Supplementary material

The multiple-mechanisms hypothesis of biodiversity–stability relationships

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iDiv Ecotron Experiment

Main objective: Study plant diversity-mediated resistance and recovery under climate extremes (*ResCUE Experiment*).

The iDiv Ecotron is a joint research platform from iDiv and the Helmholtz Centre for Environmental Research – UFZ (Eisenhauer & Türke, 2018; Schmidt et al., 2021). It is an indoor research facility housing a set of 24 identical experimental units, called EcoUnits, each of which can harbor one to four isolated ecosystems confined in compartments (**Fig. S1**). Species assemblages within ecosystems can be manipulated above and below the ground, varying horizontal diversity (*i.e.*, the number of species within a trophic level) and vertical diversity (*i.e.*, the number of trophic levels). Ecological processes can be measured with non-invasive methods, while environmental conditions within EcoUnits are either controlled for the whole set of replicates (air temperature) or for each replicate individually (*e.g.*, irrigation, illumination, soil temperature; **Fig. S1**). The Ecotron allows for the construction of complex ecosystems resembling near-natural conditions but with the possibility to eliminate or reduce the variance from unknown factors (*e.g.*, by controlling environmental conditions) and to easily measure most of the variables influencing ecological processes and, thus, the mechanisms underlying BEF.

This setup allows testing plant diversity–stability relationships under controlled conditions and as affected by a "***hot drought***". This climate extreme event was selected based on the analysis of macro- and micro-climatic data in the Jena Experiment: hot droughts have occurred repeatedly at the field site and seem to increase in frequency over time (based on the analysis of climate data in Jena between 1900 and 2000, the century before the Jena Experiment was set up) (Huang et al. *unpubl. data*).

Fig. S1. The iDiv Ecotron *at the field station of the Helmholtz-Centre for Environmental Research in Bad Lauchstädt, Germany.* ***(A)*** *Photograph of some of the EcoUnits in the Ecotron hall.* ***(B)*** *Schematic depiction of one experimental unit ("EcoUnit") with technical equipment.* ***(C)*** *Photograph of four lysimeters in an EcoUnit with internal walls.* ***(D)*** *Photograph of sowing the seed material in Petri-dishes on filter paper moistened with deionized water.* ***(E)*** *Photograph after pricking plants into plot-specific soil substrate in 96-Quickpot plates.* ***(F)*** *Photograph of all individuals to be planted in the* ***JenaTron Experiment*** *(conducted in 2022) after transport to the iDiv Ecotron.* ***(G)*** *Photograph of the species-specific marking of the positions of all plant individuals on the soil monoliths with the help of a specially-made planting stencil.* ***(H)*** *Photograph of the planting of all plant individuals on the soil monoliths using bulb planters.* ***(I)*** *Photograph of the aboveground plant biomass harvest.* ***(J)*** *Photograph of the soil sampling.* ***(K)*** *Photograph of a destructively sampled lysimeter of the JenaTron Experiment.*

We will subject half of the EcoUnits to ambient conditions and the other half to hot drought conditions for resistance and recovery assessments. Studying biodiversity–resistance and biodiversity–recovery relationships, as done here, will allow testing if resistance has higher predictive power for biodiversity–stability relationships than recovery (Isbell et al., 2015).

The soil will come from 24 plots of the *Main Experiment* that were selected to represent a gradient in plant diversity: (1) monocultures (n = 12 plots; 3 plots of each of the 4 plant functional groups), (2) 4-species mixtures (n = 6 plots; 4 plots each containing one plant species of all 4 plant functional groups, 1 plot with 4 grass species [mostly shallow-rooting, mostly thin roots], 1 plot with 4 tall herb species [mostly deep-rooting, mostly thick roots]), and (3) 16-species mixtures (n = 6; 4 plots with all plant functional groups being represented, 1 plot with 16 grass species [mostly shallow-rooting, mostly thin roots], 1 plot with 16 tall herb species [mostly deep-rooting, mostly thick roots]). We will excavate soil of each of the 24 selected plots using a digger. We will separate the top 30 cm of the soil from the bottom 50 cm of the soil to represent two soil layers in the lysimeters of the iDiv Ecotron. The top soil will be gently broken apart to remove large roots but to keep the soil meso- and macrofauna community as intact as possible. The bottom soil will be gently sieved at 1 cm to homogenize the soil. Incubation of lysimeters will be done as before in the *JenaTron Experiment* (successfully performed in the first phase of the Research Unit; Eisenhauer et al., 2019b):

the lysimeters have a depth of 0.8 m and a diameter of 0.5 m. One EcoUnit will harbor four monoliths (note that aboveground glass walls will separate the four lysimeters, as those installed in **Fig. S1c**). For each experimental plot of the *Main Experiment* (n = 24), we will establish four lysimeters in the iDiv Ecotron (n = 96): two lysimeters will be subjected to the hot droughts as defined by the *DrY Experiment* (see below), while two lysimeters will serve as ambient climate controls (**Fig. S2**). One set of lysimeters of the hot drought and the ambient treatments will be destructively harvested after the drought to study ***resistance***, respectively, while a second set of lysimeters will be destructively harvested after a ***recovery*** phase (see below; **Fig. S2**).

