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Distance-Decay Relationship for Biological Wastewater Treatment Plants

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ABSTRACT

Patterns in the spatial distribution of organisms provide important information about mechanisms underlying biodiversity and the complexity of ecosystems. One of the most well-documented spatial patterns is the distance-decay relationship, which is a universal biogeographic pattern observed repeatedly for plant and animal communities, particularly for microorganisms in natural ecosystems such as soil, ocean, and salt marsh sediment. However, it is uncertain whether the microorganisms exhibit a distance-decay pattern in engineered ecosystems. Therefore, we measured the distance-decay relationship across various microbial functional and phylogenetic groups in 26 biological wastewater treatment plants (WWTPs) in China using a functional gene array (GeoChip 4.2). We found that microbial communities of activated sludge in WWTPs exhibited a significant but very weak distance-decay relationship. The taxon-area *z* values for different functional and phylogenetic groups were <0.0065, which is about 1 to 2 orders of magnitude lower than those observed in microbial communities elsewhere. Variation-partitioning analysis (VPA) showed that the relationships were driven by both environmental heterogeneity and geographic distance. Collectively, these results provided new insights into the spatial scaling of microbial communities in engineering ecosystems and highlighted the importance of environmental heterogeneity and geographic distance in shaping biogeographic patterns.

IMPORTANCE

Determining the distance-decay relationship of microbial biodiversity is important but challenging in microbial ecology. All studies to date are based on natural environments; thus, it remains unclear whether there is such a relationship in an engineered ecosystem. The present study shows that there is a very weak distance-decay relationship in an engineered ecosystem (WWTPs) at the regional-to-continental scale. This study makes fundamental

contributions to a mechanistic, predictive understanding of microbial biogeography.

INTRODUCTION

A central goal of ecology is to understand how biodiversity is generated and maintained (1). Spatial patterns of species diversity offer insights into the mechanisms shaping biodiversity and are of practical importance for predicting the risk of biodiversity loss by environmental changes and consequently for setting up conservation priorities (2). Therefore, the spatial distribution patterns of species diversity have solicited substantial attention. Traditionally, the field of spatial distribution patterns of biodiversity has focused on plants and animals. For example, it has been well documented for plant and animal communities that community similarity decreased with geographic distance, known as the distance-decay relationship (3, 4). In recent years, a number of studies have been conducted to investigate biogeographic patterns of microorganisms, including bacteria, archaea, fungi, and other microbial eukaryotes (3, 5–10). A growing body of research has shown that microorganisms, like plants and animals, exhibited distance-decay patterns in different habitats at various taxonomic resolutions (2, 3, 11–18).

The shaping mechanisms of distance-decay patterns in microbial communities can be explained by contemporary environmental heterogeneity and historical events (19). If microbial communities are shaped mainly by contemporary environmental conditions, a distance-decay relationship could be observed because environmental factors tend to be spatially autocorrelated, and microorganisms with different niche preferences are selected from the available pool of taxa as the environment changes with distance. This is the so-called Baas-Becking hypothesis: “everything is everywhere—the environment selects” (20). However, the distance-decay relationship can also be influenced by historical contingences, which can be represented by geographic distance. For example, neutral-niche models, in which the microbial community is not influenced by its environmental conditions, can also generate a distance-decay pattern (3). Although the relative importance of environmental conditions and geographic distance is under heated debate, it is generally believed that microbial biogeographic distribution reflects the influences of both contemporary environmental conditions and geographic distance (1, 5).

To date, all microbial biogeographic studies have been carried out on natural ecosystems such as soil (2, 11), freshwater (13, 14), salt marsh sediment (3), and deep-sea surface sediments (18). The spatial distribution of microbially diverse populations in engineered ecosystems such as biological wastewater treatment plants (WWTPs) remained unknown. Engineered ecosystems are very different from natural ecosystems in that they are designed to carry out a stable function(s). Therefore, we hypothesize that the spatial distribution

patterns of biodiversity in engineered ecosystems could be different from those in natural ecosystems.

Centralized WWTPs are ideal model systems to test the distance-decay relationship since there are now more than 400,000 of them around the world, which together are estimated to process more than 730 million m^3 of wastewater daily (more than double the average flow of the Nile) (21). The WWTP bioreactor is a typical engineered system in which the functional groups of microorganisms in activated sludge are enriched to enable the efficient degradation of oxygen-depleting organics and nutrients (22). Biological WWTPs are typically similar habitats, as they often receive similar domestic wastewater and are operated under relatively similar conditions. However, microbial communities within these reactors are diverse, dynamic, and complex (23) and play a vital role in determining process efficiency and stability. Therefore, we used WWTP bioreactors to test whether and how the spatial distribution pattern of microbial communities in engineered ecosystems differs from that in natural ecosystems. We collected 78 activated sludge samples from 26 full-scale WWTP bioreactors distributed across China. We used GeoChip 4.2, a microarray containing 120,054 distinct probes to target 200,393 coding sequences related to various microbial functional processes, to determine the spatial scaling of microbial functional gene diversity in these WWTPs. Our results indicated that the distance-decay relationship existed in WWTPs, but the turnover rate was much lower than that of microorganisms in natural settings. Further analyses showed that the distance-decay relationship is shaped mainly by environmental heterogeneity, along with geographic distance.

