Light-mediated seedling responses to land use changes

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**Author contributions:** AE designed and established the full eDiValo experiment, and CW designed the seedling trait experiment. MJ, and CW collected lab and field data and conducted the analyses. All authors contributed to the writing of the manuscript and approved the final submission.

**Introduction**

Here we test the mechanisms of light limitation and species traits in seedling recruitment responses to land-use differences of grazing regime and fertilization levels. We hypothesized that light limitation would affect seedling density and richness in ungrazed and fertilized plots. Additionally, we hypothesized that species-specific responses to light limitation would depend on seedling traits, with fast-growing traits associated with stronger responses to light.

**Methods**

*Lab measurements of seedling traits*

For seedling trait measurements, plants were grown from seed in GrowBanks growth chambers, set to a 14 hour day / 10 hour night cycle. Daytime parameters were 25˚C, 30% relative humidity, and 300 µmol light; nighttime dark parameters were 18˚C and 50% relative humidity. Seedlings were grown in trays of germination cells 40 mm x 40 mm wide and 80 mm deep, filled in a 1:1 mix of potting soil and sand. 5-10 seeds of each species were planted at 0.5 cm depth and were thinned if necessary to 1-2 seedlings per cell.

Ten seedlings per species were harvested two weeks after germination for shoot length, biomass, C:N, and root trait measurements. Ten separate seedlings per species were harvested for specific leaf area measurements when two real leaves were present and the third real leaf was emerging. Trait measurements were conducted using the standard protocols outlined in Cornelissen et al (). Root traits were measured using WinRhizo software (). Carbon and nitrogen concentrations were measured using VarioEL CNHS analyzer (Elemental Analyse Systeme GmbH, Hanau, Germany).

*Study site and field data collection*

We collected the field data for this study within the Global Change Experimental Facility (GCEF), in Bad Lauchstädt, E-Germany (51°22060 N, 11°50060 E). This long-term experiment combines land-use and climate change research in a split-plot design (Schädler et al. 2019). For this study we exclusively used the areas of extensively used pastures in the GCEF which are characterized by species typical for dry Central-Germany. The grazing regime for the grazed areas (16 x 24 m) consists of one to three 24h lasting grazing events (the amount is dependent on the state of the vegetation) each vegetation period by a herd of 20 German black-headed mutton sheep. In 2016 a full factorial experiment combining herbivore exclusion, fertilization and light addition has been implemented in these grazed areas. Each possible treatment combination was replicated five times in ambient and future climate resulting in 80 experimental plots of XX x XX m. For the additional illumination which was designed to test the role of light limitation for small statured species in the vegetation understory we installed two LED lamps (C65, Valoya, Finland) mimicking the spectrum of natural sunlight in each plot assigned to light addition. The lamps added light to the understory vegetation each day from two hours after sunrise to two hours before sunset from early spring (March/April) to fall (October/November) and were taken down during the winter and for each grazing event. To implement the herbivore exclusion treatment metal mobile fence units with a mesh size of 10cm and a height of 82cm were placed around each non-grazed plot. The fences excluded sheep and naturally occurring rodents like European hare (*Lepus europaeus*) while voles and mice (mainly *Microtus arvalis* and *Apodemus sylvaticus*) could pass the fences. For the fertilization treatment we manually added a mixture of NPK fertilizer (Haifa Multicote 2 M 40-0-0 (40% N), Super Triple Phosphate TPS (45% P203), Kaliumsulfate-fertilizer (50% K2O, 45% SO3, 18% S)) to each fertilization plot twice per vegetation period resulting in a total of XX g added N, XX g added K and XX g added P per m2.

25 x 50 cm subplots were surveyed for naturally recruiting seedlings in October 2019 and March, May, and June 2020. Seedling surveys were conducted after the lab measurements, so we had personal experience observing all the species in their seedling forms. We additionally put together a seedling guide with photographs taken from the lab germinating seedlings that we used for reference in the field (Supplement xx). Seedlings that could not be identified because only cotyledons were present or they had been damaged were grouped together into an unknown category.

We additionally planted 12 seeds of six species into a separate part of each plot, marked by colored plastic toothpicks. The species used were *Plantago lanceolata*, *Dianthus carinatus*, *Daucus carota*, *Medicago falcata*, *Crpbis biennis*, and *Galium aparine*. The seeds attached to the toothpicks were placed in the plots in November 2019??? and were surveyed for emergence and survival monthly from February to July 2020.

*Statistical analyses*

To test the mechanism of light limitation in the effects of grazing and fertilization regimes on seedling recruitment, we calculated the total count and species richness of naturally regenerating seedlings in each survey month. We modelled total seedling count and richness using linear mixed effects models, with grazing treatment, nutrient treatment, light treatment, and their 2- and 3-way interactions; survey month and 2-way interactions between survey month and each treatment; and random effect of plot nested in block.

To investigate the role of species traits in light-based recruitment responses to grazing and fertilization regimes, we calculated peak abundance as maximum number of seedlings observed for each species in each plot. We modelled peak abundance using generalized linear mixed effects models with a negative binomial distribution, with a separate model for each trait. We used AIC to compare models with no trait predictor, trait as a main effect but not interactions, a full model with all 2-, 3-, and 4-way interactions between trait and predictors, and a specific model interacting trait with light treatment, grazing treatment, and their combination (model specifications and AIC values in Supplement xx). This specific model was chosen based on our observation that the fertilization treatment had minimal effects on community composition or total seedling recruitment in the study period, potentially due to it being a somewhat dry year. In most cases, our specific model fit the data best, so we present the results from this form for all the trait models. In this final model, predictors were grazing treatment, nutrient treatment, light treatment, and their 2- and 3-way interactions; trait and its 2- and 3-way interactions with grazing treatment and light treatment; and random effects for species and plot nested in block.

All data management and analyses were conducted in R, using the 'tidyverse' package (). Linear models were fit using the 'lme4' package with p-values estimated using the 'lmerTest' package (). Generalized linear models were fit using the 'glmmTMB' package, with AIC comparisons conducted using the 'bbmle' package (). Figures were made using the 'ggplot2' package (). [potential additions: 'GGally' for checking correlations between traits, 'gtsummary' for pretty tables from R markdown, 'broom.mixed' for getting glmmTMB outputs into the form needed for those pretty tables, still needs package info for the survival analyses]

**Results**

**Discussion**

**Tables**

**Figures**

**Acknowledgements**

**Data and code archiving**

We intend to archive our data and code in a permanent Figshare repository after acceptance