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# Ecological Differentiation Among Nitrous Oxide Reducers Enhances Temperature Effects on Riverine $N_2O$ Emissions

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**Received:** 29 July 2024 | **Revised:** 26 January 2025 | **Accepted:** 29 January 2025

**Funding:** This research was financially supported by the Basic Science Center Project of the Natural Science Foundation of China (53288101), National Natural Science Foundation of China (52239005, 52039001, and 52109050), and Program for Guangdong Introducing Innovative and Entrepreneurial Teams (2019ZT08L213). WHM was supported by US NSF 2215300.

**Keywords:** ecological differentiation | global warming | greenhouse gas | $N_2O$ reduction | *nosZ* clade II

## ABSTRACT

Nitrous oxide ($N_2O$) reductase, the sole natural microbial sink for $N_2O$, exists in two microbial clades: *nosZ*I and *nosZ*II. Although previous studies have explored inter-clade ecological differentiation, the intra-clade variations and their implications for $N_2O$ dynamics remain understudied. This study investigated both inter- and intra-clade ecological differentiation among $N_2O$ reducers, the drivers influencing these patterns, and their effects on $N_2O$ emissions across continental-scale river systems. The results showed that both *nosZ*I and *nosZ*II community turnovers were associated with similar key environmental factors, particularly total phosphorus (TP), but these variables explained a larger proportion of variation in the *nosZ*I community. The influence of mean annual temperature (MAT) on community composition increased for more widespread $N_2O$-reducing taxa. We identified distinct ecological clusters within each clade of $N_2O$ reducers and observed identical ecological clustering patterns across both clades. These clusters were primarily characterized by distinct MAT regimes, coarse sediment texture as well as low TP levels, and high abundance of $N_2O$ producers, with MAT-related clusters constituting predominant proportions. Intra-clade ecological differentiation was a crucial predictor of $N_2O$ flux and reduction efficiency. Although different ecological clusters showed varying or even contrasting associations with $N_2O$ dynamics, the shared ecological clusters across clades exhibited similar trends. Low-MAT clusters in both the *nosZ*I and *nosZ*II communities were negatively correlated with denitrification-normalized $N_2O$ flux and the $N_2O$:($N_2O + N_2$) ratio, whereas high-MAT clusters showed positive correlations. This contrasting pattern likely stems from low-MAT clusters being better adapted to eutrophic conditions and their more frequent co-occurrence with $N_2O$-producing genes. These findings advance our understanding of the distribution and ecological functions of $N_2O$ reducers in natural ecosystems, suggesting that warming rivers may have decreased $N_2O$ reduction efficiency and thereby amplify temperature-driven emissions.

## 1 | Introduction

Nitrous oxide ($N_2O$) is a potent greenhouse gas, with a global warming potential ~300 times that of carbon dioxide over a 100-year period (Stein and Lidstrom 2024) and significantly contributes to stratospheric ozone depletion (Ravishankara et al. 2009). Net $N_2O$ fluxes in natural ecosystems depend on the balance between its production and consumption (Tang et al. 2022). The consumption of $N_2O$ is exclusively catalyzed by $N_2O$ reductase (nosZ) found in $N_2O$ reducers, which are categorized into two clades: *nosZ*I and *nosZ*II (Hallin et al. 2018; Hiis et al. 2024). The *nosZ*I clade, mainly consisting of denitrifying microbes, has been extensively studied (Hallin et al. 2018). In contrast, the recently identified *nosZ*II clade includes a larger proportion of non-denitrifying microbes (Jones et al. 2013) and thus inherently possesses a higher genetic potential to act as a net $N_2O$ sink. This potential has been confirmed by the significant role of the *nosZ*II clade in enhancing soil $N_2O$ sink capacity (Domeignoz-Horta et al. 2018; Jones et al. 2014).

River ecosystems are significant global sources of atmospheric $N_2O$, with emissions increasing nearly three times faster than those from soils (Yao et al. 2020). Accumulating evidence underscores the critical function of $N_2O$ reduction in regulating emissions from freshwater systems (Aho et al. 2023; Soued et al. 2016). This highlights the urgent need to elucidate the distribution patterns of *nosZ*I- and *nosZ*II-type $N_2O$ reducers and their links with $N_2O$ emissions in these environments for effective mitigation. However, previous research has predominantly focused on the differences between *nosZ*I- and *nosZ*II-type $N_2O$ reducers in soil environments (Hiis et al. 2024; Maheshwari et al. 2023), with limited investigations into freshwater systems (Zhang et al. 2024). Furthermore, recent studies in laboratory cultures and controlled experiments have revealed substantial variability in the ecological niches of each *nosZ* clade (Hallin et al. 2018; Maheshwari et al. 2023). This variability is influenced by factors such as temperature (Zhou et al. 2021), organic matter (Maheshwari et al. 2023), and oxygen levels (Wang et al. 2023; Wittorf et al. 2016), which are key drivers of $N_2O$ transformation and emissions. The niche differentiation within each clade in natural systems and its implications for $N_2O$ emissions remain largely unexplored, leaving a significant gap in our understanding of their broader ecological consequences.

In the context of global warming, it is crucial to understand how temperature affects $N_2O$ transformation to project future emission scenarios (Yin et al. 2020). Most research in natural systems has focused on how $N_2O$ production responds to temperature changes (Palacin-Lizarbe et al. 2018; Tan et al. 2020). In contrast, $N_2O$ reduction remains comparatively underexplored, particularly under field conditions (Quick et al. 2019). Laboratory environments often lack the complex ecological interactions that exist in natural ecosystems (Goldenberg et al. 2018), which can skew observations of temperature response (Velthuis and Veraart 2022). Using $N_2O$ reducers as proxies for studying $N_2O$ reduction is a valuable strategy, as changes in biogeochemical function under different temperatures could be reflected by variations in the corresponding microbial communities (Hallin et al. 2012). Investigating the temperature sensitivities of $N_2O$ reducers across broad environmental gradients can provide preliminary ecological insights into how $N_2O$ reduction responds to a warming world.

To address these issues, we conducted a comprehensive study on the ecological differentiations of *nosZ*I- and *nosZ*II-type $N_2O$ reducers in river systems, focusing on their patterns and effects on $N_2O$ dynamics and the associated mechanisms. This study was conducted across six major river networks in China and targeted sediment, which is the primary site for $N_2O$ reduction in aquatic environments (Tang et al. 2022). We explored both the inter- and intra-clade ecological differentiation among $N_2O$ reducers and compared the environmental and microbial factors driving these differentiations. Using a space-for-time approach (Lugato et al. 2021), we projected the vulnerability of $N_2O$ reducers to global warming by examining the effect of mean annual temperature (MAT) on their ecological distribution on a continental scale. MAT was chosen as it represents a long-term, integrated measure of thermal conditions experienced by microbial communities, providing a consistent and comparable temperature metric across different sites. Variations in MAT would significantly influence the temperature dynamics of water bodies and their associated sediments (Alsterberg et al. 2011; Arrigoni et al. 2008; Morrill et al. 2005). Finally, we analyzed the relationship between ecological differentiation among $N_2O$ reducers and $N_2O$ dynamics, with a particular focus on MAT-related differentiation, to provide ecological insights into $N_2O$ reduction and emissions under current and future environmental conditions.

## 2 | Sampling and Methods

### 2.1 | Sampling

A total of 93 surface sediment samples (0–10 cm) were collected from six major rivers in China: Pearl River, Yangtze River, Huai River, Yellow River, Hai River, and Liao River (Figure 1a). The study area spanned latitudes from 22.70° N to 43.52° N and longitudes from 103.64° E to 125.12° E. To account for seasonal variation in $N_2O$-reducing microbial communities, sampling campaigns were conducted during spring (March–April, $n=23$), summer (August, $n=31$), and fall to early winter (October–December, $n=39$). Across these sampling periods, water temperatures ranged from 7.9°C to 34.1°C.

These rivers encompass diverse climatic, land cover, geomorphological, and hydrological conditions (Wang, Xia, et al. 2022), exhibiting significant variation in both water and sediment properties (Tables S1 and S2). The analytical procedures for measuring sediment and water properties are detailed in Text S1. The MAT across these sampling sites ranged from 5.2°C to 22.6°C, with data collected from the WorldClim database (https://www.worldclim.org). Dissolved oxygen levels in the river water ranged from 1.4 to 14.5 $mg\,L^{-1}$. Water $NO_3^-$ concentration varied from 0.01 to 16.24 $mg\text{-}N\,L^{-1}$, and sediment total nitrogen content ranged from 0.02 to 3.96 $mg\text{-}N\,kg^{-1}$ dry sediment. These factors are known to significantly influence $N_2O$ reduction (Quick et al. 2019). This extensive range of site characteristics provides a comprehensive biogeochemical context for investigating the ecological differentiation of $N_2O$ reducers and the underlying mechanisms.

