1 **Soil microbes mediate the effects of environmental variability on plant invasion**

2 Xue Zhang^{1,2}, Mark van Kleunen^{3,4}, Chunling Chang¹, Yanjie Liu^{1*}

3 ¹*Key Laboratory of Wetland Ecology and Environment, Northeast Institute of geography and*
4 *groecology, Chinese Academy of Sciences, Changchun, 130102, China*

5 ²*University of Chinese Academy of Sciences, Beijing, 100049, China*

6 ³*Ecology, Department of Biology, University of Konstanz, 78464 Konstanz, Germany*

7 ⁴*Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation,*
8 *Taizhou University, Taizhou 318000, China*

9

10 *Email of corresponding author: liuyanjie@iga.ac.cn, +86 431 82536096

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12 Running title: Soil microbes, nutrient pulses and plant invasion

13

14 **Abstract**

15 Many studies indicate that increases in resource variability promote plant invasion. However,
 16 it remains unknown to what extent these effects might indirectly be mediated by other
 17 organisms. To test this, we grew eight alien species in pot-mesocosms with five different
 18 native communities under eight combinations of two nutrient-availability, two nutrient-
 19 fluctuation and two soil-microbe treatments. We found that when plants grew in sterilized
 20 soil, nutrient fluctuation promoted the dominance of alien plants under low nutrient
 21 availability, whereas its effect was minimal under high nutrient availability. However, the
 22 opposite pattern was found when plants grew in living soil. Analysis of the soil microbial
 23 community suggests that this might reflect that nutrient fluctuation strongly increased the soil
 24 fungal pathogen diversity under high nutrient availability, but slightly decreased it under low
 25 nutrient availability. Our findings indicate that besides its direct influence, environmental
 26 variability could also indirectly affect plant invasion via changes in soil-microbial
 27 communities.

28 **Keywords:** global change, niche, plant invasion, resource competition, soil biota, trophic
 29 level

30

31 **Introduction**

32 Eutrophication, increases in environmental variability and rapid accumulation of alien
 33 species are three major characteristics of the Anthropocene ¹⁻³. Plant invasions often lead to
 34 highly undesirable changes in ecosystem structure and function ^{4,5}, and are frequently driven
 35 by changes in resource availability and environmental variability. The fluctuating resource
 36 hypothesis predicts that a plant community becomes more susceptible to invasion whenever
 37 there is a fluctuation (i.e. a temporal increase) in resource availability ⁶. Although it has
 38 become a widely accepted theory in ecology, empirical studies that tested it ⁷⁻¹² have found
 39 mixed results. For example, whereas Parepa et al. ⁷ found that a nutrient pulse increased the
 40 dominance of an invasive plant, Liu et al. ⁹ showed that a pulsed nutrient supply decreased
 41 the dominance of invasive alien plants. Therefore, it has become an urgent quest to
 42 understand when and how fluctuating resources affect alien plant invasion in native
 43 communities.

44 The fluctuating resources hypothesis rests on the assumption that the native community is
 45 resource limited ⁶. Consequently, a resource pulse could lead to shifts in the intensity of alien-
 46 native competition, increased dominance of alien species and potential competitive exclusion
 47 of the natives over time ^{8,13}. When, however, resources are not limiting, fluctuations in
 48 resource availability should not benefit the aliens, even if they can respond quicker to
 49 resource fluctuations ⁸ or have higher maximum growth rates than natives ⁷. If this is true, it
 50 logically follows that if the native community grows under more nutrient-limiting conditions,
 51 the positive effect of fluctuating resources on alien plant invasion should be stronger.
 52 However, very few studies have tested whether this expectation holds, as most previous
 53 studies tested the fluctuating resource hypothesis only under relative high nutrient conditions.
 54 This might explain why previous studies found mixed results.

55 While the fluctuating resource hypothesis explains how environmental variability in

56 general should affect alien plant invasion, it considers only the effect of resource availability.
 57 Invasion success of alien plants can also be driven by biotic factors. For example, the enemy
 58 release hypothesis predicts that the absence or reduced numbers of enemies, such as
 59 microbial pathogens, in their introduced range drives invasion success of alien plants^{14,15}. On
 60 the other hand, the missed mutualisms hypothesis predicts that alien plants suffer from the
 61 absence or reduced numbers of suitable mutualistic partners, such as arbuscular mycorrhiza
 62 fungus (AMF);¹⁶. Furthermore, the environmental context (e.g., resource availability) may
 63 alter the relative contribution of pathogens versus mutualists to plant performance, and thus
 64 shape plant-species coexistence¹⁷. Following this logic, we expect that fluctuations in
 65 resource availability should not only directly but also indirectly —through changes in soil-
 66 microbial communities— influence invasion success of alien plants into native communities.

67 To test the interactive effects among nutrient availability, resource fluctuations and soil
 68 microbes, we did a multispecies experiment (Fig. 1). We grew eight naturalized alien plants
 69 as target species in pot-mesocosms with five different synthetic native communities under
 70 eight combinations of two nutrient-availability (low vs high), two nutrient-fluctuation
 71 (constant vs pulsed) and two soil-microbe (living vs sterilized) treatments. We compared the
 72 absolute aboveground biomass production of the alien target species as well as their biomass
 73 production relative to the aboveground biomass production of the native competitors.
 74 Moreover, we used amplicon DNA sequencing of the soil in the mesocosms of the living soil-
 75 microbe treatment to assess how the nutrient treatments affect the soil bacterial and fungal
 76 communities. We addressed the following questions: (1) Does the effect of nutrient
 77 fluctuations on absolute and relative biomass of alien plants depend on the average nutrient
 78 availability level? (2) Does the presence of soil microbes change the effects of increases in
 79 nutrient availability and fluctuations therein on the absolute and relative biomass of alien
 80 plants?

81 **Material and Methods**

82 **Study location and species**

83 We conducted our multi-species experiment in a greenhouse of the Northeast Institute of
84 Geography and Agroecology, Chinese Academy of Sciences (43°59'49"N, 125°24'30"E). We
85 selected eight naturalized alien species as targets and 25 native species as native-community
86 members (Table S1). All species are herbaceous plants, and commonly co-occur in many
87 grasslands of China. To cover a wide taxonomic breadth, and thus to increase the
88 generalizability of our findings, we selected the eight alien target species from six genera of
89 three families, and the 25 native species from 22 genera of eleven families (Table S1). We
90 classified the species as naturalized alien or native to China based on the book “The Checklist
91 of the Alien Invasive Plants in China”¹⁸ and the Flora of China database (www.efloras.org).
92 Seeds of the study species were collected from wild populations or obtained from commercial
93 seeds companies (Table S1).

