Appendix IV: Design of the Ecotron Experiment

The iDiv Ecotron is a joint research platform from iDiv and the Helmholtz Centre for Environmental Research – UFZ (Eisenhauer & Türke 2018). 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. A2**). 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. A2**). 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.

Twenty-three plots of species pool 2 of the Trait-Based Experiment (TBE) in the Jena Experiment (established in 2010; Ebeling et al. 2014) were selected to cover a plant diversity gradient of 1, 2, 3, and 6 species (6 plant species mixtures serve as a higher-diversity reference). Six plant species of species pool 2 were selected due to the overlap of plant species with the Field Experiment, the equal (three species each) representation of grasses (*Anthoxanthum odoratum, Holcus lanatus, Dactylis glomerata*) and herbs (*Plantago lanceolata, Leucanthemum vulgare, Ranunculus acris*), and the dissimilarity in temporal resource acquisition traits (Ebeling et al. 2014), which may increase over time (**Fig. 1**). In spring 2021, we will excavate two monoliths of each of the 22 selected TBE plots, and additional four monoliths of the selected 6-species plot (48 monoliths in total; **Fig. 4, Table A4**). The monoliths will have a depth of 0.8 m and a diameter of 0.5 m. Those monoliths will represent Ecotron treatments with soil history (**Fig. 4**). In addition, we will excavate 48 monoliths from the bare ground plots of the Jena Experiment. These plots have been maintained without vegetation cover since 2002 and will represent treatments without soil history (**Fig. 4**). These monoliths are ideal for this purpose as they have the same soil characteristics as the experimental plots (pH, sand content etc.). One EcoUnit will harbor four monoliths (**Fig. A2c**; note that aboveground glass walls will separate the four monoliths, such as installed in **Fig. A2b**), two with plot-specific soil history (from the same TBE plot) and two without soil history (from the bare ground plots; **Fig. 4**).

The vegetation will be gently removed from the monoliths with plot-specific soil history, such as already successfully done in the Field Experiment, to start with equal conditions across soil history treatments. The two soil history treatments will be crossed with two plant history treatments by transplanting pre-grown seedlings of the respective plant community composition of the TBE at equal densities at a distance of ~5 cm among plant individuals (~360 plant individuals m^{-2}; ~70 per monolith). One treatment will be planted with pre-grown plants from seeds collected in the respective TBE plot in 2019 (with plot-specific plant history). As a reference, the same plant species will be grown from seed material that was used in 2010 (**Fig. 4**), when the TBE was set up. These plants will not have a plot-specific plant history. Plants will be pre-grown in plot-/treatment-specific soil that will also be used for the **Ecotron Experiment**. This means that in each EcoUnit, we will establish four treatments with the same plant species composition and plant density: (1) *'with plant history, with soil history'*; (2) *'without plant history, with soil history'*; (3) *'with*

plant history, without soil history'; and (4) *'without plant history, without soil history*' (**Fig. 4**). The experiment will run for ~6 months before it gets destructively sampled.

Table A4. A. *Table providing an overview of the different treatments in the planned Ecotron Experiment, covering a gradient on plant species richness and functional diversity in temporal resource use (Ebeling et al. 2014) crossed with two treatments of plant history and soil history, respectively. The number of replicates per plant species richness level is given in brackets.* ***B.*** *Representation of the different plant species (for full names see text) in the different plant species richness levels. Given is the number of occurrences in different plant communities. Each plant species can be investigated along the plant species richness gradient.*

A. Treatments	**Levels**			
Plant species richness	1 (6 reps)	2 (9 reps)	3 (7 reps)	6 (2 reps)
FD plant temporal resource use	1	1-4	2-4	4
Plant history	with history	without history		
Soil history	with history	without history		

B. Plant species	**monocultures**	**2-species mix**	**3-species mix**	**6-species mix**
Ant odo (12)	1	4	5	2
Dac glo (12)	1	4	5	2
Hol lan (9)	1	3	3	2
Leu vul (7)	1	2	2	2
Pla lan (10)	1	3	4	2
Ran acr (7)	1	2	2	2

We will establish a **phytometer experiment** in the Ecotron Experiment using genotypes of the model species *Plantago lanceolata* (led by SP10). Three mother individuals will be collected each at low (monoculture) and high (8-species mixture) diversity with 8 years selection history in the TBE. The mother individuals will be vegetatively propagated and one offspring of each genotype will be transplanted into those units of the Ecotron Experiment, which contain *P. lanceolata* in the original composition (= 10 communities x 4 treatments x 6 individuals = 240 plants). This experiment will test if genetically identical plants show adaptive phenotypic plasticity and epigenetic variation in response to the actual environment (local plant diversity, plant community and soil history) and if these responses depend on the selection history of the genotypes.

Similar to the herbivory experiment in the Field Experiment, the generalist herbivore *Spodoptera littoralis* will be used by SP5 to induce herbivory on three *Plantago lanceolata* individuals per treatment, and three individuals will serve as 'control' plants. This will be only realized in treatments that have *Plantago lanceolata* in the species pool, summing up to a total of 240 plant individuals (10 plots x 4 soil/plant history treatments x 2 herbivory treatments á 3 individuals). Shortly before the harvest of the experiment, the focal plant individuals will be screened for volatiles (SP7), metabolites and shoot traits (SP8).

