1. Summary of EcoStressWeb experiment in the iDiv Ecotron:

Topic: Joint impacts of climate and predation on multitrophic communities and ecosystem functioning
Lead: Malte Jochum (EIE, iDiv)
Setting: Temperate forest soil & litter system, above and belowground; *Fagus sylvatica* and *Quercus petraea* trees (2 each) planted in each subunit
Experimental design: 4 predator levels, 3 climate levels, each of the 12 combinations replicated 4 times (48 replicates = Ecotron Subunits)
Treatments: Climate change (drought) and predator (wolf spider, predatory mite, both, none) treatments, see text and Fig. 1.
Main hypotheses: See Fig. 2.
Prospective time plan: Experiment setup in April 2021, running for ~6 months, for details, see Fig. 3.

Measurements already planned:
- invertebrate fauna community assessment
- quantification of feeding interactions between predators, detritivores, and basal resources (amino acids, DNA-based gut content analysis, predator gut microbiome)
- tree performance
- soil PLFA, pH
- Cmic and microbial respiration
- microbial composition (litter and soil)
- decomposition (litter bags and bait lamina)

Most of these measurements will be taken once at the end of the experiment, or at the beginning and end. So far, only the bait lamina decomposition is assessed continuously.

Collaboration:
We are open to suggestions for additional measurements / sampling and welcome you to test additional hypotheses. If you are interested to collaborate, please get in touch (malte.jochum@idiv.de) as soon as possible.

2. Background and general information
Human societies depend on the stable provisioning of ecosystem services by Earth's ecosystems. These services include food production, pest control, water purification, and erosion control. Unfortunately, the stable delivery of such services is threatened by intensifying global-change drivers, such as climate change, or the direct exploitation of natural resources (Díaz et al. 2019). As a result, we see changing climate and biodiversity patterns across the globe (IPCC 2018, Díaz et al. 2019). The consequences of these changes for natural and managed ecosystems are complex and need to be studied comprehensively in order to understand, mitigate, and manage their impact on ecosystem functioning, and human wellbeing.

Forests are amongst the most threatened and most diverse ecosystem types delivering important ecosystem services and hosting a large fraction of global biodiversity (Reid et al. 2005). They are particularly important as carbon sinks (Pan et al. 2011). The functioning of forest ecosystems is heavily dependent on the decomposition of dead organic matter (mostly plant leaves and branches). This central ecosystem process is driven by the community of invertebrate animals, in interaction with bacteria and fungi, living in the litter and soil compartments of forest ecosystems (Gessner et al. 2010). The complex interplay of these organisms with their biotic and abiotic environment is key to the overall functioning and efficiency of decomposition and many other processes carried out by forest ecosystems.

Through rising temperatures and altered precipitation patterns, future climate heavily impacts levels of ecological organization from individuals to communities (IPCC 2018, Díaz et al. 2019). Besides shifts in species ranges and phenology, the seasonal timing of activity and life-cycles, the organisms are heavily impacted by physiological stress changing their metabolism (Brown et al. 2004), and subsequently consumption and competition (Lang et al. 2012, Rall et al. 2012). Biodiversity is altered through direct and indirect effects of global-change drivers (Pimm et al. 2014). Both their trophic position at the top of the food chain and their relatively large body size make predators particularly prone to these stressors. As a result, predator diversity is often more heavily affected than that of other groups (Barnes et al. 2014). Decade-long ecological research has shown that predator diversity and loss have severe consequences for community structure and ecosystem functions (Schneider and Brose 2013). Warming affects predators via both physiological and behavioral pathways. At higher temperatures, predators are expected to show higher attack rates and lower prey handling times (Rall et al. 2012), and to reduce prey coexistence (Thakur et al. 2017). Soil-invertebrate predators can increase their consumption rates with cascading effects on fungi and fungal decomposition under higher temperatures and drought (Lang et al. 2013). Such indirect effects of climate on prey populations have been related to predators seeking thermal refuge (Barton 2011). Previous research shows that the effects of warming and predation on food webs and ecosystem functions depend on the predators' vertical habitat domain (Schmitz 2007), their relative body size (Schneider and Brose 2013), and predator-prey body size ratios (Brose et al. 2019).

