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Trait–environmental relationships reveal microbial strategies of environmental adaptation

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Abstract

Microbial trait variation along environmental gradients is crucial to understanding their ecological adaptation mechanisms. With the increasing availability of microbial genomes, making full use of the genome-based traits to decipher their adaptation strategies becomes promising and urgent. Here, we examined microbial communities in water and sediments of 20 East African lakes with pH values ranging from 7.2 to 10.1 through taxonomic profiling and genome-centric metagenomics. We identified functional traits important for microbial adaptation to the stresses of alkalinity and salinity based on the significant trait–environment relationships (TERs), including those involved in cytoplasmic pH homeostasis, compatible solute accumulation, cell envelope modification, and energy requisition. By integrating these significant traits, we further developed an environmental adaptation index to quantify the species-level adaptive capacity for environmental stresses, such as high pH environments. The adaptation index of pH showed consistently significant positive relationships with species pH optima across regional and global genomic datasets from freshwater, marine, and soda lake ecosystems. The generality of the index for quantifying environmental adaptation was demonstrated by showing significant relationships with the species niche optima for the gradients of soil temperature and seawater salinity. These results highlight the importance of TERs in facilitating the inference of microbial genomic-based adaptation mechanisms and expand our understanding of ecological adaptive strategies along environmental gradients.

KEYWORDS

East African lakes, environmental adaptation, metagenome, microbial trait, trait–environment relationship

INTRODUCTION

Microbes inhabit nearly every habitat on Earth and play an important role in biogeochemical cycling as integral components of ecosystems (Gilbert et al., 2014). Biogeography

and metabolic capabilities of microbial communities are driven by multiple environmental gradients occurring naturally in ecosystems, including salinity, temperature, and pH. For instance, a decrease in microbial diversity with salinity has been consistently observed across soil

(Hollister et al., 2010; Rath et al., 2019) and aquatic ecosystems (Campbell & Kirchner, 2013; Mo et al., 2021; Tee et al., 2021). However, our understanding of functional mechanisms underlying diversity patterns along environmental gradients lags behind. Generally, environmental conditions affect the growth of microbes or their competition and select directly or indirectly against microbial lineages with variable adaptive capability to environmental changes (Cadotte & Tucker, 2017). Resolving the dynamics of species adaptation potential along environmental gradients will greatly improve our understanding of biogeography and habitat expansion for microbes.

The trait-based concept, which focuses on the fitness-influencing characteristics of individuals, is widely applied to identify the underlying mechanisms of community assembly and dynamics (Martiny et al., 2015; Wang et al., 2022; Yang, 2021). For instance, the traits inferred from microbial genomes or proteomes reflect a cascade of cellular and molecular regulation and potentially predict species ecological performance (Hu et al., 2021; Ren & Wang, 2021; Westoby et al., 2021) and their adaptive strategies for aridity (Li et al., 2022). Microbial traits are further used to explore their life history strategies, including the copiotroph–oligotroph (Fierer et al., 2007), the competitor–stress tolerator–ruderal (Ho et al., 2013; Krause et al., 2014), and the growth yield–resource acquisition–stress tolerance strategies (Malik et al., 2020).

How to identify the traits that are linked to microbial life strategies or their adaptive mechanisms remains a challenge. Trait–environment relationships (TERs), that is, how microbial traits relate to environments, are generally used to inform traits associated with environmental changes (Funk et al., 2017; McGill et al., 2006), enhancing our understanding of the functional dynamics of microbial communities along environmental gradients. Such traits identified through similar approaches could be utilized to inform environmental adaptation for microbes. For instance, the species' pH preference could be predicted through a model of the presence or absence patterns of the pH-associated traits, which are identified from representative genomes linked to conserved marker genes (Ramoneda et al., 2023). Even more complex microbial functions, including salinity preference and methanogenesis, are commonly summarized by a combination of multiple traits rather than captured by a single trait (Martiny et al., 2015). Therefore, how to define quantitatively microbial adaptation ability considering the joint effects of multiple functional traits is pivotal for predicting the responses of microbial communities to these environmental changes and understanding their implications at the global scale.

In this study, we examined the functional trait patterns of microbial communities in water and sediments

of African lakes along a pH gradient and developed a trait-based index to quantify species' capacity for environmental adaptation through a framework of three steps (Figure 1). Firstly, we determined the TER relationships between the community-weighted means of functional traits that are represented by KEGG orthologs (KOs) and lake pH by the effect size metrics, such as the correlation coefficients. Secondly, we identified the functional traits important for microbial adaptation based on the significant TERs and finally presented a novel index of a species to quantify how to adapt to an environmental stressor by calculating the mean value of the TERs across species traits weighted by the copy number of KOs. Our novel approaches and main findings in the study suggest that the utilization of functional traits inferred from microbial genomes not only moves beyond descriptive patterns in community composition but also improves our understanding of genetic mechanisms driving biogeographic distribution and their adaptation to global environmental change.

