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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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

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n/a	Confirmed
	\square 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection

WINRHIZO Pro version 2017a was used to extract root trait data from root scans.

Data analysis

All bioinformatics and statistical analyses were performed using R version 4.1.0 and associated packages (nlme version 3.1.157; vegan version 2.6.2; ggplot2 version 3.3.6; car version 3.1.0; phyloseq version 1.38.0; metagenomeseq 1.36.0.)

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 <u>data availability statement</u>. 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 <u>policy</u>

Fungal ITS2 sequence data are uploaded to NCBI under project number PRJNA952944. Plant biomass data of the main experiment, as well as root trait data are available on Figshare under DOI: 10.6084/m9.figshare.22740974. The FUNGUILD database (http://www.funguild.org) was used for fungal ASV annotation to ecological guilds.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.			
Reporting on sex and gend	r not applicable		
Population characteristics	not applicable		
Recruitment	not applicable		
Ethics oversight	not applicable		
Note that full information on the	e 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		
For a reference copy of the documer	t with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Ecological, ev	olutionary & environmental sciences study design		
All studies must disclose on t	hese points even when the disclosure is negative.		
1 1 1 1	Greenhouse plant-soil feedback study with two experimental phases. In phase 1 we conditioned a mixture of field-collected and an artificial background soil (sand + vermiculite) with 18 conditioning plant species (+ an unplanted control treatment), each with 4 pots per replicate block (N = 5 blocks; total pot number = 380). At the end of the 11-week conditioning phase, conditioned soils of each species were pooled within each block (using 3 out of 4 pots), resulting in 5 pooled replicate soils per plant species. Soil samples were taken for analysis of fungal communities (metabarcoding of fungal ITS2; focus on fungal pathogens and arbuscular mycorrhizal fungi). In the second phase, each plant species was grown on each of the pooled replicate soils of each plant species, resulting in 19 conditioned soils*18 responding species*5 experimental replicates = 1710 pots. Plant biomass responses in control and conditioned soils were used to determine species-level plant-soil feedbacks. In a seperate experiment, we determined root trait variation among these plant species, by growing individual plants (N = 3) in background soil for seven weeks, after which plants were harvested and root systems were scanned and analysed.		
· - - !	Our experiment included 18 European annual and perennial grassland plant species: Salvia pratensis, Prunella vulgaris, Clinopodium acinos, Lamium purpureum (Lamiaceae), Cichorium intybus, Centaurea jacea, Centaurea cyanus, Sonchus olereaceus (Asteraceae), Trifolium campestre, Trifolium medium, Vicia cracca, Vicia sativa (Fabaceae), Anchusa arvensis, Anchusa officinalis (Boraginaceae), Agrostemma githago, Cerastium fontanum (Caryophyllaceae), Diplotaxis tenuifolia and Sinapis arvensis (Brassicaceae). Plant species were selected based on their occurrence as a native plant in south-western Germany, their life history (annual or perennial) and their plant family (equal numbers of annuals and perennials for each included plant family).		
	With 18 plant species included, each life history group therefore had N=9 biological replicates covering most of the common nerbaceous plant families in Europe. With (18*18 =) 324 conditioning-responding species combinations, we moreover had ample replication to examine overall effects of life history (annual vs perennial) on soil conditioning effects and feedback responses, and to examine differences between conspecific and heterospecific plant-soil feedback responses.		
	At the end of phase 1, Rutger Wilschut processed root and shoot biomass samples and collected soil samples together with a student nelper. Phase 2 was set up and harvested by Rutger Wilschut with the help of multiple assistents and student helpers. Processing of soil samples and soil DNA extraction was done by Ekaterina Mamonova.		

Timing and spatial scale

Phase 1 ran from 16-03-2020 - 04-06-2020 and phase 2 from 10-06-2020 - 07-08-2020. As setting up and harvesting of the second phase took multiple times, we performed these activities according to the block design (with block 1 being set up and harvested first, etcetera).

No data were excluded, but for a number of experimental units no data was collected due to sample loss (dead plants).

Reproducibility

Data exclusions

Due to the length and size of the experiment, we could not temporally replicate the experiment.

Randomization

The experiment was set up according to a replicate randomized block design. Plant individuals were randomly assigned to pots, and to minimize the effect of plant size variation at the start of the experiment, we modelled initial plant size as a covariate in our plant-soil feedback models.

Blinding

Blinding was not relevant to our study as the main data acquisition included weighing biomass on precise scales; moreover, the high number of conditioning-responding species combinations made it impossible to infer any pattern, making blinding superfluous.

Did the study involve field work?	Yes	No.
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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		Methods	
n/a	Involved in the study	n/a Involved in the study	
\boxtimes	Antibodies	ChIP-seq	
\boxtimes	Eukaryotic cell lines	Flow cytometry	
\boxtimes	Palaeontology and archaeology	MRI-based neuroimaging	
\boxtimes	Animals and other organisms	·	
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		