

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 [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a Confirmed
- ☒

☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒

☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒

☐ 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.
- ☒

☐ A description of all covariates tested
- ☒

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☒

☐ 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)
- ☒

☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted
Give P values as exact values whenever suitable.
- ☒

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒

☐ 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 custom software or algorithms were developed or used for data collection in this study. Image acquisition was performed using manufacturer-provided microscope control software.
Data analysis	No custom code was used for data analysis. Image processing and visualization were conducted using standard, commercially available software.

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](#)

All data generated or analysed during this study are included in this published article. Additional raw SEM image data are available from the corresponding author upon reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	This study does not involve human participants; therefore, reporting on sex and gender is not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	This study does not involve human participants, and no analyses of race, ethnicity, or other socially relevant groupings were conducted.
Population characteristics	No human population characteristics were assessed in this study.
Recruitment	No human participants were recruited for this study.
Ethics oversight	Ethics approval was not required for this study as it did not involve human participants, human data, or human biological materials.

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.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

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

Study description	This study describes the development and application of a biological cross-sectional polishing scanning electron microscopy (bioCP-SEM) method to visualize intact rhizosphere structures, including plant roots, soil particles, and associated microorganisms, under natural soil conditions.
Research sample	Research samples consisted of resin-embedded plant root–soil blocks prepared from <i>Arabidopsis thaliana</i> grown in unsterilized soil and wild-collected plant root–soil systems.
Sampling strategy	Samples were selected to preserve intact root–soil–microbe associations suitable for cross-sectional polishing and electron microscopy. Multiple independent biological samples were prepared and examined to confirm the reproducibility of observed structural features.
Data collection	Data were collected as backscattered electron images acquired from polished resin-embedded cross sections using scanning electron microscopy. Representative regions showing characteristic rhizosphere structures were imaged and documented.
Timing and spatial scale	Sample preparation and imaging were conducted after plant growth under natural soil conditions. Imaging covered spatial scales ranging from millimetre-scale root–soil interfaces to submicrometre-scale cellular and microbial structures.
Data exclusions	No data were excluded from this study.
Reproducibility	Key observations were reproduced across multiple independently prepared samples and experimental replicates, confirming the robustness of the bioCP-SEM method.
Randomization	Randomization was not applicable, as this study is based on observational imaging of prepared samples rather than comparative experimental group assignments.
Blinding	Blinding was not applicable, as no subjective outcome assessment or group comparison was performed.
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Field samples were collected within the premises of the authors' research institute under ambient outdoor conditions, without experimental manipulation of the surrounding environment.
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Location	Sampling was conducted within the grounds of the authors' research institute in Japan. Exact geographic coordinates are not disclosed, as sampling was performed on institutional property and precise location information is not essential for interpretation of the results.
Access & import/export	No special permits were required for sample collection, and no regulated import or export of biological materials was involved.
Disturbance	Sampling was conducted with minimal disturbance to the surrounding environment and vegetation.

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.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	This study did not involve the use of laboratory animals.
Wild animals	This study did not involve experimental manipulation of wild animals.
Reporting on sex	Sex was not reported because this study did not involve animals used as experimental subjects.
Field-collected samples	Field-collected samples consisted of plant root–soil systems containing naturally associated microorganisms. No animals were specifically collected, handled, or experimentally manipulated.
Ethics oversight	Ethics approval was not required for this study, as it did not involve laboratory animals, wild animals as experimental subjects, or any procedures requiring animal ethics review.

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

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	Arabidopsis thaliana (Col-0) seeds were obtained from standard laboratory stocks.
Novel plant genotypes	This study did not involve the generation or use of novel plant genotypes.
Authentication	No authentication was required, as no novel plant genotypes or genetically modified lines were used in this study.