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Biogeography and Assembly of Microbial Communities in Wastewater Treatment Plants in China

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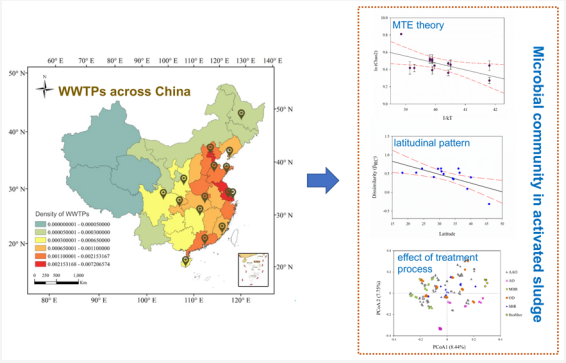
ABSTRACT: Scientific understanding of microbial biogeography and assembly is lacking for activated sludge microbial communities, even though the diversity of microbial communities in wastewater treatment plants (WWTPs) is thought to have a direct influence on system performance. Here, utilizing large-scale 16S rRNA gene data generated from 211 activated sludge samples collected from 15 cities across China, we show activated sludge microbes, whose growth and metabolism can be regulated followed with the metabolic theory of ecology with an apparent Ea value (apparent activation energy) of 0.08 eV. WWTPs at a lower latitude tend to harbor a more diverse array of microorganisms. In agreement with the general understanding, the activated sludge microbial assembly was mainly driven by deterministic processes and the mean annual temperature was identified as the most important factor affecting the microbial community structure. The treatment process types with similar microbial growth types and functions had a distinct impact on the activated sludge microbial community structure only when WWTPs were located near each other and received similar influent. Overall, these findings provide us with a deeper understanding of activated sludge microbial communities from an ecological perspective. Moreover, these findings suggest that, for a given set of performance characteristics (e.g., combined nitrification, denitrification, and phosphorus removal), it may be difficult to employ common engineering levers to control additional aspects of community structure due to the influence of natural environmental factors.



INTRODUCTION

Biogeography is the study of biodiversity distribution patterns over space and time[1] and can offer profound insights into the underlying mechanisms that maintain the biodiversity[2] and structure of microbial communities.[3] Species richness patterns are determined across large-scale geographic gradients[4,5] and the latitudinal diversity gradient, the observation of diversity from low at the pole to high at the equator, has long been recognized as one of the most generally observed biogeographical patterns.[6] According to the metabolic theory of ecology (MTE), temperature is deemed to play a central role in latitudinal diversity gradient pattern[7−10] because increasing temperature could accelerate the kinetics of biological processes, like rates of reproduction, speciation, and adaptive evolution, thereby leading to marked changes in biodiversity.[7,8,11] Elucidating the microbial community assembly is also crucial to fully understanding the patterns in biodiversity and composition.[4] It has been recognized that in some cases deterministic processes play major roles in microbial assembly,[12,13] but in other cases stochastic factors dominate.[14] Langenheder et al.[15] pointed out that both deterministic and stochastic factors are important, and these two processes can interact during microbial assembly.[13,15] However, the relative contributions of these two processes responsible for microbial assembly remain poorly understood.

Wastewater treatment plants (WWTPs), as the largest application of biotechnology in the world, utilize microorganisms to remove pollutants, and the biodiversity of microbes in the activated sludge is critical for stable and efficient WWTP operation.[16,17] Though biological WWTPs have been designed mostly from an engineering perspective, it is now recognized that many technical problems can be avoided by monitoring the microbial community structure.[18] As such, microorganisms have been becoming the focus of operation monitoring schemes in WWTPs. At the same time, as typical artificial microbial ecosystems[19] with relatively uniform design and well-defined operational parameters, WWTPs are considered a fertile testing ground for a range of fundamental ecological questions.[20] Furthermore, WWTPs

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are ideal models to address biogeographic patterns at a continental scale because of their wide distribution. To date, much work has delineated activated sludge microbial diversity, described the relative abundance of individual bacterial taxa, and compared the structure and composition of the microbial community in multiple WWTPs.[19,21−23] In addition, a worldwide survey of microbes in wastewater treatment systems has been done by MIDAS. This project will provide a reference database including all microbes (bacteria and archaea) identified in the surveys and makes it possible to compare results from different studies across the world.[24] However, few studies have attempted to describe the biogeographical distribution patterns of activated sludge microbial communities.[25,26] It is important to integrate such microbial ecology theories into WWTPs' designs and operations, as this will be beneficial for better predicting variations in an activated sludge community structure in response to environmental changes.[27] Since parameters in WWTPs can be adjusted in real time to achieve better performance, specifying which environmental factors exert the strongest influence on the activated sludge community can facilitate the operation and regulation of WWTPs. To date, many environmental factors have been revealed to be important, such as treatment processes,[28−30] influent wastewater characteristics,[31−33] and operational parameters.[33−35] Nevertheless, these finding are somewhat contradictory because of the differences in treatment systems, sampling scales. and analysis methods, thus limiting our understanding of the factors that structure an activated sludge microbial community. Moreover, not only have many deterministic factors been shown to have significant influence on microbial communities in WWTPs[22,36,37] but also stochastic assembly such as dispersal and immigration has been reported to play an important role in the population dynamics of WWTPs.[38,39] The relative importance of deterministic and stochastic processes needs to be further explored to better characterize the activated sludge microbial community.

Given the current shortage of knowledge on microbes in WWTPs, a global survey of 1186 activated sludge samples collected from 269 WWTPs around the world has been undertaken, and the valuable data obtained from this survey have been published.[39] Interestingly, several properties observed in the activated sludge microbial communities from Asia were quite different from those on other continents.[39] To better understand why the communities in Asia were so distinct, we leveraged an investigation of 211 activated sludge samples from 60 WWTPs located in 15 cities across China using high-throughput sequencing to more closely examine the biogeography and assembly of the activated sludge microbial communities.

