# nature portfolio

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## **Reporting Summary**

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	$\boxtimes$	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	$\boxtimes$	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	$\boxtimes$	The statistical test(s) used AND whether they are one- or two-sided  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	$\boxtimes$	A description of all covariates tested
	$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	$\boxtimes$	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	$\boxtimes$	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	$\boxtimes$	Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated
	'	Our web collection on statistics for biologists contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

No software was used in the data collection process. The collection of leaf samples from the mangrove sites was done manually, following standardized field sampling protocols.

Data analysis

QIIME2 (version 2022.2) was used for processing and analyzing the 16S rRNA gene sequencing data, including quality filtering, operational taxonomic unit (OTU) clustering, and taxonomic assignment.

R (version 4.1.2), an open-source statistical software, was used for statistical analyses, including diversity indices calculation and correlation analysis with environmental factors.

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States): Used for predicting functional profiles of bacterial communities.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The sequence data generated during this study are available in GenBank under the BioProject Accession number PRJNA1130664. The source data for plotting figures and tables can be archived in the supplementary material (Supplementary Data 1.xlsx). Climate variables can be obtained from the China Meteorological Data Sharing Service System (https://data.cma.cn).

#### Research involving human participants, their data, or biological material

Policy information about studies with human participants	<u>or human data</u> . S	See also policy	information	about sex,	gender (	identity/	<u>presentatio</u>	n),
and sexual orientation and race, ethnicity and racism.								

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	NVA
Recruitment	N\A
Ethics oversight	N\A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for	your research. If you are not sure,	read the appropriate sections before	making your selection.

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Behavioural & social sciences

## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Life sciences

This study investigated the ecological dynamics of generalist and specialist bacteria in the leaf endosphere of Kandelia obovata mangrove plants across coastal wetlands in China. Researchers collected 250 leaf samples from 10 sites spanning a wide geographical range, and used high-throughput 16S rRNA gene sequencing to characterize bacterial communities. Environmental factors, including climate variables and soil properties, were measured at each site. The study compared generalist and specialist bacteria in terms of community composition, diversity, responses to environmental factors (especially temperature), assembly processes, and predicted functional profiles. This design allowed for examination of biogeographical patterns and environmental responses of leaf endophytic bacterial communities across a large spatial scale, with a particular focus on comparing the ecological dynamics of generalist versus specialist taxa.

Ecological, evolutionary & environmental sciences

Research sample

The research sample consisted of leaf samples from Kandelia obovata, a common mangrove species in China. These samples were selected to represent different geographical locations, ensuring a comprehensive study of microbial diversity in varying environmental conditions. The plants sampled were naturally occurring, and no manipulations were performed on them.

Sampling strategy

Leaf samples were collected from 250 Kandelia obovata individuals across 10 different coastal mangrove wetlands. No specific sample-size calculation was performed, but the selection was aimed at capturing a broad range of environmental conditions across the study sites. This sample size was considered sufficient to provide statistically meaningful insights into the bacterial communities.

Data collection

Data collection involved the careful sampling of leaf material from each location. The leaves were surface-sterilized to remove external microbes, and the endophytic bacterial DNA was extracted for sequencing. The data collection was carried out by the research team in a standardized manner to ensure consistency across all samples.

Timing and spatial scale

All samples were collected on 2019.08.15-16. The study spanned coastal mangrove wetlands from 18.44 to 28.35°N and 108.24 to 121.18°E across China.

Data exclusions	No data were excluded from the analyses
Reproducibility	The study employed standardized and widely accepted methods for DNA extraction, sequencing, and bioinformatics analysis. These protocols ensure that the findings are reproducible by other researchers using similar techniques and sample types.
Randomization	The selection of sampling sites and plots was designed to capture natural environmental variability across a wide latitudinal gradient, rather than being strictly randomized. Within each region, five $5 \times 5$ m² plots were established to ensure comprehensive coverage of Kandelia obovata populations. The latitudinal transect spans nearly 10 degrees, enabling the study of thermal sensitivity across a diverse range of climatic conditions, from tropical to subtropical zones. The southernmost point (18.44°N) represents the tropical mangrove ecosystems of Hainan Island, while the northernmost boundary (28.35°N) in Fujian Province marks the species' upper latitudinal limit, approaching its thermal tolerance threshold. A total of 250 individual leaf samples of K. obovata were collected.
Blinding	Blinding was not applicable in this study, as the data collection and analysis were conducted by the same research team. The nature of the study did not involve interventions or treatments that required blinding, as the focus was on naturally occurring bacterial communities in the leaf endosphere of mangroves.
Did the study involve	

### Field work, collection and transport

Field conditions	The fieldwork was conducted in coastal mangrove wetlands across different regions of China. The regions varied in environmental conditions, including temperature and rainfall. The study sites are known for their tropical and subtropical climates, with considerable variation in temperature and humidity depending on the location and season. The mean annual temperature, rainfall, and other climatic factors like humidity were assessed as part of the environmental factors influencing microbial communities.
Location	The sampling was conducted at ten different coastal mangrove wetlands across China. The study covered a range of latitudes from 18.44°N to 28.35°N and longitudes from 108.24°E to 121.18°E.

Access & import/export

Permits were obtained from relevant authorities to conduct research in the mangrovThe specific permits were issued by Guangdong Provincial Forestry Bureau. These permits covered the collection, transport of the biological samples. These permits ensured that the collection of leaf samples was authorized and aligned with conservation efforts.

Disturbance

The study was designed to minimize disturbance to the natural habitat. The collection process involved taking leaf samples, which is a minimally invasive procedure. No significant disturbance to the mangrove ecosystems was caused as the sampling was carefully conducted to avoid damage to the plants and surrounding environment.

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Ma	terials & experimental systems	Me	thods
n/a	Involved in the study	n/a	Involved in the study
$\boxtimes$	Antibodies	$\boxtimes$	ChIP-seq
$\boxtimes$	Eukaryotic cell lines	$\boxtimes$	Flow cytometry
$\boxtimes$	Palaeontology and archaeology	$\boxtimes$	MRI-based neuroimaging
$\boxtimes$	Animals and other organisms		
$\boxtimes$	Clinical data		
$\boxtimes$	Dual use research of concern		
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#### **Plants**

Seed stocks

Novel plant genotypes

The plant material used in this study consisted of leaf samples from Kandelia obovata, a mangrove species. These samples were collected from various coastal mangrove wetlands across China, specifically between the geographical coordinates of 18.44°N to 28.35°N latitude and 108.24°E to 121.18°E longitude. Surface sterilization was performed immediately after collection to eliminate had surface microbes, ensuring that only endophytic bacteria were analyzed. The samples were collected and stored in sterile polyethylene bags and transported in ice-packed coolers to maintain freshness until further processing.

Authentication

N/A			