MATERIALS AND METHODS

Field sampling

We collected a total of 39 samples, including 19 water and 20 sediment samples, from 20 lakes and reservoirs in East Africa in February 2020 (Appendix S1: Figure S1). These lakes have a wide range of salinities (56 ~ 85,318 ppm) and pH (7.2 ~ 10.1). A more detailed description of the sampling sites is found in previous literature (Yao et al., 2022; Zhao et al., 2022). The total DNA of samples was extracted using the PowerSoil DNeasy Kit (QIAGEN, Germany) under sterile conditions and then subjected to metagenomic sequencing according to the manufacturer's protocol (Magigene, China). The information about sample collection, DNA extraction, and sequencing is detailed in Appendix S1: Supplementary Methods and Materials. The physicochemical parameters are shown in tab. S1 of Ren (2024).

Taxonomic profiling using metagenomic sequencing

Taxonomic profiling of microbial communities across samples was performed based on metagenomic sequencing using a custom pipeline established recently (Ren & Wang, 2022). Briefly, the raw metagenomic reads were cleaned, trimmed, and assembled into contigs for all samples. The metagenome-assembled genomes (MAGs) were finally recovered from each of these assemblies. Detailed information about sequence cleaning, assembly,

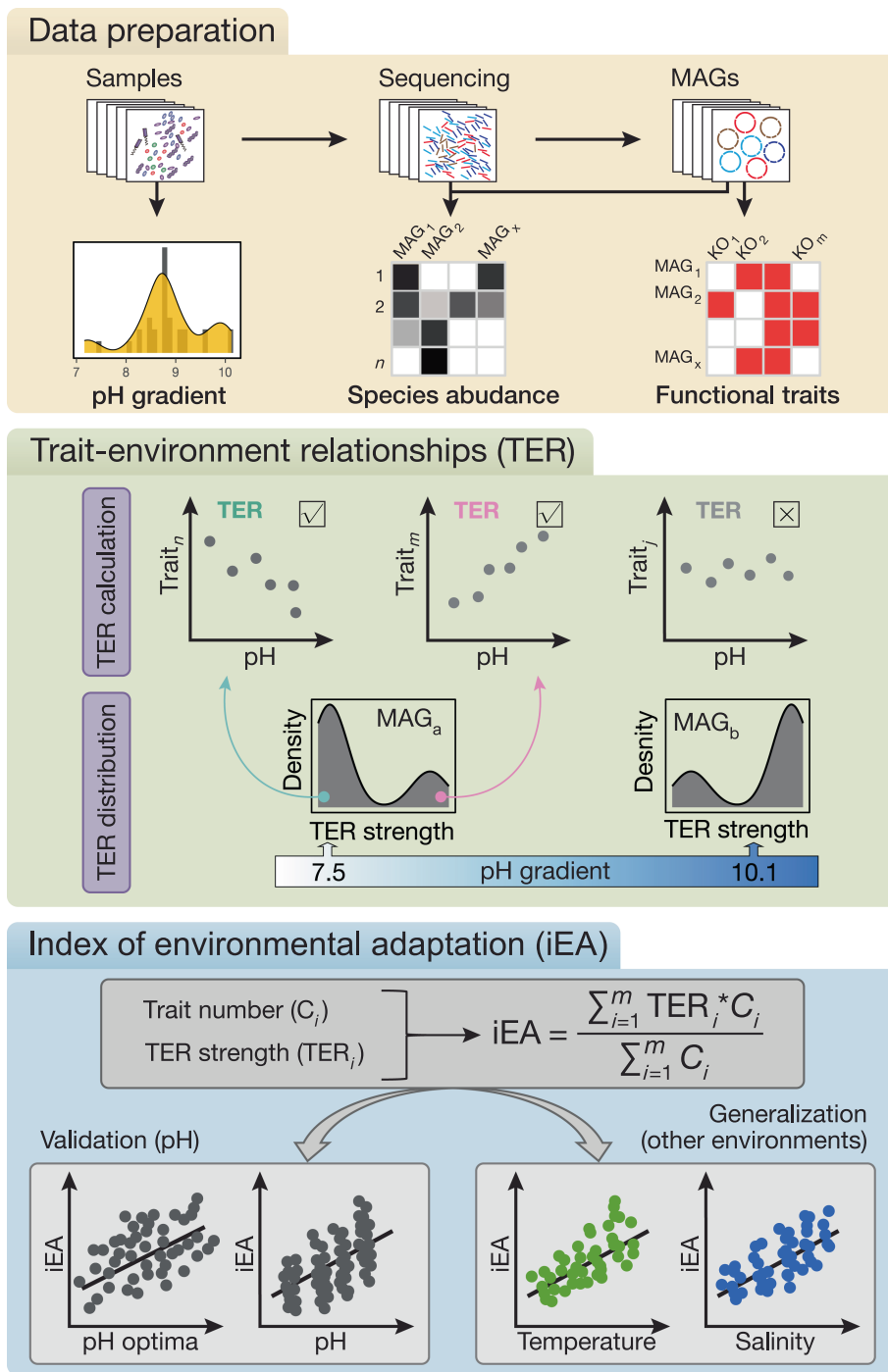


FIGURE 1 The overview of the trait-based framework in the study. The overflow of the framework consists of three modules, highlighted with rectangular boxes in distinct colors. At the top module, two data tables were prepared through taxonomic profiling and metagenomic-centric analyses, including the species abundance table and functional traits tables. At the middle module, we identified functional traits important for microbial adaptation through trait–environment relationships (TERs), and then presented an index of environmental adaptation to quantify species’ adaptive capacity to the environmental stressor at the bottom. TER was determined by the effect size metrics to represent the strength of the relationship between microbial traits and environmental stresses. The details about each step, see [Materials and methods](#).

and MAG recovery was provided in the Appendix S1: Supplementary Methods and Materials. The statistics regarding clean reads and metagenomic contigs for

39 samples are shown in tab. S2 of Ren (2024). The information about 1001 high-quality nonredundant MAGs is shown in tab. S3 of Ren (2024).