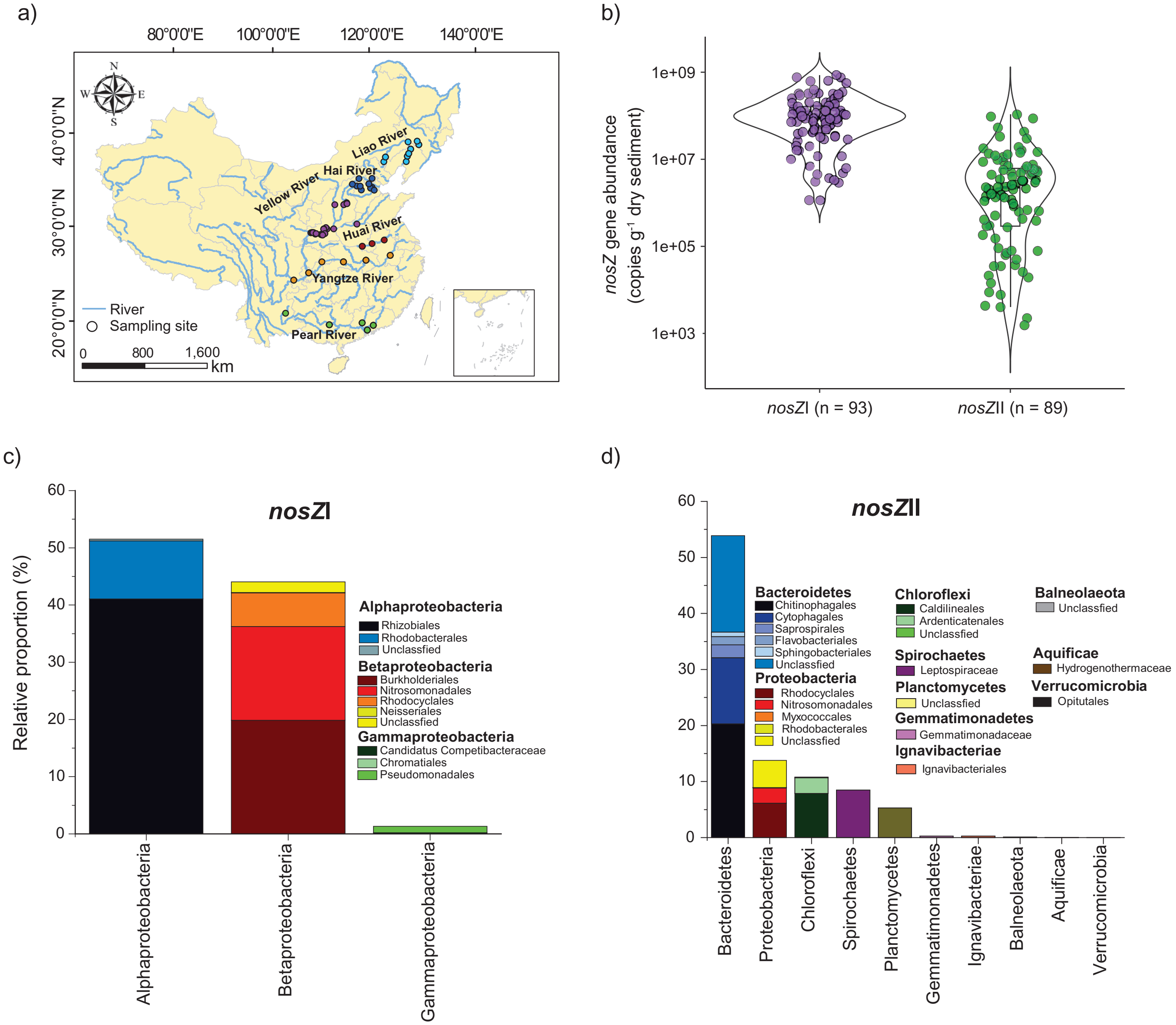

**FIGURE 1** | Community composition of $N_2O$ reducers in the sediment samples of six major rivers across China. (a) Map of the six surveyed major rivers in China. Map lines delineate study areas and do not necessarily depict accepted national boundaries. (b) The abundance of *nosZ*I and *nosZ*II in the sediment samples. Four samples had *nosZ*II abundances below the detection limit. Taxonomic composition of *nosZ*I-containing microbes at the levels of class and order (c) and taxonomic composition of *nosZ*II-containing microbes at the levels of phylum and order (d).

### 2.2 | DNA Extraction and Quantification of $N_2O$ Transformation-Related Microbial Abundance

Sediment samples designated for DNA extraction and microbial analysis were flash-frozen with liquid nitrogen in the field and stored at −80°C upon return to the laboratory. Genomic DNA was extracted in triplicate from each sampling site using the FastDNA SPIN Kit for Soil (MP Biomedicals, CA, USA), following the manufacturer's protocol. The concentration and quality of extracted DNA were assessed using a NanoDrop 2000 UV–visible spectrophotometer (Thermo Scientific).

Quantitative PCR (qPCR) assays were conducted to determine the genetic potential for $N_2O$ reduction and production in the sediment samples. To evaluate $N_2O$ reduction potential, we determined the abundance of *nosZ*I and *nosZ*II genes, which encode the two known clades of $N_2O$ reductase, using the primer pairs 1840F/2090R (Henry et al. 2006) and nosZIIF-nosZIIR (Jones et al. 2013), respectively. To evaluate the genetic potential of nitrifying microbial $N_2O$ production, we quantified the abundance of the ammonia monooxygenase subunit A (*amoA*) gene from ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (Zhang et al. 2012). Additionally, the *nirS* and *nirK* genes, which encode two types of nitrite reductases, were quantified to evaluate the genetic potential for denitrifying microbial $N_2O$ production (Putz et al. 2018). Standard curves for qPCR quantification were constructed using plasmids containing the respective marker genes. Details regarding the primer pairs, qPCR reaction mixtures, and thermal profiles are provided in Table S3. All standard curves exhibited $R^2$ values greater than 0.98, and the qPCR efficiencies ranged from 65% to 97% (Table S3).

### 2.3 | PacBio SMRT Sequencing and Sequence Processing

The *nosZ*I gene sequences were amplified using the primer pair CGCTSTTYMTIGAYAGYCAG and CAKRTGCAISGC RTGGCAGAA (Juhanson et al. 2017). For *nosZ*II, the primer pair CTIGGICCIYTKCAYAC and GCIGARCARAAITCBGTRC (Jones et al. 2013) was used. Both *nosZ*I and *nosZ*II gene amplicons (~700 base pairs) were sequenced using single-molecule real-time circular consensus sequencing by Pacific Biosciences (PacBio) platform from MajorBio Bio-pharm Technology Co. Ltd. (Shanghai, China).

We processed the *nosZ* gene sequences using a reference database composed of full-length *nosZ* sequences created from microbial genome assemblies retrieved from NCBI in June 2019 (Maheshwari et al. 2023). Initially, raw sequence reads were filtered using mothur (Schloss 2020), and chimeric sequences were detected and removed using de novo-based UCHIME methods (Edgar 2013). An operational taxonomic unit (OTU) table was constructed following the UPARSE pipeline (Edgar 2013) with a 95% nucleotide similarity cutoff. Representative sequences of the resulting OTUs were translated and screened against the *nosZ* sequence reference database (Maheshwari et al. 2023) for further identification and removal of contaminating sequences. The next-generation evolutionary placement algorithm (Barbera et al. 2019) was used to incorporate these filtered representative sequences into the reference phylogeny, which was constructed with the LG + R10 likelihood model in IQ-Tree 2 (Minh et al. 2020). Phylogenetic placement analysis was performed using the “gappa” software (Czech et al. 2020) to identify the taxonomy of $N_2O$ reducers.

### 2.4 | Analysis of Community Diversity, Drivers, and Assembly Processes

Gradient forest (GF) analysis (Ellis et al. 2012), a machine-learning regression tree-based approach, was used to evaluate the relative importance of various environmental, microbial, and spatial factors in predicting the ecological turnover of $N_2O$ reducers. Only OTUs present in at least 10% of the samples were retained for GF analysis, using the procedures available in the R package “gradientForest” (version 0.1.32) (Ellis et al. 2012). To account for potential spatial autocorrelation among environmental and microbial variables, we incorporated principal coordinates of neighbor matrices (PCNMs) as explanatory variables in the GF analysis to examine the importance of spatial heterogeneity in shaping community composition (Egidi et al. 2023). The PCNMs were calculated using the R package “vegan” (Oksanen et al. 2019) based on the latitude and longitude of the sampling sites. The first half of the PCNMs with positive eigenvalues was included in the GF analysis ($n = 12$ in this study) (Gugger et al. 2018). To avoid multicollinearity, we used an uncorrelated set of predictors ($|r| < 0.7$) (Gugger et al. 2018). The predictive performance of the GF model for each OTU was quantified using the proportion of out-of-bag data variance explained ($R^2$). The total model prediction performance from the GF analysis was partitioned into contributions from each predictor in proportion to their accuracy importance.

To validate the importance of key environmental and microbial factors identified by the GF analysis, we conducted an additional GF analysis excluding spatial factors while keeping other settings the same. This step ensured that the identified predictors were not influenced by spatial autocorrelation. Additionally, edge principal components analysis (ePCA) ordination (Matsen IV and Evans 2013) was used to exhibit phylogeny-based changes in community composition and to validate the effectiveness of key predictors identified in the GF models in indicating these changes.

