nature portfolio

Corresponding author(s):   NCOMMS-24-08554

Last updated by author(s): Feb 16, 2024

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	From mid-July to mid-August in both 2022 and 2023, we obtained data from 80 sites of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau. Moreover, we recorded geographic information (latitude, longitude, and altitude) to extract bioclimatic variables from WorldClim2 (http://www.worldclim.org/).
Data analysis	We use R (https://www.r-project.org/), ArcGIS 10.1 (https://www.arcgis.com/index.html) and Python 3.12.2 (https://www.python.org/downloads) to carry out data analysis.

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

All raw and processed data will be made available upon request, the data and code (https://figshare.com/s/bf7de9fb08b375775ced) are freely available at Figshare.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation), and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	Our research did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	Our research did not involve human participants.
Population characteristics	Our research did not involve human participants.
Recruitment	Our research did not involve human participants.
Ethics oversight	Our research did not involve human participants.

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Ecological, evolutionary & environmental sciences study design

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

Study description	The spread of woody plants into grasslands represents a worldwide concern because of its impact on species biodiversity and ecosystem operations. However, our comprehension of the influence of shrubs on herbaceous diseases in grasslands is limited. In our study, we performed extensive examinations on herbaceous populations under the shrub canopy as well as nearby grassland patches in shrubby grasslands over 4,000 kilometers. This large-scale assessment encompassed grassland regions with significant variability in both temperature and precipitation. Our findings uncovered that shrubs elevated the under-canopy pathogen load of herbaceous foliar fungal diseases by increasing the air humidity of the herbaceous assemblages alongside aboveground biomass in warmer regions. However, in colder regions, shrub expansion reduced both pathogen load and herbaceous biomass below the canopy. Temperature, rather than precipitation, was the primary driver for the variations in biomass and herbaceous pathogen load in the variable under-canopy herbaceous populations. These contrasting alterations in herbaceous biomass and diseases following shrub expansion, along with the related environmental variations, emphasize the requirement for adaptive management approaches tailored to shrub encroachment scenarios.
Research sample	We obtained data from 80 sites of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau, each site encompassed two treatments (grassland patches vs. shrub patches) and shrub patches (at least 10 meters apart) paired with adjacent grassland patches. Each treatment had four replicates, resulting in eight 0.5 m × 0.5 m herbaceous plots. In each plot, we trimmed all aboveground green herbaceous individuals, classified them to the species level.
Sampling strategy	The sampling sites were chosen at least 500 m away from cities, receiving limited human interference, and considered typical regions for local natural vegetation.
Data collection	In each plot, we trimmed all aboveground green herbaceous individuals, classified them to the species level, and oven-dried all samples at 65 ℃ for 72 hours to a constant weight and measured the biomass. The plot (community) level of herbaceous biomass (Biomass) was determined as the sum of aboveground biomass from all species collected in each plot. To characterize herbaceous community-level disease severity, we calculated pathogen load. We recorded species-level disease severity (vi) as % of the leaf area covered by fungal lesions. To characterize the soil properties of each plot, we obtained six upper mineral soil cores (0-10 cm) from random locations within each plot and homogenized them thoroughly to generate a composite sample. All soil samples were stored at 4 ℃ during transportation.
Timing and spatial scale	From mid-July to mid-August in both 2022 and 2023, we obtained data from 80 sites of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau (28.26 °N to 49.77 °N and 80.91 °E to 121.34 °E).
Data exclusions	No data were excluded from the analyses.
Reproducibility	Our study focused on the herbaceous communities below the shrub canopy (landscape mosaic established by shrub encroachment) and adjacent grassland patches, where shrub patches were interspersed with grassland patches. This mosaic enabled the establishment of ideal study plots pairing shrub patches with adjacent grassland patches.
Randomization	At each site, we established four herbaceous plots below the canopy within shrub patches, with each shrub patch plot paired with a corresponding adjoining grassland patch plot of identical size. This design resulted in a total of eight plots, corresponding to four pairs. To characterize herbaceous community-level disease severity, we recorded species-level disease severity from 25 randomly

selected leaves from at least five individuals for each herbaceous plant species in each plot. For species with fewer than 25 leaves, we obtained all available leaves.

| Blinding | Each site encompassed two treatments (grassland patches vs. shrub patches) and shrub patches (at least 10 meters apart) paired with adjacent grassland patches. Because most shrub individuals cover an area less than 1 m × 1 m and to avoid potential edge effects, we established 0.5 m × 0.5 m plots. |

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	We obtained data of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau, the mean annual temperature (°C; MAT) and mean annual precipitation (mm; MAP) at each survey site ranged from -5.6 to 9.9 °C, and from 75 to 861 mm, respectively.
Location	We obtained data of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau (28.26 °N to 49.77 °N and 80.91 °E to 121.34 °E)
Access & import/export	In our study, we performed a comprehensive field survey across China's vast grasslands, encompassing the largest contiguous natural grassland on Earth, with an area of over 4,000 square kilometers. The College of Ecology of Lanzhou University needs to collect soil and plant samples for the second comprehensive scientific investigation and research project on the Qinghai-Tibet Plateau, which will only be used for ecological research and environmental monitoring.
Disturbance	The sampling sites were chosen at least 500 m away from cities, receiving limited human interference, and considered typical regions for local natural vegetation. Each site encompassed two treatments (grassland patches vs. shrub patches) and shrub patches (at least 10 meters apart) paired with adjacent grassland patches. Each treatment had four replicates, resulting in eight 0.5 m × 0.5 m herbaceous plots. Because most shrub individuals cover an area less than 1m × 1m and to avoid potential edge effects1, we established 0.5m × 0.5m plots.

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

Plants

Seed stocks	From mid-July to mid-August in both 2022 and 2023, we obtained data from 80 sites of shrubby grasslands across the Qinghai-Tibet Plateau and Inner Mongolia Plateau (28.26 °N to 49.77 °N and 80.91 °E to 121.34 °E), each site encompassed two treatments (grassland patches vs. shrub patches) and shrub patches (at least 10 meters apart) paired with adjacent grassland patches. Each treatment had four replicates, resulting in eight 0.5 m × 0.5 m herbaceous plots. In each plot, we trimmed all aboveground green herbaceous individuals, classified them to the species level, and oven-dried all samples at 65 °C for 72 hours to a constant weight and measured the biomass. The plot (community) level of herbaceous biomass (Biomass) was determined as the sum of aboveground biomass from all species collected in each plot.
Novel plant genotypes	Our research produced no novel plant genotypes.
Authentication	Our research did not involve authentication procedures for each seed stock and novel genotype.