



# Article Temporal and Spatial Characterization of Sediment Bacterial Communities from Lake Wetlands in a Plain River Network Region

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Abstract: Sediment bacterial communities are a vital component of microbial communities in aquatic and terrestrial ecosystems and they play a critical role in lake wetlands. We aimed to investigate the effect of season, depth and regional environmental factors on the composition and diversity of bacterial communities in a plain river network area from Taihu Basin. The millions of Illumina reads (16S rRNA gene amplicons) at the surface 25 cm inside samples of the study area were examined using a technically consistent approach. Results from the diversity index, relative abundance, principal component analysis (PCA), redundancy analysis (RDA) and linear discriminant analysis effect size (LEfSe) analysis indicated that the diversity of the bacterial community in summer was generally higher than in other seasons. Proteobacteria were the most abundant phylum in the sediment samples in different seasons (43.15-57.41%) and different layers (39.66-77.97%); the autumn sediments were enriched with Firmicutes (5.67%) and Chloroflexi (12.5%); in all four seasons the sediments were enriched with Betaproteobacteria (14.98-23.45%), Gammaproteobacteria (11.98-14.36%) and Deltaproteobacteria (8.68–14.45%). In the bottom sediments (10–25 cm), Chloroflexi were abundant (average value 10.42%), while Bacteroidetes was the dominant phylum in the surface sediments; redundancy analysis found that total phosphorus (TP) (p = 0.036) was the main environmental factor influencing the sediment bacterial community in different layers. This study provides a reference for further understanding the effects of seasonal changes on sediment microorganisms in lake wetlands.

Keywords: bacterial community; lake wetland; sediment; seasonal variation

# 1. Introduction

In plain river network regions, the lake wetland is a special wetland type that includes wetland plants, microbes and animals, and these play an important ecological role in flood storage, water purification, habitat maintenance and maintaining species diversity [1–3]. Bacterial communities are prevalent in lake wetlands [4]. Sediments are a unique element in aquatic ecosystems and have significantly higher microbial species and diversity than the overlying water [5,6]. Sediment bacterial communities are the drivers of nutrient cycling and energy conversion in aquatic ecosystems, and changes in microbial physiological functions affect nutrient formation and cycling in sediments, which can lead to secondary pollution of water and lake eutrophication [7,8]. Bacterial diversity is an important index for water quality evaluation, which can reflect water quality and the migration and transformation of pollutants in sediments [9,10].

The functional diversity of microbes is influenced by environmental factors. Numerous studies of the bacterial communities in lake wetland ecosystems have shown that they are closely related to a variety of environmental factors, such as C and N availability, temperature and sediment structure characteristics [11–17]. Furthermore, the organic matter (OM) content affects the structure and function of sediment communities [18]. There



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). are differences in the physical and chemical indexes of the biogeochemical properties of the sediments, owing to the influence of spatial and seasonal factors [8,19]. Some studies have shown that sediment bacterial community structure and composition are characterized by significant seasonal and temporal variations [5,19,20]. Because of the sensitivity of sediment bacteria to small chemical and physical changes, their structure may change spatially and temporally in response to environmental variations. Season is one of the key factors affecting sediment bacterial communities [21]. The spatial heterogeneity of bacterial community richness and diversity in sediments has an impact on water quality [22,23]. There has, however, been limited exploration of the changes in the bacterial community with respect to environmental variability (season) and vertical distribution (depth) in lake wetlands.

Taihu Basin, located in China, has a subtropical monsoon climate. It also has numerous lake wetlands, which are located in the southeast parts of Taihu Basin. Numerous lake wetlands exist around several large rivers and influence the water quality in the plain river network region. The northwestern part of the study area is heavily polluted from exogenous sources due to chemical industries, aquaculture and other factors. We investigated the bacterial communities in the sediments of a plain river network area from Taihu Basin in all four seasons, focusing particularly on the vertical bacterial community characteristic of sediment samples from six typical sites in summer. Our main aim was to understand the spatiotemporal variation in the bacterial communities and to identify the driving factors at different locations in the lake wetland. Seasonal changes in the composition and diversity of microbes were analyzed by constructing clone libraries of 16S rRNA, from which potential reasons for differences in community structure were inferred. Our results provide a new perspective on and valuable reference to the bacterial community in shallow, eutrophic lake wetlands, which we hope can be applied to other shallow and eutrophic aquatic ecosystems.