MATERIALS AND METHODS

Sampling. The whole sampling task was implemented during the summer of 2014 using a uniform sampling protocol. In general, 12 activated sludge samples from aeration tanks of 4 WWTPs were collected in each city. However, there were some exceptions during sampling. For example, more than 12 samples were obtained because of multiple treatment process types in one WWTP (e.g., CNBJ, CNSZ, and CNWX). In contrast, only 9 samples were collected from SY in total. Moreover, only 2 WWTPs were allowed to do sampling in DL (CNDL1 and CNDL2). So, we sampled twice from different parallels in CNDL1 and CNDL2 to keep the minimum amounts of samples. Consequently, 211 activated sludge samples were obtained from the aeration tanks of 60 full-scale WWTPs located in 15 cities in China. Sampling cities are shown in Figure S1. For each aeration tank, samples were taken near the inlet, in the middle of the tank, and near the outlet. The 60 WWTPs were operated using different treatment processes, including: oxidation ditch (OD), anaerobic/anoxic/oxic (A^2O), inverted A^2O, anaerobic/anoxic/oxic/anoxic/oxic (AAOAO), anoxic/oxic (AO), membrane bioreactor (MBR), absorption biodegradation (AB), modified University of Cape Town (MUCT), sequencing batch reactor (SBR), modified sequencing batch reactor (MSBR), biofilter, cyclic activated sludge system (CASS), and cyclic activated sludge technology (CAST). The additional information about these treatment process types is provided in Supporting Information Table S1, and treatment process types of 60 sampled WWTPs are shown in Supporting Information Table S2. Among these WWTPs, 5 had two or more treatment processes with identical influents (CNBJ1, CNSZ3, CNSZ4, CNWX1, and CNWX3). Details of the locations, wastewater properties, and operational parameters of the 60 WWTPs are listed in Supporting Information Table S3.

All activated sludge samples were briefly settled on site and then immediately transported to the laboratory on ice. Once in the laboratory, 2 mL of activated sludge sample was removed from each sample and centrifuged at 15 000*g* for 10 min. The resultant pellets were stored at −80 °C until DNA was extracted. Another 100 mL of activated sludge was removed from each sample and centrifuged (10 000*g* for 10 min). The supernatant was collected and used to measure common chemical parameters including ammonia, nitrite, nitrate, total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD). Pollutant concentrations of influent and effluent, including ammonia, nitrite, nitrate, TN, TP, biological oxygen demand (BOD), and COD, were obtained from technicians in each WWTP. Temperature, pH, dissolved oxygen (DO), and conductivity of activated sludge were measured in situ. Other information about the WWTPs, including geographic coordinates, age of plant, hydraulic retention time (HRT), sludge retention time (SRT), volume of aeration tanks, ratio of industrial wastewater, and recycling ratio were obtained from technicians or from online sources.

DNA Extraction, Purification, and Quantification. Microbial genomic DNA was extracted using a PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA). DNA quality was assessed with a ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE). The absorbance ratios at 260 and 280 nm and at 260 and 230 nm were calculated. No samples had ratios less than 1.8 and 1.7, respectively. The DNA was purified using agarose gel electrophoresis, and final DNA was quantified with PicoGreen using a FLUO star Optima (BMG Labtech, Jena, Germany).

ILLUMINA SEQUENCING AND DATA PROCESSING

The extracted DNA samples were amplified with a set of primers targeting the V4 variable region of the 16S rRNA gene. The forward primer was 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and the reverse primer was 806R (5′-GGACTACHVGGGTWTCTMAT-3′).[40] PCR amplification was performed in a 25 μL reaction volume, containing 2.5 μL of 10× AccuPrime PCR buffer II (Invitrogen, Grand Island, NY), 1 μL of each primer (10 mM), 5 μL of template DNA (2 ng/μL), and 0.5 μL of homemade Taq polymerase by IEG lab (http://

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www.ou.edu/ieg/tools/protocols). The reaction mixture was denatured at 94 °C for 1 min, followed by 10 cycles of 94 °C for 20 s, 53 °C for 25 s, and 68 °C for 45 s and a final extension at 68 °C for 10 min. Reactions were performed in triplicate for each sample to minimize potential biases from amplification.[41] The triplicate products from the first step PCR were pooled and purified using 75 μL of bead solution (Agencourt AMPure XP) to remove primer dimers and other contaminants. Purified genes were recovered in 50 μL of water, and 15 μL were used as a template for the second round PCR. The 25 μL reaction volume contained 2.5 μL of 10× AccuPrime PCR buffer II (Invitrogen, Grand Island, NY), 2 μL of barcode primers, 15 μL of first-round PCR products, and 0.5 μL of homemade Taq polymerase. Fifteen cycles of amplification were performed following the same program as the first round PCR. PCR products from triplicate reactions were pooled and quantified by PicoGreen. Subsequently, 200 ng of DNA from each sample were combined and then loaded onto 1% agarose gel. The gel slice containing target genes was extracted using a QIAGEN Gel Extraction Kit to remove agarose. Finally, the mixture was loaded into a MiSeq reagent cartridge and run on a Miseq (Illumina, San Diego, CA) using 2× 250 bp paired-end sequencing. Sequencing was performed by The Institute for Environmental Genomics at The University of Oklahoma (Norman, OK).

Raw sequences were separated to their respective samples using barcodes. Quality trimming was done using Btrim.[42] Forward and reverse reads were merged into full length sequences by FLASH.[43] Sequences were removed if they were too short or contained ambiguous bases. In order to fairly compare the 211 samples at the same sequencing depth, normalization of the sequence number was conducted by randomly extracting 25000 sequences in each sample for the following analyses. The operational taxonomic units (OTUs) were classified using UCLUST at a 97% similarity level, and singletons were removed. Taxonomic assignment was conducted by RDP classifier.[44] Based on the instruction of RDP Classifier Web site (https://rdp.cme.msu.edu/classifier/class_help.jsp#conf), a bootstrap confidence threshold of 50% was set up because the length of sequences obtained in our study was 250 bp.

Data Analysis. Richness, estimated richness in the sequence pool (Chao2), and the Shannon−Weaver index (H) were used to evaluate microbial taxonomic diversity. Principal coordinates analysis (PCoA) was used to determine changes of the overall microbial community structure. Canonical correspondence analysis (CCA) and a Mantel test were used to explore linkages between the microbial community structure and environmental variables. Bray−Curtis and Sorensen distances were used to calculate the dissimilarity matrices from the high-throughput sequencing data, and Euclidian distances between samples were computed based on standardized values of physicochemical parameters. To create a pairwise geographic distance matrix, the geographic distance was calculated using latitudinal and longitudinal coordinates of each sampling site using the R package. To calculate the relative influence of deterministic and stochastic processes on microbial assembly, a null model developed by Stegen et al. was used.[12,45,46] In this model, deterministic processes included homogeneous selection and variable selection, while stochastic processes included dispersal limitation, homogenizing dispersal, and drift.[12] The item "Undominated" in the model was used to refer to the scenario in which neither dispersal nor selection was the primary cause. It should be noted that the process of speciation was ignored in this study due to its little influence within communities.[45] Generally, the whole process to estimate the relative contributions of these ecological assemblages can be divided into two steps. First, the influence of selection was estimated by measuring the difference between the observed between-community version of the β-mean-nearest taxon distance (βMNTD) and the mean of the null distribution. Second, the influences of drift and limitation were estimated by standardizing the deviation between the observed Bray−Curtis distance and a null distribution of Bray−Curtis values. More details of the calculation can be found in refs 45 and 46. To identify the relative importance of multiple factors contributing to the dissimilarity of microbial composition, the multiple regression on matrices (MRM) method was used.[47] The partial regression coefficients from the MRM model gave a measure of the rate of change in microbial community similarity for variables of interest when other variables were held constant.[48] A variance partitioning analysis (VPA) was carried out to disentangle the effects of distance and environmental physicochemical parameters. Chao2, permutation test, and the MRM function were performed using the R package "fossil", "lmPerm", and "ecodist", respectively. Other analyses were performed with functions in the R package "Vegan" (v.1.15-1) in R (v.3.0.1).