The *nosZ* community assembly process was evaluated using a null model analysis (Stegen et al. 2013). Briefly, the observed β-mean-nearest taxon-distance (βMNTD) index was first calculated in the iCAMP package (Ning et al. 2020). Then, abundance-weighted β-nearest taxon distance (βNTI) was calculated to describe the extent to which βMNTD deviates from the mean values of the null model. Values of $|\beta\text{NTI}| > 2$ indicated that pairwise community assembly was governed by environmental selection. This could be further differentiated into homogeneous selection ($\beta\text{NTI} < -2$) and heterogeneous selection ($\beta\text{NTI} > 2$). For pairwise comparisons not attributed to environmental selection ($|\beta\text{NTI}| < 2$), we calculated the extended Raup-Crick metric (RCbray) (Stegen et al. 2013). When $\text{RCbray} > 0.95$, the assembly was attributed to dispersal limitation, whereas $\text{RCbray} < -0.95$ indicated homogenizing dispersal. The relative proportion of pairwise comparisons with $|\text{RCbray}| < 0.95$ represented the contribution of ecological drift to community assembly. To assess the variation in community assembly processes along environmental and microbial gradients, we performed Mantel tests in the “vegan” package (Oksanen et al. 2019) by comparing the βNTI values to corresponding Euclidean distance matrices of each environmental and microbial factor.

### 2.5 | Identification of Ecological Niches for $N_2O$ Reducers

To identify ecological differentiation within each clade of $N_2O$ reducers, we first selected OTUs for which environmental and microbial variables explained more than 10% of the variance ($R^2 > 0.1$) in GF models containing only environmental and microbial variables. Following previous studies (Delgado-Baquerizo et al. 2018), we considered these OTUs to have predictable habitat preferences, making them more likely to exhibit genuine shifts in response to environmental changes. We then conducted Spearman correlation analysis to examine the relationships between these selected *nosZ* OTUs and environmental and microbial variables. Subsequently, hierarchical clustering analysis based on the correlation matrix was performed using the R package “pheatmap” (Kolde and Kolde 2015) to group *nosZ* OTUs (Delgado-Baquerizo et al. 2018). Ecological clusters were identified by examining groups of OTUs that were distinctly clustered and exhibited similar responses to environmental and microbial factors. OTUs that lacked strong evidence for similar environmental responses were not assigned to any specific cluster, even if they showed correlation with a single factor. Each ecological cluster was categorized based on the most consistently influential variables, and only OTUs with significant correlations to corresponding factors were assigned to an ecological cluster.

To validate the ecological relevance of these clusters, we aggregated the OTUs within each cluster and assessed their collective correlations with corresponding environmental and microbial variables using linear regression models. We further analyzed the taxonomic composition of the main ecological clusters and reviewed existing literature to gather reported associations between these species and corresponding factors, assessing their consistency with our identified clusters.

### 2.6 | Fluxes of $N_2O$ and $N_2$ at the Sediment-Overlying Water Interfaces

Sediment-water fluxes of $N_2O$ and $N_2$ were determined in situ using an open-bottom benthic chamber method (Wang, Xia, et al. 2022). Water samples were collected from the chamber using a micropump at 1-h intervals over a 6-h sampling period. Samples for $N_2O$ and $N_2$ concentration measurements were transferred into 120-mL glass serum vials and 12-mL Exetainer Labco vials (UK), respectively. $N_2O$ concentration was measured using a headspace equilibrium technique (Wang, Xia, et al. 2022). The headspace gas in the glass vials was created and extracted within 1 h after sampling, and the $N_2O$ was then analyzed using a gas chromatograph with an electron capture detector (Agilent 7890B GC). $N_2$ concentration was determined using the $N_2$:Ar ratio method with a membrane inlet mass spectrometry instrument (MIMS, PrismaPlus, Germany) (Wang, Xia, et al. 2022). Detailed procedures are provided in Text S2. $N_2O$ and $N_2$ fluxes at the sediment-water interfaces were calculated based on the linear increase in gas concentrations over the entire sampling period. In this study, 83% of the $N_2O$ and $N_2$ fluxes ($n = 71$) were measured in large rivers (river order $\geq 5$, Figure S1), with sampling mainly confined to their main channels. Groundwater contributes minimally to runoff in large rivers, typically less than 10% (Krause et al. 2007; Wang, Xia, et al. 2022). Additionally, the substantial water volume of large rivers can further dilute exogenous $N_2O$ inputs from groundwater and other sources. This suggests that the primary sources of sediment $N_2O$ and $N_2$ emissions in the rivers we studied are endogenous, mainly driven by in-sediment production and reduction processes.

### 2.7 | Statistical Analyses

All statistical analyses in this study were conducted at a significance level of $p < 0.05$. Spearman's correlation analysis with FDR (False Discovery Rate) correction was used to assess relationships among environmental variables, microbial variables, and sediment $N_2O$ flux. Paired sample *t*-tests were used to compare gene abundances between *nosZ*I-type $N_2O$ reducers and $N_2O$ producers. A partial least squares (PLS) path model was constructed using SmartPLS 3 software (Boenningstedt, Germany) to illustrate how MAT potentially influenced $N_2O$ dynamics. The path model was validated using 1000 bootstraps to assess the significance of path coefficients.

## 3 | Results

### 3.1 | Abundance and Community Composition of *nosZ*I and *nosZ*II Microbes

qPCR assays revealed the widespread presence of both *nosZ*I-(100% of the 93 samples) and *nosZ*II-containing microbes (96%) in sediment samples from six major rivers in China (Figure 1b). *nosZ*I gene abundance ranged from $1.15 \times 10^6$ to $8.58 \times 10^8$ copies $g^{-1}$ dry sediment, whereas *nosZ*II gene abundance varied between $1.54 \times 10^3$ and $1.07 \times 10^8$ copies $g^{-1}$ dry sediment (Figure 1b). Compared with $N_2O$-producing microbes, *nosZ*I gene abundance was lower than that of the denitrifying bacterial *nirS*, but higher than *nirK*, archaeal *amo*A, and bacterial *amo*A ($p < 0.01$) (Figure S2). These results highlight the potential significance of $N_2O$ reducers in $N_2O$ transformations within river sediments. Stepwise linear regression analysis identified total phosphorus (TP) in the water as the primary predictor of the *nosZ*II:*nosZ*I gene abundance ratio, with a significant negative correlation between TP and the ratio ($r = -0.74$).

The community diversity of *nosZ*I- and *nosZ*II-containing microbes was analyzed using PacBio SMRT sequencing. This analysis identified 1,112,933 *nosZ*I gene sequences and 1,064,115 *nosZ*II gene sequences, which were clustered into 1392 and 3718 OTUs, respectively. Phylogenetic placement analysis revealed that 96.9% of *nosZ*I sequences were classified as Proteobacterial species (Figure 1c), with the remaining 3.1% unclassified at the phylum level. Over half (51.5%) of *nosZ*I sequences were associated with alpha-proteobacterial species, mainly from the orders Rhodobacterales (41.1%) and Rhizobiales (10.2%). Another 44.1% of *nosZ*I sequences were classified as beta-proteobacterial species, primarily from the orders Burkholderiales (19.84%), Nitrosomonadales (16.39%), Rhodocyclales (5.88%), and Neisseriales (0.10%). Gamma-proteobacterial species represented a minor proportion (1.33%).

The community composition of *nosZ*II-containing microbes was more diverse (Figure 1d). A total of 93.0% of sequences were taxonomically classified at the phylum level, spanning ten phyla, with Bacteroidetes accounting for 53.9% of the total sequences. In contrast to *nosZ*I, only 13.8% of *nosZ*II sequences were associated with Proteobacteria-like species. The phyla Chloroflexi, Spirochaetes, and Planctomycetes accounted for 10.8%, 8.5%, and 5.3% of the *nosZ*II sequences, respectively. The remaining phyla, including Gemmatimonadetes, Ignavibacteriae, Balneolaeota, Aquificae, and Verrucomicrobia, each constituted less than 0.28% of *nosZ*II sequences.

### 3.2 | Ecological Differentiation Between *nosZ*I and *nosZ*II Communities

We conducted a comprehensive evaluation of the relative importance of various factors in explaining community turnover for *nosZ*I- and *nosZ*II-type $N_2O$ reducers using GF models. The models showed that the dynamics of the *nosZ*I community were more strongly explained by a combination of environmental, microbial, and spatial variables compared with the *nosZ*II community (Figure 2). This difference was mainly attributed to the greater contributions of environmental and