Plant seeds will be purchased from the commercial supplier that also provided the seeds for all other experiments in the framework of the Jena Experiment. Plants will be pre-grown in the iDiv Greenhouse (**Figu Fig. re S1d-f**). We will plant the target communities of the field plots by using a planting density per lysimeter of 55 (at a distance of ~2 cm among plant individuals), such as done in the *JenaTron Experiment* (**Fig. S1g**). After an establishment phase of six weeks, a ^{15}N label (98atom% $^{15}NH_4^{15}NO_3$) will be added as liquid solution homogeneously in the depths of 0-10 cm to all lysimeters that are set up to study resistance (n = 48). Homogeneous distribution of the ^{15}N label will be ensured by application via syringes in a grid pattern (5 by 5 cm grid size) as done in a previous labeling study in the Jena Experiment (Jesch et al., 2018). We will apply only a small amount of N with the applied ^{15}N label, however, it will be highly enriched in ^{15}N to reduce the impact of the label on N cycling rates while increasing the detectability of the label in the plant-soil system. The hot drought will be applied to half of the lysimeters per plot (**Fig. S2**). Drought conditions will be maintained for 6 weeks. At the end of the drought period, ^{13}C pulse labeling will be applied to the ***resistance*** lysimeters, and a destructive sampling of these lysimeters will be conducted 1 day later (**Fig. S2**). In the remaining 48 lysimeters, plant biomass will be harvested, and rewetting of the lysimeters will be performed to study ***recovery***. At the start of the recovery phase, a ^{15}N label will be added as described above, and the experiment will be run for another six weeks (**Fig. S2**). Prior to the final harvest, ^{13}C pulse labeling will be applied to recovery lysimeters, and a destructive sampling of these lysimeters will be conducted 1 day later (**Fig. S2**). In addition to plant community-related measurements, we will also introduce ***a common phytometer species (Plantago lanceolata)*** to all lysimeters to be able to follow the same species along the plant diversity gradient (n = 3 individuals per lysimeter). We selected this phytometer species, given its ubiquity in the experimental study systems and our experience successfully using it as a phytometer to study biotic interactions and plant-defense mechanisms in previous work (*e.g.*, Eisenhauer et al., 2009; Fontana et al., 2009). One individual will be subjected to herbivory shortly before the end of the experiment using clip cages, while a second individual will be clip-caged in the same way but will serve as a control. The third individual will serve as a back-up plant in case of mortality of another phytometer individual.

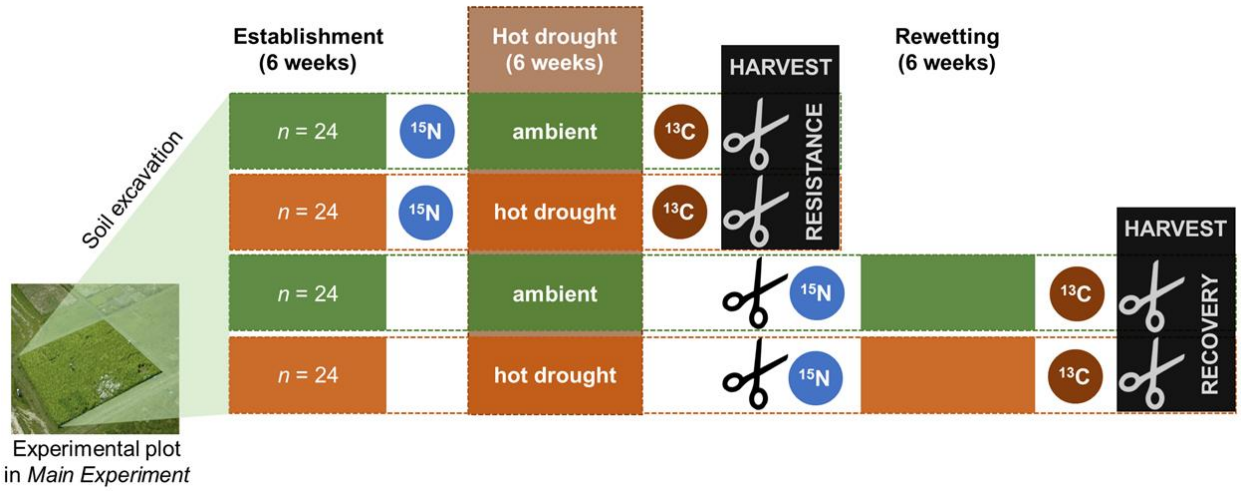

Fig. S2. Time plan of the ResCUE Experiment. *The four treatment lines will be realized for each of the 24 selected plots in the Jena Experiment that represent a plant diversity gradient of 1 (n = 12), 4 (n = 6), and 16 species (n = 6). This means that the 24 plant communities will be factorially crossed with 2 climate extreme treatments (ambient and hot drought) and 2 phases (resistance and recovery) = 96 experimental units.*