MATERIALS AND METHODS

Wastewater treatment plants and sampling.Activated sludge samples were collected from the aeration tanks of 26 full-scale wastewater treatment systems located in 10 different cities across long transects of China. The 26 systems used different treatment processes, including anaerobic/anoxic/aerobic (A^2O), oxidation ditch, and membrane bioreactor (MBR) processes, etc. Details of the locations, treatment processes, influent characteristics, and operational parameters for all the systems studied are listed in Table S1 in the supplemental material.

In the summer of 2011, we collected activated sludge samples from the end part of the aeration tank of each WWTP once a day for three consecutive days to generate triplicates. The samples were briefly settled on-site to be concentrated and then fixed in a 50% (vol/vol) aqueous ethanol solution. The fixed samples were immediately transported to the laboratory on ice, where 50 ml of each sample was dispensed into a sterile Eppendorf tube and centrifuged at 14,000 × *g* for 10 min. The supernatant was decanted, and the pellet was stored at −80°C prior to analysis.

DNA extraction and microarray hybridization.Microbial genomic DNA was extracted from the activated sludge samples by combining freeze-thawing

and sodium dodecyl sulfate (SDS) treatment for cell lysis as previously described (24). Crude DNA was purified by using the Wizard SV Genomic DNA purification kit (Promega, Madison, WI, USA) and then assessed by the ratios of the absorption at 260/280 nm and 260/230 nm measured by an ND-1000 spectrophotometer (NanoDrop Inc., Wilmington, DE, USA), agarose gel electrophoresis, and a Quant-It PicoGreen kit (Invitrogen, Carlsbad, CA, USA).

All purified DNA was labeled, concentrated, and resuspended in 10 μl of hybridization solution as described previously (25). The fluorescently labeled DNA was hybridized with a GeoChip 4.2 array on a Maui hybridization station (BioMicro, Salt Lake City, UT, USA) at 42°C with 40% formamide for 16 h. After unbound labeled DNA was washed away, microarrays were scanned (MS200; NimbleGen, Madison, WI, USA) at a laser power of 100%.

Data analysis.Low-quality spots were removed prior to statistical analysis, as described previously (26, 27). Spots with a signal-to-noise ratio of <2 and outliers of replicates were removed. The signal intensities were normalized within and across samples based on the mean signal intensity, as described previously (28). We then averaged the normalized signal intensities for the three replicates for each WWTP to conduct downstream analyses. The normalized hybridization data for individual functional gene sequences were grouped based on gene families (e.g., *nifH* and *nirS*) or functional groups (e.g., nitrification and denitrification) to calculate the *z* values of the distance-decay relationship (2).

The Sorensen index was used to construct community similarity matrices for microorganisms, whereas Euclidean distances were used to construct similarity matrices for geographic distance and environmental parameters. The *z* values of the distance-decay relationships of the bacterial communities were calculated as the slope of a linear least-squares regression on the relationship between geographic distances (log transformed) and bacterial similarity (log transformed). Because the data points (pairwise comparisons) are not independent, the significance of the distance-decay slope was tested by using bootstrapping with replacement; that is, geographic distance and microbial community similarity were randomly paired from the original data set, and the distance-decay slopes were calculated 10,000 times. A one-sample *t* test was then used to determine whether the observed slope was significantly different from the mean of the randomly generated slopes, as described previously (2). The exponent *z* values of the power law taxon-area relationship were calculated from the slope of the power law distance-decay relationship by using the equation $\log(S_s) = \text{constant} - 2z\log(D)$, where S_s is the pairwise similarity in community composition and D is the distance between two samples (12).

To separate the effects of environmental heterogeneity and geographic distance, a canonical correspondence analysis (CCA)-based variation-partitioning analysis (VPA) was performed based on the presence or absence of each gene sequence of all individual functional gene sequences (FGSs)

(29). Spatial variables measured as latitude-longitude coordinates were converted into projected coordinates and represented by a cubic trend surface polynomial to capture broad-scale spatial trends (29). A multivariate regression tree (MRT) analysis was carried out to identify important factors in shaping microbial community compositions. A 1,000-cross-validation process was used to decrease the structure complexity of the MRT. Predictive accuracy was estimated from the cross-validated relative error, which varies from 0 for a perfect tree to close to 1 for a poor tree (8). MRT analysis was carried out with the package mvpart in the R statistical programming environment.

RESULTS

Distance-decay relationship.To determine the distance-decay relationship in WWTPs, a total of 78 activated sludge samples were collected from 26 WWTPs located in 10 cities in China. A total of 55,724 functional genes were detected by GeoChip 4.2 analysis. For individual samples, the numbers of detected genes ranged from 19,922 to 47,153 (Table 1). Pairwise community similarity between samples was calculated based on the presence or absence of each functional gene by using Sorensen's index (3). The whole microbial community displayed a significant, negative distance-decay relationship ($P < 0.01$) (Fig. 1), meaning that WWTPs located in proximity to each other were more similar in composition than WWTPs located farther apart.