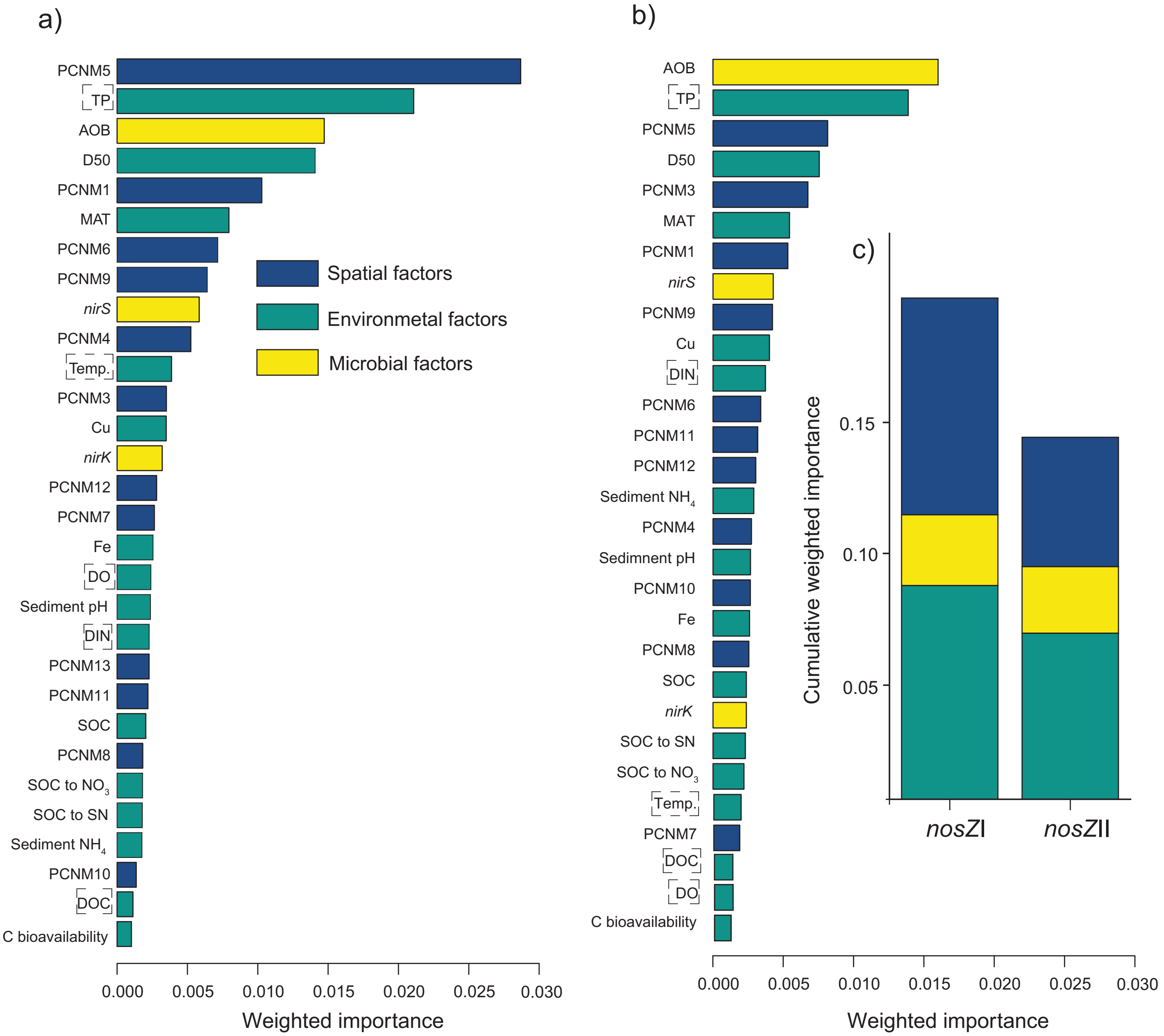

**FIGURE 2** | Predictors of community composition for *nosZ*I- (a) and *nosZ*II-containing (b) $N_2O$ reducers. (c) Relative contributions of environmental, microbial, and spatial factors to community variation in $N_2O$ reducers. PCNMs are spatial variables derived from distance matrices, designed to capture spatial structure within $N_2O$ reducer communities. Factors within dashed boxes are water properties. PCNM1-PCNM12, represent the first half of PCNMs with positive eigenvalues; TP, total phosphorus; D50, median sediment particle size; SOC to $NO_3^-$, sediment organic carbon to nitrate ratio; C bioavailability was indexed by rA1630/rA2930 from Fourier-transform infrared spectroscopy, with detailed information available in Text S1.

spatial variables to the *nosZ*I community (cumulative weighted importance: 0.17 vs. 0.12) (Figure 2c). The key environmental variables driving community composition variations were consistent across both communities, including TP, median sediment particle size (D50), and MAT. However, these factors had a stronger explanatory power for *nosZ*I microbes than for *nosZ*II microbes (Figure 2a,b). These results remained consistent even when spatial variables were excluded from the model (Figure S3). ePCA, which explained 86.3% and 82.4% of the total variation in the *nosZ*I and *nosZ*II communities, respectively, further confirmed that these environmental factors were more strongly associated with the *nosZ*I community (Figure S4).

In contrast, microbial variables, including the abundances of nitrifiers (bacterial *amoA*) and denitrifiers (*nirK* and *nirS*), exhibited nearly equivalent explanatory power for both communities (Figure 2c). When spatial factors incorporated into the GF model, the weighted importance of denitrifying *nirS* and *nirK* gene abundances was slightly higher for the *nosZ*I community, while the nitrifying bacterial *amoA* (i.e., AOB) showed similar weighted importance in explaining the *nosZ*II community (0.014 for *nosZ*I vs. 0.016 for *nosZ*II, Figure 2a,b). Edge PCA analysis revealed community separation along the first principal component (PC1), with regression analyses showing similar correlations between AOB abundance and PC1 scores for both *nosZ*I ($R^2 = 0.08$) and *nosZ*II ($R^2 = 0.10$) communities. Together, these results indicate ecological differentiation between *nosZ*I and *nosZ*II communities in rivers in response to environmental and microbial factors.

### 3.3 | Intra-Clade Ecological Differentiation and Its Relationship With MAT

We identified 90 *nosZ*I OTUs and 87 *nosZ*II OTUs with predictable habitat preferences, each achieving a cumulative model prediction performance of $R^2 > 0.1$ in GF models. These specific OTUs accounted for 21.9% of the total sequences in the *nosZ*I community and 12.2% in the *nosZ*II community. Clustering analysis identified four distinct ecological clusters within both *nosZ*I and *nosZ*II communities (Figure 3). The primary factors driving ecological differentiation within the *nosZ*I and *nosZ*II communities were D50, MAT, TP, nitrifying bacterial *amoA* abundance, and denitrifier *nirS* abundance (Figure 3), similar to those influencing the overall communities (Figure 2). Notably, within the *nosZ*II community, we consistently observed clusters with ecological niches nearly identical to those in the *nosZ*I community. These paired ecological clusters showed significant positive correlations (Figure 4).

One ecological cluster preferred river environment with a large D50 and low levels of nitrifier abundance and TP (referred to as the “large D50 cluster”), accounting for 3.4% of the *nosZ*I community and 1.0% of the *nosZ*II community (Figure 3). Another cluster was associated with high denitrifying *nirS* abundance (referred to as the “high *nirS* cluster”), and contributed more to the *nosZ*I community composition (0.7%) than to the *nosZ*II community composition (0.2%) (Figure 3). The relative proportions of the large D50 and high *nirS* clusters increased significantly with D50 and *nirS* gene abundance, respectively, within both *nosZ*I and *nosZ*II communities (Figure S5).

The remaining two ecological clusters were associated with low and high MAT levels. Stepwise linear regression analysis identified MAT as the primary predictor of the relative proportions of these clusters (Figures S5 and S6). Clusters associated with high and low MAT exhibited negative correlations with each other in both communities (Figure 4b). These MAT-related clusters accounted for the largest proportion of OTUs with predictable ecological niches in both the *nosZ*I (13.6% out of 21.9%) and *nosZ*II communities (8.9% out of 12.2%) (Figure 3), underscoring the crucial role of MAT in shaping the ecological distribution of $N_2O$ reducers in river sediments. The taxonomic composition of low MAT clusters differed substantially from that of high MAT clusters (Tables S4 and S5). Specifically, low MAT clusters within the *nosZ*I community predominantly consisted of beta-proteobacterial species (95%), whereas high MAT clusters

