistic disturbances, such as wet weather events.[16]

Biofilm-based treatment systems such as MBBR have the potential to improve the resilience of WRRFs to wet weather events. MBBR systems may be used in a compact high-rate process (<1 h total HRT) for secondary treatment[17] which can mitigate the impacts of high flow. The active biomass is held on carrier media surfaces via attached growth (i.e., biofilm). Thus, biomass washout is prevented during hydraulic overloading.[18] The biofilms harnessed in MBBR carrier media additionally offer protection to sensitive populations such as nitrifiers.[19,20] While biofilm-based MBBR treatment systems are implemented in full-scale in more than 50 countries,[21] mainly for process intensification,[22] our understanding of their performance and recovery in wet weather disturbances is limited. Few studies have quantified the impact of wet weather events (including conditions such as high flow, high loading, and power outages) on microbial communities in conventional activated sludge systems.[11−13,23,24] More research is needed to assess the functional and microbial community structure resilience of MBBR systems to high hydraulic loads, high influent loadings, and power outages.

The objectives of our study were to investigate the impact of wet weather events on (1) the function of the biofilm microbial community, (2) the structure of the biofilm and suspended biomass community, and (3) the nitrifier microbial community. To accomplish these, MBBR bench-scale reactors were subjected to three simulated wet weather disturbances: (1) a pulse disturbance of high flow and high load for 24 h (representative of flooding and a first flush); (2) a pulse disturbance of high flow and high load and no air for 24 h (representative of flooding, first flush, and simultaneous power outage); and (3) a press disturbance of prolonged starvation and no air for 3 weeks (representative of temporary plant shut down), after a pulse disturbance. A high load was created by increasing the flow rate of the influent during the simulated wet weather experiments. Biofilm function was characterized in terms of the soluble chemical oxygen demand (sCOD) and ammonia-N removal efficiency. We also assessed the combined effects of a pulse and press disturbance on the microbial community. Biofilm and suspended growth microbial communities in the reactors were characterized from samples collected pre-disturbance, during the disturbance, and post-disturbance using 16S rRNA gene amplicon sequencing.

https://doi.org/10.1021/acsestwater.4c00524
ACS EST Water 2025, 5, 575−582

■ MATERIALS AND METHODS

MBBR Reactor Setup, Operation, and Monitoring. Two parent 2L MBBR batch reactors (R1 and R2) were operated at a 60% fill ratio with virgin K3 biofilm carriers (specific surface area of 500 m^2/m^3) and inoculated with return activated sludge (RAS) from a WRRF in Houston, Texas (Figure 1). The reactors were aerated, and the carriers were submerged in RAS for 24 h. After 24 h, the HRT in the reactors was set to 5 days for 2 months, achieved by intermittently feeding a synthetic medium containing 50 mg-N/L of ammonia-N, 250 mg/L of sCOD, and trace nutrients (Table S1 provides influent composition details). The HRT of the reactors was decreased to 2 days by operating as chemostat reactors with synthetic medium continuously fed at the flow rate of 0.03 L/h for 2 months. The HRT of the reactors was further decreased to 12 h for another 2 months with synthetic medium continuously fed at a flow rate of 0.125 L/h and a loading rate of 1.67 sCOD/m^2·d of sCOD and 0.33 g NH_4^+-N/m^2·d of ammonia-N. The HRT was decreased during each stage after stable sCOD and ammonia-N removal performance was achieved. We defined stable removal as consistent removal efficiency with a standard deviation of less than 10% over a period of at least 1 week.

Simulated Wet Weather Experimental Design and Sample Collection. After the operation of the parent reactors for 156 days, they were each divided into replicate 1 L bench MBBR reactors for a total of four reactors: R1A, R1B, R2A, and R2B. Three simulated wet weather stressor experiments were conducted on replicate bench MBBR reactors: (1) a pulse disturbance of high flow and high load for 24 h, representative of flooding and a first flush (termed "high flow" disturbance); (2) a pulse disturbance of high flow, high load, and no air for 24 h, representative of flooding with power outage (termed "high flow no dissolved oxygen (DO)" disturbance). Both disturbance conditions were achieved by decreasing the HRT of the reactors from 12 h to 2 h for 24 h with the same concentration of the synthetic feed (loading rates are shown in Table S2). After 24 h, the influent flow was reverted to achieve a 12 h HRT. The reactors used in the high flow no DO test were also subjected to no aeration or stirring (DO measured below 0.5 mg/L). Following the high flow disturbance on reactors R1A and R1B, a subsequent disturbance (3) consisted of a sequential press disturbance of prolonged starvation and no air for 3 weeks, representative of a temporary plant shutdown (termed "starvation" disturbance), after a pulse disturbance (disturbance 1). This condition was achieved by halting influent, aeration, and mixing for 3 weeks.