TABLE 1 *z* values for taxon-area relationships for various functional and phylogenetic groups[a]

Group	*z*	*t*	*n*	*P*	Lower 95% CI	Upper 95% CI
Individual functional gene sequences	0.0038	−112.321	999	<0.01	−1.4E−05	1.95E−05
Functional genes	0.0011	−215.321	999	<0.01	−1.7E−05	1.83E−05
Functional groups						
Organic remediation	0.0032	−225.783	9,999	<0.01	−1.3E−05	0.000018
Carbon cycling	0.0041	−393.523	9,999	<0.01	−2.2E−05	1.11E−05
Nitrogen	0.0040	−267.513	9,999	<0.01	−8.3E−06	0.000027
Nitrogen fixation	0.0055	−120.825	9,999	<0.01	−3.4E−05	8.93E−06
Ammonification	0.0036	−268.735	9,999	<0.01	−1.8E−05	1.33E−05
Denitrification	0.0035	−225.846	9,999	<0.01	−9.5E−06	2.52E−05
Nitrification	0.0062	−178.323	9,999	<0.01	−2.1E−05	4.52E−05
Phosphorus	0.0044	−280.387	9,999	<0.01	−5.3E−06	0.000031
Sulfur	0.0046	−258.977	9,999	<0.01	−1.7E−05	2.41E−05
Metal resistance	0.0035	−265.63	9,999	<0.01	−3E−05	1.46E−06
Phylogenetic groups						
Fungi	0.0051	−286.756	9,999	<0.01	−1.8E−05	2.46E−05
Archaea	0.0050	−345.7347	9,999	<0.01	−5.5E−06	3.13E−05
Bacteria	0.0037	−278.4152	9,999	<0.01	−2.2E−05	1.02E−05
Bacteroidetes	0.0065	−305.237	9,999	<0.01	−1.8E−05	3.33E−05
Chloroflexi	0.0046	−254.281	9,999	<0.01	−1.6E−05	2.49E−05
Proteobacteria	0.0035	−251.315	9,999	<0.01	−8.7E−06	2.33E−05
Alphaproteobacteria	0.0030	−227.715	9,999	<0.01	−5.9E−06	2.23E−05
Betaproteobacteria	0.0030	−211.723	9,999	<0.01	−2.7E−05	2.02E−06
Gammaproteobacteria	0.0049	−284.513	9,999	<0.01	−3.1E−05	9.82E−06
Epsilonproteobacteria	0.0033	−264.024	9,999	<0.01	−1.8E−05	1.16E−05

[a] CI, confidence interval.

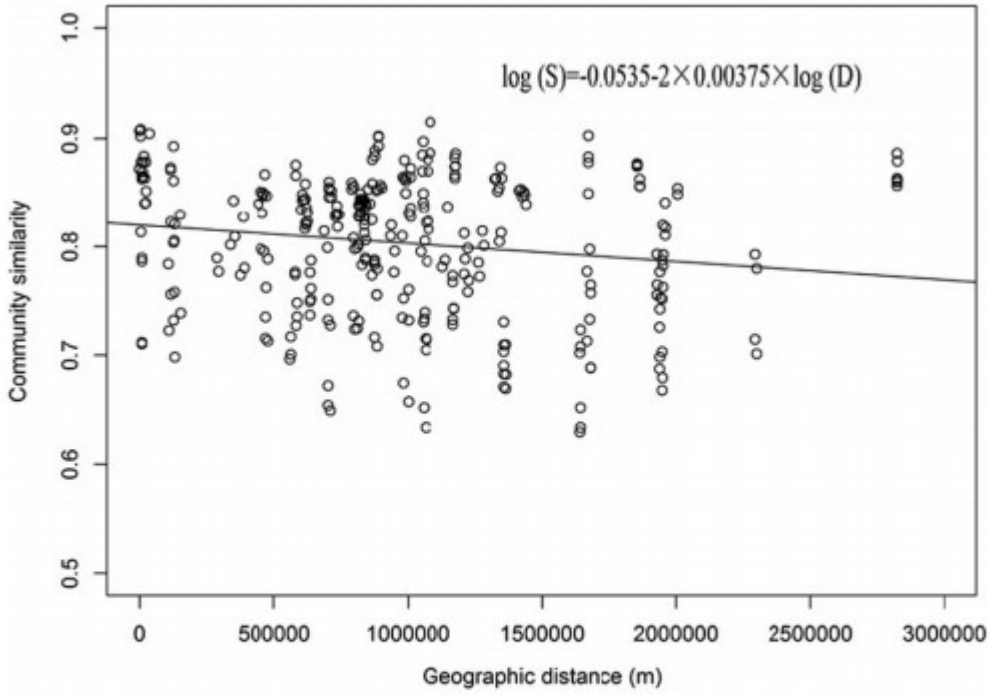

FIG 1 Distance-decay relationship for activated sludge microbial communities in WWTPs. Pairwise similarities are plotted as a function of the distance between WWTPs. Each circle represents the pairwise similarity of a microbial community (Bray-Curtis index). *S* means community similarity, and *D* means geographic distance.

Spatial scaling of microbial communities.

The slope of the distance-decay relationship reflects the rate at which OTU richness increases with distance. Therefore, the slope of the power law distance-decay relationship can be used to calculate the exponent *z* value of the power law taxon-area relationships (11). As shown in Table 1, the *z* value for the whole functional gene sequence was 0.0038. The mean *z* value was 0.0043 ± 0.0009 for different functional gene groups, which was similar to *z* values for phylogenetic groups (0.0043 ± 0.0011). In addition, it was found that the *z* values varied by taxonomic resolution. For example, the *z* value was 0.0038 based on all individual functional gene sequences but was approximately four times lower based on functional genes ($z = 0.0011$) (Table 1).