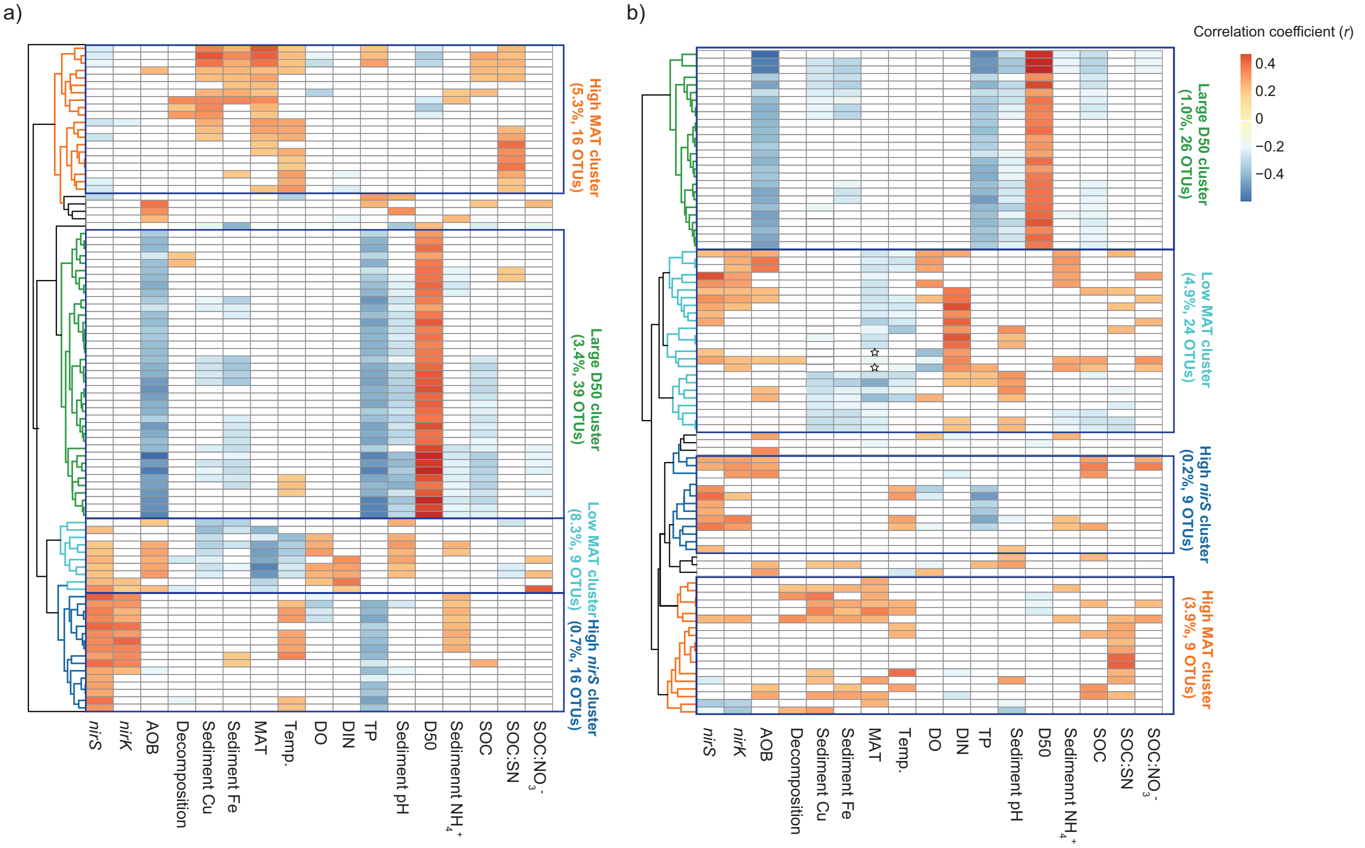

**FIGURE 3** | Clustering heatmap showing the distribution of ecological clusters and their associated factors. Each row represents an OTU within the *nosZ*I (a) and *nosZ*II (b) communities, which were selected based on an $R^2$ threshold $> 0.1$ as explained by environmental and microbial variables. Colored cells indicate correlations that are significant at the $p < 0.05$ level, whereas blank cells indicate correlations with $p > 0.05$. Star symbols (☆) denote correlations that are approaching significance at the $p < 0.06$ level. Abbreviations: TP, total phosphorus; D50, median sediment particle size; SOC to $NO_3^-$, sediment organic carbon to nitrate ratio; Temp., water temperature.

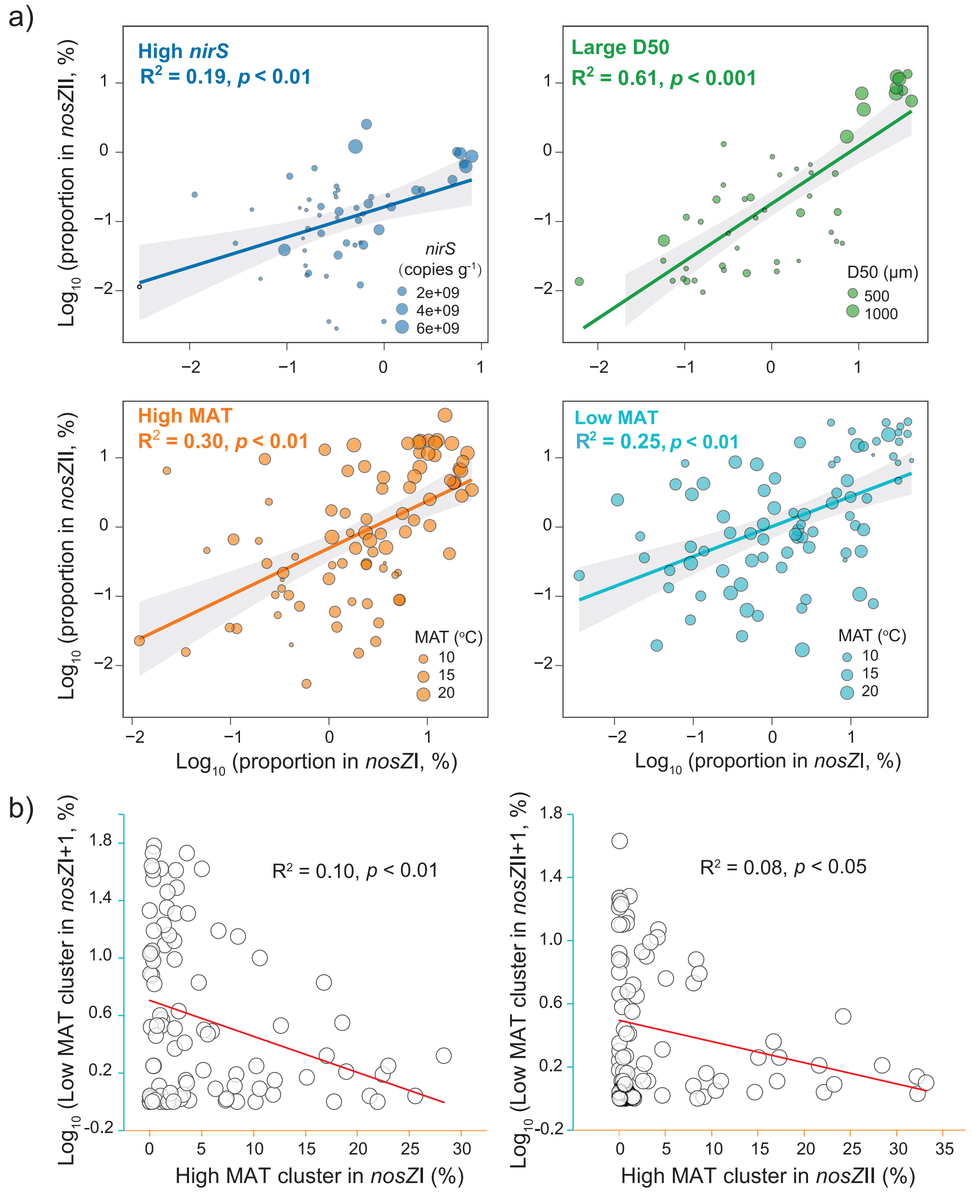

**FIGURE 4** | Relationships between the relative proportions of identified ecological clusters within the $N_2O$ reducer community. (a) Correlations between shared ecological clusters from the *nosZ*I and *nosZ*II communities in relation to high *nirS* abundance, high mean annual temperature (MAT), large median sediment particle size (D50), and low MAT. (b) Correlations between ecological clusters associated with high and low MAT within the *nosZ*I and *nosZ*II communities.

were composed mainly of alpha-proteobacterial species (92%) (Table S4).

Compared with other ecological clusters, OTUs within the MAT-related clusters were more prevalent in China's rivers ($p < 0.05$) and had an average occurrence frequency exceeding 30 in both the *nosZ*I and *nosZ*II communities (Figure S7a,b). Building on this observation, we conducted additional GF analyses on subcommunities of $N_2O$ reducers, focusing on OTUs present in at least 30 samples, and subsequently extended the analysis to those with an occurrence threshold of 50 samples. The results showed that, among the environmental and microbial factors, MAT emerged as the strongest predictor of variation in subcommunities with OTUs present in more than 30 samples (Figure S7c,d), and its explanatory power further increased for OTUs present in more than 50 samples (Figures S7c,d and S8).

### 3.4 | Assembly Processes of *nosZ*I and *nosZ*II Communities

The assembly processes of *nosZ*I and *nosZ*II communities, including environmental filtration, dispersal, and drift, exhibited significant differences (Figure 5a). Combined environmental filtration (including both homogeneous and heterogeneous selection) played a more significant role in assembling the *nosZ*I community (41.3%) than in the *nosZ*II community (23.3%) (Figure 5a). This observation aligns with the greater contribution of environmental factors to