94 **Experimental set-up**

95 From 26 June to 14 August 2020 (Table S1), we sowed the study species separately into
96 plastic trays (19.5 cm × 14.6 cm × 6.5 cm) filled with peat moss as substrate (Pindstrup Plus,
97 Pindstrup Mosebrug A/S, Denmark). Because the time required for germination were known
98 to differ among the species, we sowed them on different dates (Table S1) to ensure that all
99 species were in similar developmental stages at the start of the experiment. We placed all
100 trays with seeds in a greenhouse under natural light conditions, with a temperature between
101 20 and 28 °C.

102 On 30 August 2020, we transplanted similar-sized seedlings of each species into 2.5 L
103 circular plastic pots (top diameter × bottom diameter × height: 18.5 cm × 12.5 cm × 15 cm).
104 We transplanted one seedling of an alien target species in the center of each pot. For each of
105 the eight target species, we transplanted at total of 40 seedlings into 40 pots (i.e., one seedling

per pot). We then randomly assigned the 40 pots of each alien target species to be planted with five different native grassland communities (i.e., eight pots per alien target species per native community). We used five native communities to increase generalizability of the results. The communities were created by randomly assigning the 25 native species into five groups of five species (Table S1). We planted one seedling of each native-community member so that each pot included five individuals of native species growing at equal distance in a circle (diameter = 9 cm) around each alien target plant (Figure 1). After transplanting, we randomly assigned all pots to positions on four benches of a greenhouse under natural light conditions, with a temperature between 20 and 28 °C. After five weeks, we re-randomized the positions of all pots again. To avoid leakage of nutrient solution, as well as pot-to-pot cross contamination by soil microbes, we put a plastic dish under each pot.

To test the interactive effects of nutrient availability, nutrient fluctuations and soil microbes on alien plant invasion into resident native communities, we assigned the 40 pots of each alien target species to two levels of soil-microbe (living *vs* sterilized) treatments fully crossed with two levels of nutrient-availability (low *vs* high) and two levels of nutrient-fluctuation (constant *vs* pulsed) treatments. In other words, per alien species, we had five pots (i.e., replicates), each with a different native community, in each of the eight treatment combinations. To create different soil-microbe treatments, we had filled each pot with a substrate mixture of 37.5% (v/v) sand, 37.5% (v/v) vermiculite and 25% living or sterilized field soil. The living field soil served as inoculum to provide a soil microbial community, and was collected from the surface soil (0-20 cm depth) of a grassland site in Jinlin province (44°0'4"N, 125°24'12"E), China, on 21 August 2020. We sieved the field soil through a metal grid with a mesh size of 0.5 cm, and thereafter stored half of the soil at 4 °C until pot-filling. The other half of the soil we sterilized with a dose of 25 kGy of ⁶⁰CO_γ irradiation for four days at the Harbin Guangya Radiation New Technology Co., Ltd (Harbin, China). On 3

September 2020 (i.e., four days after transplanting), we started to apply the different nutrient treatments at weekly intervals for a total of ten weeks (Fig. 1). We applied the four nutrient treatments (2 nutrient availabilities $\times$ 2 nutrient fluctuations) using a 400%-strength Hoagland solution (for recipe details see Ref. ⁸). During the 10 weeks of the experiment, we added a total of 50 ml and 400 ml of the Hoagland solution per pot in the low and high nutrient-availability treatment, respectively. Within each nutrient-availability treatment, there were two different nutrient-supply patterns, the constant nutrient supply and the pulsed nutrient supply (Fig. 1). For the constant treatment, we supplied each pot every week with 5 ml and 40 ml of the nutrient solution for the low and high nutrient-supply treatments, respectively. For the pulsed treatment at low-nutrient availability, we first supplied each pot with 2 ml of the nutrient solution per week for three weeks, then with 9.5 ml for four weeks, and again with 2 ml for the last three weeks (Fig. 1). For the pulsed treatment at high-nutrient availability, we first supplied each pot with 10 ml of the nutrient solution per week for three weeks, then with 85 ml for four weeks, and again with 10 ml for the last three weeks (Fig. 1). To avoid that the plants would receive different amounts of water, we added extra water to the nutrient solution in each specific nutrient-supply treatment to ensure that each pot received a total of 85 ml water per nutrient application. To avoid water limitation, we checked the pots every day and watered all plants when needed by filling the dishes under the pots.

Plant harvest and measurements

On 13 November 2020 (i.e., c. 11 weeks after transplanting), we started to harvest the aboveground biomass of all pots. We first harvested the alien target species and then harvested the native community of each pot. As one alien target plants had been planted incorrectly and 10 pots had accidentally been treated with the wrong nutrient solution, we only harvested 309 of the 320 pots. All aboveground biomass samples were dried at 65 °C for 72 hours and then weighed. Based on the aboveground biomass, we calculated the total

aboveground biomass production per pot (i.e., alien target species + native community) and the biomass proportion of the alien target species in each pot (i.e., alien target species / [alien target species + native community]).

Soil sampling, DNA extraction, amplicon sequencing and bioinformatics

To gain insights into how effects of nutrient availability and fluctuations therein on plants may be mediated by soil microbes, we also tested how the nutrient treatments influenced the soil-microbial communities of the pots with living soil inoculum. On 14 November 2020 (i.e., one day after plant harvest), we first homogenized the soil in each of these pots, and then collected from each pot a random soil sample of 12 ml, which we put into sterile centrifuge tubes (15 ml). The 154 soil samples were immediately stored at -80 °C until DNA extraction.

The DNA extraction, PCR amplifications and amplicon sequencing were performed by Novogene Co., Ltd (Beijing, China). Total genomic DNA was extracted from 0.5 g of each soil sample using the Magnetic Soil And Stool DNA Kit (Tiangen Biotech [Beijing] Co., Ltd) following the cetyltrimethylammonium bromide protocol¹⁹. Then, the universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The specific fungal primers ITS1-1F-F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS1-1F-R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the ribosomal internal transcribed spacer of the ITS rDNA gene. All PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs) under the following conditions: initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s and elongation at 72 °C for 30 s, and a final extension step of 5 min at 72 °C. PCR products were mixed in equidensity ratios. Then, mixtures of PCR products were purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA),

and its quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform (Illumina, USA) and 250 bp paired-end reads were generated. As fungal DNA extraction failed for one sample, we finally had 153 samples of the ITS rDNA gene, and 154 samples of the 16S rRNA gene.