Effluent samples were collected every hour during the 24 h disturbance experiment and on days 290 and 390 of the 3 week starvation experiment. Additional samples were collected every 2 h for 48 h after the disturbance experiment to characterize the recovery of the reactors after the disturbances. Pieces of carrier media for biofilm samples and of suspended biomass samples were collected before the start of the experiment, at the highest disturbed condition, at the 24th hour of the disturbance, and after 48 h into the post-disturbed condition. Resilient performance was defined as a similar or statistically insignificant removal efficiency of ammonia-N and sCOD in the reactors as the pre-disturbed condition.

Water Quality Analysis. pH in the reactors was controlled manually via acid (concentrated HCl) or base (NaOH, 3N) addition to maintain the pH at approximately 7 ± 0.5. Influent and effluent samples were collected three times weekly and analyzed for sCOD and ammonia-N. sCOD was measured using low-range CHEMetrics COD vial kits with a potassium hydrogen phthalate blank standard curve (CHEMetrics, US). Ammonia-N was measured using Standard Method 4500.[25] Samples were filtered through 0.45 μm nitrocellulose filters and stored at 4 °C until analysis of soluble chemicals within 3 days. The influent was kept refrigerated at 4 °C, and the reactors were operated at room temperature (typically between 21 and 24 °C). The reactors were aerated constantly to maintain a DO concentration above 4 mg/L and measured daily with an optical DO probe and HQ440D benchtop meter (Hach, US).

Biofilm Extraction, DNA Extraction, PCR, and Gel Electrophoresis. Biofilm samples were collected for the analysis of the attached growth microbial community structure. To dislodge the biofilm, the carrier media containing the biofilm were submerged in 1% (w/v) NaOH solution in a conical flask and mixed at 100 rpm for 30 min at 60 °C in a hot stir plate. The carriers in NaOH solution were ultrasonicated at 40 Hz for 20 min using a Branson 5800 Series ultrasonic bath (Bransonic, US). 30 mL of the sloughed-off biofilm was then spun down, collected in pellets, and stored in a −80 °C freezer until extraction. Additionally, suspended biomass was collected from each reactor under each condition, centrifuged, and stored similarly until extraction.

DNA was extracted from the biofilm and suspended biomass samples for the three different disturbances (1, 2, and 3) and for the three different conditions (pre-disturbed, disturbed, and post-disturbed). Bead beating was carried on a BioSpec Mini-BeadBeater-24 for 1 min at 3500 oscillations/min, placed on ice for 2 min, bead beaten once more for 1 min at 3500 oscillations/min, and then placed on ice in preparation for extraction. The genomic DNA was extracted using Promega's Maxwell RSC PureFood GMO and Authentication Kit (Promega Corporation, US). Extracted DNA samples were amplified in duplicate 50 μL reactions with the following cycling parameters: 98 °C for 30 s, 35 cycles of 98 °C for 10 s, 57 °C for 15 s, and 72 °C for 30 s, and a final elongation step at 72 °C for 2 min. All amplifications were performed using the F515 (5′- GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACHVGGGTWTCTAAT-3′) primer pairs modified with partial Illumina adapters (Genewiz/Azenta Life Sciences, US) for sequencing of the V4 hypervariable region of the 16S rRNA gene. Following amplification, the PCR amplicons were visualized and purified by using gel electrophoresis on a 1.5% agarose gel. Bands of the expected size were verified, purified from the gel, and extracted using a Monarch Gel Extraction kit (New England Biolabs Inc., US). Concentrations of extracted, amplified, and purified DNA were measured using a DNA HS Assay kit on a Qubit fluorometer (Thermo Fisher Scientific, US).