To determine whether the *z* values were significantly different among various functional or phylogenetic groups, bootstrapping was performed to estimate the variances of *z* values, followed by a pairwise *t* test with Bonferroni correction. The results showed that the estimated *z* values were significantly different among various functional or phylogenetic groups ($P < 0.005$), except for the *z* values between the sulfur group and the metal resistance group and between *Chloroflexi* and *Epsilonproteobacteria*. For

instance, the *z* value for nitrification genes was 0.0062, a value much higher than that for denitrification genes (0.0035) (Table 1). Also, the *z* value for bacteria was 0.0037, which was lower than those for archaea (0.0050) and fungi (0.0051), suggesting that bacteria had a lower turnover rate in space than archaea and fungi in WWTPs. In addition, among the *Proteobacteria*, the *z* value for the *Gammaproteobacteria* (0.0049) was higher than those for the *Alphaproteobacteria* (0.0030), *Betaproteobacteria* (0.0030), and *Epsilonproteobacteria* (0.0033).

z values for individual genes varied considerably. For C-cycling genes, the *z* value of *amyX* was 0.0084, which was approximately four times higher than that of the cadherin (CDH) gene (0.0021) (see Table S2 in the supplemental material). Considerable variations were also observed for N cycling, S cycling, and organic remediation genes (see Tables S3, S4, and S5 in the supplemental material).

In order to obtain general insights into spatial scaling across different organisms, we compared the *z* values obtained in this study and all available previously reported data (715 data sets) (Fig. 2). The average *z* value was 0.27 for mammals, whereas it was 0.32 for plants. The average *z* value (data from this study were not included) was 0.09 for microbes, which was 2 to 4 times lower than those observed for plants and animals, but it was 20 times higher than that observed in this study.

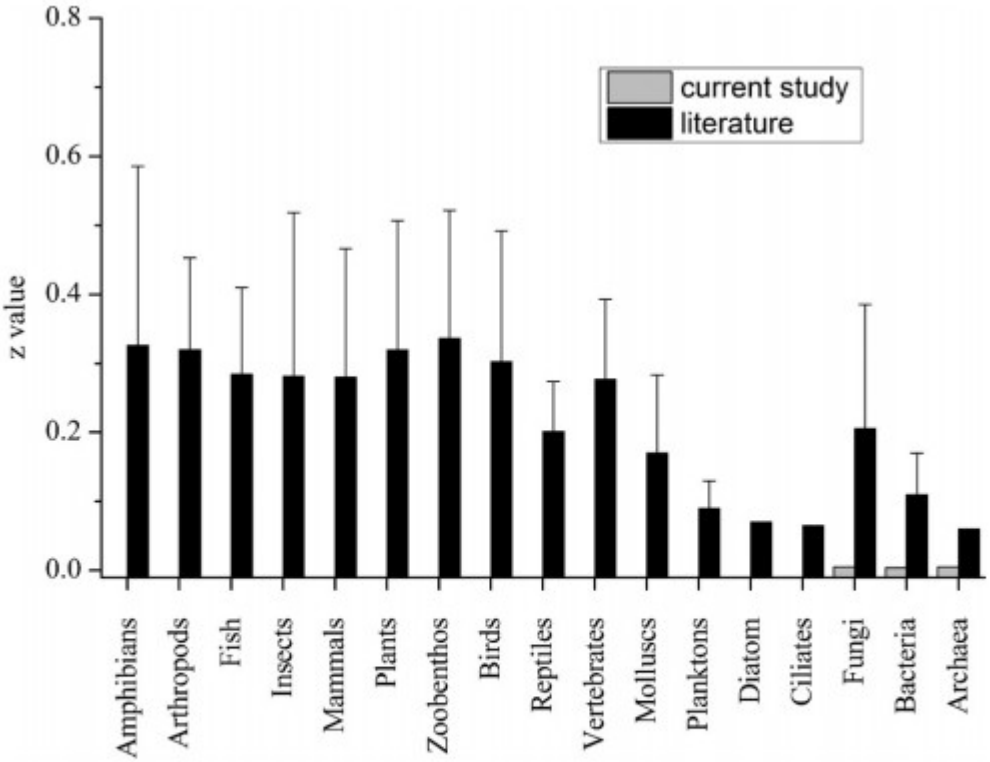

FIG 2 Comparison of *z* values for macrobial and microbial taxonomic groups. Most of the data were reported previously by Drakare et al. (43). A total of 715 recent data sets for macrobial and microbial communities are included. The bars represent average *z* values, and error bars represent standard deviations.

Effects of environmental heterogeneity and geographic distance.

Environmental heterogeneity and demographic processes (e.g., dispersal, colonization, speciation, and extinction) are important for determining the biogeographic distribution of microbes (30).

To determine whether environmental variables affected microbial community composition, partial Mantel tests were performed. When the effects of geographic distance were removed, partial Mantel tests indicated a significant correlation between the measured environmental variables and microbial functional composition ($P < 0.05$). Similarly, partial Mantel tests revealed a significant correlation between geographic distance and microbial functional composition ($P < 0.05$) (Table 2). The results of the Mantel test also showed that there was no significant correlation between treatment process and most of the functional and phylogenetic groups except for the functional groups involved in C cycling (correlation coefficient in Mantel's test [$r_M = 0.166$;] $P < 0.05$), N cycling ($r_M = 0.164$; $P < 0.05$), and organic remediation ($r_M = 0.161$; $P < 0.05$), which exhibited a significant correlation with treatment process (Table 2).

