

## Reporting Summary

Nature Research 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 Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- An indication of 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 statistics 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
- Clearly defined error bars  
*State explicitly what error bars represent (e.g. SD, SE, CI)*

*Our web collection on [statistics for biologists](#) may be useful.*

### Software and code

Policy information about [availability of computer code](#)

Data collection	NA
Data analysis	The software used in this paper is extensive. It is detailed in the methods and supplementary methods sections (with version parameters and run parameters)

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Genome assemblies and annotations are available at the JGI MycoCosm and Genoscope portals, and NCBI as detailed in the paper.

## Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

## Ecological, evolutionary & environmental sciences study design

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

Study description	Comparative genomics of full nuclear genomes and comparative transcriptomics of field-collected truffles
Research sample	Tuber aestivum fruit body, T. magnatum fruit body, Choiromyces venosus fruit body, Ascobolus immersus mycelium, Terfezia boudieri mycelium, Morchella conica mycelium
Sampling strategy	not applicable
Data collection	The genomic DNA used for sequencing T. aestivum, T. magnatum and C. venosus genomes were extracted from fruiting bodies (ascmata) sampled in truffle ground Montdoré (Haute-Saône, France), Montemagno (Piedmont, Italy) and Charmes State Forest (Grand-Est, France), respectively.
Timing and spatial scale	Samples were collected from truffle grounds in France and Italy. Sample sizes were set based on the availability of samples because truffles cannot be produced under controlled conditions. Our results and discussion are all framed within the parameters of statistical certainty.
Data exclusions	not applicable
Reproducibility	For transcriptomic analyses 3 replicates were realized
Randomization	not applicable
Blinding	not applicable
Did the study involve field work?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

## Reporting for specific materials, systems and methods

### Materials & experimental systems

- |                                     |   |
|-------------------------------------|---|
| n/a                                 | Included in the study   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Unique biological materials |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                             |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |

### Methods

- |                                     |   |
|-------------------------------------|---|
| n/a                                 | Included in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Unique biological materials

Policy information about [availability of materials](#)

Obtaining unique materials	Fruiting bodies of truffles are unique materials collected in truffle grounds and produced in environmental settings. All the sample tissues were used for DNA or RNA extraction.
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