Engineering xxx (xxxx) xxx

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Environmental Engineering—Article

Rules governing general assembly of microbial communities in engineered biotreatment processes

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ARTICLE INFO

Article history:
Received 3 December 2024
Revised 17 May 2025
Accepted 12 June 2025

Keywords:
Microbial community assembly
Engineered ecosystems
Environmental disturbances
Performance
Operation stability

ABSTRACT

The principles of microbial community assembly in engineered ecosystems (particularly biotreatment processes) exposed to high-intensity environmental disturbances remain poorly understood. In this study, we conducted a meta-analysis of microbial communities across nine datasets from bioreactors operated under varied conditions. Null model-based analysis indicated that stochastic processes predominantly govern community assembly during contaminant-free selection phases or stable operation. In contrast, deterministic processes consistently govern initial acclimation of activated sludge. These processes then shift toward stochastic dominance as the operation stabilizes. The presence of environmental disturbances—such as shock, refeeding, or stress—during operation increases the relative contribution of deterministic processes to community structuring. Notably, low bioreactor performance is associated with deterministic assembly, whereas sustained stable operation corresponds to stochastic dynamics, irrespective of reactor type or condition. These findings were integrated across four categories of biotreatment operations to illustrate how specific disturbances influence microbial community succession and performance stability. This work advances the theoretical understanding of microbial dynamics in engineered systems and offers practical insights into linking community assembly mechanisms with bioreactor function.

1. Introduction

Microorganisms have critical roles in biogeochemical cycling, human health, and environmental pollution control [1,2]. Most microbial functions are realized through communities composed of thousands of microbial taxa [3–5]. Advances in high-throughput sequencing have enabled researchers to determine community composition, substantially improving the understanding of microbial community assembly mechanisms [6]. This progress has stimulated discussion on community-assembly succession in microbial ecology [7–9].

Abiotic and biotic factors determine the presence, absence, and relative abundance of species and are typically defined as deterministic processes that affect community establishment under different conditions [10]. Stochastic processes, such as probabilistic dispersal and random fluctuations in species abundance, also play a predominant role in shaping microbial communities [11,12]. Recent studies have integrated both processes to clarify their patterns and relative effects during ecological succession. For example, Zhou et al. [13] reported time-dependent succession of deterministic and stochastic processes in groundwater microbial communities and found that stochasticity responded to emulsified vegetable oil amendment. Dini-Andreote et al. [7] analyzed 105 years of soil microbial community turnover and suggested that initial community assembly is stochastic, whereas deterministic processes are correlated with sodium concentration. Meanwhile, analyses of six successional soil datasets revealed that pH, rather

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https://doi.org/10.1016/j.eng.2025.06.041
2095-8099/

Please cite this article as: Y.-C. Wang, Y.-H. Lv, Y. Deng et al., Rules governing general assembly of microbial communities in engineered biotreatment processes, Engineering, https://doi.org/10.1016/j.eng.2025.06.041

than age, mediates the succession of stochastic and deterministic processes over a 150-year period [14]. These frameworks have enhanced understanding of microbial community assembly in large natural ecosystems under single environmental disturbances. However, the field lacks experimental results under multiple disturbances, and current frameworks provide a limited understanding of community assembly and succession in engineered micro-ecosystems, such as bioreactors.

Engineered bioreactors, in contrast to natural ecosystems, are designed to achieve specific objectives (such as pollutant removal) through microbial community acclimation and enrichment of functional bacteria [15–17]. During operation, these systems are frequently affected by environmental factors. For example, temperature decreases due to seasonal changes can cause fluctuations in the performance of moving-bed biofilm reactors and destabilize community composition [18]. Biofilm formation and microbial community development are affected by variations in inlet flow, velocity, and substrate availability [19]. These observations indicate that microbial community assembly in bioreactors is highly sensitive to various operating conditions. In engineered bioreactors, communities are more vulnerable to multiple, high-intensity disturbances, where even minor environmental changes can significantly alter micro-ecosystem structure and community succession [20–22]. Despite this, a comprehensive framework for community assembly in these systems remains lacking.

Traditionally, microbial community assembly in engineered ecosystems was assumed to be dominated by deterministic processes due to strong environmental selection, such as continuous substrate influx [23]. However, recent findings indicate that stochastic processes may dominate even under similar performance conditions [24,25]. Subsequently, studies have sought to clarify assembly mechanisms in bioreactors and to identify reasons for shifts in assembly processes. Wang et al. [26] found that whereas stochastic processes drive microbial community assembly, electrostimulation can enhance deterministic processes in bioreactors. Matar et al. [12] observed that homogeneous selection governed initial biofilm community formation, but the relative importance of stochastic processes increased at later stages in membrane bioreactors, a result corroborated by Yuan et al. [27]. These studies demonstrate succession and assembly mechanisms under different bioreactor types and operating conditions but often yield contradictory conclusions. Few studies have systematically addressed the assembly rules of microbial communities under various conditions in bioreactors. Key open questions concern the rules governing succession in microbial community assembly and the association between assembly processes and performance in bioreactors.

Here, we aimed to elucidate the succession mechanisms of microbial community assembly under environmental disturbances during bioreactor operation. General assembly rules in engineered ecosystems were derived from meta-analysis of raw amplicon sequencing data from nine bioreactors operated under different conditions (total number of community samples: N = 524). We hypothesize that community assembly in bioreactors is strongly affected by disturbance and dynamic succession between stochastic and deterministic processes. High-throughput sequencing data (N = 524) from various bioreactor types and environmental conditions were analyzed to test this hypothesis. Null models were used to quantify the relative contributions of stochastic and deterministic processes and to identify the effects of selection, dispersal, and drift. The dominant assembly processes were analyzed and compared across all samples. These analyses provide a conceptual model to guide community assembly succession and improve understanding of the association between community assembly and performance in engineered bioreactors.