# 2. Material and Methods

#### 2.1. Sediment Sampling

Thirty sampling sites were chosen in a plain river network (Figure 1), and the surface sediment samples were collected from these sites in spring (March), summer (June), autumn (September) and winter (December) using sediment sampler. A total of 120 sediment samples were obtained over the four seasons: spring (A), summer (B), autumn (C) and winter (D). We divided the sediment samples from six typical sites (3, 10, 14, 18, 19, 20) in summer into five layers (0–5, 5–10, 10–15, 15–20, 20–25 cm), and 30 sediment samples were collected. The sediment samples were homogenized, stored in sealed plastic bags and placed immediately onto dry ice. All samples were transferred to the laboratory within 24 h and kept at -80 °C until processing. Water samples from the 30 sites were also collected for analysis of the influence of environmental factors on bacterial communities in the sediments.



Figure 1. Sampling sites and the six typical sites.

# 2.2. Geochemical Analysis

The total nitrogen (TN) and total phosphorus (TP) of each sediment were measured by using standardized methods and tests [24]. The organic matter (OM) content of each sediment was calculated according to the loss on ignition to constant mass (4 h) at 550 °C [25]. The main properties of the sediment samples are shown in Figure 2.



**Figure 2.** Spatial distribution of pollutants (total nitrogen, TN; total phosphorus, TP; and organic matter, OM) in the lake wetlands.

# 2.3. DNA Extraction, PCR Amplification and Sequencing

DNA was extracted from the sediment samples using a PowerSoil DNA Isolation Kit (Mobio Laboratories Inc., San Diego, CA, USA), following the manufacturer's manual. DNA concentration and purity were checked using a NanoDrop Spectrophotometer (Thermo Scientific NanoDrop Lite, Waltham, MA, USA). DNA was diluted to 10 ng  $\mu L^{-1}$  using sterile ultrapure water and stored at  $-80\ ^\circ C$  before further analysis.

The specific primers 515F (GTGCCAGCMGCCGCGGTAA) and 926R (CCGTCAATTC MTTTRAGTTT) were used in PCR amplification of bacterial 16S rRNA V4–V5 regions. We chose these primers because of their high coverage of almost all common phyla [26,27]. The conditions for PCR amplification were: initial denaturation at 94 °C for 1 min, 30 cycles (denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s and elongation at 72 °C for 45 s) and a final extension at 72 °C for 5 min. Library quality was evaluated on a Qubit<sup>®</sup>2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system. Eventually, the library was subjected to paired-end sequencing (2 × 250 bp) on an Illumina MiSeq platform from Tiny Gene Bio-Tech Co., Ltd. (Shanghai, China).

# 2.4. Data and Statistical Analysis

The richness estimators (Chao and Abundance-based Coverage Estimator (ACE)) and the diversity indices (Shannon and Simpson) were calculated. Principal component analysis (PCA) was employed to explore and visualize the similarities between sediment samples obtained from four seasons based on Bray–Curtis dissimilarity using the package Ape [28]. Canoco 5 software was used to carry out redundancy analysis (RDA) based on population abundance and environmental factors. The sediment samples with specific indicator groups of bacteria were found using LEfSe (Linear discriminant analysis Effect Size) [29]. Independent T-test and permutational multivariate analysis of variance were used to determine the differences that exist among different groups.

# 2.5. Accession Numbers

All of the sequencing data analyzed in this study can be downloaded from the NCBI's Sequence Read Archive using the accession numbers SRP314068 for samples from four seasons (120 samples) and five layers (30 samples) in lake wetlands.

#### 3. Result

### 3.1. Spatial Distribution of Physicochemical Parameters in Sediments

The physicochemical parameters in sediments varied by region, ass shown in Figure 2. In general, there was no significant change in the mean concentrations of TN, TP and OM in sediment samples from the lake wetlands over the four seasons. Relatively high levels of TN (4847 mg kg<sup>-1</sup>), TP (1697 mg kg<sup>-1</sup>) and OM (17.66%) in sediment samples from six typical sampling sites (3, 10, 14, 18, 19, 20) were found in summer (Figure 2).

