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 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 software was used.
Data analysis	CLustal Omega and Bioedit v 7.0.5.3 was used for the alignment and manual editing of α-actin sequences of the fungus Pseudoxylaria isolated in this study and MrBayes v 3.2.7 was used for the phylogenetic analysis of these sequences. The annotation of Nanopore generated sequences was done using LAST v 973 and to estimate the sequencing depth, rarefaction curves were generated using the Vegan package v 2.5-4 in R v 3.6.2. The estimated community richness (Chao1) and diversity indices (Evenness, Shannon, Simpson, and Inv Simpson) were also calculated in R v 3.6.2.

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 ITS gene fragments obtained have been submitted to GenBank (accession number MN913749–MN913772) and Nanopore sequencing generated reads have been submitted to NCBI Sequence Read Archive (SRA) under the BioProjects PRJNA665655 (SAMN16262892 - SAMN16262897), PRJNA758817 (SAMN21036816 – SAMN21036821) and PRJNA758827 (SAMN21037110 – SAMN21037115).

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 research does not involve human subjects and hence no information has been collected.
Reporting on race, ethnicity, or other socially relevant groupings	This research does not involve human subjects and hence no information has been collected.
Population characteristics	This research does not involve human subjects and hence no information has been collected.
Recruitment	This research does not involve human subjects and hence no information has been collected.
Ethics oversight	This research does not involve human subjects and hence no information has been collected.

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

Life sciences study design

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

Sample size	*Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data exclusions	*Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic*

Research sample	*information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	The fungus-growing termites cultivate Termitomyces on a special spongy substrate called fungus comb which is made by mixing the plant substrate with the fungus nodules that act as the inoculum for the new comb. Usually, Termitomyces is the only fungus visible on the comb but as these combs are continuously augmented with foraged plant materials which also increases the threat of introducing weedy and pathogenic fungi capable of displacing Termitomyces. Termitomyces in healthy combs indicate that the termites are employing some mechanism by which the growth of other non-specific fungi are suppressed. Currently, the exact mechanism by which the growth of the weedy fungi remain suppressed is largely unknown. This study identifies pathogenic load by establishing the pan-mycobiota of O. obesus from the fungus comb and termite castes. In addition, there were samples from the decaying stages of the fungus comb, taken every 24 hours for 120 hours, to maximize the identification of these fungal invaders. The myco- and microbiota of these decaying combs showed a possible interaction between the crop fungus Termitomyces, the weedy fungus Pseudoxylaria and some bacterial mutualists. These were confirmed using in vitro interactions of these microbes against other contaminating fungi isolated from this symbiosis. The results indicate that Termitomyces, Pseudoxylaria and bacterial mutualists all possess the capability to keep the fungal crop free of diseases.
Research sample	The research sample was a fungus-growing termite, Odontotermes obesus from the mounds of IISER Mohali campus (host Institution). Different castes (major workers, minor workers, male and female alates and nymphs) and fungus combs of O. obesus were collected from two different mounds for this study.
Sampling strategy	This study presents the analysis of the different fungal and bacterial genera found within two colonies of O. obesus. Two separate sets of nanopore runs were done. The first was to identify the pan-mycobiota and the second was to identify the microbiota of the degrading combs. To obtain a comprehensive assessment of fungal diversity, three separate DNA extractions per mounds were pooled in equimolar concentrations. This was done for all the termite castes, except alates, where three individual DNA extractions from males and females were pooled. Five different DNA samples (major worker, minor worker, nymph, male alate and female alate) were thus prepared for mycobiota estimation and the other six comb samples (0 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs) were prepared for both micro- and mycobiota estimation on the Nanopore platform. We included two mounds as part of our plan to have a more in-depth and long-term monitoring to build up a more holistic picture of the dynamics of this symbiosis across several years.
Data collection	Sequencing data collection: Data for sequencing was collected by Renuka Agarwal and Manisha Gupta. After the completion of the run, sequences were first separated according to their barcodes by using the program ont-albacore (Oxford Nanopore Technologies Ltd.). These were then converted to FASTA and FASTQ files using Poretools (v 0.5.1) for downstream analysis6. High-quality reads (average read quality score ≥ 10) were selected using the program Nanofilt. The obtained high-quality sequences were then further processed where the barcodes and adaptors were removed using Porechop (https://github.com/rrwick/Porechop). The fungal OTU's were identified by comparing them against the fungus repository from NCBI FTP site and the bacterial sequences generated from the six comb samples (0 hrs- 120 hrs) were identified by comparing them against the customized microbial repository made by combining 16S rRNA gene fragments from NCBI FTP site and the DictDb v 3.0 database. Annotation was done using LAST v 973, with the following parameters: match score of 1, gap opening penalty of 1, and gap extension penalty of 1. The identified reads were sorted from the phylum to the genus level and their relative abundances were calculated. In vitro interaction experiments data collection: All the interaction plates were evaluated by Renuka Agarwal for growth inhibition, by taking photographs using the Panasonic Lumix G2 camera. Photographs from the 7th day were taken as the final data and were analyzed in Adobe Photoshop CS6 by measuring the area of test fungi growth in their respective interactions (both test and control plates) in pixels and converting it into millimeters (100 pixels = 1 mm).
Timing and spatial scale	Reproductive were collected in June 2018. DNA was extracted from the individual reproductives immediately after the collection. Fresh fungus combs, to study the micro- and mycobiota of degrading fungus comb, were collected across three months (August-October, 2018) from two mounds while other castes were collected along with these fungus combs. For each incubation assay, three