2. Materials and methods

2.1. Experimental setup and dataset collection

To evaluate microbial community assembly in bioreactors under normal operating conditions, a laboratory-scale waste gas biofilter was operated for 95 days to treat gaseous toluene, as described previously [28]. The total volume of the biofilter was 1.3 L (45 cm height, 6 cm inner diameter), packed with wooden balls to a height of 33 cm (0.94 L). Inoculated sludge, sourced from the Tianjin Jinnan Wastewater Treatment Plant (China), was cultivated for 7 days. Biofilm samples were collected on days 0, 25, 55, and 90. At each time point, six individual fillers were removed and biofilm collected for DNA extraction and 16S rRNA gene sequencing (N = 6). Raw sequence data (four time points × six replicates) are available in National Center for Biotechnology Information (NCBI) GenBank (Accession Number: PRJNA882197).

To further explore the effects of operational conditions and environmental disturbances on microbial community assembly, eight datasets representing different biotreatment processes (total number of community samples: N = 500) were compiled (Table S1 and Text S1 in Appendix A). There were no restrictions regarding the type of biotreatment process included. Each selected dataset contained both operational conditions and microbiome data at multiple time points. Based on these criteria, two datasets from stably operated wastewater treatment plants (AS 1–2) with 24 months of microbial amplicon data were included [29–31]. Four datasets—sequencing batch reactor (SBR-1), moving bed biofilm reactor (MBBR-1), biofilter (BF), and MBBR-2—documented microbial community succession from start-up to stable operation in various bioreactor types [32,33]. Three datasets (SBR-2, semi-continuous stirred tank reactor (CSTR), and SBR-3) represented succession in bioreactors exposed to additional environmental disturbances, such as extra pollutant loading, severe ammonia shock, or 3-chloroaniline disturbance during operation [34–36]. Except for the BF dataset, sequence and metadata were obtained from public repositories (NCBI Sequence Read Archive; Text S1). These samples represent typical operating patterns or conditions of bioreactors.

Environmental disturbance was defined as any physical force, agent, or process—either abiotic or biotic—that causes a perturbation (including stress) in an ecological component or system, relative to a specified reference state, as described in previous studies [37]. Acclimation of the microbial community was regarded as an environmental disturbance because it generally alters community composition and diversity. Changes in condition during operation were considered additional environmental disturbances, as they modify the system state. Scenarios SBR-1, MBBR-1, BF, and MBBR-2 were considered to experience a single disturbance, whereas SBR-2, CSTR, and SBR-3 experienced multiple disturbances. AS 1–2 datasets were included for analysis of activated sludge assembly in engineered ecosystems. Notably, scenarios with continuously increasing environmental disturbances, such as enrichment and separation of target bacteria, were excluded due to insufficient microbiome data. The type definition diagram and operating conditions are shown in Fig. S1 in Appendix A.

2.2. High-throughput sequencing, processing, and quality control

For the gas biofilter microbial community, DNA was extracted from different samples using the E.Z.N.A.® Stool DNA Kit (D4015, Omega, Inc., USA) following the manufacturer's instructions. The V3–V4 region of the bacterial 16S ribosomal RNA (rRNA) gene was amplified using primers 341F and 805R. Sequencing was performed on a NovaSeq PE250 platform (LC-Bio Technology Co., Ltd, China) as described previously [38].

Due to differences in the 16S rRNA gene regions analyzed (Table S1), raw data from each dataset were processed separately. For each dataset, paired-end reads were merged using FLASH, assigned to samples based on unique barcodes, and truncated to remove barcodes and primer sequences [13,23]. Quality filtering of raw reads was conducted using fqtrim (v0.94) to generate high-quality clean tags. Chimeric sequences were removed with Vsearch software (v2.3.4). After dereplication with UPARSE at a 97 % similarity threshold, a feature table and representative sequences were generated. Sequencing depth was normalized across samples by rarefaction to the minimum read count in each dataset before downstream analyses (Table S1 for rarefied OTU counts).

2.3. Statistical analysis

Microbial community assembly was quantitatively assessed using the ''Picante'' R package, based on a null model as described in previous studies [39,40]. In this context, the expectation in the null model refers to the average outcome observed under the assumption of no underlying pattern or mechanism, serving as a baseline against which actual data are compared to identify random or non-random factors in community assembly [13,14]. The β-nearest taxon index (βNTI) was calculated as the difference between the observed mean nearest taxon distance (βMNTD) and the mean of the null distribution of βMNTD, normalized by its standard deviation. Based on βNTI values, community assembly processes were classified as either stochastic or deterministic. The classification criteria were as follows: ①$\beta\text{NTI} > 2$ indicates assembly driven by variable selection; ②$\beta\text{NTI} < -2$ indicates homogeneous selection is predominant; and ③$|\beta\text{NTI}| < 2$ indicates assembly is primarily stochastic. The Bray–Curtis-based Raup–Crick metric (RC_{bray}) was calculated to further distinguish specific assembly processes. The relative importance of each process was quantified as the percentage contribution to overall community assembly. A detailed description of the processes is provided in Text S2 in Appendix A.

Nonmetric multidimensional scaling (NMDS) analysis was used to visualize dissimilarities among microbial communities. Linear regression analysis was performed to test the association between microbial community assembly processes and bioreactor performance. Here, performance refers to the target functions of the bioreactor (such as contaminant removal), as defined in the original sources; removal efficiency was selected for fitting analysis. To ensure accuracy, the fitting analysis included only removal efficiency data reported in the reference literature for AS-1, AS-2, SBR-1, BF, MBBR-2, and SBR-3. Differences between microbial community assembly processes were analyzed using one-way analysis of variance (ANOVA) in SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Microbial community assembly of activated sludge without environmental disturbance

As the most common biological wastewater treatment technology, the activated sludge (AS) process uses complex microbial communities to degrade organic pollutants [41,42]. Therefore, the microbial community assembly in AS-related biotechnologies was analyzed using a null model (Fig. 1). Two stably operating wastewater treatment plants (WWTPs; AS process) were selected as scenarios to analyze microbial community assembly. Analysis of microbiome data sampled over 24 months identified the importance of stochastic processes in microbial community assembly (Fig. 1(a) and (b)). Most of the βNTI values were distributed between −2 and 2 during the entire operation. Meanwhile, the performance of these two WWTPs remained stable and almost reached 100 %. These results suggest that stochastic processes may dominate microbial community assembly when influent and performance remain stable. In this condition, ecological drift and homogenizing dispersal became the dominant assembly pattern (Fig. S2 in Appendix A), as further reflected in the succession of microbial community diversity (Fig. 1(c) and (d)). It was observed that the β diversity of the microbial community in AS displayed a similar composition. This phenomenon can be attributed to the homogenizing dispersal process, wherein microbial communities consistently tended toward similarity and maintained stable functional performance over time.