RESULTS

Diversity Distribution Patterns. A total of 33849 OTUs were recovered from 211 samples after resampling on the basis of the rarefaction curve (Figure S2). Individual samples contained from 1286 to 3464 OTUs (Table S4). The observed richness of the activated sludge microbial communities in China fell within the range of global activated sludge richness (592−3801 OTUs)[39] but was quite lower than the microbial richness in many other ecosystems. For example, the richness of microbial communities ranged from 5000 to 11000 OTUs in soil[49] and from 3500 to 6000 OTUs in the rhizosphere.[50] The bacterial richness in freshwater, intertidal wetlands, and marine sediments was 8331, 6761, and 6059 OTUs, respectively.[51] To reasonably estimate the pool richness at the city level, 9 samples were randomly selected each time in each city to calculate the Chao2 index, and final Chao2 was the average of 1000 random samplings. Chao2 values varied from 10642 ± 386 to 18219 OTUs at the city level. Although there was no latitudinal diversity gradient pattern observed at the global scale,[39] our results showed that the estimated richness (Chao2) at the city level had a significantly negative correlation with latitude ($r = -0.56$, $P < 0.05$, significance was verified by a permutation test). To exclude the influences of a hierarchy of process types on latitudinal diversity gradient pattern, samples from the AAO process were selected to verify whether a latitudinal gradient pattern still existed. A significant latitudinal diversity gradient pattern ($r = -0.42$, $P < 0.05$, significance was verified by a permutation test; Figure S3) was observed within the single type of treatment process, indicating that treatment process types had a very slight effect on the latitude gradient pattern. These results indicated that the activated sludge microorganisms in China are supportive of latitudinal diversity gradient pattern and WWTPs at a lower latitude tend to harbor a more diverse array of microorganisms.

Whether microbes in activated sludge follow MTE was also explored, and our statistical analyses identified a strong linear relationship between the log-transformed estimated taxon

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richness (Chao2) from individual samples at the city level and the reciprocal temperature ($1/kT$) for microbes in WWTPs, suggesting that temperature plays a major role in latitudinal diversity gradient in WWTP (Figure 1, $r = -0.58$, $P < 0.05$, permutation test). The apparent activation energy of activated sludge communities (the slope of the plot, apparent Ea) was 0.08 eV.

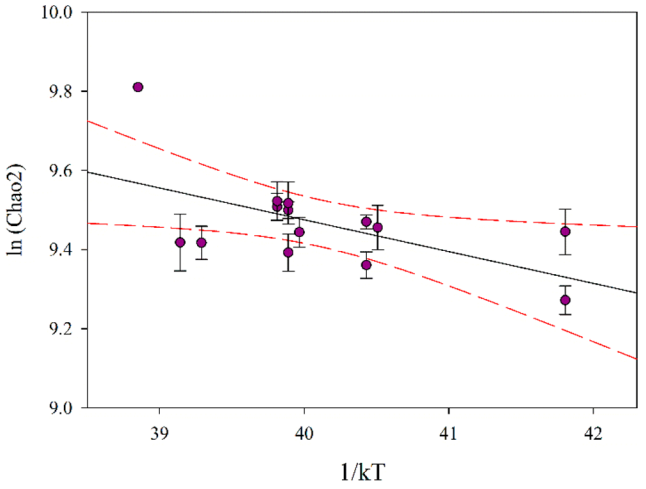

Figure 1. Relationships between activated sludge richness and mean annual temperature.

β-diversity is central to understanding the forces responsible for the magnitude and variability of biodiversity, and diversity distribution patterns can offer valuable clues to the relative influences of dispersal limitation, environmental heterogeneity, and environmental and evolutionary changes in shaping the structure of ecological communities.[52−54] Considering that the distinctly negative gradient in α-diversity observed with latitude may pose effects on the β-diversity gradients, a null model approach was utilized to disentangle variation in community dissimilarity from variation in α-diversity. This approach has been demonstrated to effectively discern whether variation in community dissimilarity results more from changes in the underlying structure by which communities vary or instead simply from difference in α-diversity among localities.[55] A modified Raup−Crick metric (β_{RC}) was calculated in this approach to represent both of dissimilarity among pairwise communities per se and dissimilarity among two communities relative to the null expectation.[55] The R code about calculations of β_{RC} can be found in ref 56 Our results showed that dissimilarity (β_{RC}) between pairwise activated sludge microbial communities within cities had strong negative correlations with latitude ($r_{BC} = -0.66$, $P < 0.01$, permutation test) (Figure 2a) and positive correlation with mean annual temperature ($r_{BC} = 0.7$, $P < 0.01$, permutation test) (Figure 2b). This suggests that temperature could affect the microbial community structure in WWTPs via metabolic processes and that activated sludge microbial communities within cities at lower latitudes and higher mean annual temperatures tend to be more dissimilar.

Because there was a quite strong correlation between latitude and mean annual temperature ($r = 0.96$, $P < 0.01$), a partial Mantel test was performed to determine whether the influence of latitude results from temperature. Partial Mantel results showed that latitude had a significant effect on the microbial community structure after excluding the influence of temperature (0.432, $P = 0.001$), indicating that the influences of latitude on the community structure did not entirely result from the covarying air temperature.

Changes in composition similarity among the activated sludge communities with pairwise geographic distances across China was then investigated to reveal the distance−decay relationship in WWTPs. Distance−decay relationship is one of the most well-documented spatial patterns for plants, animals, and some microorganisms in natural ecosystems, which means the community similarity decreases with increasing geographic distance.[57,58] Overall, activated sludge communities exhibited a significant distance−decay relationship ($P < 0.001$), meaning that bacterial communities located closely together were more similar in composition than communities located farther apart (Figure S4, slope = 0.0584 for Sorensen distance and slope = 0.0334 for unweighted Unifrac distance). The turnover rate, or z value, was calculated by the equation log (Ss) = constant − $2z$ log (D), where Ss is the pairwise similarity in community composition and D is the pairwise geographic distance between two samples.[59] Accordingly, the turnover rate (based on taxonomic diversity) for the activated sludge microbial communities across China was 0.0292 (Sorensen) and the z value (based on phylogenetic diversity) was 0.0167 (unweighted Unifrac), indicating that phylogenetic compositions were much more similar than taxonomic compositions among WWTPs.