Sequencing and Bacterial Community Analysis. The purified extracts were sent for Amplicon-EZ Next Generation Sequencing (NGS) (Genewiz/Azenta Life Sciences, US). Sequence analysis was conducted using the QIIME2 v. 2023.2 pipeline.[26] Pair-end, demultiplexed reads for all samples were combined, quality filtered, trimmed of poor-quality bases, dereplicated, chimera filtered, pair merged, and identified as amplicon sequence variants (ASVs) using the DADA2 plug-in ref 27. Taxonomy was assigned by training a naïve-Bayes classifier on the V4 region of the 16S gene in the SILVA database. Unrarefied sequences were also aligned to a custom

16S rRNA gene sequence database of known nitrifiers obtained from (Sulfide inhibition of nitrite oxidation in activated sludge depends on microbial community composition).[28] This additional step was used to identify ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) ASVs in the samples. All aligned nitrifier ASVs had over 248 base pair alignment lengths, e-values below 2.3×10^{-115}, and bit scores above 405. In addition, the aligned nitrifier ASVs were compared with the Bacterial 16S Ribosomal RNA RefSeq Targeted Loci project database by using BLAST to ensure that the nitrifying taxonomic assignment was the best available assignment. From this analysis, 79 confirmed nitrifier ASVs were detected in the samples. Rarefied ASV tables (rarefied to 86,000 reads per sample) were used to calculate alpha diversity metrics using QIIME2.

Statistical Analysis. A Shapiro−Wilk statistical test[29] was applied to check the assumption of normality in the distribution of the data. When the test showed a non-normal distribution, then nonparametric tests like the Kruskal−Wallis test by ranks[30] were used to test for significant differences in alpha-diversity by conditions (pre-disturbed, disturbed, and post-disturbed). Dunn's post hoc[31] test was used to identify the significant differences between pairs of conditions if a significant effect was detected. Dunn's post hoc test was selected as some sample sizes were unequal. All of the statistical analysis was done in the R environment.

RESULTS

Resilience of Process Performance in Response to Wet Weather Disturbances. Process performance resilience was measured via two metrics: (1) the deviation of performance from baseline performance and (2) the recovery time for the performance to return to baseline performance levels. The baseline performance (i.e., the pre-disturbed condition) of the parent reactors was evaluated for 36 days. The parent bench reactors, R1 and R2, had a baseline sCOD removal performance of 81.6% ± 5.58% (R1) and 82.6% ± 8.78% (R2); and baseline ammonia-N removal performance of 62.6% ± 5.72% (R1) and 59.4% ± 5.07% (R2) over 36 days (Figure S1A−D: 0 to 888 h; Figure S1E,F: 0 to 16 h). After splitting the parent reactors into R1A and R1B and R2A and R2B, the replicate reactors were subjected to two different wet weather pulse disturbances: (1) high flow, and (2) high flow no DO. R1A and R1B were subjected to high flow and high load for 24 h, during which the HRT was decreased from 12 to 2 h for 24 h and then reverted to a 12 h HRT. During the 2 h HRT, the influent sCOD load and ammonia-N load were increased from 2.16 to 14.8 g sCOD/m^2·d and 0.36 to 2.42 g NH_4^+-N/m^2·d (Table S2). During this period, the effluent sCOD concentration increased and the reactor's removal performance declined (below 5% sCOD removal), as shown in Figure 2A,B. The disturbance overloaded the reactors for a short period of time, exceeding the capacity of the bioreactor's maximum substrate utilization rate and thus resulting in the discharge of untreated substrate in the effluent. The sCOD removal performance of R1A recovered to 75.5% ± 3.48% with a recovery time of 8 h, comparable to the pre-disturbed condition (p = 0.103). Reactor R1B recovered to 84.2% ± 8.09% with a recovery time of 1 h after removing the disturbance, similar to the sCOD removal efficiency in the pre-disturbed condition (p = 0.838). Details of recovery time are shown in Table S3, and removal rates are shown in Table S4. Thus, the sCOD removal performance of the bench scale MBBR reactors was resilient to high flow disturbance, as the performance recovered back to the baseline performance within hours after the disturbance subsided.