3.2. Microbial community assembly in biotreatment processes with single environmental disturbance

The acclimation of the microbial community and various stress factors are considered disturbances in the biotreatment process and often result in alterations in community composition and diversity [26,32]. Therefore, the microbial community assembly process under environmental disturbance was analyzed (Fig. 2). In SBR-1, the inoculated sludge was derived from a bioreactor that had been operating stably for seven years, and nitrite stress was applied on day 78 [32]. During the start-up period, the community assembly showed a high degree of stochasticity, and the reactor exhibited high performance (Fig. 2(a)). The observed result may be attributed to the inherent properties of the inoculated sludge source, which enabled consistent high performance during system startup under uniform conditions. Meanwhile, the relative importance of deterministic processes increased with the addition of nitrite stress and led to a heterogeneous selection process (day 78; Fig. 2(a); Fig. S3 in Appendix A). Subsequently, microbial community assembly returned to a stochastic process-dominated condition on day 186. The dissimilarity of the microbial community showed a similar trend with the succession of the assembly pattern (Fig. 2(b)). The microbial communities presented significant differences with the addition of nitrite stress and then clustered together after day 186. These results suggest that microbial community assembly responds to environmental disturbances (the occurrence of deterministic processes) and then tends to revert to a stochastic process.

To further explore assembly succession in biotreatment processes with environmental disturbance, three datasets were analyzed during the entire operation (Figs. 2(c)–(h)). For MBBR-1, carriers were added into an anammox reactor to study colonization and biofilm formation [33]. Deterministic processes occurred in the first month of the biofilm community (Fig. 2(c)). This may be induced by the carrier's selection effect, whereby different pioneer species composed different microbial communities [28]. Ecological drift, dispersal limitation, and heterogeneous selection contributed to community assembly at this stage (Fig. S3). Functional bacteria (such as nitrite-oxidizing and anammox bacteria) appeared on the carrier surface and gradually became the dominant species (Fig. S4 in Appendix A). The relative importance of the stochastic assembly process then increased, and dispersal limitation dominated community assembly (Fig. 2(c)). Meanwhile, the microbial community succeeded in a specific direction (Fig. 2(d)). These results indicate that as biotreatment processes advanced, community assembly gradually shifted toward a more random structure, with the environmental selection effect weakening and functional species becoming dominant. Analysis of community assembly mechanisms in a biofilter and MBBR further supported this hypothesis. As shown in Fig. 2(e) and (g), microbial community acclimation

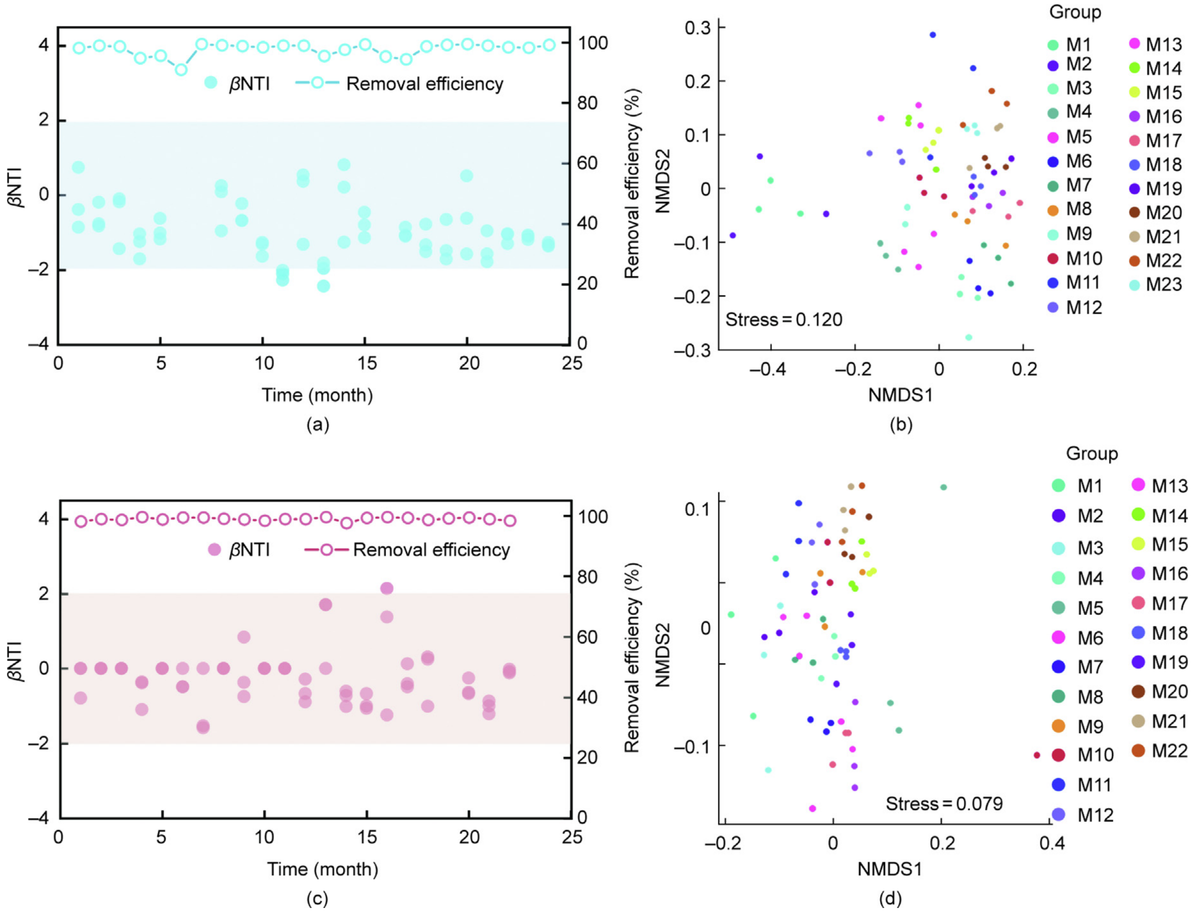

Fig. 1. Dynamic changes of *β*NTI values during the succession of the microbial community in activated sludge over time in (a) AS-1 and (b) AS-2. The samples were taken monthly and consisted of mixed liquor collected from the three functional stages. The performance in AS-1 and AS-2 indicates the removal efficiency of biochemical oxygen demand. (c) and (d) indicate the *β* diversity of the microbial community in the two WWTPs during 24 months of operation. The labels M1 through M23 represent the monthly sampling intervals (Month 1 to 23) for the collected specimens.