### 3.2. Diversity Indices

A total of 6,234,741 reads were obtained after tag merge and quality control. Table 1 shows the diversity indices of the sediment samples from the four seasons. The sediment samples from summer and autumn had a greater diversity than those from the other seasons. The sediment samples in summer had the highest Chao (4825) and ACE (5276) indices, while the sediment samples in spring had the lowest Chao (4282) and ACE (5166) indices. The Shannon values of the sediment samples over the four seasons showed a similar trend in variation.

**Table 1.** Diversity indices of the sediment samples from the four seasons (A for spring, B for summer, C for autumn and D for winter).

6 I T	Diversity						
Sample Type	Chao	ACE	Shannon	Simpson			
А	$4282\pm34$	$5166\pm48$	$5.84\pm0.62$	$0.016\pm0.010$			
В	$4825\pm52$	$5276 \pm 112$	$6.19\pm0.74$	$0.014 \pm 0.004$			
С	$4581 \pm 101$	$5172\pm87$	$6.18\pm0.41$	$0.012\pm0.006$			
D	$4308\pm57$	$5271\pm68$	$6.09\pm0.58$	$0.010\pm0.007$			

Sediment samples from six typical sampling sites (3, 10, 14, 18, 19, 20) were chosen, and the samples from each site were divided into five layers (0–5, 5–10, 10–15, 15–20, 20–25 cm). Figure 3 shows the diversity indices of the sediment samples from different layers. The results indicated that most of the sediment samples from the surface layer (0–10 cm) had greater diversity (Chao, ACE, Shannon) than the sediment samples from the bottom layer (15–20 cm). The surface (0–5 cm) sediment samples from site 3 had the lowest Chao, ACE and Shannon values compared to the sediment samples from other layers, and the bottom sediment (20–25 cm) samples had the highest Simpson value (0.06). Sediment samples from site 18 had a greater diversity than the other sediment samples, and sediment samples from the 5–10 cm layer had the highest Chao (3765), ACE (3745) and Shannon (6.2).



**Figure 3.** The diversity indices of the sediment samples from six typical sites (3, 10, 14, 18, 19, 20) were divided into five layers (0–5, 5–10, 10–15, 15–20, 20–25 cm) in summer, respectively.

# 3.3. Temporal and Spatial Comparisons of Sediment Bacterial Community Based on Taxonomy

The taxonomic assignment results indicated that there were 22 phyla and 29 classes (total percentage > 95%) at the 97% sequence identity level (Figure 4). Figure 4a shows the main phyla with high relative abundance (>0.1%) in sediment samples from the four seasons. *Proteobacteria* had the highest relative abundance (43.15–57.41%) in the sediment samples from four seasons. At the phylum level, the bacterial community was also largely dominated by *Bacteroidetes* (12.14–18.08%), *Chloroflexi* (6.19–12.50%), *Acidobacteria* (2.94–4.71%), *Ignavibacteriae* (1.38–2.60%), *Nitrospirae* (1.82–3.68%) and *Firmicutes* (1.33–5.67%) and contained a small amount of, for example, *Chlorobi*, *Aminicenantes*, *Verrucomicrobia* and *Spirochaetae*. *Chloroflexi* was more abundant in the sediments from summer (9.3%) and autumn (12.5%), and the sediment samples from summer had the most abundant *Nitrospirae* (3.68%). The sediment samples from autumn also had more abundant *Firmicutes* (5.67%) and *Actinobacteria* (2.61%).

The changes in the bacterial community at the class level are shown in Figure 4b. *Betaproteobacteria* (14.98–23.45%), *Gammaproteobacteria* (11.98–14.36%), *Deltaproteobacteria* (8.68–14.45%), *Alphaproteobacteria* (1.18–4.04%) and *Epsilonproteobacteria* (0.70–1.64%) were the most abundant classes of *Proteobacteria*. Such classes as *Flavobacteria*, *Bacteroidia*, *Holophagae*, *Nitrospira* and *Anaerolineae* were also the main classes in the sediment samples from the four seasons. The sediment samples from winter and spring had more *Betaproteobacteria* (14.45%). *Nitrospira* in the sediment samples from summer was also more abundant (3.68%) than in the three other seasons.