The raw paired-end reads were assigned to samples, truncated by removing the barcode and primer sequence, merged using FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) to get splicing sequences. According to the QIIME (Quantitative Insights Into Microbial Ecology, V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html)²⁰, the raw reads were quality filtered under specific filtering conditions to obtain high-quality clean reads. The clean reads were obtained after detecting and removing the chimera sequence by UCHIME algorithm²¹. All effective reads were clustered and classified into the same OTUs (Operational Taxonomic Units) with an identity of 97% similarity using the UPARSE software (Uparse v7.0.1001; <http://www.drive5.com/uparse/>). The Mothur method and the SSUrRNA database²² of SILVA138 (<http://www.arb-silva.de>) were used to annotate taxonomic information for each representative sequence of bacteria (with a threshold value of 0.8-1). The blast method²³ and the UNITE database (v8.2, <https://unite.ut.ee/>)²⁴ were used for sequence annotation analysis to obtain taxonomic information of fungal OTUs. To account for differences in sequencing depths, bacteria and fungi were rarefied to 38,678 and 47,773 reads, respectively, using the RAM package²⁵. Putative fungal functional groups (e.g., AMF and plant pathogens) with “highly probable” and “probable” confidence were identified using the FUNGuild database (v1.1)²⁶.

Statistical analyses

To test the effects of the nutrient-availability, nutrient-fluctuation and soil-microbe treatments, and their interactions on aboveground biomass production of the alien target

species, biomass production of the native communities and biomass proportion of the alien target species in each pot, we fitted Bayesian multilevel models using the function `brm` of the R package `brms`²⁷ in R 4.0.2²⁸. To improve normality and homoscedasticity of the residuals, aboveground biomass production of the alien target species and biomass production of the native communities were natural-log-transformed, and biomass proportion of the alien target species in each pot was logit-transformed prior to analyses. In all models, we included the nutrient-availability (low *vs* high), nutrient-fluctuation (constant *vs* pulsed) and soil-microbe (living *vs* sterilized) treatments, and all their interactions as fixed factors.

To test the effects of nutrient availability, nutrient fluctuations and their interaction on the true Shannon diversity (i.e., effective number of species;²⁹⁻³² of soil bacteria, soil fungi and soil pathogenic fungi, as well as on the ratio between the diversity of pathogenic fungi and the diversity of AMF, we also fitted Bayesian multilevel models using the function `brm` of the R package `brms`²⁷ in R 4.0.2²⁸. The true Shannon diversity of soil bacteria, all soil fungi and soil pathogenic fungi were calculated by the function `true.diversity` of the `RAM` package²⁵. To improve normality and homoscedasticity of the residuals, the diversity of complete soil fungi, soil pathogenic fungi and the ratio between the diversity of pathogenic fungi and AMF were natural-log-transformed prior to analyses. In all models, we included nutrient availability (low *vs* high), nutrient fluctuations (constant *vs* pulsed) and their interaction as fixed factors.

For all models mentioned above, we included identities of the plant family and the alien target species as random factors to account for non-independence of species belonging to the same family and for non-independence of replicates of the same species. In addition to account for variation among the five different native communities, we also included identity of the native community as a random factor in all models. To relax the homogeneity of variance assumption in all models, we allowed the residual standard deviation σ to vary

by the identity of the alien target species³³. We used the default priors set by the brms package, and ran four independent chains for all models. The total number of iterations per chain was 8000, and the number of warm-up samples was 4000. To directly test hypotheses about the main effects and interactive effects based on each coefficient's posterior distribution, we used the sum coding, which effectively 'centers' the effects to the grand mean (i.e., the mean value across all data observations)³⁴. To implement this in brms, we used the functions contrasts and contr.sum of the stats package in R. We considered the fixed effects nutrient availability, nutrient fluctuations and soil microbes, and their interactions as significant when their 95% credible interval of the posterior distribution did not overlap zero, and as marginally significant when the 90% credible interval did not overlap zero.

To visualize and analyse any effects of the nutrient treatments on the composition of the microbial soil communities, we did non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity distances and permutational analysis of variance (PERMANOVA), using the functions metaMDS and adonis of the vegan package in R 4.0.2²⁸.

Results

Plant performance

Averaged across the nutrient-fluctuation and soil-microbe treatments, an increase in nutrient availability significantly enhanced the biomass production of alien target species (+98.2%) and the biomass production of native communities (+142.2%), but decreased the relative biomass production of target alien species in the native communities (-13.9%; Extended data Table 1; Fig. 2 and Fig. S1). Although a pulsed nutrient supply did not significantly affect the biomass production of alien target species and native communities, it tended to increase the relative biomass production of alien target species (+9.7%; 90% CIs: [0.009, 0.157] in Extended data Table 1; Fig. 2 and Fig. S1). Inoculation with living soil significantly decreased the biomass production of native communities (Fig. 2 and Fig. S2), particularly under low nutrient availability (low vs high: -37.6% vs -1.6%; Extended data Table 1; Fig. 2 and Fig. S3), and significantly increased the biomass production of alien target species (+28.8%; Extended data Table 1; Fig. 2 and Fig. S2). Consequently, the living soil microbiome significantly increased the relative biomass production of alien target species (Fig. 2 and Fig. S2), in particular under low nutrient availability (low vs high: +55.8% vs +15.1 %; Extended data Table 1; Fig. 2 and Fig. S3).

For biomass production of the alien target species, we also found a significant three-way interaction of the soil-microbe, nutrient-availability and nutrient-fluctuation treatments (Extended data Table 1). Specifically, when plants grew on substrate with sterilized soil inoculum, the pulsed nutrient supply tended to increase the biomass production of alien target species when the overall nutrient availability was low (+35.2%), whereas its effect was minimal when the overall nutrient availability was high (+0.5%; Fig. 2). However, when plants grew on substrate with living soil inoculum, the pulsed nutrient-supply effect tended to decrease the biomass production of alien target species when the overall nutrient availability

was low (-13.3%), whereas the opposite was true when the overall nutrient availability was high (+19.5%; Fig. 2). Another three-way interaction was found for the relative biomass production of alien target species (Extended data Table 1). When plants grew on substrate with sterilized soil inoculum, the pulsed nutrient supply tended to increase the relative biomass production of alien target species when the overall nutrient availability was low (+27.2%), whereas its effect was minimal when the overall nutrient availability was high (-1.2%; Fig. 2). However, when plants grew with living soil inoculum, this pattern was reversed (pulsed nutrient-supply effect under low vs high nutrient availability: +0.5% vs +19.1%; Fig. 2).