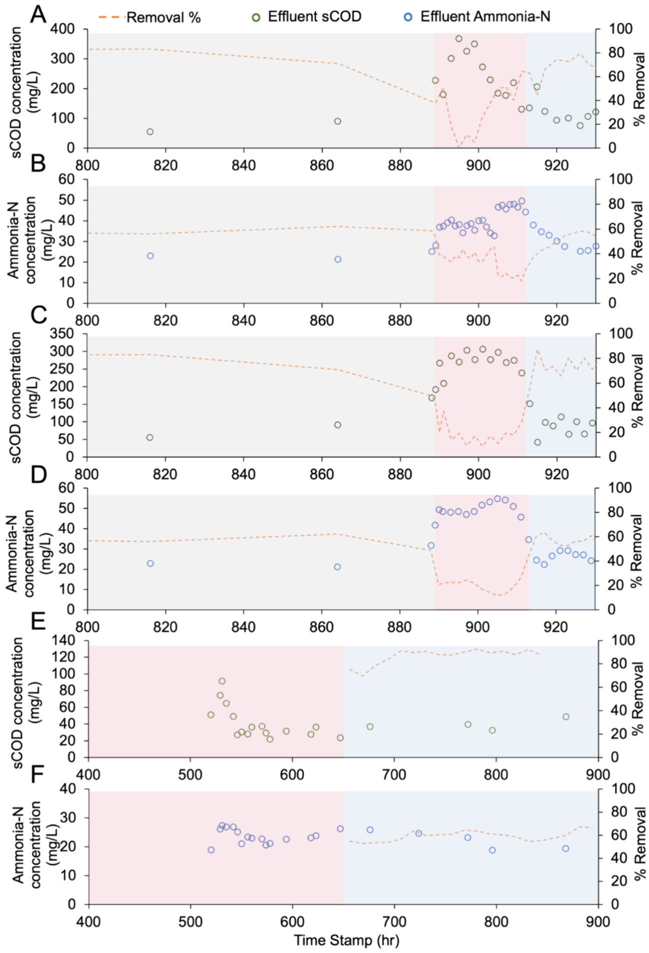

Figure 2. Functional resilience of bench-scale MBBR reactors subjected to three wet weather disturbances based on sCOD and ammonia-N removal performance. Gray shading denotes the pre-disturbed period, red shading denotes the disturbance period, and blue shading denotes the post-disturbed period. Ammonia-N and sCOD effluent concentrations and removal performances are shown in (A, B) for high flow; (C, D) for high flow no DO; (E, F) for starvation.

The ammonia-N removal was also resilient to the High Flow disturbance. During the 2 h HRT period, the ammonia-N concentration in the effluent increased, and the removal performance decreased to the lowest efficiency of 18 and 9% ammonia-N removal in R1A and R1B, respectively. The ammonia-N removal performance of R1A recovered to 56.8% ± 2.01% with a recovery time of 13 h, and R1B recovered to 56.8% ± 2.01% with a recovery time of 10 h after the disturbance ceased. The ammonia-N removal performance was not significantly different between the post-disturbance and pre-disturbed periods (p = 0.153 for R1A and p = 0.221 for R1B), indicating functional resiliency in terms of ammonia-N removal. On average, the recovery time for ammonia-N removal was longer (10 h) than the recovery time of sCOD removal (8 h), likely due to the slower growth rate of nitrifiers as compared to heterotrophs.[32]

For the high flow and no DO disturbance, we subjected the reactors R2A and R2B to high flow, high load, and no aeration

https://doi.org/10.1021/acsestwater.4c00524
ACS EST Water 2025, 5, 575−582

for 24 h. The decrease and recovery of sCOD and ammonia-N removal efficiency and recovery time were similar to those observed in high flow disturbance (Figure 2B). The sCOD and ammonia-N removal efficiency in the post-disturbed condition was not significantly different compared to the pre-disturbed condition, and therefore, the performance was resilient (Tables S5 and S6). Thus, the additional stress caused by the loss of aeration had a negligible effect on the reactors.

For the third simulated wet weather disturbance, we used reactors R1A and R1B after 2 days of recovery following the high flow disturbance, at which point they demonstrated similar sCOD and ammonia-N removal efficiency to baseline conditions. In this experiment, we subjected the reactors to an additional press disturbance that consisted of no influent flow and no aeration for 3 weeks (starvation). After 3 weeks, the reactors were operated with a 12 h HRT and the sCOD removal performance recovered to 86.5 ± 6.8% (R1A) and 79.2 ± 8.9% (R1B) over the subsequent 2 weeks, and sCOD removal performance increased as compared the pre-disturbed condition (p = 0.0254 for R1A and p = 0.0124 for R1B). Ammonia-N removal efficiency also recovered after the starvation disturbance and increased to significantly higher levels as compared to the pre-disturbed performance, at 59.3 ± 4.4% (R1A, p = 0.00342) and 62.1 ± 4.2% (R1B, p = 0.000305). Therefore, the sCOD and ammonia-N removal performance of the biofilm reactors was improved during the starvation disturbance, as the removal efficiency was increased by the sequential press disturbance.