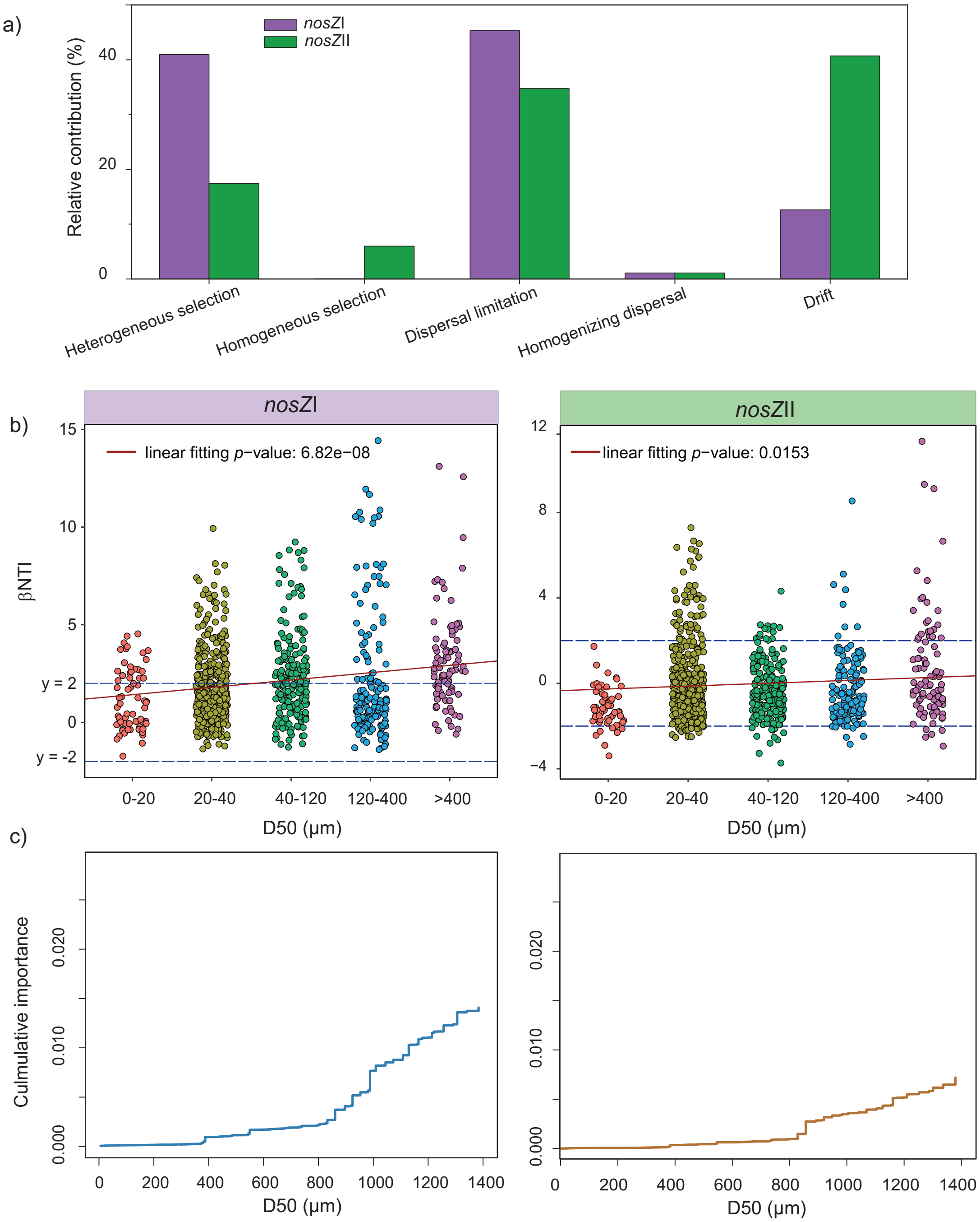

**FIGURE 5** | Correlations between $N_2O$ reducers community assembly patterns and median sediment particle size (D50). (a) Differences in community assembly processes between *nosZ*I- and *nosZ*II-containing microbes. (b) Patterns of βNTI across D50 categories for the *nosZ*I and *nosZ*II communities. Linear regression models (red lines) and associated correlation coefficients are shown in each panel. βNTI > 2 indicates heterogenous selection, whereas βNTI < −2 indicates homogeneous selection. (c) Cumulative importance of D50 in explaining *nosZ* community composition across different D50 values.

compositional variation in the *nosZ*I community (Figure 2c). The greater importance of heterogeneous selection over homogeneous selection within both the *nosZ*I and *nosZ*II communities (41.3% vs. < 0.1% for *nosZ*I; 16.9% vs. 6.4% for *nosZ*II) corresponded to their significant intra-clade ecological differentiations (Figure 3). Drift, a completely stochastic process, predominantly influenced the assembly of the *nosZ*II community (40.4%), while its contribution to the *nosZ*I community was considerably lower (12.0%).

To evaluate shifts in community assembly patterns across specific environmental gradients, we analyzed the relationships between βNTI and environmental variables, with |βNTI| > 2 indicating deterministic assembly. Mantel test results showed that D50, a key factor accounting for *nosZ* community differentiation (Figure 2), was the most effective predictor of assembly dynamics in both the *nosZ*I and *nosZ*II communities (Table S6). The significant role of D50 was likely due to its negative correlation with carbon and nutrient levels and nitrifier abundance

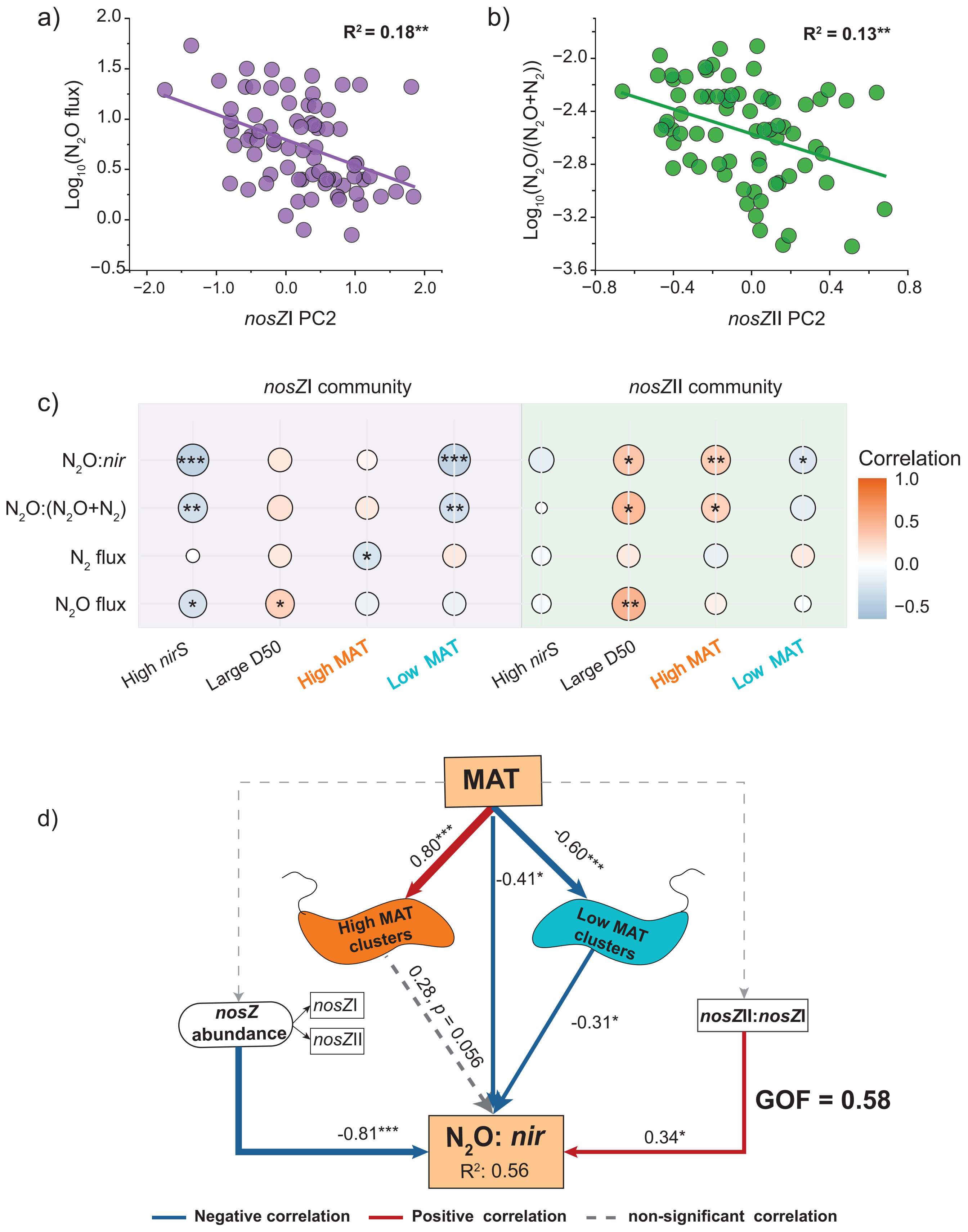

**FIGURE 6** | Legend on next page.

in the sediments (Figure S9). Pairwise comparisons of β-nearest taxon distance (βNTI) values (−4.6 to 28.4) showed a significant positive correlation with D50 differences between samples for both *nosZ*I and *nosZ*II communities (Figure S10), suggesting that larger D50 differences increased the contribution of environmental filtration in both communities. Further analysis of

**FIGURE 6** | Relationships between the community composition of $N_2O$ reducers and $N_2O$ dynamics. (a, b) Linear regression relationships between $N_2O$ dynamics and specific variations within $N_2O$ reducer communities. (c) Correlations of identified ecological clusters with $N_2O$ dynamics (log-transformed data). (d) PLS pathway models illustrating how mean annual temperature (MAT) potentially affects $N_2O$ dynamics. *Nir* is defined as *nirS* + *nirK*, indicating the $N_2O$ production potential within denitrifiers. Solid lines (significant, $p < 0.05$) are colored red for positive correlations and blue for negative correlations, whereas dashed lines represent non-significant correlations ($p > 0.05$). GOF (Goodness of Fit) reflects the predictive capability of constructed models, with values above 0.5 generally indicating an acceptable model fit. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

βNTI across subgroups based on D50 values showed that as D50 increased, the proportion of deterministic assembly (primarily via heterogeneous selection) increased in both communities (Figure 5b), with this trend being more evident in the *nosZ*I community. Accordingly, the contribution of D50 to *nosZ* community turnover was generally higher in sediment subsets with larger D50 values (Figure 5c).