There were 2677 representative species identified from assembled contigs of all samples based on the conserved ribosomal protein *rpS3* using a modified pipeline (Diamond et al., 2019). The species here are defined as the representatives of ribosomal protein-coding *rpS3* genes that clustered at 99% similarity (Sharon et al., 2015). The abundance of these representative species in a sample was calculated as the total bases mapped on the representative sequence divided by the length of the representative sequence and the total number of sequences in the corresponding sample. The taxonomy for these species was determined by sequence alignment against a custom *rpS3* reference database, which was derived from the NCBI RefSeq prokaryotic genome database. The taxonomy was further validated and carefully corrected according to a phylogenetic tree of *rpS3* genes identified above. Detailed information about species identification, abundance estimation, and taxonomic assignment was provided in Appendix S1: Supplementary Methods and Materials.

The genomes for representative species were retrieved to obtain functional traits for microbes through the *rpS3*-containing sequence shared between the reconstructed MAGs and the *rpS3*-represented species, resulting in 647 representative genomes. The functional traits were represented as orthologous genes from the most commonly used annotation databases, such as KOs. The KO annotation links the orthologs to molecular-level functions and higher-level metabolic processes that provide a high resolution for the biochemical role of traits (Kanehisa et al., 2016). The protein-coding gene sequence of each representative genome was predicted by Prodigal v2.6.3 (Hyatt et al., 2010), then annotated against the eggNOG database v5.0 (Huerta-Cepas et al., 2018) using eggNOG mapper v2.1.6 with DIAMOND as the seed ortholog search engine (Cantalapiedra et al., 2021). The assignments of KOs for each species were retrieved from the annotation results of eggNOG mapper using custom Perl scripts.

Development of the functional trait-based framework

A trait-based framework was developed to qualify the species' capacity to adapt to environmental stresses by considering its key functional traits and their relationships with environments. There were three primary steps in the framework, which were illustrated based on the microbial communities in African lakes along a pH gradient as below. Three dataset tables were used in the following analyses: (1) the species abundance table with 2677 species from 39 samples; (2) a list of environmental factors for 39 samples; (3) the functional traits represented by KOs for 647 representative species with

genomes available. All statistical analyses were performed in the R language v4.1.1.

Firstly, the TERs were assessed by the fourth-corner method. Since last century, a variety of statistical tests have been developed to assess the TERs (Grime, 1974), including the species-level and community-level approaches (Kleyer et al., 2012; Lepš & Bello, 2023). Among these methods, the fourth-corner method represents one of the most integrated and efficient methods to analyze the TER. The method combines the three tables (i.e., the traits of the species, species abundance across sites, and environment variables in sites) into a matrix describing the TER, and provides parameters to measure the TER and permutation procedures to evaluate the significance of these relationships (Dray & Legendre, 2008; Legendre et al., 1997). The method with the row- and column-based permutation test strategy is proposed to control for the potential Type I error rates (Peres-Neto et al., 2017), which was implemented by the “modeltype = 6” in the function “fourthcorner” in the “ade4” package v1.7-22. We performed the trait and environment analysis with 49,999 permutations and adjusted the *p* values for multiple testing using the false discovery rate method. The correlation coefficient in the “D2” in the output of the function indicates the strength of the association between the KO-represented trait and pH. Further discussion about the potential effect of the nonrandom KOs on the TER is provided in Appendix S1: Supplementary Material.

Second, potential adaptive mechanisms were inferred by the genomic and functional traits significantly correlated with pH. The functional traits important for microbial adaptation were identified based on the significant TERs. The important roles of selected traits in mediating species adaptation to environmental stresses were confirmed by the consistency of these functions with expert opinions or current knowledge in the literature (tab. S4 in Ren, 2024). Further inspection of the other significant functional traits with curated annotation was applied to provide novel insights into putatively adaptive mechanisms but rarely reported in previous literature.

Finally, we presented an index of environmental adaptation (iEA) to quantify the species' adaptive capacity for environmental stresses by considering its key traits and their relationships with environments (TERs). The

iEA is calculated using the formula
$$iEA = \frac{\sum_{ko=1}^n P_{ko} \times C_{ko}}{TC},$$

where P_{ko} is the coefficient of Spearman rank correlation between pH and a functional trait represented by KO; C_{ko} , which is the copy number of the KO, and TC, which is the total count of KO copy number in the species. The extent of the iEA to the species optimum niche was accessed by the relationship between the index of a species and its pH optima through a linear regression model.

The pH optima of an individual could reflect the optimum niche where the species is expected to be most abundant and was calculated based on its abundance across samples and lake pH of samples where the species was identified.

The formula is shown as $\text{pH optima} = \frac{\sum_i \text{Abund} \times \text{pH}}{\sum_i \text{Abund}}$, where “Abund” is the abundance of the species in sample i and “pH” is the pH of sample i .