The Disturbances Did Not Shift the Microbial Community Diversity in the Bioreactors. To investigate the impact of the disturbances on bioreactors' microbial community structure, we collected biofilm and suspended biomass samples at three different time points during the disturbance experiments, pre-disturbance, during the disturbance, and post-disturbance and characterized the microbial communities using 16S rRNA gene sequencing. We quantified alpha diversity using the chao1 index,[33] a species richness index of the microbial communities (Figure 3). For all three disturbances, we observed no significant differences in alpha diversity between biofilm samples collected at the pre-disturbed, disturbed, and post-disturbed time points (Table S7). This indicates that the biofilm microbial community structure was resistant in the face of wet weather disturbances. This is consistent with the performance results that we observed (Figure 1), as the integrity of the biofilm microbial community structure resulted in the preservation of microbial community function in terms of ammonia-N and sCOD removal along with structural resilience of the microbial community following exposure to the disturbances. These results support that biofilm-based treatment can protect the microbial community and its function during both pulse and press disturbances.

The microbial community structure in the suspended biomass was also resistant to the pulse disturbance, as there were no significant differences in alpha diversity between the pre-disturbed, disturbed, and post-disturbed conditions (Table S7). However, there was a significant increase in the chao1 index between the pre-disturbed and post-disturbed suspended biomass microbial communities from the starvation disturbance experiment (p = 0.04763, Table S8).

Nitrifiers Were Resilient to Wet Weather Disturbances. As nitrifiers are a critical yet sensitive and relatively low-abundance microbial population, we wanted to investigate the effect of wet weather events on the abundance of nitrifiers in the biofilm. Figure 4 shows the changes in the relative abundance of nitrifiers in the biofilm microbial communities between the pre-disturbed vs disturbed and the pre-disturbed vs post-disturbed conditions from the three disturbance experiments. The dashed line represents no change in total nitrifier ASV relative abundance as compared to the pre-disturbed sample. We observed that the abundance of nitrifiers in the biofilm decreased during the disturbance (i.e., the dots fell below the dashed line for the disturbed compared to pre-disturbed) but recovered after the disturbance (i.e., the dots fell above the dashed line for post-disturbed conditions). This indicates that the nitrifier community was resilient to pulse disturbances. In the high flow disturbance, the nitrifier ASVs in

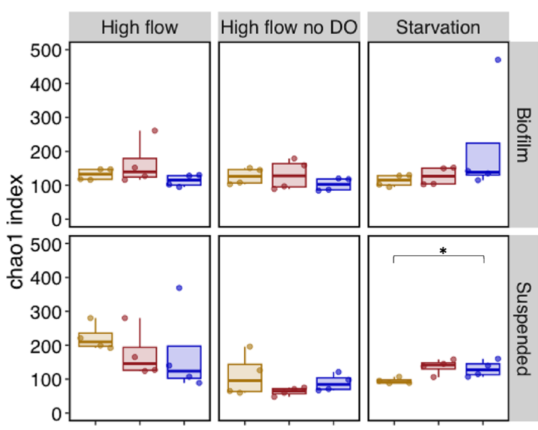

Figure 3. Biofilm (top panels) and suspended biomass (bottom panels) microbial community diversity (chao1 index) of samples collected during pre-disturbed (yellow), disturbed (red), and post-disturbed (blue) conditions from the three simulated wet weather experiments. Asterisks (*) indicate significant differences between the conditions.