In concerted campaigns, SP1 will study species-specific mycorrhization rates and mycorrhizal diversity as well as mycorrhizal and fungal diversity at the community level; SP2 will investigate species-specific protist communities; SP3 will sequence the microbiome of the *Plantago*

phytometers and the root microbiome of a subset of the other plant species; SP7 will study constitutive (repeated measurements) and herbivore-induced VOC emission; SP8 will perform metabolomics analyses on leaf and root tissue, phloem and root exudates of *Plantago* phytometers, and will study species-specific traits of all plant species; SP9 will study community-level DOM and DOC (repeated measurements); SP4 will study plant species-specific phenology, soil invertebrate feeding activity and community composition and traits, root growth and turnover, and soil microbial biomass (all repeated measurements); SP5 will examine herbivore effects on plant performance in a common *Plantago* experiment (where SP7 and SP8 will study the plant's volatile emissions, metabolome, and defense traits, respectively); SP6 will study soil food webs to infer belowground herbivory and the dominance of different soil energy channels; SP10 will lead the *Plantago* phytometer study; and SPZ will study plant species-specific shoot biomass, community-level root biomass, soil and plant community-level C, N, and P concentrations, and will coordinate the field component of the study (e.g., selection of plots and sampling locations, collection of seeds, excavation of soil cores).

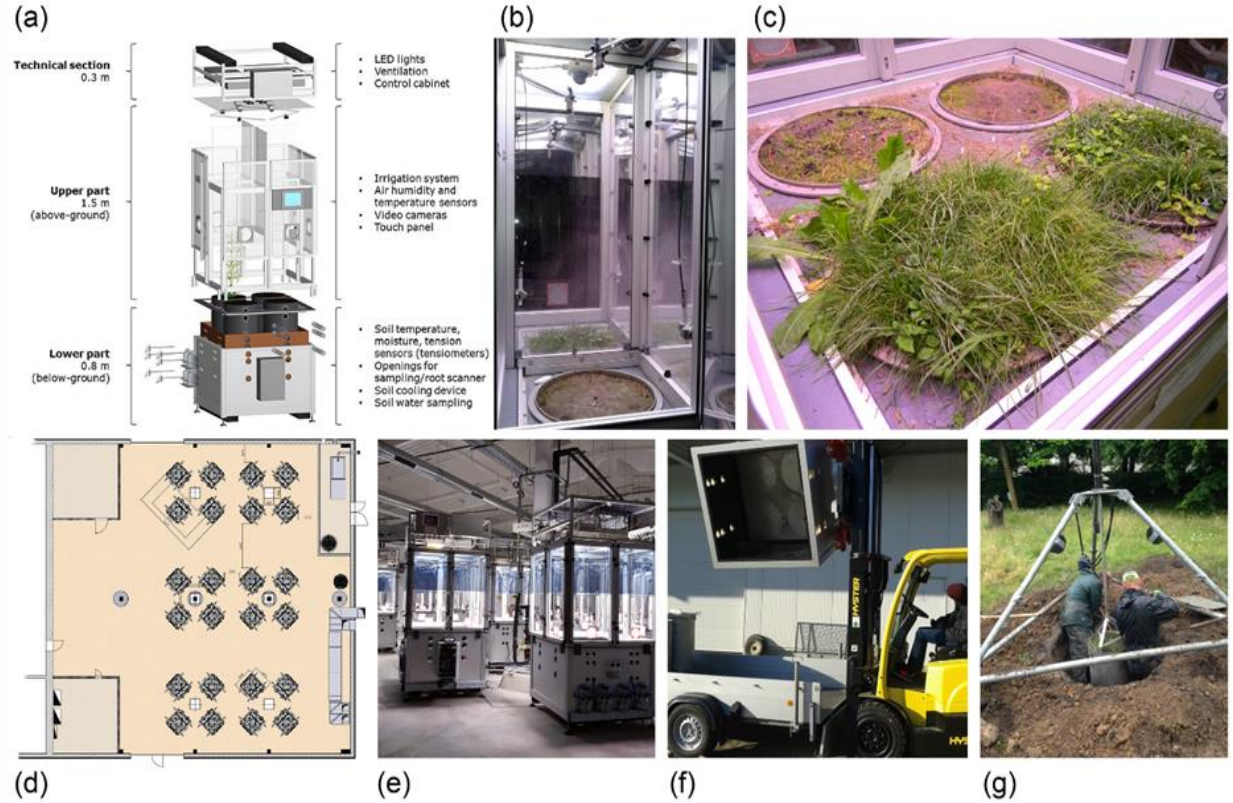

Figure A2. *Experimental infrastructure in the iDiv Ecotron in Bad Lauchstädt, Germany.* ***(a)*** *Schematic depiction of one experimental unit ("EcoUnit") with technical equipment.* ***(b)*** *Photograph showing the automated irrigation system, camera system, sensors, and inner walls of one half of an EcoUnit.* ***(c)*** *Photograph of four lysimeters in one EcoUnit without inner walls. Lysimeters can be manually filled with new soil and plants (the two lysimeters in the back where germination just started) or intact monoliths can be excavated in the field and incubated in the Ecotron (the two lysimeters in the front with the dense vegetation).* ***(d)*** *Schematic depiction of the spatial arrangement of the 24 EcoUnits of the iDiv Ecotron (from above) in the climate-controlled Ecotron hall.* ***(e)*** *Photograph of some of the EcoUnits in the Ecotron hall.* ***(f)*** *Photograph showing one exemplary lower part of an EcoUnit that is transported and turned with the help of a forklift.* ***(g)*** *Photograph of the excavation of a soil monolith in the Botanical Garden in Leipzig in preparation of this proposal.*

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