Validation and generalization of the index

The utility of the adaptation index for microbial species was validated using three independent genome datasets with explicit pH gradients. (1) The first one is a set of 345 MAGs, which were reconstructed in the study but not included in any of the data analyses above. (2) The second dataset consists of 530, 464, and 606 species-level MAGs from freshwater, marine, and alkaline habitats, respectively. (3) The third dataset contains 830 high-quality species-level MAGs from freshwater and soda lakes with pH values available. The accession number and relevant environmental information of the last two datasets are shown in tab. S6 and S7 in Ren (2024). Detailed information about these three genomic datasets was provided in the Appendix S1: Supplementary Methods and Materials.

The generality of the framework for other environmental gradients was examined by two sets of metagenomic datasets along salinity and temperature gradients. The first dataset contains 12 soil samples collected from a slow-burning coal-seam fire around Pennsylvania (USA), with soil temperatures ranging from 13.3 to 54.2°C (Sorensen et al., 2019). The second dataset comprises 10 surface marine water samples followed by a 0.2- μm filtration, from the Baltic Sea with salinities ranging from 2440 to 28,100 ppm (Alneberg et al., 2018). The procedures for performing sequence analysis and the trait-based framework were the same as in the case of the African lake microbial community along a pH gradient above. The accession number and relevant metadata for the samples in both gradient datasets are shown in tab. S8 in Ren (2024).

RESULTS

Multilevel adaptive strategies revealed by the trait–environmental relationships

Before investigating how functional traits relate to the pH gradient, we identified pH as an important environmental driver of the lake microbiomes based on the

following analyses. (1) The Mantel test analysis indicated that microbial communities were significantly correlated with the difference in lake pH, with the highest Mantel r value of 0.35 and 0.34 for water and sediment communities, respectively ($p < 0.0001$, Mantel test, Appendix S1: Figure S2a). (2) The random forest model result revealed that pH was the most important among measured environmental variables for the Shannon diversity of water communities and water temperature for sediment communities (Appendix S1: Figure S2b).

We identified the functional traits important for microbial adaptation based on the significant relationships between the community-weighted means of traits and lake pH, that is, the TERs (Table 1, see the details in *Materials and methods*). Briefly, the TERs were examined with the fourth-corner method (Dray & Legendre, 2008; Legendre et al., 1997), and the significance was tested with a row- and column-based permutation strategy to control for inflated Type I error in the statistical analyses of TERs (Peres-Neto et al., 2017). We found that there were 170 significantly pH-associated functional traits represented by KOs, including 40 KOs for planktonic microbes, 120 KOs for benthic microbes, and 10 KOs shared by both communities (Figure 2a; tab. S4 in Ren, 2024). These KOs accounted for 2.58% of all 6600 KOs identified from all 647 representative genomes from African lakes, while the remaining 6430 KOs were not significantly correlated with pH. When inspecting the distribution of these genes across individuals, the number of pH-related genes ranged from one to 126 per species, with a median value of 137.

Based on the significant TERs, we further identified two fundamental properties for each functional trait: strength and sign. Specifically, the strength of TER is represented by the absolute value of the correlation coefficient and indicates the extent to which the functional trait contributes to microbial adaptation to pH stress. For instance, a TER value of 1 indicates a constant increase or decrease of traits at the community level along a pH gradient, while 0 indicates no variations. The signs of TER inform whether the trait is for or against pH stress, and the positive and negative correlation coefficients indicate the alkaline-tolerant and alkaline-sensitive traits, respectively.

Among the 170 functional traits, there were 161 alkaline-tolerant traits with their TER strength ranging from 0.221 to 0.516, 19 alkaline-sensitive traits with their strength ranging from 0.219 to 0.393, and two traits with contrasting TER signs between water and sediments (Figure 2a; tab. S4 in Ren, 2024). Among these functional traits, there were at least 38 KO-represented traits (tab. S5 in Ren, 2024), which are well known for their involvement in microbial pH adaptation (Banciu & Muntyan, 2015; Sorokin et al., 2014). These traits could be grouped into

TABLE 1 Glossary of the relevant terms referred to in the study.

Terms	Explanation
Environmental adaptation	The adjustment of an organism's features in order to enhance its survival in specific environments, which have been shaped by evolutionary forces.
Functional traits	The characteristics that affect the fitness of a microorganism under certain environmental conditions. They could be defined as orthologous gene families encoded by microbial genomes, such as KEGG orthologs (KOs).
Community-weighted mean (CWM)	A community-level aggregated measure of traits, reflecting mean features of microbial communities through combining the information of species traits and their relative abundance.
Trait–environment relationship (TER)	TER describes the relationship between the community-weighted mean values of traits and environmental conditions. For a functional trait, the strength and sign of TER are determined by effect size metrics, such as Spearman rank correlation coefficient between a community-weighted mean of the trait and a major environmental factor. The strength of TER is represented by the absolute value of correlation coefficient, whereas the sign of TER informs whether the trait is for or against environmental stress.
Index of environmental adaptation (iEA)	The iEA is to quantify a microbial species' capacity to adapt to an environmental stressor by considering its key traits and their relationships with the environment (that is, TER). The iEA is calculated as the mean value of the TERs across species traits weighted by the copy number of KOs.

four main strategies, including cytoplasmic pH homeostasis, cell envelope modification, compatible solute accumulation, and energy acquisition. For example, eight functional traits associated with cytoplasmic pH homeostasis showed significant positive relationships with pH, including the Mrp-type $\text{Na}^+:\text{H}^+$ antiporter (*mrpABCDEFG*) and the Na^+

symporters. There were at least seven well-annotated traits relevant to the biosynthesis and uptake of compatible solutes showing significant positive relationships with pH ($p < 0.05$), including the biosynthesis of trehalose (i.e., *treP*) and transport systems such as the osmoprotectant ABC transport system (*opuABC*) and glycine betaine/proline ABC transport system (*proV*).