such containers were set up from each mound with 12 such incubation assays in all. DNA was extracted from these combs consecutively for six days, i.e., 0 hrs to 120 hrs. For 0 hrs of incubation, DNA was extracted from each portion separately within 3 hours of the collection. DNA extraction of termite castes was done immediately after separating them from the fresh fungus comb. These DNA samples were then used for Nanopore Sequencing and qPCR analysis. Nanopore sequencing was done in the end of year 2019 and qPCR was done in the mid of the year 2020.

Data exclusions: No data were excluded from the analyses.

Reproducibility: All the interaction assays were repeated and were successful. The culture of Paraconiothyrium-MN913759 was lost during this study and therefore, bacterial interactions against this fungus could not be done.

Randomization: No randomization of collection was done as DNA from samples from both mounds were used.

Blinding: Blinding was not possible as the fungal and microbial cultures were distinctive enough.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions: Sampling was started during monsoon season in the year 2018. Reproductives were collected from the two termite mounds during swarming, which began after the first rains in June 2018 with 90% humidity and a temperature of 40 degree celsius. Fresh fungus combs were collected across three months (August-October, 2018) from the same two mounds while other castes (major workers, minor workers and nymphs) were collected along with the fungus combs.

Location: The two different mounds chosen for the study were located within the IISER Mohali (30.6650° N, 76.7300° E) campus which is in Punjab, India.

Access & import/export: All DNA samples used in the study is available for further analysis. Import of these is subject to the regulations prescribed by the Department of Science & Technology, Department of Biotechnology and the Department of Environment & Forestry of the Government of India. No samples have been imported for this study.

Disturbance: Termites were harvested sustainably as it was done only during the monsoon and post-monsoon season. No major disturbance was done during sampling. While collecting the fungus comb, a small portion of mound soil was dug, fungus comb was taken out and then the soil was refilled. Termites usually take less than 24 hrs to completely reseal their mounds.

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

Antibodies

Antibodies used: *Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Validation: *Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): *State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*

Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

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	No laboratory animals were used
Wild animals	Different castes (major workers, minor workers, male and female alates and nymphs) and fungus combs of O. obesus were collected from two different mounds within the IISER Mohali campus. Male and female alates were collected from these mounds during swarming, which began after the first rains in June 2018, while other castes (major workers, minor workers and nymphs) were collected along with the fungus combs. DNA was extracted from the different castes by using whole organisms.
Reporting on sex	Sex was considered only for the swarming alates but not for the other castes of termites.
Field-collected samples	Different castes (major workers, minor workers, male and female alates and nymphs) and fungus combs of O. obesus were collected from two different mounds within the IISER Mohali campus. Male and female alates were collected from these mounds during swarming, which began after the first rains in June 2018, while other castes (major workers, minor workers and nymphs) were collected along with the fungus combs.
Ethics oversight	Use of termites for research do not require the approval of the Institutional Ethics Committee.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

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	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*

Field strength *Specify in Tesla*

Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

(See Eklund et al. 2016)

Correction *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*