Typically, controlled experiments are used to study the mechanisms behind these ecosystem processes and their sensitivity to global change. However, such experiments are often confined to very small spatial and temporal scales, to small subsets of natural communities, to single global-change drivers, or to either the above- or belowground compartment. Due to the often non-additive nature of the effects of multiple simultaneously-acting global-change drivers on ecosystems, research on multi-stressor systems needs to be expanded (Rillig et al. 2019). Furthermore, joint above-belowground systems in larger experimental units are urgently needed in global-change research (Eisenhauer and Türke 2018). Given that experiments combining climate change and predator loss are rare (Lang et al. 2013), and that investigating above-belowground interactions in forest ecosystems will significantly improve our ability to understand and manage future global-change impacts, there is an urgent need for more-realistic experimental research to study the impact of global-change drivers on forest ecosystem processes.

This project aims to answer the question how simultaneous climate change and predator-community manipulation will jointly impact experimental forest litter and soil-invertebrate communities and their ability to control ecosystem processes. We will test how the treatments will affect i) community structure i.e. the relative importance of different species and functional groups, their vertical stratification, and the trophic interaction network, and ii) ecosystem functioning measured as decomposition, predation, and the main energy channels in the system. To answer these questions, we will run the **EcoStressWeb** experiment (**Fig. 1**) in the iDiv Ecotron (https://www.idiv.de/en/research/platforms-and-networks/idiv-ecotron.html), a novel, high-end, experimental facility to manipulate climate and multitrophic biodiversity in above-belowground communities and simultaneously study the consequences for multiple ecosystem processes (Eisenhauer and Türke 2018). The EcoStressWeb experiment will full-factorially cross an invertebrate-predator treatment (4 levels: no predators, ground spider only, predatory mite only, both predators) with a climate-change treatment (precipitation manipulation from current to projected future climate for central Germany during summer (Schädler et al. 2019); three levels: current, intermediate, future, with decreasing soil moisture), resulting in 12 treatment combinations, each of which will be replicated 4 times across overall 48 Ecotron subunits (**Fig. 1**). The experiment will be set up as an above-belowground temperate forest litter and soil ecosystem. It will help us understand how combined climate and predator-community change will affect temperate forest systems and their ability to withstand future perturbations to reliably support ecosystem processes that humanity depends on (for hypotheses, see next section and **Fig. 2**).