TABLE 2 Influence of geographic distance and environmental heterogeneity on microbial community composition

Group	Effect of environmental similarity, controlling for geographic distance		Effect of geographic distance, controlling for environmental similarity		Effect of treatment process, controlling for environmental similarity	
	r	*P*	*r*	*P*	*r*	*P*
Functional genes	0.13	0.013	0.14	0.037	−0.03	0.664
Functional groups						
C cycling	0.21	0.001	0.05	0.043	0.170	0.010
N cycling	0.21	0.001	0.05	0.041	0.16	0.014
Denitrification	0.22	0.001	0.05	0.036	−0.06	0.820
Nitrification	0.03	0.286	0.05	0.031	−0.06	0.841
P cycling	0.14	0.017	0.04	0.073	−0.03	0.671
Sulfur cycling	0.15	0.008	0.04	0.045	−0.02	0.551
Organic remediation	0.22	0.001	0.05	0.048	0.16	0.012
Metal resistance	0.13	0.019	0.05	0.079	−0.06	0.798
Phylogenetic groups						
Archaea	0.14	0.009	0.07	0.071	0.00	0.506
Fungi	0.15	0.004	0.09	0.005	0.01	0.457
Bacteria	0.13	0.013	0.09	0.04	−0.04	0.682
Alphaproteobacteria	0.11	0.027	0.09	0.02	−0.06	0.853
Betaproteobacteria	0.12	0.026	0.09	0.002	−0.07	0.868
Gammaproteobacteria	0.15	0.008	0.09	0.05	0.01	0.424
Epsilonproteobacteria	0.12	0.025	0.06	0.02	−0.07	0.839

Because partial CCA has been shown to be more appropriate to correctly partition the beta diversity values among sites than the partial Mantel test (31), a CCA-based VPA was further performed to separate the effects of environmental heterogeneity and geographic distance. Environmental heterogeneity was further split into wastewater characteristics (chemical oxygen demand [COD], total nitrogen [TN] level, ammonia level, total phosphorus [TP] level, pH, and conductivity) and operational parameters (dissolved oxygen [DO], temperature, hydraulic retention time [HRT], and mixed-liquor suspended solids [MLSS]). As shown in Table 3, 41.5% of the variance could be explained by these three components. Wastewater characteristics, operational parameters, and geographic distance could independently explain 10.7, 9.2, and 16.3% of variations, respectively. Therefore, both environmental heterogeneity (including wastewater characteristics and operational parameters) and geographic distance played important roles in shaping microbial biogeographic patterns in biological WWTPs.

TABLE 3 Variation-partitioning analysis of all FGSs

Group[a]	% of explained variation
W	10.7
O	9.2
G	16.3
W × O	0.3
W × G	0.7
O × G	2.9
W × O × G	1.4

[a] W, wastewater characteristics; O, operational parameters; G, geographic location; W × O, interactions of wastewater characteristics and operational parameters; W × O × G, interactions of all three factors.

MRT analysis was further used to determine which environmental factors were important in shaping microbial community compositions. We showed that the most important factor in explaining the variances of microbial community components in the 26 samples was pH (Fig. 3). The coefficient of variation (CV) error and standard error (SE) for the MRT analysis were 0.506 and 0.007, respectively, indicating greater reliability of the model. The samples were divided into two main groups: group A, with a pH lower than 7.22, and group B, with a pH higher than 7.22. Group A was further split by conductivity into samples with high conductivity levels (≥1,235 μS/cm) and those with low conductivity values (<1,235 μS/cm). Group B was then split into two groups by TN. Finally, all the samples were further split into six subgroups by temperature and latitude.

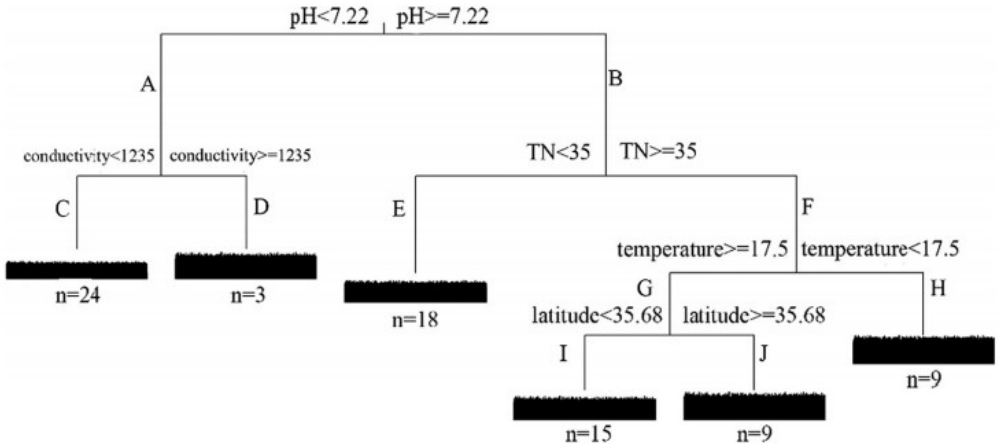

FIG 3 MRT analysis of environmental and spatial variables and GeoChip data for 78 samples. A, B, C, D, E, F, G, H, I, and J are clusters.

DISCUSSION

A central goal in community ecology is to determine the distribution patterns of microorganisms and the relative influence of contemporary environmental

factors versus the legacies of historical events on distribution patterns (8). Today, several studies have demonstrated that there are biogeographic patterns for microbes in natural habitats such as soil, freshwater, and the ocean. However, no previous studies have focused on the microbial distribution patterns in engineered ecosystems.