**Figure 4.** Relative abundances of different phyla (**a**) and classes (**b**) in 120 sediment samples from four seasons (A for spring, B for summer, C for autumn and D for winter).

The bacterial composition of the six typical sediments in different layers is shown in Figure 5. *Proteobacteria* were still the most abundant (39.66–77.97%) phylum in the different layers of the sediments. The surface sediments (0–15 cm) in sites 14, 18 and 20 had more *Proteobacteria* than the bottom sediments (15–25 cm), while the bottom sediments (20–25 cm) had more *Proteobacteria* than the surface sediments (0–10 cm) in sites 3, 10 and 19. More *Chloroflexi* was found in the bottom sediments than in the surface sediments, and it was most abundant in sediments from the 10–25 cm layer (average value 10.42%). Sediments from the 0–5 cm layer had a greater abundance of *Bacteroidetes* than the other layers. *Firmicutes* in surface sediments (0–10 cm) from site 3 were in greater abundance than in the bottom sediments (10–25 cm), while the bottom sediments (15–25 cm) from site 10 had more *Firmicutes* than the other layers.



**Figure 5.** Relative abundances of different phyla in 30 sediment samples from six typical sites ((**a**) for site 3, (**b**) for site 10, (**c**) for site 14, (**d**) for site 18, (**e**) for site 19, (**f**) for site 20) divided into five layers (0–5, 5–10, 10–15, 15–20, 20–25 cm).

PCA was used to study the grouping of the sediment samples from the four seasons (Figure 6a) and six typical sediments from different layers (Figure 6b) according to their bacterial community structure. The first two PCA axes explained 87.9% of total variation in the microbial community structure. Bacterial communities in the sediment samples displayed seasonal clustering. Summer and autumn bacterial communities were gathered on the second and third quadrants, and communities from spring and winter appeared in the first and fourth quadrants. The first two PCA axes explained 86.2% of the total variation in the bacterial community structure of the six typical sediment samples from different layers (Figure 6b). The bacterial communities in the sediments from layers (0–15 cm) were gathered on the first, second and fourth quadrants, while the bacterial communities in the bottom sediments (15–25 cm) were gathered only on the third quadrant.



**Figure 6.** (a) Ordination plot showing grouping of sediment samples from the four seasons according to their bacterial community structure; (b) ordination plot showing grouping of sediment samples from different depths according to their bacterial community structure.

# 3.5. Redundancy Analysis

The RDA results for the relationship between bacterial community composition and environmental factors (physicochemical parameters) at the phylum level are shown in Figure 7. Samples were taken from different sediment layers in six typical areas: the interpretation rates of the first two RDA axes were 67.05% and 20.3%, respectively, and the cumulative explanation rate was 87.35%. OM, TP and TN were positively correlated with each other, but pH and DO showed a negative correlation. Some physicochemical parameters influence the bacterial community in lake wetland sediments. Monte Carlo variable tests and permutation experiments indicated that the environmental variable (TP) was statistically significant (p = 0.036, pseudo-F = 4.1) and an important determinant of bacterial community structure. *Aminicenantes* showed the highest positive correlation with TP concentration. In contrast, *Bacteroidetes* (p < 0.01) was negatively correlated with TP concentration (Table 2).



**Figure 7.** Redundancy analysis (RDA) of the bacterial communities as affected by environmental conditions and based on the relative abundance of dominant bacterial phyla (dots of different colors represent different depths).

**Table 2.** Pearson rank correlation analysis of sediment environmental factors with the abundance of the sediment bacterial community.