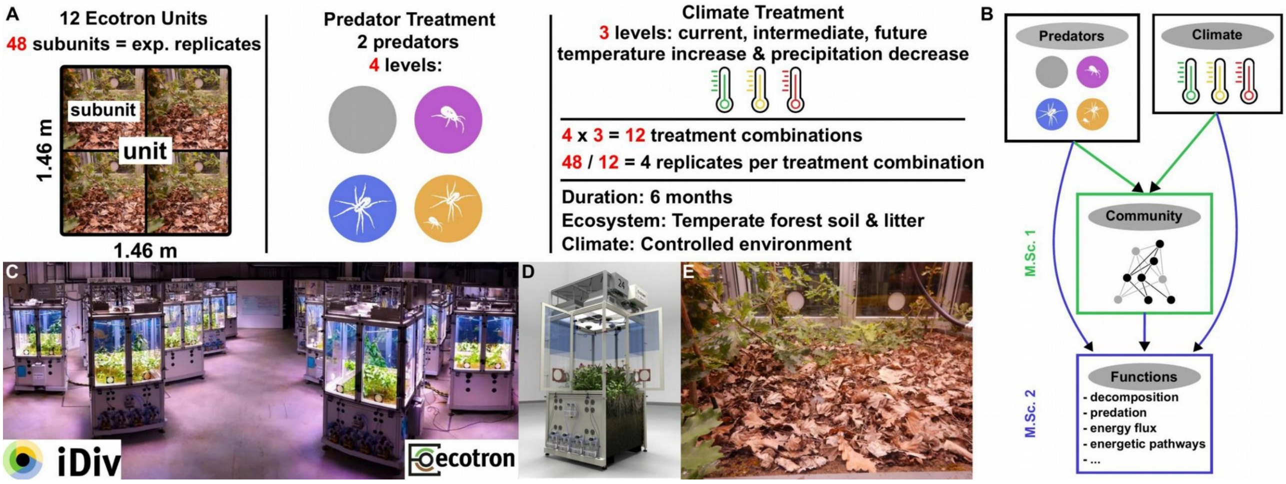

Figure 1. Overview over the proposed iDiv Ecotron experiment **EcoStressWeb**. Experimental design (A) including the predator and climate treatments, the 12 treatment combinations and replication, as well as duration, ecosystem type, and climate control setup. Panel B illustrates expected pathways of treatment impacts on the ecological community (green) and ecosystem functions (blue), the two foci of the core M.Sc. projects. For hypotheses, see Fig. 2. Other panels show the Ecotron hall in Bad Lauchstädt, Germany (C), a technical drawing of an Ecotron unit (D), and an in-situ view from a previous Ecotron experiment in a forest-litter environment (E), respectively.

This project was designed to involve two core M.Sc. students at Leipzig University (see **Fig. 3**). M.Sc. 1 will focus on treatment effects on the faunal communities and feeding relationships among the taxa. M.Sc. 2 will focus on the effect of treatments and changing faunal communities on ecosystem functions (decomposition, predation), energy flux, and energetic pathways. Other thesis projects focus e.g. on tree performance, microbial composition of soil and litter, and primer design for the DNA-based gut content analysis.

3. Hypotheses

Two predators will be used: a litter-based wolf spider (*Pardosa sp.*) and a predatory mite (*Stratiolaelaps scimitus)* able to hunt above and belowground. To track the predators' vertical habitat domain, we will add two springtail species (decomposers), which are separated in their vertical distribution: *Heteromurus nitidus* (predominantly litter-dwelling) and *Folsomia candida* (predominantly soil-dwelling). These species have been combined in previous experiments of iDiv groups (Schneider and Brose 2013, Thakur et al. 2017). Relative feeding on the springtails will allow us to assess the predator habitat-domain shift in response to the treatments. We expect the predator treatment to impact competition and intraguild predation via downward-shifts in vertical habitat domains and reduced niche breadth (**Fig. 2**). Feeding relationships and intraguild predation will be assessed by DNA-based gut content analysis combined with a multiplex PCR assay (Sint et al. 2014, Eitzinger et al. 2018).

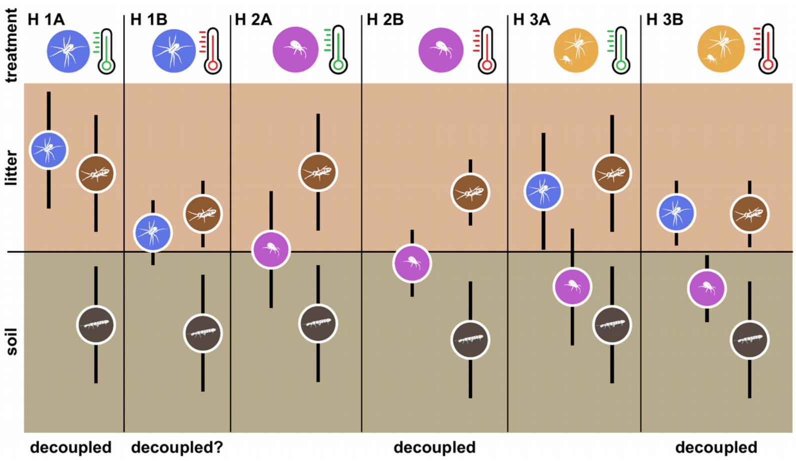

Figure 2. Hypothesized effects of predator (spider – blue, H1; mite – pink, H2; both – orange, H3) and climate (current, future; hypotheses A/B) treatments on predator and prey (litter-dwelling springtail – brown; soil-dwelling springtail – black) vertical stratification (black bars). The "no predator" and "intermediate climate" levels are not shown but needed to set predator effects into context, and enable picking up intermediate climate-change effects, respectively.