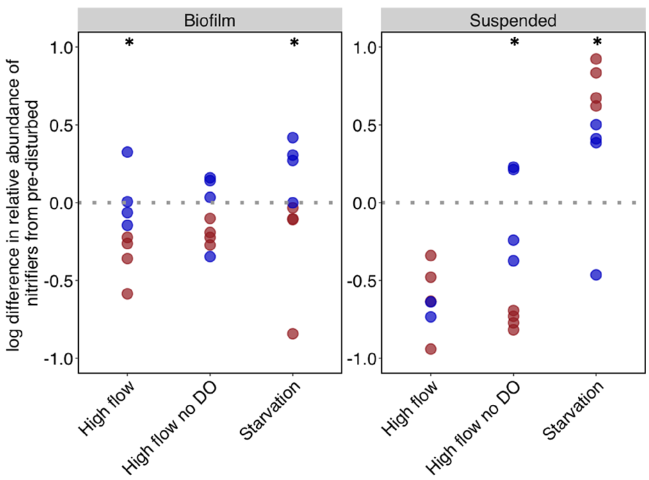

Figure 4. Changes in the biofilm and suspended biomass relative abundance of nitrifiers (log-transformed) from the MBBR bench scale reactors from pre-disturbed (gray line), disturbed (red), and post-disturbed (blue) conditions, during the three disturbance experiments. The gray dotted line at zero indicates no change in the relative abundance of nitrifier ASVs as compared to the pre-disturbed sample and thus corresponds to a resilient nitrifier community. Asterisks (*) indicate significant differences between the disturbed and post-disturbed conditions.

https://doi.org/10.1021/acsestwater.4c00524
ACS EST Water 2025, 5, 575−582

the biofilm decreased in relative abundance during the disturbance and they returned to significantly higher relative abundances in the post-disturbance samples ($p = 0.0209$). In the high flow, no DO disturbance experiment, nitrifier ASVs in the biofilm decreased in relative abundance during the disturbance and then recovered post-disturbance. Overall, these results are consistent with the functional resilience results and show that the biofilm nitrifier communities were resilient to the pulse disturbances as they recovered to similar relative abundances as observed in pre-disturbed samples.

In the starvation disturbance, we observed an unexpected improvement in nitrification performance post-disturbance that may have been caused by several factors. One possibility is that the nitrifiers survived despite the starvation conditions of no feed or air,[34] whereas other microbial community members died, resulting in a higher relative abundance of nitrifiers post-disturbance and less competition. Another factor that may explain the persistence of nitrifiers during the disturbance is that the decay rates of AOB are reduced in the absence of oxygen. A prior study of a month-long starvation experiment reported that AOBs have a lower decay rate in anaerobic (0.06 d^{-1}) and anoxic (0.1 d^{-1}) conditions as compared to aerobic conditions (0.2 d^{-1}).[35] Another possibility is that the nitrifiers increased in relative abundance because long SRTs favor the growth of slow-growing nitrifiers.[36] The nitrifiers may have efficiently utilized the protein and polysaccharides resulting from cellular lysis for growth[37] in the long SRT condition, allowing them to increase in abundance and improving performance efficiency. Previous studies show that slow-growing microbes are more resistant to press disturbances and also efficient in resource use under stress.[11,38]

In contrast to the biofilm nitrifiers, the suspended growth nitrifiers were less resilient to wet weather disturbances. In the high flow disturbance suspended biomass samples, we observed a decrease in the relative abundance of nitrifier ASVs between the pre-disturbed vs disturbed and the pre-disturbed vs post-disturbed conditions, indicating that the nitrifier microbial community was not resilient in the suspended biomass. The disturbed and post-disturbed relative abundance compared to pre-disturbed were not significantly different (Table S9). Importantly, the nitrifiers in the biofilm microbial community were present at a significantly higher relative abundance compared to the suspended biomass community ($p = 0.00556$ for pre-disturbed and $p = 0.000218$ for post-disturbed) (Table S10).

■ DISCUSSION

In our study, nitrification performance was resilient in the biofilm wastewater treatment systems to wet weather disturbances. Nitrification is a critical biotransformation process in wastewater treatment that converts ammonia to nitrite and nitrate.[39] Disturbances such as low HRTs can hamper nitrification irrevocably in conventional activated sludge systems.[12] In this study, we show that by using a biofilm-based system, low HRT conditions only temporarily disrupted nitrification performance during the disturbance, and the system quickly recovered after the disturbance ceased. These results corroborate the results of prior studies that explored the effects of elevated hydraulic loading on system performance with biofilm-based technology.[38,40–42] We build on prior work by examining the effects of disturbances on both system function and microbial community structure across several different wet weather disturbances. We demonstrate that the microbial community structure and composition of nitrifiers responsible for nitrification within a biofilm were protected from washout across the disturbances, consistent with previous studies.[38] The biofilm system may have provided a shielding effect that protected the active microbial biomass from washout, also maintaining the system's microbial diversity. In contrast, in suspended activated sludge systems, the microbial community and slow-growing microbial populations like nitrifiers can shift significantly due to disturbances.[3,11] A previous study showed that disturbances of increased flow rate by 50% and 100% over 100 days decreased nitrifier abundance in two pilot-scale activated sludge biological nutrient removal systems.[12] Biofilm configuration can thus aid nitrifiers' growth and additionally provide enhanced protection to nitrifiers during wet weather disturbance, conserving nitrification performance, which can be difficult to attain in a conventional activated sludge system.