	ТР	TN	pН	ОМ	DO	Proteobacteria	Firmicutes	Bacteroidetes	Chloroflexi	Nitrospirae
TP	1	0.508 **	0.642 **	0.677 **	-0.051	-0.209	-0.033	-0.544 **	-0.194	-0.094
TN		1	0.1	0.046	-0.097	-0.095	0.262	-0.35	-0.171	0.066
pН			1	0.581 **	0.208	-0.018	-0.461 *	-0.286	0.007	0.099
ŌМ				1	0.410 *	-0.031	-0.027	-0.467 **	-0.167	-0.148
DO					1	0.242	0.037	-0.124	0.022	-0.172
Proteobacteri	ia					1	0.205	-0.067	-0.233	-0.185
Firmicutes							1	-0.058	-0.119	-0.398 *
Bacteroidetes	3							1	-0.119	0.133
Chloroflexi									1	0.177
Nitrospirae										1

\* Correlation is significant at the 0.05 level; \*\* Correlation is significant at the 0.01 level n = 30.

# 3.6. LEfSe Analysis Based on Community Abundance

Figure 8 shows that the bacterial communities dominant in spring were *Gammaproteobacteria* (the class and orders of *Oceanospirillales* and *Pseudomonadales*), *Betaproteobacteria* (the class and orders of *Hydrogenophilales* and *Nitrosomonadales*) and *Erysipelotrichia* (the class and order of *Erysipelotrichales*).

The bacterial lineages enriched in summer were *Bacteroidia*, *Deltaproteobacteria*, *Nitrospirae* (from phylum to family), *Ignavibacteria* (the class and order of *Ignavibacteriales*), *Elusimicrobia* (from phylum to order) and *Opitutales* (the class and order of *Opitutaceae*).

Eleven groups of bacteria were abundant in autumn: *Clostridia, Bacilli* (a class from *Firmicutes*), *Planctomycetacia* (from class to family), *Alphaproteobacteria* (the class and orders of *Rhodobacterales* and *Rhizobiales*), *Caldilineae* (the class and order of *Caldilineales*), *Anaerolineae* (from class to genus), *Flavobacteriia* (from class to genus), *Thermoleophilia, Coriobacterila, Actinobacteria* and *Verrucomicrobiae* (a class from *Verrucomicrobia*).

Nine groups of bacteria mainly belonged to five phyla (Latescibacteria, Cyanobacteria, Caldiserica, Aminicenantes and Spirochaetae) and four orders (Xanthomonadales, Arenicellales, Myxococcales and Desulfobacterales) in winter.





**Figure 8.** Cladograms showing the differences in relative abundance of bacterial communities in the four seasons according to the linear discriminant analysis effect size (LEfSe) analysis with LDA values of 3. Differences are represented by the color of the most abundant class (A for spring, B for summer, C for autumn and D for winter). The diameter of each circle is proportional to a taxon's abundance. The circles from inner region to outer region represent the phylogenetic levels from domain to genus.

# 4. Discussion

#### 4.1. Overview of the Study Area in the Yangtze River Delta Region, China

The study area was located in a typical plain river network in the Yangtze River Delta region, China. Industry and the economy are developed, and the value of GDP in this area is the highest in China. Exogenous pollution was one of the most significant sources of pollution in this area. Most pollutants were discharged into the lake wetland, and large amounts were deposited on the lake wetland sediments. The microbial communities in the sediment have a great influence on the pollutants [30,31]. There was considerable variation in the bacterial sediment communities, and they differed between seasons and layers.

#### 4.2. The Pollution Sources and Characteristics of the Study Area

The main sources of pollution were printing and dyeing industries, agriculture and livestock waste, especially around sites 14, 18, 19 and 20. There was frequent pollutant entrance and deposition in these areas, and the relative abundance of the bacterial communities in the sediment samples from these four sites was higher than in sediments from sites 3 and 10 [32,33]. The average concentrations of TN, TP and OM did not vary significantly in lake wetland sediments from different seasons. The TN, TP and OM in the different regions were higher in winter than in other seasons. This may have been because the low temperature in winter inhibited the activity of the bacterial communities. Reduced rainfall in winter also reduced the concentrations of TN, TP and OM through dilution and biological interception, which weakened the biological removal of TN, TP and OM [8,34].