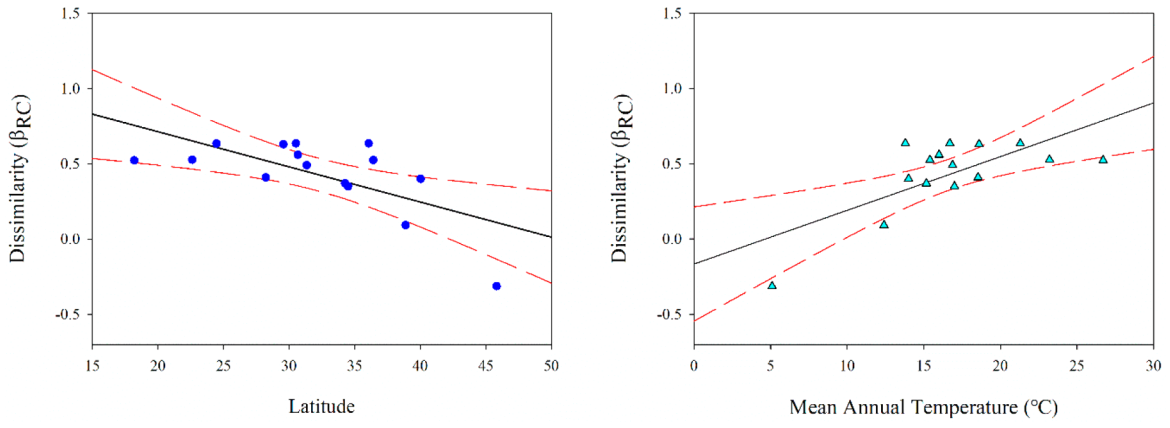

Figure 2. Relationships between dissimilarities (β_{RC}) and (a) latitude and (b) mean annual temperature.

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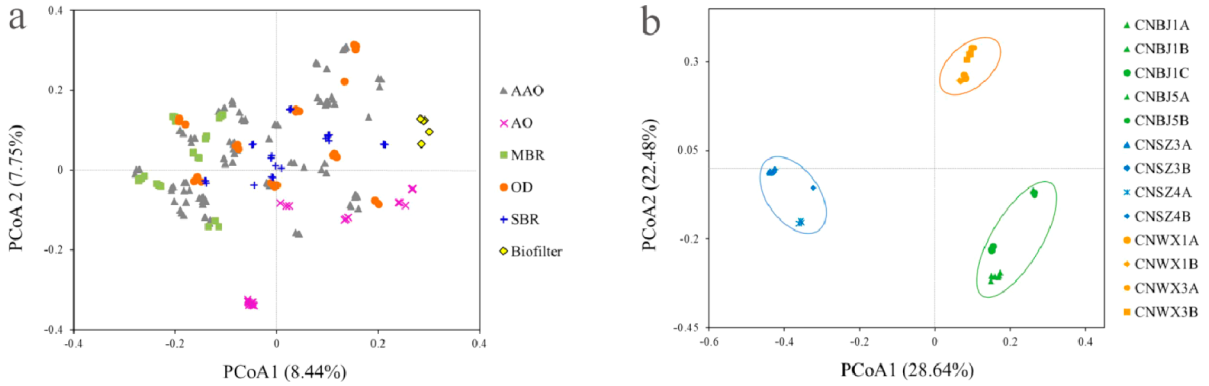

Figure 3. Principal coordinates analysis (PCoA) based on (a) treatment process types and (b) treatment process types from the same WWTP.

Although microbial community composition in WWTPs changed with temperature and geographic distance, there was a core community containing 63 OTUs detected in all WWTPs across China, which accounted for 24.8% of total abundance. The members of the core community mainly belonged to a variety of genera including *Dokdonella*, *Ferruginibacter*, *Lewinella*, and *Paludibacter* (Table S5). *Nitrospira,* a crucial participant in nitrogen cycling, was also found in the core community. As expected, the core community had very low turnover in a space with a *z* value as low as 0.0043 (Sorensen). The turnover rate of the community excluding the core (keeping each of sample having 15000 sequences) was higher than that of the whole community across China (0.0313 vs. 0.0292, Sorensen).

Microbial Assembly in WWTPs. Only very few studies have made the explicit attempt to study both deterministic and stochastic mechanisms in microbial assembly in WWTPs,[38,60] and the relative contributions of these two mechanisms are still poorly understood. In this study, a null model was used to understand the forces that structure activated sludge communities. Overall, consistent with typical understanding, deterministic processes related to environmental selection contributed more to the activated sludge microbial assembly than stochastic processes (Table S6). Deterministic processes accounted for 72.55% of the assembly process across China, which is consistent with a previous study.[60] Stochastic processes, including drift and dispersal, affected microbial community assemblage in WWTPs more deeply than expected, accounting for 25.14%. Among stochastic processes, dispersal limitation was predominant.

The impacts of treatment process types on microbial structure were examined using principle coordinate analysis (PCoA) and clustering. PCoA showed that most of the samples could not be separated on the basis of treatment process types except AO and biofilm, which were consistent with clustering results (Figure 3a and Figure S5a). This is consistent with a previous study, which indicated that samples from the same plant, but exposed to different treatment processes, were clustered together, while samples from different locations, but having the same treatment process, were not.[37] In contrast, Hu et al.[29] observed significant effects of a treatment process on the microbial communities exposed to identical influents within the same WWTP. This same phenomenon was also observed by Xia et al.,[22] who found significant differences in the functional gene structures of microbial communities under two different treatment processes at the same WWTP. To gain a deeper understanding of the effects of a treatment process on the microbial communities, more analyses were used, including redundancy analysis (RDA), dissimilarity test, canonical correspondence analysis (CCA), and linear discriminant analysis. RDA results showed that the whole influence of all treatment process types was much weaker than those of the latitude and wastewater characteristics (e.g., pollutant concentration of influent) (Figure S6). Linear discriminant analysis, which tries to detect if the within-group covariance matrix is singular, was also used. However, the error of prediction for original data was as high as 40%, indicating that the differences between different process types were not very distinct, and therefore it was hard to distinguish the microbial community by treatment process types effectively. Moreover, the CCA model showed that treatment process types could only explain 13.77% of the total variance, indicating that treatment process types had a weak influence on microbial compositions (Figure S7). Particularly, AO and biofilm had the most effects on microbial structures among these treatment process types. A dissimilarity test (ANOSIM) showed that samples from SBR, AO, and biofilm were significantly different from others (Table S7). These results indicated that only microbial communities with different microbial growth types (e.g., suspended growth type and attached growth type) and targeted functions (e.g., nitrification and denitrification, etc.) were significantly different at large scales. Accordingly, our results suggest that the impacts of treatment process types with similar microbial growth types and targeted functions may be covered by great variations of geographic locations and wastewater characteristics. Hence, samples from different treatment process types (e.g., AAO, MBR, OD) with identical wastewater characteristics and geographic locations were selected for further analysis. Results showed that activated sludge communities clustered into three groups on the basis of sampling city. Within these "city groups", the communities further separated by treatment process (Figure 3b, Figure S5b).