Soil microbial community

For the soil bacterial community, we did not find any significant effects of nutrient availability, nutrient fluctuations and their interaction on the true Shannon diversity (Extended data Table 2; Fig. 3). For the complete soil fungal community, an increase in nutrient availability decreased the true Shannon diversity significantly (-14.6%; Extended data Table 2; Fig. 3 and Fig. S4). For the subset of soil pathogenic fungi, an increase in nutrient availability significantly decreased the true Shannon diversity (Fig. 3 and Fig. S4), particularly under a constant nutrient supply (constant vs pulsed: -14.9% vs -1.8%; Extended data Table 2; Fig. 3). In other words, a constant nutrient supply decreased the diversity of soil pathogenic fungi under high nutrient availability, but increased it under low nutrient availability. As we found no significant effects of nutrient treatments on the diversity of AMF (Fig. S4), the pulsed nutrient supply consequently tended to decrease the diversity of soil pathogenic fungi relative to AMF under low nutrient availability (-20.3%), whereas the reverse was true under high nutrient availability (+51.1%; Fig. 3). This was reflected in a marginally significant two-way interaction between nutrient availability and fluctuations (90% CIs: [0.002, 0.222] in Extended data Table 2). In line with the abovementioned findings, non-

296 metric multidimensional scaling analysis (NMDS) and permutational multivariate analysis of
297 variance (PERMANOVA) also showed that the nutrient treatments affected the soil microbial
298 community composition significantly (Table S2; Extended Data Fig. 1).

299

Discussion

Our multispecies experiment showed that the presence of a living soil microbiome increased the dominance of alien plants in the native communities. An increase in average nutrient availability also promoted the dominance of naturalized alien plants, but only when they grew on substrate with sterilized soil inoculum. Interestingly, we found that when plants grew in the absence of soil microbes, nutrient fluctuations promoted the dominance of alien plants under low average nutrient availability, but hardly affected it under high average nutrient availability. However, opposite patterns were found when plants grew in the presence of soil microbes. These different patterns may be driven by the fact that nutrient fluctuations increased the diversity of pathogenic soil fungi under high nutrient availability, but not under low nutrient availability. Our findings thus partly support the idea that environmental variability promotes plant invasion, and indicate that pathogenic soil fungi could mediate the interactive effect of nutrient availability and fluctuation therein on the dominance of alien plants in native communities.

In support of the predictions of the enemy release hypothesis, we found that the presence of soil microbes decreased the biomass production of native communities while there was a positive effect on the alien target species. As a consequence the soil microbes increased the dominance of the alien plants. This most likely reflects that the alien plants escaped from heavy pathogen loads in their native range, whereas the native communities still suffered from their soil microbial pathogens^{15,35,36}. Our results also showed that an increase in nutrient availability significantly decreased the diversity of pathogenic soil fungi. This decrease in pathogen diversity may have led to a stronger biomass increase for native communities (+142.2%) than for alien target species (+98.2%) in response to an increase in nutrient availability. As a consequence, the increase in nutrient availability tended to suppress the dominance of alien target species in the pots with living soil inoculation. However, when

soil microbes were absent, our finding is in line with many theoretical^{6,37} and empirical studies^{8,38} showing that an increase in nutrient availability promotes alien plant invasion in native communities. These findings underline that integrating disparate invasion hypotheses will help to better understand alien invasions³⁹.

Due to higher growth rates, naturalized alien species are often more dominant than native species⁴⁰. Our results corroborate this, and showed that although alien plant accounted for only one-sixth of the number of individuals per pot, they accounted for about one-third of the biomass production. We also found that, averaged over all treatments, this dominance tended to increase with a pulsed nutrient supply. This finding supports the prediction of the fluctuating resource hypothesis⁶ and is in line with results of previous empirical studies^{7,12}. However, it should be noted that in our experiment, the effect of nutrient fluctuations on alien plant invasion depended on the interaction between nutrient-availability and the presence of soil microbes.

When plants grew in substrate with sterilized soil inoculum, the dominance of alien plants was, as expected, increased by the pulsed nutrient supply under low nutrient availability. However, its effect was minimal under high nutrient availability. This most likely reflects that native species were also released from the intense belowground alien-native competition under a relative high overall nutrient availability. Consequently, the five native plants in each pot were able to take advantage of the pulsed supply pattern under high average nutrient availability, and thus their relative biomass production increased and the dominance of the alien target species was suppressed. When plants grew in substrate with living soil inoculum, however, the dominance of alien plants was only increased by the pulsed nutrient supply under high average nutrient availability. The response pattern of aliens' dominance in response to nutrient fluctuations coincides with the changes of soil pathogen diversity, as well as the relative diversity changes between soil pathogens and AMF. A

possible explanation is that the increase of soil-pathogen diversity and the relative decrease of mutualism diversity induced by the nutrient pulse under high nutrient availability suppressed the native communities but benefited the alien target species. As a consequence, alien plant dominance was promoted under these conditions. Similarly, soil pathogens most likely also mediated the effects of nutrient fluctuations on alien plant invasion when plants grew under low nutrient availability.

Our finding that soil pathogenic fungi could significantly mediate the effects of environmental variability on plant invasion has several implications. First, it suggests that the findings of previous studies that only considered direct effects of environmental variability — and nutrient fluctuations in particular— on alien plant invasion need to be further verified after accounting for indirect effects of soil microbes. Second, besides the pathogenic soil microbes, many other enemies, such as various above- and below-ground herbivores, as well as mutualisms, such as AMF and pollinators, may also indirectly steer global change driven alien plant invasion. Therefore, with the forecasted increase in environmental variability³, more studies are needed to test the indirect effects of environmental variability on alien plant invasion via biotic factors.

Conclusions

The fluctuating resource hypothesis suggests that resource fluctuations could promote alien plant invasion in native communities. Our study suggests that this is particularly the case when plants grow in the absence of soil microbes and under relatively low nutrient conditions. This finding highlights that besides the direct influence, environmental variability may also indirectly affect alien plant invasion via biotic factors such as soil microbes. Moreover, our results emphasize that to better understand alien plant invasion, integration of different invasion hypotheses is needed.

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Author contributions

YL conceived the idea and designed the experiment. XZ performed the experiment. XZ, CC and YL analyzed the data. XZ and YL wrote the draft of the manuscript, with major inputs from MvK, and further inputs from CC.

Data accessibility

Should the manuscript be accepted, the data supporting the results will be archived in Dryad and the data DOI will be included at the end of the article.