In this study, we showed that the microbially diverse populations in activated sludge in WWTPs exhibited a significant but very weak distance-decay relationship. The *z* value for different functional and phylogenetic groups was <0.0066, which is 1 to 2 orders of magnitude lower than those reported in previous studies (3, 11–18). For example, Martiny et al. (3) demonstrated that *z* values for *Nitrosomonadales* in salt marsh sediments varied significantly among spatial scales, with *z* values of 0.02 within marshes, *z* values of 0.14 within regions (across marshes), and no significant *z* value at the continental scale (across regions), using 16S rRNA gene-based PCR cloning and sequencing approaches. Zhou et al. (2) suggested that, based on data from GeoChip analysis, the *z* value for microbial communities in forest soil was 0.0624, and the *z* values varied considerably across different functional and phylogenetic groups ($z = 0.0475$ to 0.0959). Other studies also showed that the *z* values are typically between 0.01 and 0.1 for microorganisms in various habitats (2, 3, 11–17). Activated sludge is a unique microbial ecosystem, and it has high diversity, with over 700 genera and thousands of OTUs (32, 33). Given the high diversity of bacterial communities in activated sludge systems, the turnover rate (*z* value) in this study is so low that it could be negligible. Actually, WWTPs are always designed to be predictable and reproducible independent of geographical distance.

Why are the *z* values in this study much lower than those reported in previous studies? One explanation is that our samples were from WWTPs with similar functions, resulting in lower spatial turnover rates for taxa. All the WWTPs in this study treated domestic wastewater, and they had similar influent characteristics. The operational conditions among the WWTPs were also relatively similar. For example, the concentration of dissolved oxygen in all bioreactors was kept above 2 mg/liter. This relatively low environmental heterogeneity may explain the low *z* values. Another explanation is that biological WWTPs are nutrient rich because the influent COD levels of most of the plants were >300 mg/liter. This environment may cause high functional redundancy that reduced the *z* values.

A theme of biogeography is the relative influence of contemporary environmental factors versus the legacies of historical events on distribution patterns. In this study, the biogeography of microorganisms reflects the influence of both contemporary environmental variation and geographic distance. Our results appear to be consistent with data from previous studies on natural ecosystems (5). Hanson et al. (5) conducted a review of 56 studies that attempted to disentangle the relative effects of contemporary environmental factors versus historical processes on the distance-decay

relationship, and they found that most studies reported that both contemporary environmental factors and geographic distance shaped microbial biogeographic patterns. However, some researchers thought that the distance effect was probably almost overestimated if any spatially autocorrelated environmental factors were not accounted for by the measured environmental variables (5, 34). In fact, a complete quantification of all environmental variables is practically impossible to achieve.

In our study, about 58.5% of the functional gene variance could not be explained by environmental heterogeneity and geographic distance. It is possible that some unmeasured biotic or abiotic environmental variable plays an influential role in affecting the microbial community in WWTPs. Previous studies showed that protozoan grazing (20) and phage predation (8) played vital roles in shaping microbial communities. Alternatively, stochastic processes (35, 36) might play a major role in shaping microbial communities.

The treatment process could be one of the factors influencing the microbial community structures in WWTPs. In our study, the 26 wastewater treatment systems used different treatment processes, including A^2O, oxidation ditch, and MBR processes, etc. Different treatment processes are usually operated with different operational parameters. For example, compared to conventional activated sludge, an MBR is typically operated at high MLSS concentrations, long sludge retention time, and high DO concentrations to control membrane fouling. Previous studies showed that different treatment processes could harbor distinct microbial community structures (37–40). However, our study revealed that treatment process was not significantly correlated with most of the functional and phylogenetic groups except for the functional groups involved in C and N cycling and organic remediation. One of the main reasons for this could be that in the partial Mantel test, the effects of some operational parameters (such as DO, temperature, HRT, and MLSS) underlying treatment processes were removed. We think that operational conditions underlying the treatment process, rather than the treatment process itself, affect the bacterial community structures in wastewater treatment systems.

In this study, a microarray hybridization-based approach (GeoChip) was used to determine the spatial scaling of microbial functional gene diversity in WWTPs. The GeoChip array has several advantages for examining microbial biogeographic patterns by minimizing sampling artifacts such as undersampling, unequal sampling, random sampling, and taxonomic lumping (2, 41). Specifically, the GeoChip array contains 84,000 probes targeting 152,000 genes involved in major microbial biogeochemical processes so that many microbial populations and functional groups can be simultaneously detected at the whole-community-wide scale, which could ameliorate the undersampling problem. Another main advantage of the GeoChip approach is that it can generally provide high resolution in differentiating various microorganisms (42), and hence, the lumping problem can be ameliorated. However, as with other microarray hybridization-based approaches, the

GeoChip approach has disadvantages. It is a closed-format detection approach and provides information only for the genes included on the microarrays (41). Also, most functional genes could provide less robust information on phylogenetic relationships among various organisms if they are distantly related, especially because many functional genes are susceptible to horizontal gene transfer.

In conclusion, understanding the spatial scaling of organisms and the underlying mechanisms shaping communities is a central goal in community ecology. Our results illustrate that microbial communities of activated sludge in WWTPs exhibit a significant but very weak distance-decay relationship. The negligible *z* values across different functional and phylogenetic groups in activated sludge were <0.0065, which is 1 to 2 orders of magnitude lower than those observed in natural environments such as soil, freshwater, and salt marsh sediment. It appears that the spatial scaling of activated sludge microbial communities was driven by both environmental heterogeneity and geographic distance.