MRM was then conducted to reveal the relative importance of the variables to the microbial taxonomic structure. The overall MRM model, with all selected variables, was significant ($P < 0.01$) and the explainable portion (R^2) was 0.748 (Table 1). The mean annual temperature had the largest regression coefficient for a single factor, approximately an order of magnitude higher than other variables. The coefficient of geographic distance was also relatively high, indicating that the mean annual temperature and geographic distance are the top two drivers in shaping the microbial community composition (Table 1). Other significant variables were HRT, conductivity

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Table 1. Relative Importance of Environmental Factors Contributing to the Microbial Taxonomic Structure

environmental factors	coefficient[a]
ln(distance)	0.0846[c]
ln(mean annual temperature)	0.1635[c]
ln(MLSS[b])	0.0198
ln(temperture of activated sludge)	0.0240[c]
ln(pH of activated sludge)	0.0324[c]
ln(conductivity of activated sludge)	0.0446[c]
ln(DO of activated sludge)	0.0120
ln(HRT)	0.0492[c]
ln(SRT)	0.0386[c]
ln(B/C[d])	0.0354[c]
ln(influent BOD)	−0.0575
ln(influent COD)	0.0212
ln(influent TN)	0.0280[c]
ln(volume of aeration tanks)	−0.0218
ln(COD loading)	0.0015

[a]Coefficient values from the multiple regression on matrices (MRM analysis) correlation. [b]MLSS is the abbreviation for mixed liquor suspended solids. [c]means $P < 0.01$. [d]means biodegradability of influent.

of activated sludge, SRT, biodegradability of influent, pH of activated sludge, TN of influent, and temperature of activated sludge (ranked high to low based on the coefficient). CCA results also indicated that the latitude, mean annual temperature, and conductivity had the most effects on an activated sludge microbial community (Figure S8).

DISCUSSION

On the basis of our results, activated sludge microbial communities in WWTPs across China, like organisms in natural ecosystems, exhibit a latitudinal diversity gradient pattern and follow the MTE. However, these findings are contrary to conclusions drawn from a global study on activated sludge microbial communities,[39] which indicated that there was no latitudinal gradient at the global scale. This is not surprising because activated sludge microbial communities in Asia are quite different from those in other continents, especially at the phylogenetic level.[39] A possible reason for this is that the majority of WWTPs in China utilize an enhanced wastewater treatment system, and 86.7% of collected samples came from WWTPs that take nitrification and denitrification into consideration. In contrast, only 41.4% of samples were collected from WWTPs with denitrification in other countries (data was provided by ref 39). Moreover, latitudinal diversity gradient patterns are influenced by many factors, like sampling scale, habitat, and longitude.[5] These factors may interact with one another, and the total effects may cover the latitudinal diversity gradient pattern when the scale of survey is global. Although microbes in WWTPs follow the MTE, the apparent Ea value was quite low compared with those of communities in natural ecosystems. For example, trees in China had apparent Ea values ranging from 0.93 to 1.02 eV, on the basis of the spatial scale.[61] Apparent Ea values for bacteria using 16S rRNA genes, fungi using ITS genes, and N fixers using *nifH* genes, all at 97% similarity, in soil were 0.184, 0.169, and 0.467 eV, respectively.[49] The apparent Ea value for zooplankton was 0.26 eV[62] and for phytoplankton was 1.0 eV[63] (Table 2). The lower apparent Ea value of bacteria in WWTPs indicated that microbes in WWTPs are less temperature dependent and

Table 2. Reported Apparent Ea Values for Organisms in Different Environments

organisms	environment	apparent Ea value (eV)
bacteria	WWTPs	0.08
trees		0.93−1.02[61]
bacteria	soil	0.184[49]
fungi	soil	0.169[49]
nitrogen fixers	soil	0.467[49]
zooplankton	lakes	0.26[62]
phytoplankton	lakes	1.0[63]

temperature had less influence in highly engineered and controlled systems compared with natural systems.

In agreement with the global survey of activated sludge microbial communities,[39] a distance−decay relationship was also observed in China. However, the distance−decay relationship slope for China (0.0292, Sorensen) was closer to that of the global scale (0.03, Sorensen) rather than the continental scale (0.065, Sorensen), another peculiarity of activated sludge microbial communities in China. Moreover, the z value calculated from 16S rRNA genes, representing the turnover rate of taxonomic composition in our study, was almost five-times larger than that from functional genes (0.0066, Sorensen),[26] which was calculated based on samples collected from the aeration tanks of 26 full-scale WWTPs located in 10 different cities across long transects of China. This suggests that microbial communities among different WWTPs were more similar functionally than taxonomically. This makes sense because WWTPs usually have similar functions and more stable performance,[64] in spite of some differences in microbial compositions.[19,37] In addition, the functional redundancy of microorganisms in WWTPs[26] may increase the functional similarity between WWTPs. The core WWTP community, whose members present in almost all of the WWTPs examined, had a low turnover rate, illustrating their stability regardless of location, treatment process, wastewater characteristics, and operational parameters and their critical roles in system function and stability. The spatial turnover of the activated sludge community across China mainly resulted from differences in rare species. The z value of microbial communities in WWTPs was half of that of microbes in soil (0.0626, Sorensen distance)[58] and was an order of magnitude less those observed in plants and animals (0.306 and 0.274, respectively).[58] Higher immigration rates in an open biological system like WWTPs[38] and less dispersal limitation of microbes may contribute to the lower turnover rates in WWTPs. Moreover, high functional redundancy caused by a nutrient-rich environment in biological WWTPs may also reduce the z values.[26]

As a practical application of biological technology, WWTP operation can be regulated depending on particular environmental conditions. A lot of effort has gone into revealing correlations between microbial community composition and environmental factors[21,65,66] to improve system performance of WWTPs. However, treatment process types with similar microbial growth types (e.g., suspended growth type or attached growth type) and similar functions did not show great impacts on activated sludge microbial communities at a large spatial scale (country level). In contrast, microbial communities from different treatment process types but with similar locations and influent properties separated well from each other. A partial Mantel test showed that the correlations