Microcosm Experiments - *DrY Experiment* and *CoMic Experiments*

Main objectives: (1) Identify the conditions and thresholds of climate extremes to inform the ResCUE and other microcosm experiments. (2) Study the potential non-linearity of drought effects on BEF relationships. (3) Study abiotic and biotic stressors affecting plant diversity–stability relationships in subproject-specific experiments.

What is a drought? At which severity threshold does a drought become detrimental for different plants and microbes? Are effects of increasing drought severity on BEF relationships non-linear (Baert et al., 2018)? To prepare the *ResCUE Experiment* and subproject-specific microcosm experiments, we need to (1) determine the conditions that cause stress in plant and soil communities in realistic ways (*e.g.*, such as seen under extreme climatic conditions in the field) and (2) develop a *Common Research Platform* with standardized soil as well as common plant species and communities (*e.g.*, to compare the results of different microcosm studies performed at different locations). We will set up a microcosm experiment (*Drought Intensity Experiment*; short: *DrY Experiment*) that varies the (a) number of plant species (monocultures, 2 and 4-species mixtures; realistic diversity levels at the small spatial scale of a microcosm) and (b) hot drought (a gradient of 5 levels of precipitation/water availability under warm temperature conditions). Based on common and functionally distinct plant species in the *Main Experiment*, we will select eight species (2 grasses, 2 legumes, 2 small herbs, 2 tall herbs). Within each plant functional group, we selected one plant species that is more vulnerable to drought stress and one species that is resistant to drought stress (**Table S1**). These eight species will either be grown in replicated monocultures (=16 microcosms), in 18 2-species mixtures, or in 12 4-species mixtures (**Table S2**). The 46 sets of microcosms will be subjected to the five drought treatments. To study resistance and recovery by conducting destructive measurements directly after the drought and after a recovery phase (*e.g.*, of root biomass

and soil microbial communities), we will set up the full setup twice. Taken together, this will result in 46 plant communities x 5 drought levels x 2 harvests = 460 microcosms.

Table S1. Selected species for the DrY Experiment. *We selected eight common plant species in the Main Experiment that belong to four plant functional groups and represent either drought-sensitive or drought-tolerant species.*

Plant species	Plant functional group	Response to drought
Holcus lanatus	Grass	Drought-sensitive
Bromus erectus	Grass	Drought-tolerant
Trifolium pratense	Legume	Drought-sensitive
Lotus corniculatus	Legume	Drought-tolerant
Rumex acetosa	Tall herb	Drought-sensitive
Knautia arvensis	Tall herb	Drought-tolerant
Bellis perennis	Small herb	Drought-sensitive
Plantago lanceolata	Small herb	Drought-tolerant

Table S2. Plant communities in the DrY Experiment *with replicated monocultures of all eight plant species (n = 16); 2-species mixtures (n = 18; all different communities) containing either only drought-tolerant (n = 6), only drought-sensitive (n = 6), or a drought-tolerant and a drought-sensitive species (n = 6) and considering equal representation of all plant functional groups; and 4-species mixtures (n = 12; all different communities) with different combinations of drought-tolerant and drought-sensitive species considering equal representation of all plant functional groups.*