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REFERENCES

1. Green J, Bohannan BJM. 2006. Spatial scaling of microbial biodiversity. Trends Ecol Evol 21:501–507. http://dx.doi.org/10.1016/j.tree.2006.06 .012.

2. Zhou JZ, Kang S, Schadt CW, Garten CT. 2008. Spatial scaling of functional gene diversity across various microbial taxa. Proc Natl Acad Sci USA 105:7768 –7773. http://dx.doi.org/10.1073/pnas.0709016105.

3. Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC. 2011. Drivers of bacterial beta-diversity depend on spatial scale. Proc Natl Acad Sci U S A 108:7850 –7854. http://dx.doi.org/10.1073/pnas.1016308108.

4. Tobler WR. 1970. A computer movie simulating urban growth in the Detroit region. Econ Geogr 46:234 –240. http://dx.doi.org/10.2307 /143141.

5. Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Microbiol 10:497–506. http://dx.doi.org/10.1038 /nrmicro2795.

6. Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. Proc Natl Acad SciUSA 103:626 –631. http://dx.doi .org/10.1073/pnas.0507535103.

7. Robeson MS, King AJ, Freeman KR, Birky CW, Martin AP, Schmidt SK. 2011. Soil rotifer communities are extremely diverse globally but spatially autocorrelated locally. Proc Natl Acad SciUSA 108:4406 –4410. http://dx.doi.org/10.1073/pnas.1012678108.

8. Wu B, Tian J, Bai C, Xiang M, Sun J, Liu X. 2013. The biogeography of fungal communities in wetland sediments along the Changjiang River and other sites in China. ISME J 7:1299 –1309. http://dx.doi.org/10.1038/ismej .2013.29.

9. Barton AD, Pershing AJ, Litchman E, Record NR, Edwards KF, Finkel ZV, Kiorboe T, Ward BA. 2013. The biogeography of marine plankton traits. Ecol Lett 16:522–534. http://dx.doi.org/10.1111/ele.12063.

10. Lear G, Washington V, Neale M, Case B, Buckley H, Lewis G. 2013. The biogeography of stream bacteria. Glob Ecol Biogeogr 22:544 –554. http: //dx.doi.org/10.1111/geb.12046.

11. Green JL, Holmes AJ, Westoby M, Oliver I, Briscoe D, Dangerfield M, Gillings M, Beattie AJ. 2004. Spatial scaling of microbial eukaryote diversity. Nature 432:747–750. http://dx.doi.org/10.1038/nature03034.

12. Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM. 2004. A taxa-area relationship for bacteria. Nature 432:750 –753. http://dx.doi.org /10.1038/nature03073.

13. Astorga A, Oksanen J, Luoto M, Soininen J, Virtanen R, Muotka T. 2012. Distance decay of similarity in freshwater communities: do macroand microorganisms follow the same rules? Glob Ecol Biogeogr 21:365– 375. http://dx.doi.org/10.1111/j.1466-8238.2011.00681.x.

14. Wetzel CE, Bicudo DDC, Ector CL, Lobo EA, Soininen J, Landeiro VL, Bini LM. 2012. Distance decay of similarity in neotropical diatom communities. PLoS One 7:e45071. http://dx.doi.org/10.1371/journal.pone .0045071.

15. Cho JC, Tiedje JM. 2000. Biogeography and degree of endemicity of fluorescent Pseudomonas strains in soil. Appl Environ Microbiol 66: 5448 – 5456. http://dx.doi.org/10.1128/AEM.66.12.5448-5456.2000.

16. Soininen J, Korhonen JJ, Karhu J, Vetterli A. 2011. Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. Limnol Oceanogr 56:508 –520. http://dx.doi.org/10.4319 /lo.2011.56.2.0508.

17. Casteleyn G, Leliaert F, Backeljau T, Debeer AE, Kotaki Y, Rhodes L, Lundholm N, Sabbe K, Vyverman W. 2010. Limits to gene flow in a cosmopolitan marine planktonic diatom. Proc Natl Acad SciUSA 107: 12952– 12957. http://dx.doi.org/10.1073/pnas.1001380107.

18. Schauer R, Bienhold C, Ramette A, Harder J. 2010. Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. ISME J 4:159 –170. http://dx.doi.org/10.1038/ismej.2009.106.

19. Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Ovreas L, Reysenbach AL, Smith VH, Staley JT. 2006. Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112. http://dx.doi.org/10.1038/nrmicro1341.

20. Baas Becking L. 1934. Geobiology: or introduction to environmental science. (In Dutch.) WP Van Stockum & Zoon, The Hague, The Netherlands.

21. Pennisi E. 2012. Water reclamation going green. Science 337:674 –676. http://dx.doi.org/10.1126/science.337.6095.674.

22. Wells GF, Park HD, Yeung CH, Eggleston B, Francis CA, Criddle CS. 2009. Ammonia-oxidizing communities in a highly aerated full-scale activated sludge bioreactor: betaproteobacterial dynamics and low relative abundance of Crenarchaea. Environ Microbiol 11:2310 –2328. http://dx .doi.org/10.1111/j.1462-2920.2009.01958.x.

23. Shade A, Caporaso JG, Handelsman J, Knight R, Fierer N. 2013. A meta-analysis of changes in bacterial and archaeal communities with time. ISME J 7:1493–1506. http://dx.doi.org/10.1038/ismej.2013.54.