Reference

- 1 Seebens, H. *et al.* No saturation in the accumulation of alien species worldwide. *Nature Communications* 8, 14435, doi:10.1038/ncomms14435 (2017).
- 2 Seebens, H. *et al.* Projecting the continental accumulation of alien species through to 2050. *Global Change Biology* 27, 970-982, doi:10.1111/gcb.15333 (2020).
- 3 Zhang, W. X. *et al.* Increasing precipitation variability on daily-to-multiyear time scales in a warmer world. *Science advances* 7, eabf8021, doi:10.1126/sciadv.abf8021 (2021).
- 4 Vilà, M. *et al.* Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14, 702-708, doi:10.1111/j.1461-0248.2011.01628.x (2011).
- 5 Linders, T. E. W. *et al.* Direct and indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive tree affects ecosystem functioning. *Journal of Ecology* 107, 2660-2672, doi:10.1111/1365-2745.13268 (2019).
- 6 Davis, M. A., Grime, J. P. & Thompson, K. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88, 528-534, doi:10.1046/j.1365-2745.2000.00473.x (2000).
- 7 Parepa, M., Fischer, M. & Bossdorf, O. Environmental variability promotes plant invasion. *Nature Communications* 4, 1604, doi:10.1038/ncomms2632 (2013).
- 8 Liu, Y. J. & van Kleunen, M. Responses of common and rare aliens and natives to nutrient availability and fluctuations. *Journal of Ecology* 105, 1111-1122, doi:10.1111/1365-2745.12733 (2017).
- 9 Liu, Y. J., Zhang, X. Q. & van Kleunen, M. Increases and fluctuations in nutrient availability do not promote dominance of alien plants in synthetic communities of common natives. *Functional Ecology* 32, 2594-2604, doi:10.1111/1365-2435.13199 (2018).

- 414 10 Zheng, Y. Y., Burns, J. H., Liao, Z. Y., Li, W. T. & Li, L. Nutrient fluctuation has
415 different effects on a tropical invader in communities from the native and non-native
416 range. *Environmental and Experimental Botany* 178, 104193,
417 doi:10.1016/j.envexpbot.2020.104193 (2020).
- 418 11 Tao, Z. B. *et al.* Magnitude and timing of resource pulses interact to affect plant
419 invasion. *Oikos*, doi:10.1111/oik.08381 (2021).
- 420 12 Gao, F. L. *et al.* Interactive effects of nutrient availability, fluctuating supply, and plant
421 parasitism on the post-invasion success of *Bidens pilosa*. *Biological Invasions* 23,
422 3035-3046, doi:10.1007/s10530-021-02555-y (2021).
- 423 13 Davis, M. A., Wragge, K. J. & Reich, P. B. Competition between tree seedlings and
424 herbaceous vegetation: support for a theory of resource supply and demand. *Journal of*
425 *Ecology* 86, 652-661, doi:10.1046/j.1365-2745.1998.00087.x (1998).
- 426 14 Keane, R. M. & Crawley, M. J. Exotic plant invasions and the enemy release
427 hypothesis. *Trends in Ecology & Evolution* 17, 164-170, doi:10.1016/s0169-
428 5347(02)02499-0 (2002).
- 429 15 Callaway, R. M., Thelen, G. C., Rodriguez, A. & Holben, W. E. Soil biota and exotic
430 plant invasion. *Nature* 427, 731-733, doi:10.1038/nature02322 (2004).
- 431 16 Mitchell, C. E. *et al.* Biotic interactions and plant invasions. *Ecology Letters* 9, 726-740,
432 doi:10.1111/j.1461-0248.2006.00908.x (2006).
- 433 17 Gundale, M. J. & Kardol, P. Multi-dimensional as a path forward in plant-soil
434 feedback research. *Journal of Ecology* 109, 3446-3465, doi:10.1111/1365-2745.13679
435 (2021).
- 436 18 Ma, J. S. & Li, H. R. *The Checklist of the Alien Invasive Plants in China*. Higher
437 Education Press, (2018).
- 438 19 Allen, G. C., Flores-Vergara, M. A., Krasynanski, S., Kumar, S. & Thompson, W. F. A

439 modified protocol for rapid DNA isolation from plant tissues using
440 cetyltrimethylammonium bromide. *Nature Protocols* 1, 2320-2325,
441 doi:10.1038/nprot.2006.384 (2006).

442 20 Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community
443 sequencing data. *Nature Methods* 7, 335-336, doi:10.1038/nmeth.f.303 (2010).

444 21 Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. UCHIME improves
445 sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194-2200 (2011).

446 22 Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid
447 assignment of rRNA sequences into the new bacterial taxonomy. *Applied &*
448 *Environmental Microbiology* 73, 5261-5267, doi:10.1128/AEM.00062-07 (2007).

449 23 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local
450 alignment search tool. *Journal of molecular biology* 215, 403-410 (1990).

451 24 Kõljalg, U. *et al.* Towards a unified paradigm for sequence-based identification of
452 fungi. *Molecular Ecology* 22, 5271-5277, doi: 10.1111/mec.12481 (2013).

453 25 Chen, W., Simpson, J. & Levesque, C. A. *RAM: R for amplicon-sequencing-based*
454 *microbial-ecology*, (2015).

455 26 Nguyen, N. H. *et al.* FUNGuild: An open annotation tool for parsing fungal community
456 datasets by ecological guild. *Fungal Ecology* 20, 241-248,
457 doi:10.1016/j.funeco.2015.06.006 (2016).

458 27 Bürkner, P. C. brms: An R package for Bayesian multilevel models using Stan. *Journal*
459 *of Statistical Software* 80, doi:10.18637/jss.v080.i01 (2017).

460 28 R Core Team. R: A language and environment for statistical computing. R foundation
461 for statistical computing. Retrieved from <https://www.R-project.org/>. (2020).

462 29 MacArthur, R. H. Patterns of species diversity. *Biological reviews* 40, 510-533 (1965).

463 30 Jost, L. Entropy and diversity. *Oikos* 113, 363-375 (2006).

- 464 31 Lundberg, D. S. *et al.* Defining the core *Arabidopsis thaliana* root microbiome. *Nature*
465 488, 86-90 (2012).
- 466 32 Merlos, F. A. & Hijmans, R. J. The scale dependency of spatial crop species diversity
467 and its relation to temporal diversity. *Proceedings of the National Academy of Sciences*
468 117, 26176-26182 (2020).
- 469 33 Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. *Mixed effects models*
470 *and extensions in ecology with R.* (Springer Science & Business Media, 2009).
- 471 34 Schad, D. J., Vasishth, S., Hohenstein, S. & Kliegl, R. How to capitalize on a priori
472 contrasts in linear (mixed) models: A tutorial. *Journal of Memory and Language* 110,
473 104038, doi:10.1016/j.jml.2019.104038 (2020).
- 474 35 Dawson, W. Release from belowground enemies and shifts in root traits as interrelated
475 drivers of alien plant invasion success: a hypothesis. *Ecology and Evolution* 5, 4505-
476 4516, doi:10.1002/ece3.1725 (2015).
- 477 36 Callaway, R. M. & Lucero, J. E. Soil biota and non-native plant invasions. *Plant*
478 *Invasions: The Role of Biotic Interactions* 13, 445-457 (2020).
- 479 37 Sher, A. A. & Hyatt, L. A. The disturbed resource-flux invasion matrix: a new
480 framework for patterns of plant invasion. *Biological Invasions* 1, 107-114 (1999).
- 481 38 Seabloom, E. W. *et al.* Plant species' origin predicts dominance and response to nutrient
482 enrichment and herbivores in global grasslands. *Nature Communications* 6, 7710,
483 doi:10.1038/ncomms8710 (2015).
- 484 39 Enders, M. *et al.* A conceptual map of invasion biology: Integrating hypotheses into a
485 consensus network. *Global Ecology and Biogeography* 29, 978-991,
486 doi:10.1111/geb.13082 (2020).
- 487 40 Li, Y. J., Gao, Y. Z., van Kleunen, M. & Liu, Y. J. Interactive effects of herbivory and
488 the level and fluctuations of nutrient availability on dominance of alien plants in

489 synthetic native communities. *BioRxiv*, doi:10.1101/2021.07.26.453898 (2021).

