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	Data were initially recorded on paper data sheets or in a lab notebook. Values were entered into Excel prior to analysis.
Data analysis	All statistical analyses were conducted in R 4.3.0, except partial least squares regression, which was conducted using JMP Pro statistical software (V 16.1.0). Existing/published packages were used and are named throughout the main text (Methods section).

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 fungal trait, SOM pool, and SOM chemistry data that support the findings of this study have been deposited in the Environmental Data Initiative repository, https://doi.org/10.6073/pasta/cf2a305c7d21938ddad3cb9430d22ed3. Source data associated with each main text and supplementary figure are also available as a supplementary Source Data file.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
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.

☐ 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

Ecological, evolutionary & environmental sciences study design

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

Study description	Saprotrophic fungal isolates were incubated in model soils that were initially organic matter-free. Fungal isolates were provided a simple carbon substrate, such that any organic matter that formed could be directly linked to the fungal isolate and its associated traits. This study evaluated relationships between a suite of physiological, morphological and biochemical fungal traits and the formation of different soil organic matter pools ranging in stability. Fungal species (or phylum) was analyzed as the treatment factor using one-sided ANOVA, MANOVA and PERMANOVA tests. Four replicates per fungal isolate were included in statistical analyses (except for one fungal isolate, Panellus stipticus, for which only three replicate samples were available). For certain statistical analyses of fungal trait values, only three replicates were able to be included, based on the minimum number of replicate values available for certain trait measurements.
Research sample	Saprotrophic fungal cultures were isolated from leaf litter, wood and sporocarps at the Harvard Forest Long-term Ecological Research (LTER) site in Petersham, MA, USA using standard isolation approaches. Fungal cultures were initially stored on a potato dextrose agar media at 4C until experiment initiation. Fungal isolates were then grown axenically in model soils (four replicates per isolate/species). Fungal isolates included four Ascomycota (Trichoderma koningii, Hypocrea minutispora, Phacidium lacerum, Cylindrium elongatum), two Basidiomycota (Panellus stipticus, Gymnopus sp.) and two Mucoromycotina (Mucor mucedo, Mucor abundans).The research samples that were analyzed in this experiment were thus homogenized subsamples of the model soil combined with fungal biomass and other extracellular products that had been produced throughout the course of the incubation experiment. Replicates are intended to represent a population of each of the specific fungal isolates that we included in this study. As explained in the manuscript, our focus in this study was on the relationships between fungal trait values and relative contributions to SOM functional pools; therefore, our results do not represent innate or immutable characteristics of the fungal isolates studied, but rather their relative trait expression and contributions to SOM pools under the specific experimental conditions of this study.
Sampling strategy	Replicates were grown in separate 50 g soil specimen cups and harvested at designated time points based on the growth dynamics of each fungal species. Contamination for slower-growing species was a challenge and we conducted pilot experiments to reduce the potential/risk of contamination. We initially established the experiment with eight replicates per isolate, but were only able to include 4 replicates per isolate in the final statistical analyses (3 for Panellus stipticus) due to contamination challenges with slower growing species. Due to the controlled nature of this experiment, there was less variation between replicates of the same species than among fungal species, and we were therefore able to detect significant statistical differences among fungal isolates in terms of their trait values and contributions to different SOM functional pools.
Data collection	Data were collected in the lab at multiple time points; raw data were recorded in a lab notebook and entered into Excel spreadsheets in a timely fashion; data resulting from sample analyses (e.g., carbon/nitrogen analysis, detailed chemical composition) were transferred using a thumb drive to the lead author's personal computer and were also stored on Dropbox. Carbon use efficiency and growth rate data collected in a previous study (Morrison et al., 2022) for the eight fungal isolates included in our experiment were also included in our final dataset to provide approximations of optimum carbon use efficiency and growth rate values for each isolate (values measured in liquid culture under ideal growth conditions).
Timing and spatial scale	Data were collected in the lab between 2020-2023. The long-term incubation experiment was conducted between 2020-2021. Separate shorter-term incubations to characterize fungal traits were conducted periodically between 2020-2022. Samples harvested

from the long-term incubation experiments were processed and analyzed between 2021-2023, based on instrument and personnel availability.

Data exclusions	For certain isolates, more replicates remained at the end of the long-term incubation than for others (free of contamination). Since all but one species (P. stipticus) had at least four replicates remaining, we chose to include four replicates in statistical analyses for these species, and three for P. stipticus. For certain analyses, more replicate samples were analyzed and a subset of four replicates was randomly selected for inclusion within statistical analyses.
Reproducibility	While the incubation experiment was only conducted once, we established more replicates than was needed for statistical analyses, inoculating each experimental unit with a separate fungal "plug." We used randomization procedures (see below) to ensure that any potential minor variation in model soil or substrate media composition was randomly distributed across taxa. We measured a large suite of fungal traits, and included multiple independent measurements of fungal carbon use efficiency and growth rates using distinct methods. We characterized fungal contributions to multiple soil organic matter pools, to avoid biasing conclusions about fungal contributions to soil organic matter formation and stabilization (writ large), based on measurements of only 1-2 SOM pools. We conducted the experiment under controlled laboratory conditions, and used a model soil mixture which is considerably more homogeneous than a natural field soil. We included detailed descriptions of our methodology, such that other researchers should be able to repeat the same or a similar experiment in the future.
Randomization	Model soil mixtures were created and weighed into specimen cups (50 g each). Fungal isolates were grown in Petri dishes and fungal "plugs" were removed from the growing front of each Petri dish using a glass tube (to standardize the size of each plug). Three Petri dishes were grown for each fungal isolate, and plugs from the separate Petri dishes were randomly paired with the cups of model soil (50 g) during inoculation. While the model soil mixtures and substrate media should, in theory, be identical across our entire experiment, this ensured that any minor variation would be distributed across experimental units of all fungal isolates. For shorter-term incubations to measure specific fungal traits (e.g., carbon use efficiency, growth rate), a random number generator was used to select sample IDs for harvest on each harvesting day.
Blinding	In our experiment, it was necessary to monitor experimental units for possible contamination (visually and via respiration measurements). Thus, it was not possible to fully "blind" the researcher from viewing individual experimental units. We used the randomization approaches above to ensure that bias in sampling was prevented. For isolates that had more than 4 replicates remaining (axenic conditions, no contamination) at the end of the incubation period, a subset of four samples was randomly selected to be included in downstream data analysis.

Did the study involve field work? ☐ Yes ☒ No

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Non-human, fungal cultures isolated from soil, sporocarps and plant litter
Authentication	Isolate identities were confirmed by ITS2 sequencing and comparison against NCBI nucleotide database using BLAST (Morrison et al. 2022; cited in main text)
Mycoplasma contamination	N/A
Commonly misidentified lines (See ICLAC register)	N/A

Plants

Seed stocks	N/A (plants not involved in study)
Novel plant genotypes	N/A
Authentication	N/A