24. Zhou JZ, Bruns MA, Tiedje JM. 1996. DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316 –322.

25. Lu Z, Deng Y, Van Nostrand JD, He Z, Voordeckers J, Zhou A, Lee YJ, Mason OU, Dubinsky EA, Chavarria KL, Tom LM, Fortney JL, Lamendella R, Jansson JK, D'Haeseleer P, Hazen TC, Zhou J. 2012. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. ISME J 6:451–460. http://dx.doi.org/10.1038/ismej.2011.91.

26. He ZL, Xu MY, Deng Y, Kang SH, Kellogg L, Wu LY, Van Nostrand JD, Hobbie SE, Reich PB, Zhou JZ. 2010. Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO2. Ecol Lett 13:564 –575. http://dx.doi.org/10.1111/j .1461-0248.2010.01453.x.

27. Tu Q, Yu H, He Z, Deng Y, Wu L, Van Nostrand JD, Zhou A, Voordeckers J, Lee YJ, Qin Y, Hemme CL, Shi Z, Xue K, Yuan T, Wang A, Zhou J. 2014. GeoChip 4: a functional gene-array-based highthroughput environmental technology for microbial community analysis. Mol Ecol Resour 14:914 –928. http://dx.doi.org/10.1111/1755-0998 .12239.

28. He Z, Deng Y, Van Nostrand JD, Tu Q, Xu M, Hemme CL, Li X, Wu L, Gentry TJ, Yin Y, Liebich J, Hazen TC, Zhou J. 2010. GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. ISME J 4:1167–1179. http://dx.doi.org /10.1038/ismej.2010.46.

29. Liang Y, Van Nostrand JD, Deng Y, He Z, Wu L, Zhang X, Li G, Zhou J. 2011. Functional gene diversity of soil microbial communities from five oil-

contaminated fields in China. ISME J 5:403–413. http://dx.doi.org/10 .1038/ismej.2010.142.

30. Rittmann BE, Hausner M, Loffler F, Love NG, Muyzer G, Okabe S, Oerther DB, Peccia J, Raskin L, Wagner M. 2006. A vista for microbial ecology and environmental biotechnology. Environ Sci Technol 40:1096 – 1103. http://dx.doi.org/10.1021/es062631k.

31. Ramette A, Tiedje JM. 2007. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proc Natl Acad SciUSA 104:2761–2766. http://dx.doi.org/10.1073/pnas .0610671104.

32. Li B, Yang Y, Ma L, Ju F, Guo F, Tiedje JM, Zhang T. 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J 9:2490 –2502. http: //dx.doi.org/10.1038/ismej.2015.59.

33. Wang X, Hu M, Xia Y, Wen X, Ding K. 2012. Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. Appl Environ Microbiol 78:7042–7047. http://dx.doi.org/10 .1128/AEM.01617-12.

34. Nekola JC, White PS. 1999. The distance decay of similarity in biogeography and ecology. J Biogeogr 26:867–878. http://dx.doi.org/10.1046/ j .1365-2699.1999.00305.x.

35. Zhou JZ, Deng Y, Zhang P, Xue K, Liang YT, Van Nostrand JD, Yang YF, He ZL, Wu LY, Stahl DA, Hazen TC, Tiedje JM, Arkin AP. 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proc Natl Acad SciUSA 111:E836 –E845. http://dx.doi.org/10 .1073/pnas.1324044111.

36. Zhou JZ, Liu WZ, Deng Y, Jiang YH, Xue K, He ZL, Van Nostrand JD, Wu LY, Yang YF, Wang AJ. 2013. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. mBio 4:e00584-12. http://dx.doi.org/10.1128/mBio.00584-12.

37. Silva CC, Jesus EC, Torres APR, Sousa MP, Santiago VMJ, Oliveira VM. 2010. Investigation of bacterial diversity in membrane bioreactor and conventional activated sludge processes from petroleum refineries using phylogenetic and statistical approaches. J Microbiol Biotechnol 20: 447–459.

38. Gomez-Silvan C, Molina-Munoz M, Poyatos JM, Ramos A, Hontoria E, Rodelas B, Gonzalez-Lopez J. 2010. Structure of archaeal communities in membrane-bioreactor and submerged-biofilter wastewater treatment plants. Bioresour Technol 101:2096 –2105. http://dx.doi.org/10.1016/j .biortech.2009.10.091.

39. Baek SH, Pagilla K. 2009. Microbial community structures in conventional activated sludge system and membrane bioreactor (MBR). Biotechnol Bioprocess Eng 14:848 –853. http://dx.doi.org/10.1007/s12257-008 -0303-1.

40. Munz G, Gualtiero M, Salvadori L, Claudia B, Lubello C. 2008. Process efficiency and microbial monitoring in MBR (membrane bioreactor) and CASP (conventional activated sludge process) treatment of tannery wastewater. Bioresour Technol 99:8559 –8564. http://dx.doi.org/10.1016/j .biortech.2008.04.006.

41. Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-Cohen L. 2015. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. mBio 6:e02288-14. http://dx .doi.org/10.1128/mBio.02288-14.

42. Tiquia SM, Wu LY, Chong SC, Passovets S, Xu D, Xu Y, Zhou JZ. 2004. Evaluation of 50-mer oligonucleotide arrays for detecting microbial populations in environmental samples. Biotechniques 36:664 –675.

43. Drakare S, Lennon JJ, Hillebrand H. 2006. The imprint of the geographical, evolutionary and ecological context on species-area relationships. Ecol Lett 9:215–227. http://dx.doi.org/10.1111/j.1461-0248.2005.00848.x.