led to partial deterministic processes at the startup stage. The overall removal performance of the biofilter and MBBR-2 showed a similar trend (Figs. S5 and S6 in Appendix A). This may be attributed to the selective pressure of initial pollutant inflow on the microbial community, which caused low reactor performance in the adaptation period. Afterwards, the relative importance of the stochastic process increased and homogenizing dispersal dominated community assembly as performance improved. A significant positive correlation (p < 0.01) was observed between performance and the community assembly process (|*β*NTI|), indicating that performance improvement may be accompanied by the stochastic assembly of microbial communities (Fig. S7 in Appendix A). The dissimilarity of the microbial community in this condition showed a similar succession trend, and the microbial community *β* diversity exhibited a significant difference between the startup period and stable operation (Fig. 2(f) and (h)).

3.3. Microbial community assembly in biotreatment processes with multiple environmental disturbances

The operation of biotreatment processes typically experiences changes in operating conditions, such as sudden increases in inlet loading, starvation, or recovery, which introduce environmental disturbances to the microbial community. Therefore, the assembly of microbial communities exposed to multiple disturbances during operation was analyzed (Fig. 3). In SBR-2, AS was collected from a stably operating WWTP and inoculated into six SBRs, with additional chemical oxygen demand (COD) and total Kjeldahl nitrogen added from day 54 [34]. The initial sludge community assembly was primarily governed by stochastic processes (Fig. 3(a)), consistent with observations from AS-1 and AS-2, where stochastic processes also dominated in stably operated systems. The relative importance of deterministic processes increased on day 11, then gradually declined, suggesting a selection effect during acclimation that disappeared following community adaptation. Notably, the relative importance of deterministic processes increased again with the exogenous stress on day 54 (Fig. 3(a)). A clear transition in microbial *β* diversity succession was observed on day 56, confirming the substantial effect of environmental disturbances on the microbial community (Fig. 3(b)).

Similar phenomena were observed in other biotreatment processes operated under multiple environmental disturbances (Figs. 3(c)–(f)). Ammonia shock increased the relative importance of deterministic processes in six semi-continuous stirred tank reactors (Fig. 3(c)). After community adaptation, stochastic processes became more important, and subsequent assembly was affected by periods of stopped and resumed feeding. These environmental disturbances produced various microbial communities and affected community succession during operation (Fig. 3(d)). Besides, the addition of xenobiotic 3-chloroaniline to the stably operated SBR increased the deterministic process (Fig. 3(e)). Microbial diversity succession in this reactor was divided into three stages: acclimation, stable operation, and xenobiotic 3-chloroaniline addition (Fig. 3(f)). These results provide direct