A TER-based index to quantify how microbes adapt to environmental stress

We further presented an index of environmental adaptation (iEA) to quantify the adaptive capacity of a species to environmental stress by considering its key traits and their relationships with environments (Figure 1, Table 1). Specifically, the iEA is calculated as the mean value of the TERs across the species' traits weighted by the copy number of KOs (see *Materials and methods*). The index is a unitless metric regardless of environmental factors considered and has values ranging from -1 to 1 . Using lake pH as an example, a species with a higher iEA has stronger adaptation capacity for high pH environments, whereas a lower value of iEA indicates that the species is more sensitive to high pH. Positive and negative iEA values indicate that a microbial species is dominated by alkaline-tolerant and alkaline-sensitive traits, respectively, while an iEA of zero suggests the two kinds of traits equally dominate. More details about the calculation of the iEA were provided in *Materials and methods*.

For the microbes in African lakes, the iEAs of pH had considerable variations ranging from -0.393 to 0.431 across habitats and phyla. The microbes had lower iEA values in water (0.125 ± 0.157) than in sediments (0.151 ± 0.170 ; Wilcoxon rank sum test, $W = 52,842$, $p = 1.03e-3$), although the species' iEAs of pH showed similar ranges in both habitats. Moreover, the iEAs of pH showed distinct distributions across phyla in lakes, such as the phylum Bacteroidetes with 0.212 ± 0.077 and Betaproteobacteria with -0.071 ± 0.152 (Appendix S1: Figure S3a). The changes in the species' iEAs of pH across habitats and phyla are likely associated with the differences in trait number and TER strength of species, which could reflect their distinct lifestyles across habitats and evolutionary histories among phyla.

Interestingly, we found that the species' iEAs of pH were significantly positively correlated with their pH optima across the water and sediments with R^2_{adj} values of 0.413 and 0.183 , respectively (Figure 2c). These correlations confirmed that the iEA could quantitatively represent species adaptive capacities to the environmental gradient (i.e., pH). The ecological significance of these correlations could be explained by a clear reorganization of