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Figures

Figure 1 Graphical illustration of the experimental design. The upper panel shows the amounts of nutrient solution supplied to each combination of the nutrient-availability and nutrient-fluctuation treatments during the ten weeks of the experiment. The lower panel shows an overview of the soil-microbe treatment and the locations of the alien target species (plant symbol with yellow flowers) and native community plants in the pots. At the end of the experiment, soil was collected from the pots that had been inoculated with living soil for microbiome analysis.

Figure 2 Modelled mean values (±SE) of biomass production of the alien target species (a) and the native communities (b), and biomass proportion of the alien target species (c) under each combination of two nutrient-availability (low vs high), two nutrient-fluctuation (constant vs pulsed) and two soil-microbe (living vs sterilized) treatments. Model terms (NA: nutrient availability, NF: nutrient fluctuation, S: soil microbiome) whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not overlap with zero are indicated with daggers (†).

Figure 3 Mean values ($\pm$ SE) of the true Shannon diversity for soil bacteria (a), all soil fungi (b) and the subset of pathogenic soil fungi (c), as well as the ratio of the Shannon diversity of pathogenic soil fungi and AMF (d) under each combination of two nutrient-availability (low vs high) and two nutrient-fluctuation (constant vs pulsed) treatments. Model terms (NA: nutrient availability, NF: nutrient fluctuation) whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not overlap with zero are indicated with daggers (†).

Extended data Tables

Extended data Table 1 Output of the Bayesian multilevel models testing effects of the nutrient-availability (low vs high), nutrient-fluctuation (constant vs pulsed) and soil-microbe (living vs sterilized) treatments, and their interactions on aboveground biomass production of the alien target species, aboveground biomass production of the native communities and aboveground biomass proportion of the alien target species in each pot. Shown are the model estimates and standard errors (SE), as well as the lower (L) and upper (U) values of the 95% and 90% credible intervals (CI).

		Estimate	SE	L95% CI	U95% CI	L90% CI	U90% CI
Biomass production of alien target species (natural-log-transformed)	Intercept	-0.835	0.791	-2.398	0.744	-2.022	0.387
	Nutrient availability (NA)	0.346*	0.040	0.269	0.424	0.281	0.411
	Nutrient fluctuation (NF)	0.042	0.037	-0.030	0.114	-0.019	0.103
	Soil (S)	0.140*	0.054	0.035	0.246	0.052	0.230
	NA × NF	0.009	0.037	-0.064	0.082	-0.052	0.070
	NA × S	-0.025	0.038	-0.101	0.050	-0.088	0.037
	NF × S	-0.033	0.038	-0.108	0.042	-0.096	0.029
	NA × NF × S	0.081*	0.038	0.008	0.155	0.020	0.143
Biomass production of native communities (natural-log-transformed)	Intercept	0.366	0.474	-0.578	1.344	-0.359	1.101
	NA	0.455*	0.020	0.415	0.495	0.421	0.489
	NF	-0.03	0.020	-0.070	0.010	-0.063	0.004
	S	-0.122*	0.020	-0.162	-0.082	-0.156	-0.088
	NA × NF	0.024	0.021	-0.016	0.064	-0.010	0.058
	NA × S	0.113*	0.021	0.072	0.153	0.079	0.146
	NF × S	-0.028	0.020	-0.069	0.011	-0.062	0.005
	NA × NF × S	-0.021	0.021	-0.061	0.020	-0.055	0.014
Biomass proportion of alien target species (logit-transformed)	Intercept	-1.202	0.930	-3.038	0.733	-2.668	0.320
	NA	-0.121*	0.047	-0.213	-0.029	-0.199	-0.044
	NF	0.082†	0.044	-0.004	0.169	0.009	0.157
	S	0.262*	0.057	0.149	0.375	0.169	0.356
	NA × NF	-0.009	0.045	-0.099	0.082	-0.085	0.066
	NA × S	-0.140*	0.045	-0.228	-0.050	-0.215	-0.065
	NF × S	-0.006	0.044	-0.092	0.080	-0.077	0.066

	NA × NF × S	0.088*	0.044	0.002	0.177	0.016	0.161
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525 Model estimates whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not
526 overlap with zero are indicated with daggers (†). Residual standard deviations *sigma* for individual alien species are given in Table S3.

Extended data Table 2 Output of the Bayesian multilevel models testing effects of the nutrient-availability (low vs high) and nutrient-fluctuation (constant vs pulsed) treatments, and their interactions on the true Shannon diversity of soil bacteria, all soil fungi and the subset of pathogenic soil fungi, as well as the ratio of the Shannon diversity of pathogenic soil fungi and AMF in each pot. Shown are the model estimates and standard errors (SE) as well as the lower (L) and upper (U) values of the 95% and 90% credible intervals (CI).

		Estimate	SE	L95% CI	U95% CI	L90% CI	U90% CI
Shannon diversity of soil bacteria	Intercept	639.536*	41.416	557.021	725.848	575.918	706.156
	NA	-2.444	18.923	-39.929	34.828	-33.037	28.477
	NF	-4.817	18.811	-42.377	31.883	-36.117	25.728
	NA×NF	0.035	18.425	-36.669	35.901	-29.945	30.258
Shannon diversity of soil fungi (square-root-transformed)	Intercept	3.614*	0.145	3.317	3.896	3.388	3.828
	NA	-0.115*	0.057	-0.228	-0.002	-0.210	-0.021
	NF	-0.032	0.060	-0.152	0.085	-0.132	0.066
	NA×NF	0.063	0.057	-0.048	0.174	-0.030	0.157
Shannon diversity of soil pathogenic fungi (natural-log-transformed)	Intercept	1.617*	0.125	1.376	1.868	1.424	1.814
	NA	-0.076*	0.035	-0.146	-0.007	-0.134	-0.018
	NF	0.053	0.035	-0.015	0.122	-0.004	0.110
	NA×NF	0.075*	0.036	0.003	0.145	0.015	0.134
Ratio of Shannon diversity between soil pathogenic fungi and AMF (natural-log-transformed)	Intercept	-0.643*	0.156	-0.964	-0.333	-0.897	-0.395
	NA	-0.057	0.069	-0.195	0.077	-0.169	0.057
	NF	0.087	0.068	-0.047	0.219	-0.025	0.198
	NA×NF	0.112†	0.068	-0.020	0.246	0.002	0.222

Model estimates whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not overlap with zero are indicated with daggers (†). Residual standard deviations *sigma* for individual native community are given in Table S4.