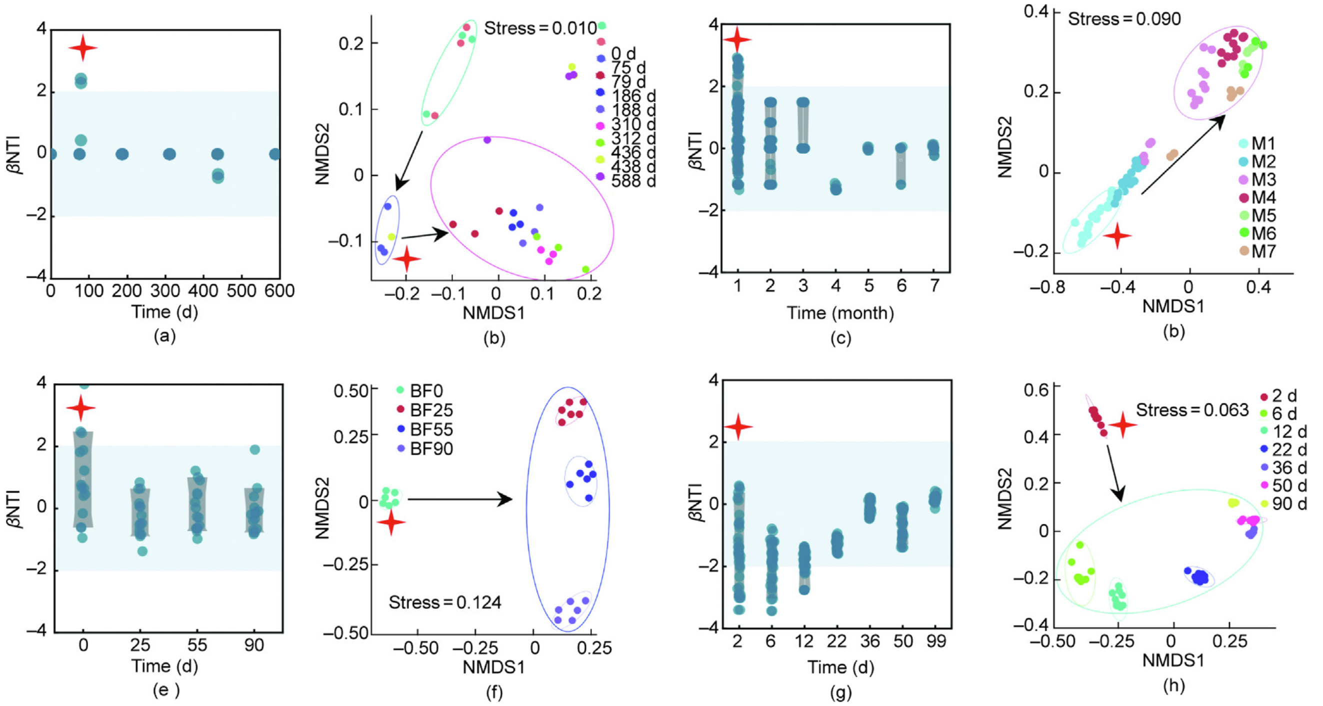

Fig. 2. Dynamic changes in βNTI values and community assembly stochasticity during microbial succession in bioreactors under single environmental disturbance. (a) Dynamic changes of βNTI values in SBR-1 with the acclimation of the startup period. (b) The dissimilarity of microbial communities in SBR-1. (c) The changes of βNTI values of anammox community during the operation in MBBR-1. (d) The dissimilarity of microbial communities in MBBR-1. The changes of βNTI values of the microbial community in (e) BF and (g) MBBR-2 from the startup to stable operation. (f) The dissimilarity of microbial communities in BF. (h) The dissimilarity of microbial communities in MBBR-2. The shaded area represents the stochastic process. The red star means the point of the environmental disturbance (the acclimation of the startup period). The labels M1 through M7 represent the monthly sampling intervals (Month 1 to 7) for the collected specimens. The arrows represent the temporal successional trajectories of microbial communities, illustrating their directional changes under specific environmental conditions over time. Notably, each point reflects the βNTI between two communities.

evidence that the relative importance of deterministic processes increases significantly regardless of the specific environmental disturbances encountered in different biotreatment processes. The alternating succession of stochastic and deterministic processes may be a general pattern for community assembly under multiple environmental disturbances.

To further explore the association between microbial community assembly and bioreactor performance, |βNTI| values were integrated with removal efficiency data (Fig. 4 and Fig. S7). The |βNTI| values tended to decrease as removal efficiency increased, as indicated by the fitting curve ($p < 0.001$). When removal efficiency was stable (above 80 %), |βNTI| values were typically less than two. This finding was also evident during stable operation of WWTPs, where |βNTI| values in two years of microbial community data remained below 2 (Fig. 1). In contrast, low removal efficiency was associated with a scattered distribution of |βNTI| values ranging from 0 to 4. These results suggest that low performance may be associated with deterministic assembly of the microbial community, potentially due to acclimation or environmental disturbances, such as shock or additional stress. Performance changes in SBR-1, SBR-3, and CSTR after disturbances further support this conclusion (Fig. S6). Stochastic processes dominated community assembly during stable operation, likely reflecting microbial adaptation to operating conditions.

3.4. Major microbial community assembly processes in biotreatment processes

The microbial community assembly processes were compared to reveal the dominant assembly process and potential mechanisms (Fig. 5). Although the biotreatment processes are usually continuously fed, the selection process is not the dominant factor (Fig. 5(a)). In contrast, the dispersal process seemed significantly more important than the other processes ($p < 0.05$), followed by ecological drift (Fig. 5(a)). The deterministic assembly behaved more positively when environmental disturbances occurred (Fig. 5(b)). The relative importance of the stochastic process decreased but the deterministic process increased ($p < 0.05$) after the environmental disturbances. This might be due to the selection effect caused by environmental disturbances, which improves the importance of homogeneous/heterogeneous selection.The schematic in Fig. 5(c) explains the possible mechanisms. In biotreatment processes, potential stresses would lead to the environmental selection of microbes in the community and species that cannot adapt to the environment will be weeded out, which causes a homogeneous selection effect (Fig. 5(c)). However, heterogeneous selection occurs when some species are acclimated, but others are not. This phenomenon is common because acclimation or start-up is not instantaneous but usually over a period of time [12,17,24]. In addition, an uneven distribution of pollutants is often encountered, and the concentration of pollutants decreases from inlet to outlet [43,44]. With the maturity of biofilm, the oxygen distribution of biofilm with different thickness will be uneven, which led to the heterogeneity of microenvironment in space [16,33]. All these conditions could lead to the heterogeneous selection of microbial communities. Dispersal is regarded as a fundamental process in community assembly and is particularly important in both biofilm formation and community succession [45]. During the biofilm formation process, the species would always proliferate and spread continuously when it first arrived at the carrier, leading to homogeneous dispersal (Fig. 5(c)). The homogenized community may also make the function more stable to better achieve the functional goal of the reactor, which is reflected in the biofilm-based bioreactors (Fig. S5–S7). Moreover, activated sludge