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between latitude and microbial compositions were much stronger than correlations between treatment process types and microbial compositions (r = 0.42 for latitude vs. r = 0.07−0.20 for treatment process types). Taken together, these findings suggest that the impacts of treatment process types with similar growth types and functions on microbial community structure were weaker than geographic locations and other environmental factors. Moreover, it indicated that it is important to consider the effect of process type on the AS microbial community structure when the WWTP is designed to treat a certain sewage in a certain place. Although most of the environmental factors, particularly activated sludge properties, and operational parameters, such as hydraulic retention time (HRT), conductivity of activated sludge, and sludge retention time (SRT), did not have significant effects on the α-diversity, they played an important role in the activated sludge microbial community structure and composition. Some studies have revealed that conductivity can greatly influence bacterial community composition.[37,67] For example, a study by Lozupone[67] using 21 752 16S rRNA gene sequences from 111 studies of diverse physical environments found that the major environmental determinant of microbial community composition was salinity rather than extremes of temperature, pH, or other physical or chemical factors. DO, reported as an important variable to microbial communities in WWTPs[22,37,68] did not show a significant effect in this study. Gao et al.[36] also found that DO had the least influence on bacterial community composition. A plausible reason for this is that we took all samples from aeration tanks with DO concentrations in the proper range and that could meet the demands of the associated microbial communities. Therefore, there were no substantial differences in DO concentration between the microbial communities.

Our results revealed that deterministic and stochastic processes simultaneously influenced microbial assembly in WWTPs and that deterministic processes contributed a greater influence on community assembly than stochastic processes. Again, these findings were inconsistent with a global survey of activated sludge communities,[39] which indicated that stochastic processes played a more important role than deterministic processes. Regardless, the findings of the current study indicate that stochastic processes do play an important role in activated sludge microbial assembly. Noticeably, among stochastic processes, dispersal limitation was a predominant driver. As WWTPs are inherently open systems that rely on different types of bacteria coming together to form a microbial community,[38] immigration could take place from incoming wastewater and consequently increase stochastic assembly in WWTPs. In addition, most microbes in the sewer system are associated with sewage infrastructure and are influenced by the local ambient environment and climate.[69] These sewer system microbes are source microorganisms and potential residents for activated sludge.[70] Therefore, differences among the microorganisms in sewer systems from different cities may increase the dissimilarities in WWTPs because of dispersal limitation. The great variation of influent wastewater and sewer systems among different continents may be one of the reasons why activated sludge microbial assembly in China is inconsistent with that across the world. Although our results preliminary have revealed some geographic distribution patterns in the WWTPs, it should be noted that our findings above generated from AS samples were collected only once from each plant because of the great amount of work at such a large sampling scale. Given that one-time sampling could result in limited representativeness of the samples for their source plants, a deep analysis based on temporal sampling is merited in a future study.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b07950.

Tables of sampled treatment process types, additional information on treatment processes, basic environmental information on 211 samples, α-diversity of 211 samples, members in the core community, and dissimilarity test between different treatment process types and figures of geographical distributions, rarefaction curves, relationship between Chao2 and latitude based on samples from AAO process, relationship between taxonomic dissimilarity and geographic distance, clustering results of samples, RDA analysis, and CCA analysis (PDF)

Table of results of assembly processes (XLSX)

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Notes

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REFERENCES

(1) Martiny, J. B. H.; Bohannan, B. J. M.; Brown, J. H.; Colwell, R. K.; Fuhrman, J. A.; Green, J. L.; Horner-Devine, M. C.; Kane, M.; Krumins, J. A.; Kuske, C. R.; Morin, P. J.; Naeem, S.; Ovreas, L.; Reysenbach, A. L.; Smith, V. H.; Staley, J. T. Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* **2006**, *4* (2), 102−112.

(2) Koch, F.; Brown, J. H.; Lomolino, M. V. *Biogeography*. 2nd ed.; Sinauer Associates, Inc. Publishers: Sunderland, MA, 2000.

(3) Green, J.; Bohannan, B. J. Spatial scaling of microbial biodiversity. *Trends Ecol Evol.* **2006**, *21* (9), 501−7.

(4) Pavoine, S.; Bonsall, M. B. Measuring biodiversity to explain community assembly: a unified approach. *Biol. Rev. Camb Philos. Soc.* **2011**, *86* (4), 792−812.

(5) Hillebrand, H. On the generality of the latitudinal diversity gradient. *Am. Nat.* **2004**, *163* (2), 192−211.

(6) Whittaker, R. W.; Willis, K. J.; Field, R. Scale and Species Richness: Towards a General, Hierarchical Theory of Species Diversity. *Journal of Biogeography.* **2001**, *28*, 453−470.

(7) Rohde, K. Latitudinal Gradients in Species Diversity: The Search for the Primary Cause. *Oikos* **1992**, *65* (3), 514−527.

(8) Allen, A. P.; Brown, J. H.; Gillooly, J. F. Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* **2002**, *297* (5586), 1545−8.

(9) Sanders, N. J.; Lessard, J. P.; Fitzpatrick, M. C.; Dunn, R. R. Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology & Biogeography.* **2007**, *16* (5), 640−649.

(10) Tittensor, D. P.; Mora, C.; Jetz, W.; Lotze, H. K.; Ricard, D.; Berghe, E. V.; Worm, B. Global patterns and predictors of marine biodiversity across taxa. *Nature* **2010**, *466* (7310), 1098−101.

(11) Brown, J. H.; Gillooly, J. F.; Allen, A. P.; Savage, V. M.; West, G. B. Toward a Metabolic Theory of Ecology. *Ecology* **2004**, *85* (7), 1771−1789.

(12) Stegen, J. C.; Lin, X.; Konopka, A. E.; Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **2012**, *6* (9), 1653−64.

(13) Dumbrell, A. J.; Nelson, M.; Helgason, T.; Dytham, C.; Fitter, A. H. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME J.* **2010**, *4* (3), 337−45.

(14) Caruso, T.; Chan, Y.; Lacap, D. C.; Lau, M. C.; McKay, C. P.; Pointing, S. B. Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *ISME J.* **2011**, *5* (9), 1406−13.

(15) Langenheder, S.; Székely, A. J. Species sorting and neutral processes are both important during the initial assembly of bacterial communities. *ISME J.* **2011**, *5* (7), 1086−1094.

(16) Briones, A.; Raskin, L. Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. *Curr. Opin. Biotechnol.* **2003**, *14* (3), 270−276.

(17) Miura, Y.; Hiraiwa, M. N.; Ito, T.; Itonaga, T.; Watanabe, Y.; Okabe, S. Bacterial community structures in MBRs treating municipal wastewater: relationship between community stability and reactor performance. *Water Res.* **2007**, *41* (3), 627−37.

(18) Shchegolkova, N. M.; Krasnov, G. S.; Belova, A. A.; Dmitriev, A. A.; Kharitonov, S. L.; Klimina, K. M.; Melnikova, N. V.; Kudryavtseva, A. V. Microbial Community Structure of Activated Sludge in Treatment Plants with Different Wastewater Compositions. *Front. Microbiol.* **2016**, *7*.

