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# **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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistics	
	es, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a Confirmed	
	ple size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	n whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
The statistical Only common te	test(s) used AND whether they are one- or two-sided states 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 descripti	on of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
For null hypotl	hesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted exact values whenever suitable.
For Bayesian a	nalysis, information on the choice of priors and Markov chain Monte Carlo settings
For hierarchical	al and complex designs, identification of the appropriate level for tests and full reporting of outcomes
Estimates of e	ffect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated
1	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Software and c	ode
Policy information abou	ıt <u>availability of computer code</u>
Data collection	In house python and R code
Data analysis	R code
For manuscripts utilizing custo	om algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. leposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data	
<ul><li>Accession codes, uni</li><li>A list of figures that I</li></ul>	nt <u>availability of data</u> nclude a <u>data availability statement</u> . This statement should provide the following information, where applicable: que identifiers, or web links for publicly available datasets nave associated raw data restrictions on data availability
All data is available in the	manuscript or upload to a sequence database and is freely available.
Field-speci	fic reporting
Please select the one be	elow 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

For a reference copy of the document with all sections, see  $\underline{\mathsf{nature}.\mathsf{com}/\mathsf{documents}/\mathsf{nr}-\mathsf{reporting}-\mathsf{summary-flat}.\mathsf{pdf}}$ 

# Ecological, evolutionary & environmental sciences study design

	<u> </u>	
All studies must disclose or	these points even when the disclosure is negative.	
Study description	Changes in the belowground microbiome of range-expanding plant species in central Europe.	
Research sample	The soil and rhizosphere microbes were characterized of 11 plant species were collected with their plant populations were sampled from 6 countries in Europe – Greece, Montenegro, Slovenia, Austria, Germany and the Netherlands - in the summer growing seaso of 2013 and 2014. All plants were flowering at the time of sampling.	
Sampling strategy	At each sampling site, environmental parameters, including weather conditions at sampling dates, were recorded. For each sampling location of a single species, 3 individuals of 3 distinct populations (in most cases at least 400m separation) were chosen, totaling 9 plant individuals for each location (see Supplementary Figure 1 for sample numbers).	
Data collection	illumina sequencing data and soil parameter data was collected by KSR, FTH, LJB, CW, DvR	
Timing and spatial scale	Collected in summer of 2013/2014	
Data exclusions	mples where sequences did not meet predetermined quality checks were excluded.	
Reproducibility	This is a survey of the belowground plant microbiome. Reproducibility is possible as we have recorded locations of all plant populations that were sampled.	
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.	
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.	
Did the study involve fiel		
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ield work, collec	tion and transport	
Field conditions	Samples were collected in natural systems between May and September. Samples were not collected during rain events.  Temperatures ranged from 12 - 28C depending on the location and the day.	
Location	Greece, Montenegro, Slovenia, Austria, Germany, The Netherlands	
Access and import/expor	t In all relevant locations permits were obtained to collect samples or permissions from private landholders.	
Disturbance	Small disturbances were made when soils were collected, but were approved by permits and permissions.	
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Reporting to	r specific materials, systems and methods	
	authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each materia evant 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 & experime	ental systems Methods	
n/a Involved in the study	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
Palaeontology	MRI-based neuroimaging	
Animals and other	organisms	
Human research pa		
	ruspuns	
Clinical data		
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

Cell line source(s)

State the source of each cell line used.

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 <u>ICLAC</u> register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

#### Palaeontology

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).

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.

## Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

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

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

## Human research participants

Policy information about studies involving human research participants

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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.

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Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

### ChIP-sea

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Data	denc	osition

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

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and

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 Acquisition Specify: functional, structural, diffusion, perfusion. Imaging type(s) Specify in Tesla Field strength Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, Sequence & imaging parameters 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.). If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types Normalization 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 Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first Model type and settings and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation). Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether Effect(s) tested 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 Models & analysis n/a Involved in the study

Graph analysis

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).

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.