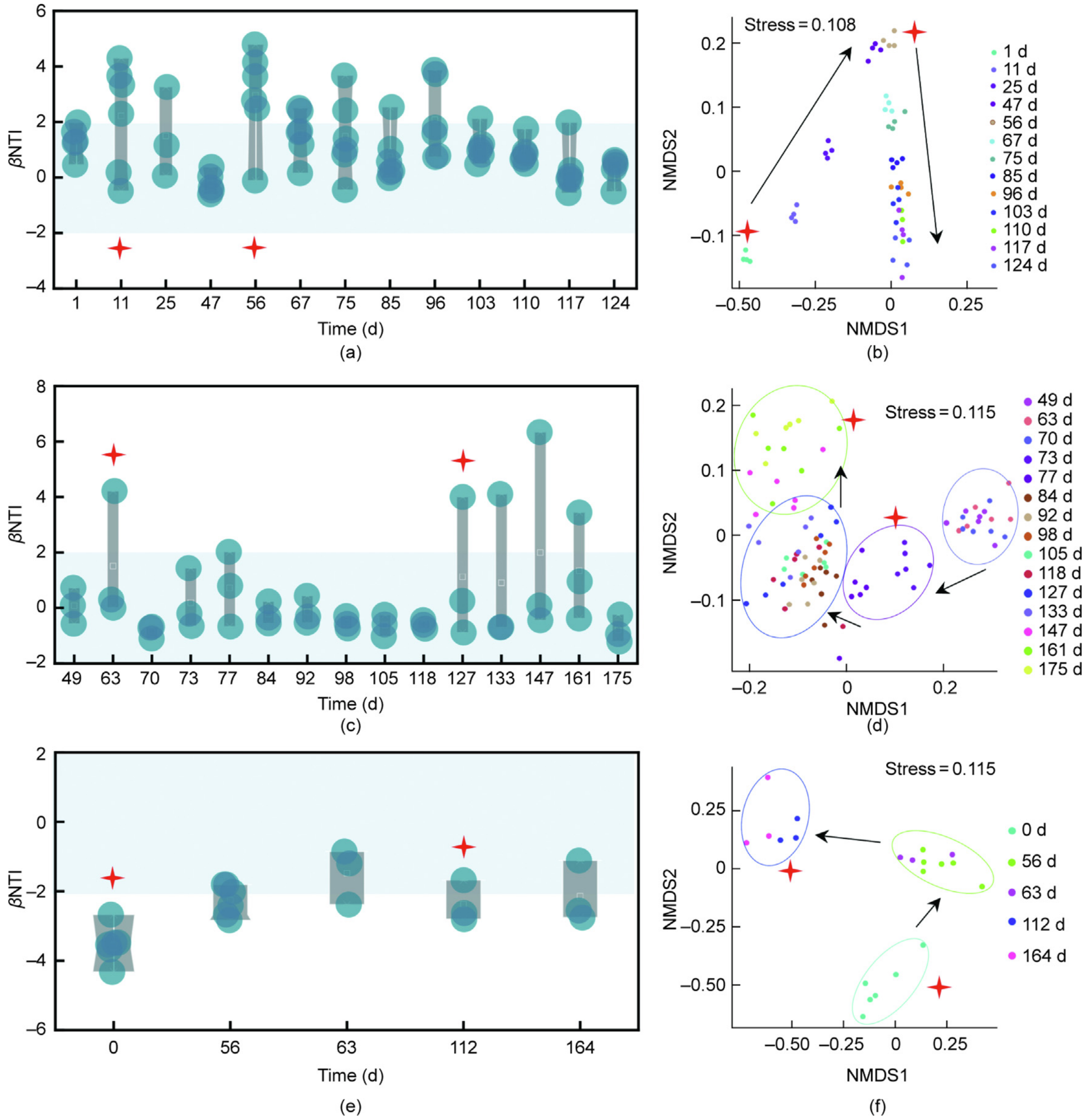

Fig. 3. Dynamic changes of βNTI values and stochasticity during the succession of microbial communities in bioreactors with multiple environmental disturbances. (a) Dynamic changes of βNTI values in an anammox reactor; acclimation during startup and high loading shock produced environmental disturbances. (b) Dissimilarity of microbial communities in SBR-2. (c) Dynamic changes of βNTI values in semi-continuous stirred tank bioreactors exposed to ammonia shock and feeding interruption. (d) Dissimilarity of microbial communities in CSTR. (e) Dynamic changes of βNTI values in SBR during acclimation and after addition of xenobiotic 3-chloroaniline. (f) Dissimilarity of microbial communities in SBR-3. The shaded area represents stochastic processes. The red star denotes the timing of environmental disturbances (acclimation, high loading shock, ammonia shock, and xenobiotic 3-chloroaniline addition). Arrows indicate temporal successional trajectories of microbial communities, illustrating their directional changes over time under specific environmental conditions.

can be regarded as a regional species pool in WWTPs owing to its process characteristics. Community succession is dominated by trophically similar individuals and species, which might also be the result of a homogenous dispersal process [46]. However, microbial movement and dispersal may be limited in mature and aging biofilms, resulting in low community turnover. This phenomenon usually occurs in the middle and late stages of biofilm formation, corresponding to the maturation and aging of the biofilm (Fig. S4–S6). Meanwhile, drift may occur throughout the bioreactor operation because the ratio of living and dead cells always changes over time.

4. Discussion

This study analyzed the succession patterns of microbial community assembly in engineered biotreatment processes using a null model based on nine meta-analysis amplicon datasets across different conditions and process types. Due to the diversity of operating conditions in engineered bioreactors, all results were integrated to clarify how different types of environmental disturbances affect community assembly succession and performance stability (Fig. 6). When an environmental disturbance occurs, a stable community undergoes adaptation and subsequently transitions to a new stable state [47,48]. Accordingly, the communities in the bioreactors were categorized into three stages: initial, adaptive, and restored communities. Meta-analysis of the datasets provided strong support for this framework. For example, activated sludge in two scenarios (AS-1 and AS-2) under normal conditions exhibited a dominance of stochastic processes in community assembly, supporting conceptual model type A. In contrast, scenarios in SBR-1, MBBR-1, BF, and MBBR-2 experiencing a single environmental disturbance, and SBR-2, CSTR, and SBR-3

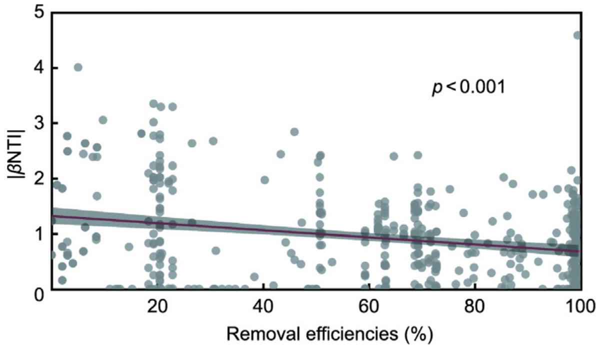

Fig. 4. The relationship between |βNTI| and performance of biotreatment processes (N = 441). For the fitting analysis, we strictly utilized the removal efficiency data documented in the reference literature (AS-1, AS-2, SBR-1, BF, MBBR-2, and SBR-3). Detailed information about the performance and the contaminants is shown in Fig. S6.

experiencing multiple environmental disturbances, supported conceptual model types B and C, respectively. These datasets, representing various conditions and disturbance regimes, collectively supported the conceptual model and provided meaningful bioreplicates (Fig. 6). Notably, microbial community assembly in bioreactors under different scenarios, including stable operation, acclimation, substrate changes, nitrite stress, ammonia shock, and 3-chloroaniline stress, supported the development and application of general rules in bioreactors exposed to different disturbances.