Extended data Figures

Extended data Figure 2 Effects of nutrient treatments on soil-community composition of bacteria (a), all fungi (b), pathogenic fungi (c) and AMF (d). Nonmetric multidimensional scaling (NMDS) was used to visualize differences in the soil microbial communities of the nutrient treatments. Data points represent soil samples. The different colors of the points indicate different nutrient treatments. Ellipses represent means $\pm$ 1 SDs for soils under constant low (green), pulsed low (purple), constant high (red) or pulsed high nutrient supply (orange).

542 Supporting information

543 **Table S1** Details of the study species used in the experiment.

Species	Family	Status	Community group	Sowing date	Life history	Propagule source
<i>Bidens frondosa</i> L.	Compositae	Alien	/	9.08.2020	Annual	wild population
<i>Bidens pilosa</i> L.	Compositae	Alien	/	09.08.2020	Annual	wild population
<i>Erigeron annuus</i> (L.) Pers.	Compositae	Alien	/	26.06.2020	Annual/Biennial	wild population
<i>Erigeron canadensis</i> L.	Compositae	Alien	/	26.06.2020	Annual	wild population
<i>Lolium perenne</i> L.	Poaceae	Alien	/	14.08.2020	Perennials	commercial
<i>Paspalum notatum</i> Flügge	Poaceae	Alien	/	06.08.2020	Perennials	wild population
<i>Phytolacca americana</i> L.	Phytolaccaceae	Alien	/	26.06.2020	Perennials	commercial
<i>Solidago canadensis</i> L.	Compositae	Alien	/	03.08.2020	Perennials	wild population
<i>Cynanchum chinense</i> R.Br.	Apocynaceae	Native	NC1	14.08.2020	Annual	wild population
<i>Digitaria sanguinalis</i> (L.) Scop	Poaceae	Native	NC1	07.08.2020	Annual	wild population
<i>Potentilla tanacetifolia</i> Willd. ex Schldl.	Rosaceae	Native	NC1	07.08.2020	Perennials	wild population
<i>Dontostemon micranthus</i> C.A.Mey.	Brassicaceae	Native	NC1	07.08.2020	Annual	wild population
<i>Cleistogenes squarrosa</i> (Trin. ex Ledeb.) Keng	Poaceae	Native	NC1	11.08.2020	Perennials	wild population
<i>Echinochloa crus-galli</i> (L.) P.Beauv.	Poaceae	Native	NC2	07.08.2020	Annual	wild population
<i>Artemisia gmelinii</i> Weber ex Stechm.	Compositae	Native	NC2	07.08.2020	Perennials	wild population
<i>Crepidiasrum sonchifolium</i> (Maxim.) Pak & Kawano	Compositae	Native	NC2	07.08.2020	Annual/Biennial	wild population
<i>Eragrostis pilosa</i> (L.) P.Beauv.	Poaceae	Native	NC2	14.08.2020	Annual	wild population
<i>Gypsophila licentiana</i> Hand. Mazz.	Caryophyllaceae	Native	NC2	07.08.2020	Perennials	wild population
<i>Geum aleppicum</i> Jacq.	Rosaceae	Native	NC3	07.08.2020	Perennials	wild population
<i>Lappula myosotis</i> V. Wolf	Boraginaceae	Native	NC3	07.08.2020	Annual/ Biennial	wild population
<i>Chloris virgata</i> Sw.	Poaceae	Native	NC3	14.08.2020	Annual	wild population
<i>Euphorbia humifusa</i> Willd.	Euphorbiaceae	Native	NC3	07.08.2020	Annual	wild population
<i>Bromus inermis</i> Leyss	Poaceae	Native	NC3	07.08.2020	Perennials	wild population
<i>Malva verticillata</i> L.	Malvaceae	Native	NC4	07.08.2020	Biennial	wild population
<i>Achillea asiatica</i> Serg.	Compositae	Native	NC4	07.08.2020	Perennials	wild population
<i>Poa annua</i> L.	Poaceae	Native	NC4	07.08.2020	Annual	wild population
<i>Dysphania schraderiana</i> (Schult.) Mosyakin & Clemants	Amaranthaceae	Native	NC4	07.08.2020	Annual	wild population
<i>Setaria pumila</i> (Poir.) Roem. & Schult.	Poaceae	Native	NC4	14.08.2020	Annual	wild population
<i>Rumex crispus</i> L.	Polygonaceae	Native	NC5	07.08.2020	Perennials	wild population
<i>Setaria viridis</i> (L.) P.Beauv.	Poaceae	Native	NC5	14.08.2020	Annual	wild population
<i>Artemisia rubripes</i> Nakai	Compositae	Native	NC5	11.08.2020	Perennials	wild population
<i>Eragrostis minor</i> Host	Poaceae	Native	NC5	14.08.2020	Annual	wild population
<i>Potentilla longifolia</i> Willd. ex Schldl.	Rosaceae	Native	NC5	07.08.2020	Perennials	wild population

544 **Table S2 Effects of nutrient treatments on soil-community composition of bacteria, all fungi, pathogenic fungi and AMF.**

	Bacteria				All fungi				Pathogenic fungi				AMF			
	Df	F	R ²	P	Df	F	R ²	P	Df	F	R ²	P	Df	F	R ²	P
Nutrient availability (NA)	1	3.213	0.021	0.010*	1	2.944	0.019	0.001*	1	3.989	0.026	0.002*	1	5.514	0.035	0.001*
Nutrient fluctuation (NF)	1	1.162	0.008	0.221	1	0.855	0.006	0.669	1	0.750	0.005	0.597	1	0.598	0.004	0.789
NA × NF	1	1.393	0.009	0.147	1	0.967	0.006	0.405	1	1.530	0.010	0.091†	1	1.456	0.009	0.138
Residuals	150		0.963		149		0.969		149		0.960		149		0.952	
Total	153		1.000		152		1.000		152		1.000		152		1.000	

545 Significant effects ($P < 0.05$) are indicated with asterisks (*), and marginally significant effects ($0.05 \leq P < 0.1$) are indicated daggers (†).