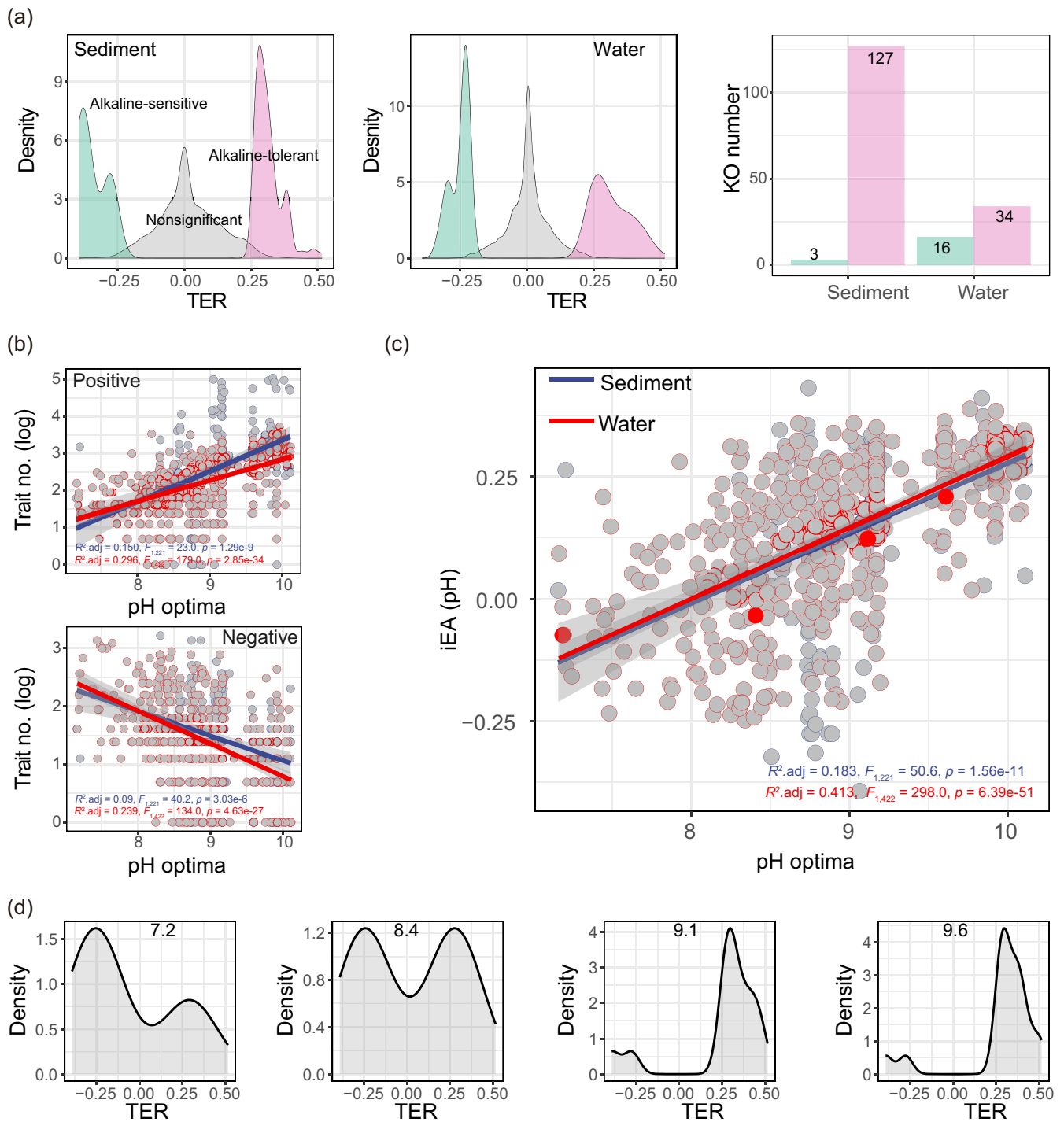


FIGURE 2 The index of environmental adaptation (iEA) and their associations with species niche optimum. (a) The distribution of trait–environment relationship (TER) for all functional traits across distinct habitats. Functional traits were classified into three groups based on the significance of TERs as shown in the plot. TERs were tested by the Spearman correlation test followed by the row- and column-based permutation test. (b) The number of alkaline-sensitive traits occurring in species decreased with their pH optima, and the number of alkaline-tolerant traits increased with their pH optima. (c) The relationships between the species’ iEA index and pH optima across water and sediments were fitted using a linear regression model. (d) Four species from the phylum Chloroflexi were selected to illustrate the reorganization of alkaline-tolerant or alkaline-sensitive traits in microbial species for their adaptation to high pH stress.

alkaline-tolerant or alkaline-sensitive traits in microbial species for their adaptation to high pH stress, which were illustrated by the distribution of functional traits (i.e., their

signs and strengths) in the four species selected from the phylum Chloroflexi along the pH optima gradient (Figure 2d). For instance, the species with the lowest and

highest pH optima values of 7.2 and 9.6 had iEA values of -0.074 and 0.202 , respectively, and expectedly showed contrasting percentages of alkaline-tolerant traits among all traits that were significantly associated with lake pH. The former species contained 11 alkaline-tolerant traits, whereas the latter had 32, nearly three times as many. Consistently, Chloroflexi species with lower iEA values are predominantly found in neutral pH conditions, such as aquifer sediments or deep lakes (Hug et al., 2016; Mehrshad et al., 2018), whereas species with higher iEA values are reported in alkaline hot springs (Hanada et al., 2002; Meer et al., 2010). These examples highlight the important roles of reorganizing distinct traits (i.e., alkaline-tolerant and alkaline-sensitive) and their TER strength for environmental adaptation along gradients.

Further, the species' iEAs of pH were also correlated with their pH optima across nine dominant phyla, including Verrucomicrobia and Gammaproteobacteria. For instance, the bacteria in the phylum Verrucomicrobia were significantly associated with pH optima and showed the highest explained variation with an R^2_{adj} of 0.689 ($F_{1,23} = 54.3$, $p = 1.72\text{e-}7$), whereas Deltaproteobacteria had the lowest explained variation with an R^2_{adj} of 0.133 ($F_{1,21} = 4.37$, $p = 4.89\text{e-}2$, Appendix S1: Figure S3b). These differences in iEA values and their explained variation in pH optima among phyla are likely explained by their preferring adaptation strategies associated with distinct functional traits. These results not only suggest that the species' iEA of pH appears to be phylogenetically conserved (Martiny et al., 2015), but also provide additional evidence for the quantification of species environmental adaptation using iEA at the broad level of taxonomic classification.

It should be noted that the number of traits in microbes was also found to be important for their pH optima, but the inclusion of the TER strength in iEA could enhance its predictive power for species niche optima more than trait number. We found that species with more alkaline-tolerant traits exhibited higher pH optima, whereas species harboring more alkaline-sensitive traits had relatively lower pH optima (Figure 2b). Specifically, the number of alkaline-tolerant traits that each species carries was positively correlated with their pH optima across water and sediments, with R^2_{adj} values of 0.296 and 0.150, respectively (Figure 2b). Conversely, the number of alkaline-sensitive traits was negatively correlated with pH optima in water and sediments, with R^2_{adj} values of 0.239 and 0.090, respectively (Figure 2b). However, the trait number performed relatively worse in explaining the variation in pH optima compared with the iEA, which considers TER strength, highlighting the important role of TER strength in predicting the species' niche optima.

Validating the utility and generality of the index of environmental adaptation

To validate the utility of the iEA and demonstrate the independence of iEA on pH, we applied it to three genome datasets with explicit pH gradients at both regional and global scales (tab. S6 and S7 in Ren, 2024). The first dataset was the redundant genomes ($n = 345$) reconstructed from the same metagenomes of the African lakes as the representative genomes (647), which were not used in any previous analyses, including the KO identification and CWM calculation. When we calculated the iEA for these redundant genomes based on the selected KOs (972) and their relevant TER values, we found that the iEA still correlated with the pH values of the samples in which these species were present (Figure 3a). The second dataset consisted of 1600 genomes from global freshwater, marine, and soda lakes (Zhao et al., 2020). Based on the same set of selected KOs, we calculated the iEA for these independent genomes and found that the iEA for the species in soda lakes was significantly higher than that of other aquatic environments (Figure 3b). The final genome dataset was retrieved from lakes with pH values ranging from 4.1 to 10.3 (Buck et al., 2021). Based on the selected KOs, we found that the iEA values for these genomes also correlated with lake pH with an R^2_{adj} of 0.298 (Figure 3c).