The results demonstrated relatively high integrity because the microbial communities were traceable from the initial to the fully operational stages of the bioreactors. Assembly of the initial communities was confirmed for each scenario (Figs. 1 and 5), which is often difficult to observe directly in natural ecosystems [7,8,13]. Inheritance and validation distinguish this model from previous ecological models. Zhou et al. [13] proposed basic conceptual frameworks for four ecosystem scenarios and confirmed one model in groundwater microbial communities. Similarly, Dini-Andreote et al. [7] described the balance between stochastic and deterministic processes based on 105 years of soil microbial assembly. Validating these conceptual models is challenging due to the large spatial and temporal scales in natural ecosystems [7]. For example, Na^+ affects the community assembly at small spatial (15 m) and temporal (6 months) scales, whereas at larger scales (8 km and 105 years), soil organic matter content has a more significant effect [7]. These limitations can be addressed in engineering bioreactors [49]. Furthermore, previously established frameworks support the conceptual model of this study across different ecosystems. Tripathi et al. [14] suggested that, compared to successional age, pH is the principal factor affecting community assembly. Extremely acidic or alkaline conditions promote deterministic processes, whereas neutral pH favors stochasticity [14]. This finding is consistent with Fig. 6, in which pH acts as an environmental disturbance that drives communities toward deterministic assembly. Besides, the selection of datasets from different bioreactor types and operational conditions provided bioreplicates that reinforce the general assembly rules proposed in this study.

In addition to revealing the general patterns for community assembly in engineered bioreactors, this study also identifies a potential association between community assembly and performance (Figs. 4 and 6). Environmental disturbances, such as additional pollutant stress and severe shock, are known to affect

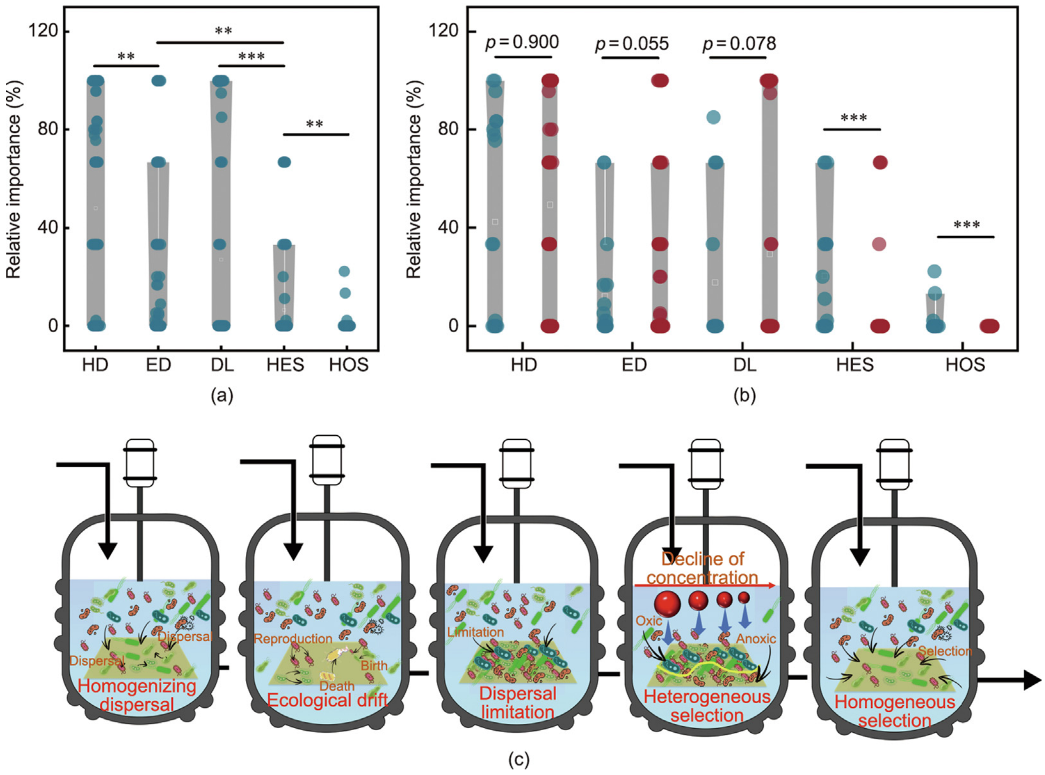

Fig. 5. The conceptual model for microbial community assembly and succession in engineered ecosystems. In engineered bioreactors, the initial microbial community is usually derived from activated sludge from stable operating WWTP or soil microorganisms. Phase 1: Initial community. And then the community undergoes an acclimation process based on the functional requirements of the bioreactor, such as nitrogen and phosphorus concentrations and organic matter removal, and so forth. Phase 2: Adaptive community. Finally, microbial community succession will restore stability. Phase 3: Restored community. The operation of bioreactors can be divided into 4 types (no environmental disturbance, single disturbance, multiple disturbances and continuously increased disturbances) according to the operating conditions. It should be noted that the vertical coordinate does not indicate the magnitude of the value, but only represents the strength.