546 **Table S3** Output of the Bayesian multilevel models' estimates of the residual standard-deviation sigmas for individual alien species. Shown are
547 the model estimates and standard errors as well as the lower (L) and upper (U) values of the 95% and 90% credible intervals (CI).

	Species	Estimate	SE	L95% CI	U95% CI	L90% CI	U90% CI
Biomass production of alien target species	<i>Bidens frondosa</i> L.	-0.635*	0.128	-0.877	-0.376	-0.840	-0.421
	<i>Bidens pilosa</i> L.	-0.522*	0.124	-0.750	-0.267	-0.715	-0.310
	<i>Erigeron annuus</i> (L.) Pers.	-0.266†	0.134	-0.523	0.006	-0.481	-0.042
	<i>Erigeron canadensis</i> L.	-0.186	0.125	-0.417	0.070	-0.383	0.027
	<i>Lolium perenne</i> L.	-0.492*	0.141	-0.760	-0.207	-0.717	-0.252
	<i>Paspalum notatum</i> Flügge	-0.713*	0.136	-0.970	-0.436	-0.931	-0.484
	<i>Phytolacca americana</i> L.	0.005	0.124	-0.230	0.258	-0.191	0.215
	<i>Solidago canadensis</i> L.	-0.253†	0.125	-0.487	0.002	-0.450	-0.041
Biomass production of native communities	<i>Bidens frondosa</i> L.	-1.095*	0.118	-1.315	-0.849	-1.282	-0.896
	<i>Bidens pilosa</i> L.	-1.032*	0.120	-1.258	-0.786	-1.221	-0.831
	<i>Erigeron annuus</i> (L.) Pers.	-0.810*	0.120	-1.035	-0.561	-0.998	-0.606
	<i>Erigeron canadensis</i> L.	-0.934*	0.121	-1.158	-0.683	-1.126	-0.727
	<i>Lolium perenne</i> L.	-1.164*	0.120	-1.386	-0.918	-1.356	-0.958
	<i>Paspalum notatum</i> Flügge	-1.106*	0.121	-1.332	-0.859	-1.299	-0.900
	<i>Phytolacca americana</i> L.	-0.755*	0.120	-0.979	-0.507	-0.943	-0.552
	<i>Solidago canadensis</i> L.	-1.168*	0.121	-1.390	-0.917	-1.360	-0.962
Biomass proportion of alien target species	<i>Bidens frondosa</i> L.	-0.457*	0.125	-0.689	-0.201	-0.656	-0.245
	<i>Bidens pilosa</i> L.	-0.230†	0.122	-0.457	0.017	-0.423	-0.023
	<i>Erigeron annuus</i> (L.) Pers.	-0.021	0.127	-0.259	0.239	-0.224	0.194
	<i>Erigeron canadensis</i> L.	-0.022	0.124	-0.255	0.228	-0.220	0.186
	<i>Lolium perenne</i> L.	-0.376*	0.133	-0.627	-0.109	-0.589	-0.153
	<i>Paspalum notatum</i> Flügge	-0.533*	0.131	-0.779	-0.265	-0.741	-0.312
	<i>Phytolacca americana</i> L.	0.233*	0.121	0.005	0.479	0.038	0.440
	<i>Solidago canadensis</i> L.	-0.203	0.125	-0.437	0.056	-0.401	0.012

548 Model estimates whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not
549 overlap with zero are indicated with daggers (†).

Table S4 Output of the Bayesian multilevel models' estimates of the residual standard-deviation sigmas for each community. Shown are the model estimates and standard errors as well as the lower (L) and upper (U) values of the 95% and 90% credible intervals (CI).

	Community group	Estimate	SE	L95% CI	U95% CI	L90% CI	U90% CI
Shannon diversity of soil bacteria	NC1	5.468*	0.140	5.210	5.753	5.246	5.706
	NC2	5.518*	0.134	5.271	5.801	5.306	5.750
	NC3	5.332*	0.147	5.056	5.633	5.099	5.583
	NC4	5.310*	0.141	5.045	5.604	5.086	5.553
	NC5	5.484*	0.135	5.232	5.759	5.270	5.711
Shannon diversity of soil fungi	NC1	-0.297*	0.132	-0.537	-0.020	-0.504	-0.071
	NC2	-0.074	0.133	-0.324	0.202	-0.283	0.152
	NC3	-0.572*	0.142	-0.837	-0.276	-0.794	-0.329
	NC4	-0.278†	0.137	-0.533	0.000	-0.493	-0.046
	NC5	-0.477*	0.142	-0.743	-0.183	-0.701	-0.235
Shannon diversity of soil pathogenic fungi	NC1	-0.887*	0.137	-1.139	-0.601	-1.101	-0.653
	NC2	-0.843*	0.137	-1.096	-0.561	-1.059	-0.611
	NC3	-0.941*	0.146	-1.213	-0.642	-1.175	-0.694
	NC4	-0.906*	0.147	-1.178	-0.610	-1.137	-0.659
	NC5	-0.584*	0.140	-0.848	-0.292	-0.803	-0.346
Ratio of Shannon diversity of soil pathogenic fungi and AMF	NC1	-0.184	0.137	-0.435	0.095	-0.400	0.048
	NC2	-0.091	0.132	-0.339	0.178	-0.301	0.134
	NC3	-0.234	0.144	-0.501	0.063	-0.461	0.009
	NC4	-0.253†	0.146	-0.527	0.046	-0.485	-0.008
	NC5	-0.17	0.138	-0.426	0.115	-0.390	0.065

Model estimates whose 95% credible intervals do not overlap with zero are indicated with asterisks (*), and those whose 90% credible intervals do not overlap with zero are indicated with daggers (†).

Figure S1 Modelled mean values ($\pm$ SE) of biomass production of the alien target species (a) and the native communities (b), and biomass proportion of the alien target species (c) under low and high nutrient availability, as well as biomass proportion of the alien target species under constant and pulsed nutrient supply (d).

Figure S2 Modelled mean values ($\pm$ SE) of biomass production of the alien target species (a) and the native communities (b), and biomass proportion of the alien target species (c) growing in substrate inoculated with living and sterilized field soil.

Figure S3 Modelled mean values ($\pm$ SE) of biomass production of native communities (a) and biomass proportion of alien target species (b) under each combination of the nutrient-availability (low vs high) and soil-microbe (living vs sterilized) treatments.

Figure S4 Mean values of the true Shannon diversity ($\pm$ SE) of all soil fungi (a) and the subset of pathogenic soil fungi (b) under low and high nutrient availability.