Plant community/ replicate	Monocultures		2-species mixtures		4-species mixtures
1	1 drought-tolerant grass	17	1 drought-tol. grass + 1 drought-tol. legume	35	4 drought-tolerant species (one of each PFG)
2	1 drought-sensitive grass	18	1 drought-tol. small herb + 1 drought-tol. tall herb	36	4 drought-sensitive species (one of each PFG)
3	1 drought-tolerant legume	19	1 drought-tol. grass + 1 drought-tol. small herb	37	2 drought-tolerant + 2 drought-sensitive (gr/le + sh
4	1 drought-sensitive legume	20	1 drought-tol. legume + 1 drought-tol.tall herb	38	2 drought-tolerant + 2 drought-sensitive (sh/le + gr
5	1 drought-tolerant small herb	21	1 drought-tol. grass + 1 drought-tol. tall herb	39	2 drought-tolerant + 2 drought-sensitive (th/gr + le
6	1 drought-sensitive small herb	22	1 drought-tol. legume + 1 drought-tol. small herb	40	2 drought-tolerant + 2 drought-sensitive (sh/th + le
7	1 drought-tolerant tall herb	23	1 drought-sen. grass + 1 drought-sen. legume	41	2 drought-tolerant + 2 drought-sensitive (gr/sh + le
8	1 drought-sensitive tall herb	24	1 drought-sen. small herb + 1 drought-sen. tall herb	42	2 drought-tolerant + 2 drought-sensitive (le/th + sh
9	1 drought-tolerant grass	25	1 drought-sen. grass + 1 drought-sen. small herb	43	3 drought-tolerant + 1 drought-sensitive (gr/le/sh +
10	1 drought-sensitive grass	26	1 drought-sen. legume + 1 drought-sen. tall herb	44	3 drought-tolerant + 1 drought-sensitive (le/sh/th +
11	1 drought-tolerant legume	27	1 drought-sen. grass + 1 drought-sen.tall herb	45	1 drought-tolerant + 3 drought-sensitive (sh + gr/le
12	1 drought-sensitive legume	28	1 drought-sen. legume + 1 drought-sen. small herb	46	1 drought-tolerant + 3 drought-sensitive (le + gr/sh
13	1 drought-tolerant small herb	29	1 drought-tol. grass + 1 drought-sen. legume		
14	1 drought-sensitive small herb	30	1 drought-tol. small herb + 1 drought-sen. tall herb		
15	1 drought-tolerant tall herb	31	1 drought-sen. grass + 1 drought-tol. small herb		
16	1 drought-sensitive tall herb	32	1 drought-tol. legume + 1 drought-sen. tall herb		
		33	1 drought-sen. grass + 1 drought-tol.tall herb		
		34	1 drought-tol. legume + 1 drought-sen. small herb		

We will use mixed soil from the edge of the Jena Experiment site (next to Block 3; Roscher et al., 2004) that will also be available to the other Microcosm Experiments in the individual subprojects. The soil will be sieved at 1 cm to remove large roots and stones. We will use 3 l-microcosms and transplant four pre-grown plant individuals per microcosm (such as shown in **Fig. S1d-h**). The drought treatment will follow a gradient design, which optimizes the use of experimental units to determine non-linear responses and thresholds (Kreyling et al., 2018; Ingrisch et al., 2023). We will create a gradient of increasing drought intensity ranging from well-watered controls (100% precipitation) to ~60%, ~35%, ~20%, and ~10% of precipitation relative to the control. The different levels of water availability will be established using an automated irrigation system with differing outlet valves per drought intensity gradient enabling us to fit 23 microcosms per drought intensity in one greenhouse chamber with a total of 115 microcosms. We will use four greenhouse

chambers to fit the full experiment and will distribute replicates equally across chambers. The factor "chamber" will be used as a random factor in statistical analyses. We will simulate hot summer conditions (30 °C/26 °C day/night for 16/8 h, respectively), and air humidity will be set at 70%. After an establishment phase of the plants of 6 weeks under control conditions (100% precipitation), the experiment will run for 6 weeks with the experimental treatments applied. Soil moisture and temperature (measured in two microcosms per treatment, additionally set up with SMT-100 Sensors (Truebner) with data logger TrueLog200 (Truebner)) will be measured throughout the experiment to identify appropriate study conditions and sets of species that will be used in the *ResCUE Experiment* and subproject-specific microcosm experiments, respectively. We note that drought-induced variations in soil water availability might be modulated by plant biomass production. After the drought period, half of the microcosms will be destructively sampled for ***resistance*** assessments. We will assess plant species-specific shoot biomass and community-specific root biomass of the 230 microcosms. Leaf material of each species x pot combination will be used for measurements of SLA as well as leaf carbon and nitrogen concentrations. Moreover, we will assess the flowering success of each plant individual. Further plant and soil material will be provided for subproject-specific analyses. Afterwards, all microcosms will be watered at 100% precipitation for 6 weeks. At the end of the experiment, we will repeat the sampling campaign as done for resistance assessments to determine ***recovery***.

Based on the insights gained in the *DrY Experiment*, subprojects will perform *Complementary Microcosm* (*CoMic*) *Experiments* to study abiotic and biotic stressors affecting plant diversity–stability relationships like drought, above- and belowground herbivory, perform repeated destructive measurements, determine the effect of plant and microbial traits related to drought resistance and recovery and antagonists on drought resistance making use also of synthetic microbial communities, determine standardized above- and belowground traits including hydrological traits to determine trait correlations and how these predict drought stability as well as quantify the effect of drought on biomass allocation belowground.

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