To further support the generality of this index, we extended the approach to two additional environmental gradients (tab. S8 in Ren, 2024), namely a salinity gradient ranging from 2.44 to 28.1 ppt in a seawater dataset (Alneberg et al., 2018) and a temperature gradient ranging from 13.3 to 54.2°C in fire-affected soil data (Sorensen et al., 2019). On the one hand, functional traits significantly associated with temperature or salinity were identified using our approach, including those known in original studies and the novel traits that were rarely discussed previously. For instance, the abundance of functional traits involved in sulfur metabolism increased toward hot soils, whereas the abundance of traits involved in two-component regulatory systems decreased, which is consistent with previous findings (Sorensen et al., 2019). The novel traits enriched in thermophiles in hot soils, including the CRISPR-associated protein that provides adaptive immunity against phages in thermophilic bacteria (Munson-McGee et al., 2018; Paez-Espino et al., 2015) and spore biogenesis and germination, may also play key roles in the resistance of mesophiles and/or thermophiles to thermal stress (Huang & Hull, 2017; Ursem et al., 2021). On the other hand, the species' iEA of salinity and temperature also showed positive relationships with their corresponding niche optima, with an R^2_{adj} of 0.283 for the seawater salinity gradient (Figure 3d), and R^2_{adj} values of

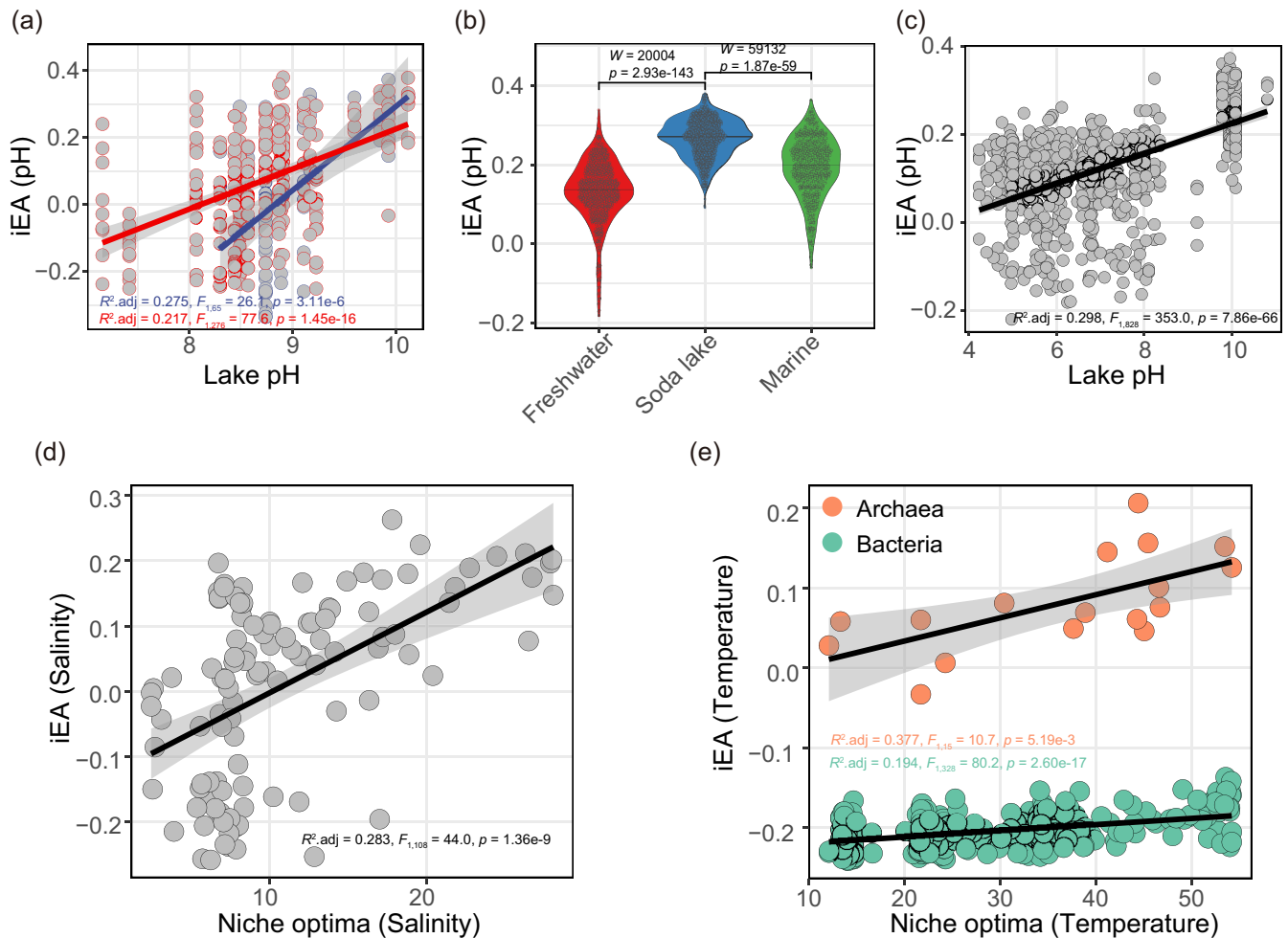


FIGURE 3 The validation and generalization of strong associations between the species' index of environmental adaptation (iEA) and their niche optimum. The relationships between the species' iEA index and pH optima or lake pH were validated across three regional (a) or global genomic datasets (b, c). The generality of the species adaptation index across a soil temperature gradient (d) and a marine salinity gradient (e). The iEA of temperature for soil bacteria and archaea was plotted separately against niche optima. The calculation of species' iEA in (a–c) was the same as that for pH, using the same trait–environment relationship (TER) strength and sign derived from the East African lake, whereas the calculation of species' iEA of two gradients (d, e) was the same as that for pH in the study. The relationship between the adaptation index and niche optimal was fitted using a linear regression model (a, c–e), and the significance of the difference in the index across habitats was tested by the Wilcoxon signed rank test (b). The complete statistical reporting for these tests was shown on each subpanel. Detailed information about three validation genomic datasets of a pH gradient is in tab. S6 and S7 in Ren (2024), and detailed information about two datasets of salinity and temperature gradients is in tab. S8 in Ren (2024).

0.194 and 0.377 for soil bacteria and archaea along the soil temperature gradient, respectively (Figure 3e). Collectively, these consistent relationships between the indices and niche optima across multiple environmental factors indicate that we could potentially extend the index to quantify species' adaptation capacity along any environmental gradients of interest.

DISCUSSION

Our study presented a novel index to quantify microbial adaptive capacity to environmental stresses by

integrating both the strength and direction of the relationships between microbial functional traits and the driving environment. The trait-based index of environmental adaptation has important implications for predicting taxon-specific biogeographic distribution and its response to global climate change, given the high proportion of uncultured species (Steen et al., 2019) and the increasing number of available microbial genomes.

The adaptation index has a robust ability to evaluate species' adaptive capacity for environmental stress, as shown by its consistent associations with species niche optima or environments across freshwater, marine, and