### 4.3. The Differences in Sediment Bacterial Communities and Their Seasonal Effects

Most surface sediments in the plain river network area were from the deposition of the pollutants, and the sediments from some sites (such as 3 or 10) have been removed by dredging in recent years. The vertical diversity distribution results indicated that there was greater diversity in the bacterial community in surface sediment samples (Chao, ACE, Shannon) than in the bottom sediments. In addition, pollutant contents in the bottom sediments were low, and the bottom sediments were clearer than the surface sediments, which is one of the main reasons for greater diversity in the surface sediments. On the basis of the richness estimators (Chao and ACE) and the diversity indices (Shannon and Simpson), there were differences in the richness and diversity of the sediment bacterial communities in different seasons. The bacterial community diversity in summer was generally higher than in other seasons owing to the warm environment that favors the growth of microorganisms during this humid season [10].

# 4.4. The Influence of Bacterial Communities Was Due to Environmental Factors

*Proteobacteria* were commonly found to have the greatest relative abundance in sediments, and sedimentary degradation and metabolism mainly relied on this phylum [35]. *Proteobacteria* were also the most abundant phylum in all sediment layers and at greater abundance in the surface sediments than in the bottom sediments. RDA analysis showed that *Proteobacteria* were negatively correlated with the contents of other environmental variables (TP, TN, OM, DO, PH). *Firmicutes* predominated in some sediment sites (19); this can generate spores that endure dehydration and harsh environmental states [31].

LEfSe analysis showed that *Firmicutes* were enriched in autumn, and this may have been related to DO and TN levels. The phylum *Firmicutes* has the ability to perform denitrification processes under anaerobic conditions [36]. In the bottom sediments, *Chloroflexi* was abundant, while *Bacteroidetes* was the dominant phylum in the surface sediments. Members of Chloroflexi are facultative anaerobes and have been identified from freshwater sediments [37]. They can fit into the changing redox conditions of an aquifer and participate in the degradation of organics, and this action may lead to the low OM content in the sediment [24,38]. Bacteroidetes is widespread in a variety of environments, such as surface sediments [39–41]. It is thought to play a vital role in ecosystems because its members process complex molecules into simpler compounds in predominantly anaerobic habitats [32,42]. They are in relatively high concentrations in surface sediment owing to nearby sewage discharge into the lake wetlands, and some of the pollutants from the towns and cities have been deposited into the surface sediments [32]. *Nitrospira* is also an important phylum in the lake wetland sediments and was more abundant on the surface sediments than on the bottom sediments because it is largely dependent on higher oxygen availability [43]. The RDA result indicated that *Nitrospira* was negatively correlated with depth, confirming that the phylum prefers aerobic environments.

The 120 surface sediment samples collected in all four seasons and the 30 sediment samples from different layers were obtained in a plain river network in lake wetlands. Large amounts of pollutants were mixed, and some were deposited into the surface sediments [32]. The highest levels of TN (4847 mg kg<sup>-1</sup>), TP (1697 mg kg<sup>-1</sup>) and OM (17.66%), and the relative abundance of the bacterial communities in the sediment samples from sites 14, 18, 19 and 20, were higher than in sediments from sites 3 and 10. The results indicated that the bacterial communities were influenced by environmental factors and that environmental conditions in these areas were complex [44].

# 5. Conclusions

The mean pollutants in sediment samples from the lake wetlands did not change significantly over the four seasons. We found a clear difference in microbial diversity, with seasonal shifts, and this diversity was strongly related to sediment depth. We investigated the bacterial communities of sediments from lake wetlands in the plain river network area via a high-throughput sequencing method. *Proteobacteria* were the most abundant phylum

in sediments throughout the four seasons. The phylum *Chloroflexi* was more abundant in the bottom sediments, whereas in the surface sediments, the phylum *Bacteroidetes* was dominant. The higher the levels of TN (4847 mg kg<sup>-1</sup>), TP (1697 mg kg<sup>-1</sup>) and OM (17.66%), the higher the relative abundance of the bacterial communities in sediments. The physicochemical properties of sediments have a strong influence on the components of a bacterial community. The level of TP had a great influence on the structure of the bacterial community in sediments from lake wetlands, because TP is an important nutrient for living organisms.

**Author Contributions:** Y.C.: Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation). X.D. and X.Y.: Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data. H.C. and H.Y.: Provision of study materials, reagents, materials, laboratory samples, instrumentation, computing resources, or other analysis tools. W.H.: Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision, including pre- or post-publication stages. All authors have read and agreed to the published version of the manuscript.

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