### 3.5 | Relationships Between Ecological Differentiation and $N_2O$ Dynamics

In our studied samples, sediment $N_2O$ flux to the overlying water ranged from 0.70 to 54.20 μmol $N_2O$ $m^{-2}$ $day^{-1}$, whereas $N_2$ flux varied from 0.20 to 13.5 mmol $N_2$ $m^{-2}$ $day^{-1}$ (Figure S1b). Although no significant correlations were found between the abundance of any $N_2O$ producers or reducers and the $N_2O$ flux ($p > 0.1$, Figure S9), the structure of $N_2O$ reducer communities played a significant role. Specifically, sediment $N_2O$ flux was negatively correlated with variations in the *nosZ*I community along the PC2 axis in edge PCA biplots ($R^2 = 0.18$, $p < 0.01$, Figure 6a). These *nosZ*I community variations were associated with a significant decrease in $NO_3^-$ levels and an increased abundance ratio of *nosZ*I to *nir* (*nirS* + *nirK*) genes (Table S7). In contrast, the $N_2O/(N_2O + N_2)$ ratios, where a lower ratio indicates a higher reduction of $N_2O$ to $N_2$, showed negative correlations with community variations in the *nosZ*II community along the PC2 axis (Figure 6b). These community variations were significantly associated with higher *nirS* abundance, lower *nirK* abundance, and elevated levels of dissolved organic matter, as well as reduced water inorganic nitrogen content and lower sediment pH (Table S7). Overall, these results indicate that *nosZ*I and *nosZ*II communities have significant but differential relationships with $N_2O$ dynamics, with specific compositions of $N_2O$ reducers playing disproportionately important roles.

To further understand microbial effects on $N_2O$ reduction and flux, we analyzed the relationships between ecological differentiation within each clade and $N_2O$ dynamics. We found that the identified ecological clusters among $N_2O$ reducers were closely correlated with $N_2O$ dynamics, with shared clusters across both communities showing consistent correlation directions (Figure 6c). Certain correlations between ecological differentiations and $N_2O$ dynamics were stronger than those observed in overall community variations (Figure 6a–c). The large D50 clusters in both communities showed a significant positive correlation with $N_2O$ flux, whereas high *nirS* clusters were negatively correlated, with significant correlations particularly observed in the *nosZ*I community (Figure 6c). Low MAT clusters, although not directly linked to $N_2O$ flux, showed negative correlations with denitrifier abundance-normalized $N_2O$ flux (i.e., $N_2O$:*nir*) (Figure 6c). In contrast, high MAT clusters in the *nosZ*I ($p < 0.05$) and *nosZ*II communities ($p = 0.18$) exhibited positive relationships with the normalized $N_2O$ flux. Additionally, the high MAT clusters were negatively correlated with $N_2$ flux, the product of $N_2O$ reduction ($N_2O \rightarrow N_2$) (Figure 6c). Focusing exclusively on $N_2O$ reducers, the PLS pathway model revealed that the significant decrease in normalized $N_2O$ flux with increasing MAT was largely attributed to a decline in the proportion of low-MAT clusters (Figure 6d). These results highlight the significant role of intra-clade ecological differentiation among $N_2O$ reducers in explaning $N_2O$ dynamics in river systems.

## 4 | Discussion

### 4.1 | Significant Ecological Differentiation Between and Within *nosZ*I and *nosZ*II Communities

This study revealed significant ecological differentiation among $N_2O$ reducers across continental-scale river ecosystems (Figures 2 and 3). Our findings demonstrated that *nosZ*I community was more responsive to environmental factors than *nosZ*II community (Figure 2). This pattern has also been observed in terrestrial ecosystems (Hallin et al. 2018), where environmental factors such as soil texture and nutrient availability significantly shape the *nosZ*I community, while no specific environmental factor has been consistently identified as driving the *nosZ*II community. Consistent with previous studies (Juhanson et al. 2017), nitrifying bacteria showed a comparable correlation level with the *nosZ*I and *nosZ*II community, whereas denitrifying bacteria were more strongly associated with the *nosZ*I community (Figure 2). Previous studies have shown that ~83% of *nosZ*I-type species harbor the *nir* genes (Hallin et al. 2018), which catalyze the precursor step in $N_2O$ production during denitrification, leading these species to more readily reduce autogenously produced $N_2O$. In contrast, > 50% of *nosZ*II-type species lack *nir* genes (Hallin et al. 2018), forcing more reliance on extracellular $N_2O$ pools generated through processes like nitrification (Quick et al. 2019). These differing associations suggest that *nosZ*I- and *nosZ*II-type $N_2O$ reducers may play distinct roles with respect to different $N_2O$ sources.

Among the environmental factors studied, TP was a key driver of community turnover for both clades of $N_2O$ reducers (Figure 2), a link rarely documented in aquatic systems. Elevated TP levels stimulate biological activity and growth, which accelerates oxygen consumption and subsequently reduces oxygen levels (Finlay et al. 2013). $N_2O$ reducers exhibit marked interspecies variability in oxygen sensitivity (Wittorf et al. 2016). Some species sustain $N_2O$ reduction under suboxic conditions, whereas others are strictly anaerobic (Wang et al. 2023). Therefore, TP-driven oxygen fluctuation might alter the community composition of $N_2O$ reducers. Concurrently, phosphorus enrichment may intensify biological competition for bioavailable nitrogen (Shen and Zhu 2022), restricting nitrogen accessibility for $N_2O$ producers (Wells et al. 2018) and thereby reducing the $N_2O$ pool available to reducers. Given the divergent substrate affinities of $N_2O$ reducers (Hiis et al. 2024), changes in $N_2O$ supply could reshape the composition of $N_2O$ reducer communities. Future research should prioritize validating these TP-mediated community assembly mechanisms and exploring the relationship between TP-driven shifts in $N_2O$-reducer communities and aquatic $N_2O$ flux (León-Palmero et al. 2023).

Ecological niche partitioning was also observed within both the *nosZ*I and *nosZ*II communities (Figure 3), a phenomenon that has rarely been reported in natural environments (Maheshwari et al. 2023). Beta-proteobacterial species, dominant in low-MAT clusters within the *nosZ*I community (Table S4), have been shown to decrease significantly with rising temperatures across various soils (Fierer et al. 2007; Rui et al. 2015). In contrast, alpha-proteobacterial species, prevalent in high-MAT clusters, tend to increase with temperature (Rui et al. 2015). Similarly, psychrophilic *Leptospira* species within the *nosZ*II community (Bierque et al. 2020) disappeared under high-MAT conditions (Table S5). The exclusive dominance of alpha-proteobacteria in large-D50 clusters of the *nosZ*I community (Tables S8 and S9) aligns with their adaptive traits for oligotrophic environments (Fierer et al. 2007; Newton Ryan et al. 2011). These previous findings confirmed the clade-specific niche differentiation among $N_2O$ reducers (Figure 3). Importantly, although demonstrating divergent compositions between *nosZ*I and *nosZ*II communities (Tables S4 and S5 and S8–S10), the paired ecological clusters exhibited consistency in modulating $N_2O$ flux (Figure 6). This underscores the importance of ecological niches in shaping the relationship between microbes and ecosystem functions (Kahmen et al. 2006). Building on this, ecological differentiation within $N_2O$-reducing microbial communities serves as a critical predictor of $N_2O$ dynamics (Figure 6c). These findings suggest categorizing $N_2O$ reducers by ecological niche, in addition to the traditional *nosZ*I and *nosZ*II classification, to improve our understanding of their distribution and functions.

### 4.2 | Associations of Sediment Texture With Community Assembly and Function of $N_2O$ Reducers

A mechanistic understanding of microbial community variations requires identifying the factors that influence the relative contributions of stochastic and deterministic processes in community assembly (Jiao and Lu 2020). Consistent with our findings (Figure 5a), previous soil research has shown that *nosZ*II community assembly is more stochastic, whereas *nosZ*I community assembly is more deterministic (Juhanson et al. 2017). This distinction may be partly attributed to the greater diversity within the *nosZ*II community, which is composed of a dozen different phyla, whereas *nosZ*I primarily consists of Proteobacteria (Hallin et al. 2018). Functional differences between these two clades further contribute to this divergence. Unlike *nosZ*I microbes, for which $N_2O$ reduction is more closely associated with energy production, many *nosZ*II microbes use $N_2O$ reduction as an electron sink during transitions from oxic to anoxic conditions (Hallin et al. 2018; Shan et al. 2021). Thus, the ecological distribution of *nosZ*II microbes may be governed by a broader range of ecological functions and environmental factors, rather than solely by conditions controlling $N_2O$ reduction.