soil ecosystems. The evaluation of species' adaptive capacity based on the index could help guide how to optimize media formulations for uncultivated taxa with the increasing availability of genomic data (Lewis et al., 2021). The index could be used as a quantitative trait of microorganisms to complement their limited phenotypic traits (Wang et al., 2022; Westoby et al., 2021) and be further incorporated as key species attributes in meta-analyses or modeling across ecosystems (Allison, 2012; Barberán et al., 2014).

The adaptation index could be generalized to typical environmental factors across ecosystems, such as pH, temperature, and salinity, as shown in the study. Because the index is a unitless and standardized metric, with its values ranging from -1 to 1 , regardless of environmental factors, we could easily compare the indices concerning a single environment or even the component of multiple environmental factors (i.e., PCA) within a study. Taking the microbes in the African lake as an example, we calculated the indices of all other environmental factors for the species in the same way as for pH. As expected, these indices showed distinct distributions within a constrained range (Appendix S1: Figure S4a) and were significantly associated with the species niche optima of the corresponding factor (Appendix S1: Figure S4b). Interestingly, we found that, compared with other iEA indices, the index of pH had the highest correlation with species pH optima, showing an R^2_{adj} of 0.332 (Appendix S1: Figure S4c). The results demonstrate the generality of the index for various environmental variables and also confirm the major role of pH in shaping taxonomic and functional compositions of microbial communities in the African lakes.

We further expect that the establishment of a quantitative environment-associated trait database could be helpful in exploring microbial adaptation capacities along environmental gradients across different ecosystems. The functional traits, represented by KOs and their relationships with the driving environmental factors, could be generally transferred to a wide range of studies to quantify microbial adaptive capacity across spatial scales. This was demonstrated by our three independent validation datasets, in which the iEA indices of pH were calculated using the TERs derived from the African lakes with a pH gradient. With the increasing number of ecologically relevant traits from microbial genome sequences (Cébron et al., 2021; Karaoz & Brodie, 2022; Madin et al., 2020), we expect that the efforts to establish a database of functional traits with both strength and signs in their relationships with typical environments, such as temperature and nutrients, would facilitate evaluating the independent or additive responses of the species to global climate changes.

AUTHOR CONTRIBUTIONS

The research was conceived by Jianjun Wang. Sample collection was performed by Lu Zhang, Xiaolong Yao, and Jianjun Wang. Data analyses and drafting of the manuscript were undertaken by Minglei Ren with the contribution of Jianjun Wang. All authors were involved in the discussion and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequence data, including metagenomic reads for all samples and high-quality MAGs, are available in the National Omics Data Encyclopedia (NODE) database under accession number OEP00003641 at <https://www.biosino.org/node/project/detail/OEP00003641> and in the National Center for Biotechnology Information (NCBI) BioProject database under accession number PRJNA857294 at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA857294>. Code, scripts, and tables with supplementary data (Ren, 2024) are available in Figshare at <https://doi.org/10.6084/m9.figshare.24196512.v2>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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