We expect the spider alone (H 1A, **Fig. 2**) to forage in the litter layer, with the above- and belowground compartments (litter and soil compartments) being energetically decoupled. If it manages to reach the soil-dwelling springtails under future climate conditions (H 1B), this might couple the subcompartments. The mite alone will hunt both above and below the ground (subcompartments coupled; H 2A). Future climate might lead to a decoupling, should the mite shift belowground and stop foraging on aboveground springtails (H 2B). With both predators present (H 3A), the spider will drive the mite downwards, but systems will still be coupled and we would expect trophic-cascade effects. Future climate might decouple the systems if the spider cannot forage on the vertically-shifting mite and the soil-dwelling springtail (H 3B). Energetic coupling of above- and belowground compartments will be assessed by energy-flux calculations (Barnes et al. 2018) and molecular gut content analysis (Eitzinger et al. 2018). We expect strong treatment effects on intraguild predation, due to varying physiological response and habitat-domain shifts of differently-sized predators (Barton 2011, Schneider and Brose 2013).

Future climate will decrease decomposer feeding (assessed by bait lamina and litter bags, Butenschön et al. 2011, Lang et al. 2013) and change the vertical stratification of the predators (Barton 2011). Warming will directly and indirectly (via changes in faunal communites) impact decomposition, predation, and energetic pathways. Specifically, future climate will increase total predation (energy flux to predators, prey responses, Thakur et al. 2017), decrease decomposition (Butenschön et al. 2011), and affect microbial biomass, respiration, and community composition (the latter assessed by bacterial 16S and fungal 18S rRNA gene high-throughput amplicon sequencing, Hugerth and Andersson 2017), favoring fungal over bacterial energy pathways (assessed via PLFA measurements).

In addition to assessing predator and decomposer density, biomass, body mass-structure, and feeding interactions (molecular gut contents, stable-isotope analysis), we will measure multiple biotic and abiotic properties allowing additional insights.

4. Project time frame and milestones

The overall project was scheduled to run from 01.04.2021 to 31.12.2022 and is based on the EcoStressWeb experiment in the iDiv Ecotron. The project timeline and major milestones are shown in **Fig. 3**.

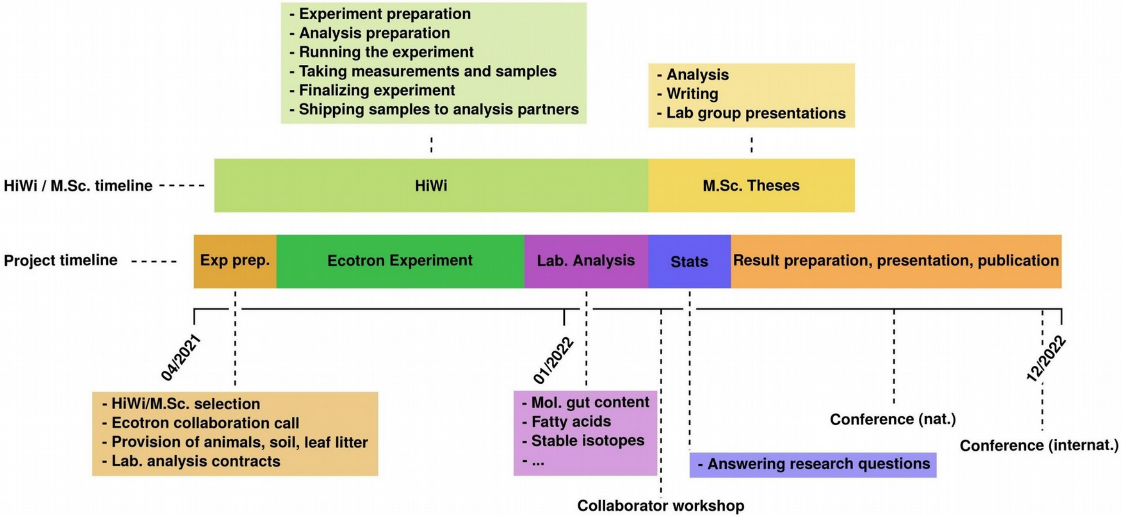

Figure 3. Timeline and milestones for the proposed research project **between 04/2021 and 12/2022**. The timeline is split into a project timeline (lower bar) and a student helper (HiWi) / M.Sc. student timeline (upper bar). Milestones are presented in rectangles that are color-coded according to the sections on the timelines and linked to those sections via dashed vertical lines. The collaborator workshop and two conferences are inserted along the timeline.