(19) Zhang, T.; Shao, M. F.; Ye, L. 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. *ISME J.* **2012**, *6* (6), 1137−47.

(20) Daims, H.; Taylor, M. W.; Wagner, M. Wastewater treatment: a model system for microbial ecology. *Trends Biotechnol.* **2006**, *24* (11), 483−489.

(21) Xia, Y.; Wang, X.; Wen, X.; Ding, K.; Zhou, J.; Yang, Y.; Zhang, Y. Overall functional gene diversity of microbial communities in three full-scale activated sludge bioreactors. *Appl. Microbiol. Biotechnol.* **2014**, *98* (16), 7233−42.

(22) Xia, Y.; Hu, M.; Wen, X.; Wang, X.; Yang, Y.; Zhou, J. Diversity and interactions of microbial functional genes under differing environmental conditions: insights from a membrane bioreactor and an oxidation ditch. *Sci. Rep.* **2016**, *6*, 18509.

(23) Yang, C.; Zhang, W.; Liu, R. H.; Li, Q.; Li, B. B.; Wang, S. F.; Song, C. J.; Qiao, C. L.; Mulchandani, A. Phylogenetic Diversity and Metabolic Potential of Activated Sludge Microbial Communities in Full-Scale Wastewater Treatment Plants. *Environ. Sci. Technol.* **2011**, *45* (17), 7408−7415.

(24) McIlroy, S. J.; Kirkegaard, R. H.; McIlroy, B.; Nierychlo, M.; Kristensen, J. M.; Karst, S. M.; Albertsen, M.; Nielsen, P. H. MiDAS 2.0: an ecosystem-specific taxonomy and online database for the organisms of wastewater treatment systems expanded for anaerobic digester groups. *Database.* **2017**, *2017* (1).

(25) Zhang, B.; Xia, Y.; Wen, X.; Wang, X.; Yang, Y.; Zhou, J.; Zhang, Y. The Composition and Spatial Patterns of Bacterial Virulence Factors and Antibiotic Resistance Genes in 19 Wastewater Treatment Plants. *PLoS One* **2016**, *11* (12), e0167422.

(26) Wang, X.; Wen, X.; Deng, Y.; Xia, Y.; Yang, Y.; Zhou, J. Is there a distance-decay relationship in biological wastewater treatment plants? *Appl. Environ. Microbiol.* **2016**, *82* (16), 4860−4866.

(27) Cydzik-Kwiatkowska, A.; Zielinska, M. Bacterial communities in full-scale wastewater treatment systems. *World J. Microbiol. Biotechnol.* **2016**, *32* (4), 66.

(28) Sheng, X.; Liu, R.; Song, X.; Chen, L.; Tomoki, K. Comparative study on microbial community in intermittently aerated sequencing batch reactors (SBR) and a traditional SBR treating digested piggery wastewater. *Front. Environ. Sci. Eng.* **2017**, *11* (3).

(29) Hu, M.; Wang, X.; Wen, X.; Xia, Y. Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresour. Technol.* **2012**, *117* (10), 72.

(30) Hall, E. R.; Monti, A.; Mohn, W. W. A comparison of bacterial populations in enhanced biological phosphorus removal processes using membrane filtration or gravity sedimentation for solids−liquid separation. *Water Res.* **2010**, *44* (9), 2703−2714.

(31) Chen, Y.; Lan, S.; Wang, L.; Dong, S.; Zhou, H.; Tan, Z.; Li, X. A review: Driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems. *Chemosphere* **2017**, *174*, 173−182.

(32) Liu, H.; Yang, F.; Shi, S.; Liu, X. Effect of substrate COD/N ratio on performance and microbial community structure of a membrane aerated biofilm reactor. *J. Environ. Sci.* **2010**, *22* (4), 540−546.

(33) Han, H.; Zhang, Y.; Cui, C.; Zheng, S. Effect of COD level and HRT on microbial community in a yeast-predominant activated sludge system. *Bioresour. Technol.* **2010**, *101* (10), 3463−3465.

(34) Stadler, L. B.; Love, N. G. Impact of microbial physiology and microbial community structure on pharmaceutical fate driven by dissolved oxygen concentration in nitrifying bioreactors. *Water Res.* **2016**, *104*, 189−199.

(35) Kim, Y. M.; Cho, H. U.; Lee, D. S.; Park, D.; Park, J. M. Influence of operational parameters on nitrogen removal efficiency and microbial communities in a full-scale activated sludge process. *Water Res.* **2011**, *45* (17), 5785−95.

(36) Gao, P.; Xu, W.; Sontag, P.; Li, X.; Xue, G.; Liu, T.; Sun, W. Correlating microbial community compositions with environmental factors in activated sludge from four full-scale municipal wastewater treatment plants in Shanghai, China. *Appl. Microbiol. Biotechnol.* **2016**, *100* (10), 4663−4673.

(37) Wang, X.; Hu, M.; Xia, Y.; Wen, X.; Ding, K. Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. *Appl. Environ. Microbiol.* **2012**, *78* (19), 7042−7.

(38) Ofiteru, I. D.; Lunn, M.; Curtis, T. P.; Wells, G. F.; Criddle, C. S.; Francis, C. A.; Sloan, W. T. Combined niche and neutral effects in a microbial wastewater treatment community. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (35), 15345−50.

(39) Wu, L.; Ning, D.; Zhang, B.; Li, Y.; Zhang, P.; Shan, X.; Zhang, Q.; Brown, M.; Li, Z.; Van Nostrand, J. D.; Ling, F.; Xiao, N.; Zhang, Y.; Vierheilig, J.; Wells, G. F.; Yang, Y.; Deng, Y.; Tu, Q.; Wang, A.;

https://dx.doi.org/10.1021/acs.est.9b07950
Environ. Sci. Technol. 2020, 54, 5884−5892