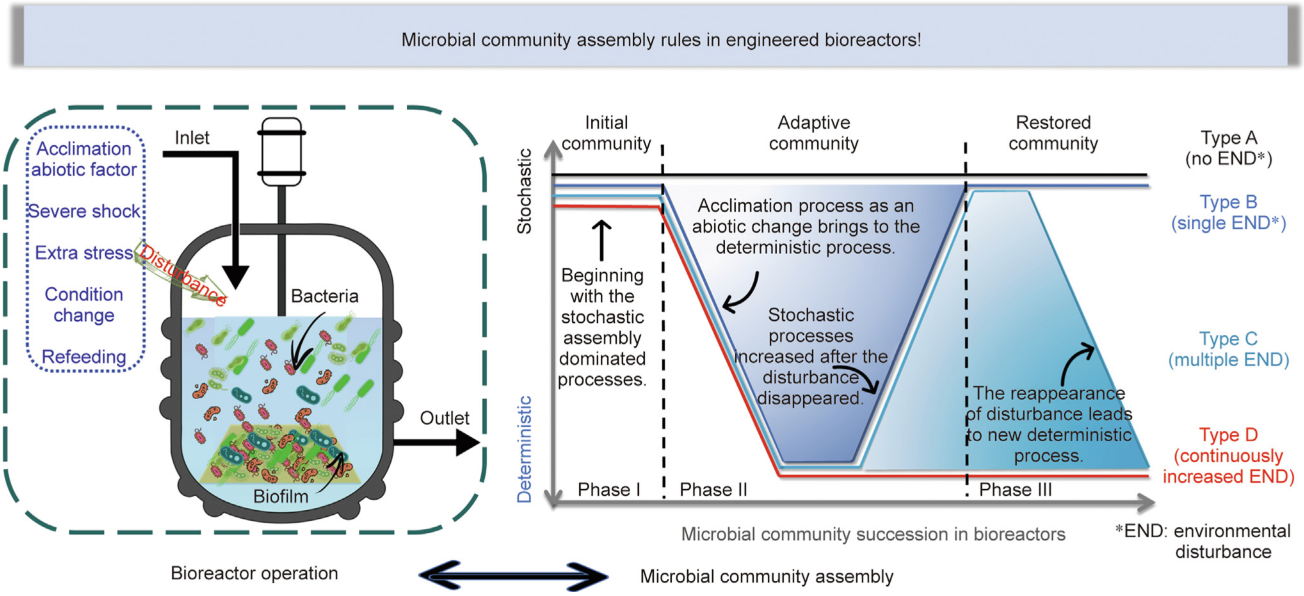

Fig. 6. (a) The relative importance of microbial community assembly processes governed primarily by deterministic (homogeneous selection (HOS) and heterogeneous selection (HES)) and stochastic processes (dispersal limitation (DL), homogenizing dispersal (HD), and ecological drift (ED)) (N = 92 groups). (b) The relative importance of the specific assembly processes in microbial communities with (N = 18 groups) and without (N = 74 groups) environmental disturbances. The bioreactors that operated stably were regarded as without environmental disturbance. In contrast, acclimation and external shocks are environmental disturbances. The vertical axis means the percentage of microbial community assembly processes in that community. Note that the relative importance of a process was quantitatively measured as the percentage for each process in the community assembly. (c) The schematic diagram shows five specific assembly processes of microbial community assembly in biotreatment processes.

bioreactor stability [50,51]. Considering the role of microbes in bioreactor function, it is necessary to correlate performance with community dynamics. As expected, our results demonstrated an inverse linear relationship between $|\beta\text{NTI}|$ values and performance metrics (Fig. 4). This finding suggested that the improvement of bioreactor performance coincided with a shift from deterministic to stochastic assembly processes, which might be the result of interactions between species and the environment. During bioreactor disturbances, the selection and filter effects spontaneously emerge, promoting deterministic assembly. Both performance and community stability are consequently impacted. by these disturbances. However, the community exhibit self-regulatory capacity [52,53], whereby stochastic process regain dominance as communities adapt to disturbances. This phenomenon in bioreactors is reflected in performance improvement and community stability. A recent study supports this result that the microbial community remains unstable due to various disturbances [54].

Moreover, this study investigated the interactions and function of microbial communities in engineering bioreactors, with particular emphasis on assembly mechanisms. These insights offer practical value for bioreactor construction and regulation. For instance, it was demonstrated that stochastic assembly predominance correlated with stable bioreactor performance, while deterministic processes associated with performance fluctuations (Fig. 4). This correlation suggested a potential indicator of bioreactor system stability through community changes. Meanwhile, this also supported using functionally adapted activated sludge inoculate from established bioreactors to accelerate start-up time in a new bioreactor. Importantly, this observation emphasized that modifications to organic carbon loading or operational parameters must be carefully optimized. Although such adjustments can enhance target functions, they may compromise microbial community stability. Besides, the results advances fundamental understanding of microbial ecology in bioreactor systems. Despite being managed ecosystems, the community assembly in bioreactors appeared to be driven most probably by stochastic processes (Fig. 5), including homogenizing dispersal and dispersal limitation, as confirmed by recent research [55]. Above all, these findings highlighted the need to balance community dynamics with reactor performance, which could help formulate a stable operation strategy for bioreactors. Although the bioreactors vary in design and function, the microbes act as the core to bring them together. Operational parameters including temperature, pH, and substrate composition would significantly influence microbes assembly processes. The microbial community assembly changes in bioreactors in different scenarios, including stable operation, acclimation, substrate changes, nitrite stress, ammonia shock, and 3-chloroaniline stress, support the development of general assembly rules and conceptual models. Hence, diverse environmental conditions (substrates, temperatures, mixing types, and so on) could be integrated through microbial community dynamics and further presented by the community assembly rules in this study.

5. Conclusions

This study elucidates the rules governing the general assembly of microbial communities in engineered bioreactors through systematic analysis of typical bioreactor scenarios. A conceptual model was developed to guide the influence of different types of environmental disturbances to both community assembly succession and performance stability. The above results have relatively high integrity and building upon and extending current understanding in this field. Moreover, the potential relationship between community assembly and reactor performance was verified and discussed. In general, these results greatly advance our understanding of community succession in engineered bioreactors and provide theoretical and experimental guidance for connecting engineering performance and community assembly.

List of abbreviations

AS-1/2: Activated sludge community succession in WWTPs.
SBR-1: Anammox community in sequencing batch reactor.

SBR-2: Microbial community succession with environmental stress in sequencing batch reactor.

SBR-3: Microbial community succession with the 3 Chloroaniline (3-CA) disturbance in sequencing batch reactor.

MBBR-1: Anammox community succession in moving bed biofilm reactor.

MBBR-2: Microbial community succession in normal operated moving bed biofilm reactor.

BF: Microbial community succession in normal operated gas biofilter.

CSTR: Microbial community succession with a severe ammonia shock in semi-continuous stirred tank reactor.

Availability of data and materials

Sequence data of all samples are available at: NCBI GenBank. Accession Number: PRJNA588045, PRJNA719992, PRJNA564893, PRJNA636402, PRJNA882197, PRJNA640191, PRJNA559245, PRJNA676039, and PRJNA720804.

CRediT authorship contribution statement

Yong-Chao Wang: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ya-Hui Lv:** Visualization, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Ye Deng:** Writing – review & editing, Methodology, Conceptualization. **Yu-Ting Lin:** Visualization, Resources, Methodology, Formal analysis, Conceptualization. **Guan-Yu Jiang:** Visualization, Resources, Methodology, Formal analysis, Conceptualization. **John C. Crittenden:** Writing – review & editing. **Can Wang:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (52170114); Projects of Science and Technology, Tianjin (21JCJQJC00080); and Natural Science Foundation of Hebei Province-Key Project (B2021208033). The authors acknowledge the support by the Brook Byers Institute for Sustainable Systems, Hightower Chair and the Georgia Research Alliance at the Georgia Institute of Technology.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2025.06.041.

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