Our results suggest that in an actual storm event, a biofilm system would likely experience a loss of performance throughout the duration of the wet weather event, which can result in potential effluent permit violations. Excluding reactor performance during disturbances, the findings from the MBBR wet weather disturbances demonstrate that the sCOD and ammonia-N removal performance of the biofilm reactors was resilient, i.e., no change between pre- and post-disturbance. This is consistent with a previous study by Vuono, which showed that the system function in activated sludge wastewater treatment was not altered post-disturbance.[13] However, the disruption in the function of the treatment systems during disturbance was not evaluated in this prior study, possibly attributed to their use of a lower sampling frequency as compared to that in our work. In our study, we were able to collect samples with high temporal resolution and thus capture the performance before, during, and after wet weather disturbances. Our observations indicate that the duration of impaired performance of the wastewater system is directly related to the duration of the wet weather event.

Biofilm-based treatment systems such as MBBR can thus be an approach to improving the resilience of WRRFs to wet weather events. There are additional benefits of utilizing biofilms in wastewater treatment systems compared to conventional suspended growth activated sludge systems, such as process intensification, smaller space requirements, higher biomass retention, and low HRT operations. Biofilm-based systems are also good retrofit options in space-limited localities. Future research should include cost-benefit analyses to assess the economic viability of integrating biofilm-based treatments to enhance the resilience of wastewater systems during periods of wet weather. Further research is also needed to explore the long-term effects of wet weather events on biofilm-based treatment systems, including their resilience over multiple disturbance events and under varying environmental conditions. Additionally, exploring novel strategies for enhancing the stability and functionality of biofilm-based treatment systems, such as incorporating advanced monitoring and control techniques[43,44] or utilizing innovative materials for biofilm support matrices,[45] could further improve their suitability for mitigating the impacts of wet weather events on wastewater treatment processes.

■ CONCLUSIONS

In this study, we demonstrated that the ammonia-N and sCOD removal performance of bench scale MBBR reactors was

https://doi.org/10.1021/acsestwater.4c00524
ACS EST Water 2025, 5, 575−582

resilient to three different wet weather disturbances as the reactor performance recovered back to baseline operation after the disturbances subsided. The results showed that the biofilm microbial community structure was resistant to all the wet weather disturbances, and the nitrifiers in the biofilm community were resilient to the pulse wet weather disturbances. The selection of nitrifiers occurred during the press disturbance in both the biofilm and suspended biomass communities, likely due to longer retention times during the disturbance. Results from our study suggest that biofilm-based treatment systems are able to quickly recover to baseline performance post-disturbance. Thus, biofilm-based systems are viable wastewater treatment solutions for increasing resilience in regions that experience frequent wet weather events.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.4c00524.

Synthetic influent wastewater composition, concentrations, and loadings; recovery times and removal rates sCOD and ammonia-N in pre, during, and post disturbance states; statistical significance values; relative abundance values of nitrifiers in pre, during, and post disturbance states (PDF)

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Author Contributions

CRediT: **Priyanka Ali** conceptualization, data curation, formal analysis, investigation, methodology, project administration, writing - original draft; **Guomin Xu** investigation; **Russell Carlson-Stadler** methodology; **Jeseth Delgado Vela** conceptualization, writing - review & editing; **Lu Liu** conceptualization, writing - review & editing; **Andrew Shaw** conceptualization, writing - review & editing; **Lauren B. Stadler** conceptualization, supervision, visualization, writing - review & editing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

P.A. was supported by the Water Environment Federation Canham Graduate Studies Scholarship while completing this work. This project was supported by NSF GOALI award (1932000 and 931937). The authors would like to thank Houston Water, Isaac G. Musaazi, Jarrett Morrison, and Dr. Dylan Christenson. The graphical abstract and Figure [1] were created with Biorender.com.

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