Zhang, T.; He, Z.; Keller, J.; Nielsen, P. H.; Alvarez, P. J. J.; Criddle, C. S.; Wagner, M.; Tiedje, J. M.; He, Q.; Curtis, T. P.; Stahl, D. A.; Alvarez-Cohen, L.; Rittmann, B. E.; Wen, X.; Zhou, J. Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nat. Microbiol.* **2019**, *4* (7), 1183−1195.
(40) Peiffer, J. A.; Spor, A.; Koren, O.; Jin, Z.; Tringe, S. G.; Dangl, J. L.; Buckler, E. S.; Ley, R. E. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (16), 6548−53.
(41) Zhou, J.; Wu, L.; Deng, Y.; Zhi, X.; Jiang, Y. H.; Tu, Q.; Xie, J.; Van Nostrand, J. D.; He, Z.; Yang, Y. Reproducibility and quantitation of amplicon sequencing-based detection. *ISME J.* **2011**, *5* (8), 1303−13.
(42) Kong, Y. Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. *Genomics* **2011**, *98* (2), 152−153.
(43) Magoč, T.; Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27* (21), 2957−2963.
(44) Wang, Q.; Garrity, G. M.; Tiedje, J. M.; Cole, J. R. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microbiol.* **2007**, *73* (16), 5261−5267.
(45) Stegen, J. C.; Lin, X. J.; Fredrickson, J. K.; Chen, X. Y.; Kennedy, D. W.; Murray, C. J.; Rockhold, M. L.; Konopka, A. Quantifying community assembly processes and identifying features that impose them. *ISME J.* **2013**, *7* (11), 2069−2079.
(46) Stegen, J. C.; Lin, X.; Fredrickson, J. K.; Konopka, A. E. Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* **2015**, *6*, 370.
(47) Legendre, P.; Lapointe, F. J.; Casgrain, P. Modeling Brain Evolution from Behavior: A Permutational Regression Approach. *Evolution* **1994**, *48* (5), 1487−1499.
(48) Martiny, J. B.; Eisen, J. A.; Penn, K.; Allison, S. D.; Horner-Devine, M. C. Drivers of bacterial beta-diversity depend on spatial scale. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (19), 7850−7854.
(49) Zhou, J.; Deng, Y.; Shen, L.; Wen, C.; Yan, Q.; Ning, D.; Qin, Y.; Xue, K.; Wu, L.; He, Z.; Voordeckers, J. W.; Nostrand, J. D.; Buzzard, V.; Michaletz, S. T.; Enquist, B. J.; Weiser, M. D.; Kaspari, M.; Waide, R.; Yang, Y.; Brown, J. H. Temperature mediates continental-scale diversity of microbes in forest soils. *Nat. Commun.* **2016**, *7*, 12083.
(50) Shi, S.; Nuccio, E.; Herman, D. J.; Rijkers, R.; Estera, K.; Li, J.; da Rocha, U. N.; He, Z.; Pett-Ridge, J.; Brodie, E. L.; Zhou, J.; Firestone, M. Successional Trajectories of Rhizosphere Bacterial Communities over Consecutive Seasons. *mBio* **2015**, *6* (4), e00746.
(51) Wang, Y.; Sheng, H. F.; He, Y.; Wu, J. Y.; Jiang, Y. X.; Tam, N. F.; Zhou, H. W. Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. *Appl. Environ. Microbiol.* **2012**, *78* (23), 8264−71.
(52) Condit, R.; Hubbell, S. P. Beta-diversity in tropical forest trees. *Science* **2002**, *295* (5555), 666−669.
(53) Harrison, S. How natural habitat patchiness affects the distribution of diversity in Californian serpentine chaparral. *Ecology* **1997**, *78* (6), 1898−1906.
(54) Nekola, J. C.; White, P. S. The distance decay of similarity in biogeography and ecology. *Journal of Biogeography* **1999**, *26* (4), 867−878.
(55) Chase, J. M.; Kraft, N. J. B.; Smith, K. G.; Vellend, M.; Inouye, B. D. Using null models to disentangle variation in community dissimilarity from variation in alpha-diversity. *Ecosphere* **2011**, *2* (2), art24.
(56) Vellend, M.; Verheyen, K.; Flinn, K. M.; Jacquemyn, H.; Kolb, A.; Calster, H. V.; Peterkent, G.; Graae, B. J.; Bellemare, J.; Honnay, O. Homogenization of Forest Plant Communities and Weakening of Species-Environment Relationships Via Agricultural Land Use. *J. Ecol.* **2007**, *95* (3), 565−573.
(57) Morlon, H.; Schwilk, D. W.; Bryant, J. A.; Marquet, P. A.; Rebelo, A. G.; Tauss, C.; Bohannan, B. J. M.; Green, J. L. Spatial patterns of phylogenetic diversity. *Ecol Lett.* **2011**, *14* (2), 141−149.
(58) Zhou, J.; Kang, S.; Schadt, C. W.; Charles, T.; Garten, J. Spatial scaling of functional gene diversity across various microbial taxa. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (22), 7768−7773.
(59) Horner-Devine, M. C.; Lage, M.; Hughes, J. B.; Bohannan, B. J. A taxa-area relationship for bacteria. *Nature* **2004**, *432* (7018), 750−753.
(60) Griffin, J. S.; Wells, G. F. Regional synchrony in full-scale activated sludge bioreactors due to deterministic microbial community assembly. *ISME J.* **2017**, *11* (2), 500−511.
(61) Wang, Z.; Brown, J. H.; Tang, Z.; Fang, J. Temperature dependence, spatial scale, and tree species diversity in eastern Asia and North America. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (32), 13388−13392.
(62) Pinel-Alloul, B.; Andre, A.; Legendre, P.; Cardille, J. A.; Patalas, K.; Salki, A. Large-scale geographic patterns of diversity and community structure of pelagic crustacean zooplankton in Canadian lakes. *Global Ecology & Biogeography* **2013**, *22* (7), 784−795.
(63) Segura, A. M.; Calliari, D.; Kruk, C.; Fort, H.; Izaguirre, I.; Saad, J. F.; Arim, M. Metabolic dependence of phytoplankton species richness. *Global Ecology & Biogeography* **2015**, *24* (4), 472−482.
(64) Ju, F.; Guo, F.; Ye, L.; Xia, Y.; Zhang, T. Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. *Environ. Microbiol. Rep.* **2014**, *6* (1), 80−89.
(65) Barberan, A.; Bates, S. T.; Casamayor, E. O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2012**, *6* (2), 343−351.
(66) Pholchan, M. K.; Baptista, J. D.; Davenport, R. J.; Curtis, T. P. Systematic study of the effect of operating variables on reactor performance and microbial diversity in laboratory-scale activated sludge reactors. *Water Res.* **2010**, *44* (5), 1341−1352.
(67) Lozupone, C. A.; Knight, R. Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (27), 11436−11440.
(68) Yuan, Z.; Blackall, L. L. Sludge population optimization: a new dimension for the control of biological wastewater treatment systems. *Water Res.* **2002**, *36* (2), 482−90.
(69) Shanks, O. C.; Newton, R. J.; Kelty, C. A.; Huse, S. M.; Sogin, M. L.; McLellan, S. L. Comparison of the microbial community structures of untreated wastewaters from different geographic locales. *Appl. Environ. Microbiol.* **2013**, *79* (9), 2906−2913.
(70) McLellan, S. L.; Huse, S. M.; Mueller-Spitz, S. R.; Andreishcheva, E. N.; Sogin, M. L. Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. *Environ. Microbiol.* **2010**, *12* (2), 378−392.

https://dx.doi.org/10.1021/acs.est.9b07950
Environ. Sci. Technol. 2020, 54, 5884−5892