D50 shaped the community assembly patterns for both clades of $N_2O$ reducers, with heterogeneous environmental selection becoming increasingly important as D50 increased (Figure 5b). Larger D50 enhances sediment permeability (Wildhaber et al. 2014), facilitating deeper penetration of oxygen-rich overlying water into the sediment. This creates stressful conditions for $N_2O$ reducers, a significant number of which are sensitive to oxygen inhibition (Wang et al. 2023). In combination with the oligotrophic conditions present in larger D50 environments (Wildhaber et al. 2014), $N_2O$ reducers in these settings seek out patchy microenvironments with relatively higher carbon and nutrient levels. In these microenvironments, increased aerobic respiration depletes oxygen, sustaining localized anaerobic zones essential for their survival (Battin et al. 2016). However, these patchy microenvironments are expected to exhibit greater heterogeneity than the bulk environment (Battin et al. 2016). Such abiotic heterogeneity allows different species to occupy specific niches according to their ecological preferences and tolerances, thereby increasing the contribution of heterogeneous environmental selection.

Deterministic processes are generally assumed to result in communities well-adapted to specific environmental conditions (Ernakovich et al. 2022). However, heterogeneous environmental selection did not alleviate all inhibitory effects experienced by $N_2O$ reducers in large D50 conditions. Although species richness (i.e., OTU count) was higher in the large D50 cluster compared with other ecological clusters, their overall relative proportion within the community remained low (Figure 3). This trend was particularly evident in the *nosZ*I community, where the *nosZ*I gene abundance significantly decreased with larger D50 (Figure S9). This pattern likely accounts for the significant variation in $N_2O$ emissions associated with river sediment texture across river systems (Quick et al. 2019). Understanding the influence of sediment texture on $N_2O$ reducer communities is especially important in the context of increasing extreme rainfall events, which are expected to become more frequent under future climate scenarios (Masson-Delmotte et al. 2021). These events can enhance sediment scouring, leading to coarser sediment conditions that could alter the community structure and function of $N_2O$ reducers.

### 4.3 | MAT-Driven Ecological Differentiation Significantly Contributes to $N_2O$ Dynamics

Building on previous findings that identified MAT as a critical driver of *nosZ*I community variations in soils (Wu et al. 2018; Xing et al. 2021), our study extends this understanding to river systems, demonstrating that MAT significantly contributed to the community turnover for both *nosZ*I and *nosZ*II microbes (Figure 2, Figure S7). Notably, the role of MAT in shaping both communities intensified with the prevalence of $N_2O$ reducers (Figures S7 and S8). This pattern aligns with earlier findings showing that abundant bacterial subcommunities are more sensitive to changes in MAT than rarer ones (Liu et al. 2015; Zhang et al. 2022). These results imply that $N_2O$ reducers are very likely to experience significant shifts under warming conditions, and future research should prioritize close monitoring of their potential changes.

Our findings suggest that high-MAT conditions may favor $N_2O$ reducers with lower efficiency in converting $N_2O$ to $N_2$ (Figure 6). Consistent with the dominance of beta-proteobacteria in the low-MAT clusters of the *nosZ*I community (Table S4) and their adaptation to eutrophic conditions (Fierer et al. 2007; Newton Ryan et al. 2011), certain $N_2O$ reducers within this cluster were positively correlated with nitrogen levels (Figure 3). This correlation was absent in the high-MAT cluster, where the dominant species, alpha-proteobacteria, tend to prefer oligotrophic conditions (Fierer et al. 2007). Similar patterns were also observed in the *nosZ*II community (Figure 3). These patterns aligned with the negative relationship between MAT, as well as water temperature, and dissolved inorganic nitrogen (Figure S9), possibly due to enhanced terrestrial vegetation nitrogen retention and accelerated microbial metabolism at higher temperatures (Wang, Wang, et al. 2022). Although elevated nitrogen concentrations inhibit $N_2O$ reduction (Hallin et al. 2018; Quick et al. 2019), $N_2O$ reducers in low-MAT clusters may maintain higher $N_2O$ reduction efficiency through their better adaptation to eutrophic conditions.

Additionally, low-MAT clusters were found in environments with lower water temperatures compared with high-MAT clusters (Figure 3). This temperature difference is important because $N_2O$ has lower solubility in warmer water, making it more likely to escape from the rivers and thus reducing the $N_2O$ reduction efficiency. On the other hand, $N_2O$ reducers in low- and high-MAT clusters showed distinct characteristics in their $N_2O$ reduction pathways. Beta-proteobacteria, which dominated in low-MAT clusters, are more likely to perform $N_2O$ reduction simultaneously with $N_2O$ production within the same cell compared with alpha-proteobacteria enriched in high-MAT clusters (Graf et al. 2014). Supporting this pattern, our analysis revealed that species within low-MAT clusters often showed positive correlations with *nirS* or *nirK* gene abundance, contrasting with the pattern observed in high-MAT clusters (Figure 3). This efficient intracellular coupling of $N_2O$ production and reduction pathways could enhance the $N_2O$ reduction efficiency in low-MAT clusters. These finding suggest that warming rivers may experience decreased $N_2O$ reduction efficiency, potentially amplifying temperature effects on $N_2O$ emissions (Velthuis and Veraart 2022). This should be carefully considered when modeling $N_2O$ transformation in warming rivers.

### 4.4 | Uncertainty and Limitations

In this study, we identified various ecological clusters within the $N_2O$ reducer community by selecting OTUs with evident ecological niches, followed by clustering analysis. To test the robustness of these clusters, we adjusted the selection criteria for OTUs with evident niches ($R^2 > 0.1$), a critical parameter that potentially introduce some level of certainty, by applying alternative thresholds of 0.05 and 0.2. We found that, even under these modified criteria, niche differentiation persisted within $N_2O$ communities (Figures S11 and S12). The types of ecological clusters identified were consistent with those recognized at the $R^2 = 0.1$ threshold (Figure 3, Figures S11 and S12), although the high *nirS* clusters in both the *nosZ*I and *nosZ*II community were less evident at the $R^2 = 0.2$ threshold (Figure S11). Furthermore, the associations between these clusters and $N_2O$ dynamics were largely consistent across the different selection criteria, with only minor variations (Figure 6c, Figure S13).

To provide ecologically relevant insights into the dynamics of $N_2O$ reducers, this study primarily relied on field data and statistical methods, including GF and linear regression models. Although these approaches are effective for identifying patterns and associations within ecological data, they have inherent limitations in establishing direct causal relationships. We presented supporting evidence for ecological differentiation within each clade of $N_2O$ reducers and proposed mechanistic assumptions linking this differentiation to $N_2O$ dynamics. However, further research, particularly controlled experiments, is needed to verify these assumptions and bridge the gap between field observations and causal understanding. Moreover, future studies should focus on discerning the hierarchical importance of environmental drivers in shaping ecological niches for $N_2O$ reducers. For example, what is the role of TP and the abundance of AOB in the formation of the large D50 clusters (Figure 3). Such efforts will enhance our understanding, prediction, and modeling of $N_2O$ reducers and the reduction process.

## 5 | Conclusions

This study investigated the ecological differentiation both between and within the *nosZ*I and *nosZ*II communities in continental-scale river systems and explored their associations with $N_2O$ dynamics and underlying mechanisms. Our findings revealed significant ecological differentiation within each clade of $N_2O$ reducers, which played a significant role in shaping $N_2O$ dynamics. Additionally, shared ecological clusters across both clades displayed similar correlation patterns with $N_2O$ dynamics. These findings suggest categorizing $N_2O$ reducers by ecological niche to improve our understanding of their distribution and functions. Sediment texture and the associated changing nutrient levels significantly shaped the assembly pattern for both *nosZ*I and *nosZ*II communities and their functions, warranting consideration in studies linking sediment texture to $N_2O$ emissions. Our study also suggests that warming may reduce $N_2O$ reduction efficiency in rivers, thereby enhancing the influence of temperature on $N_2O$ emissions, which should be carefully considered when modeling $N_2O$ transformation and dynamics in warmer environments. Overall, this study contributes to a deeper understanding of the distribution and function of $N_2O$ reducers in river systems, supporting more accurate predictions of $N_2O$ dynamics in the context of global change.

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### Author Contributions

**Sibo Zhang:** conceptualization, formal analysis, investigation, methodology, writing – original draft. **Meijuan Yang:** formal analysis, investigation, methodology, resources. **Xinghui Xia:** conceptualization, funding acquisition, writing – review and editing. **Qinyuan Gu:** formal analysis, investigation, methodology. **Qun Gao:** writing – review and editing. **Junfeng Wang:** data curation, formal analysis, investigation, methodology. **Shaoda Liu:** writing – review and editing. **Xiaokang Li:** investigation, writing – original draft. **Yingjie Li:** formal analysis. **William H. McDowell:** writing – original draft, writing – review and editing. **Zhifeng Yang:** conceptualization, funding acquisition, project administration.

## Acknowledgements

This research was financially supported by the Basic Science Center Project of the Natural Science Foundation of China (53288101), National Natural Science Foundation of China (52239005, 52039001, and 52109050), and Program for Guangdong Introducing Innovative and Entrepreneurial Teams (2019ZT08L213). WHM was supported by US NSF 2215300.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The raw *nosZ* gene sequences used in this study have been deposited in the NCBI Sequence Read Archive (SRA) under the project accession number PRJNA886309. The data that support the findings of the relationships between $N_2O$ reducers and $N_2O$ dynamics are available in figshare at https://doi.org/10.6084/m9.figshare.28329842.v3.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.