

## RESEARCH ARTICLE

# Litter accumulation, not light limitation, drives early plant recruitment

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**Abstract**

1. Theory predicts a decline in grassland diversity under nutrient enrichment and loss of herbivory, and one possible cause is hampered seedling recruitment. Two potential drivers for reduced diversity at the seedling level are diminished light availability caused by surrounding vegetation and accumulation of dead biomass.
2. To test the importance of these two mechanisms on early recruitment, we added seeds of 15 herbaceous grassland plant species and monitored sown and natural seedling emergence during one growing season in a full factorial field experiment with light addition and litter removal under fertilization and exclusion of mammalian herbivores in an experimental grassland in Central Germany. We used modern LED lamps, mimicking the spectrum of natural sun light, to provide light to small-statured understorey plants. This novel experimental set-up allowed us to specifically disentangle the roles of light limitation and litter accumulation independently and in combination.
3. In general, herbivore exclusion, but not fertilization increased the amount of litter biomass. Litter removal increased seedling number and richness by 83% and 33%, respectively, while light addition had no significant main effect on seedling recruitment, nor did it interact with any other factors, and did not affect recruitment even when litter was removed. In addition, fertilization had a negative and herbivore exclusion a negligible impact on recruitment, and these effects were independent of litter removal. Furthermore, seedling number and richness were unrelated to light intensity and quality, litter depth, soil moisture, temperature and C:N ratio.
4. *Synthesis:* These results provide novel insights into the role of light limitation versus litter accumulation driving early recruitment and help understanding the mechanisms that affect diversity in grassland communities via recruitment. Our results highlight the detrimental role of litter accumulation as opposed to surrounding vegetation induced light deficiency driving early recruitment from seeds and call for management actions that reduce the amount of litter when maintaining or restoring diversity.

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## KEYWORDS

diversity decline, germination, LED lamps, litter removal, nutrient enrichment, recruitment, seed addition, sheep grazing

## 1 | INTRODUCTION

Anthropogenic and experimental nutrient enrichment can substantially reduce plant diversity (Bobbink et al., 2010; Borer, Seabloom, et al., 2014; Clark & Tilman, 2008; Stevens, Duse, et al., 2004). Nutrient enrichment-induced species loss can be explained by a shift in limiting resources from nutrients to light (Eskelinen et al., 2022; Hautier et al., 2009) and diminished number of resource niches (Harpole & Tilman, 2007), that lead to extinctions depending on species' initial abundance and traits (Suding et al., 2005). Besides causing extinctions, nutrient enrichment can affect species gains, and has been shown to be an important impediment for colonization by seed (Fayolle et al., 2009; Foster & Gross, 1998; Lamb, 2008; Myers & Harms, 2009; Stevens, Bunker, et al., 2004; Tilman, 1993).

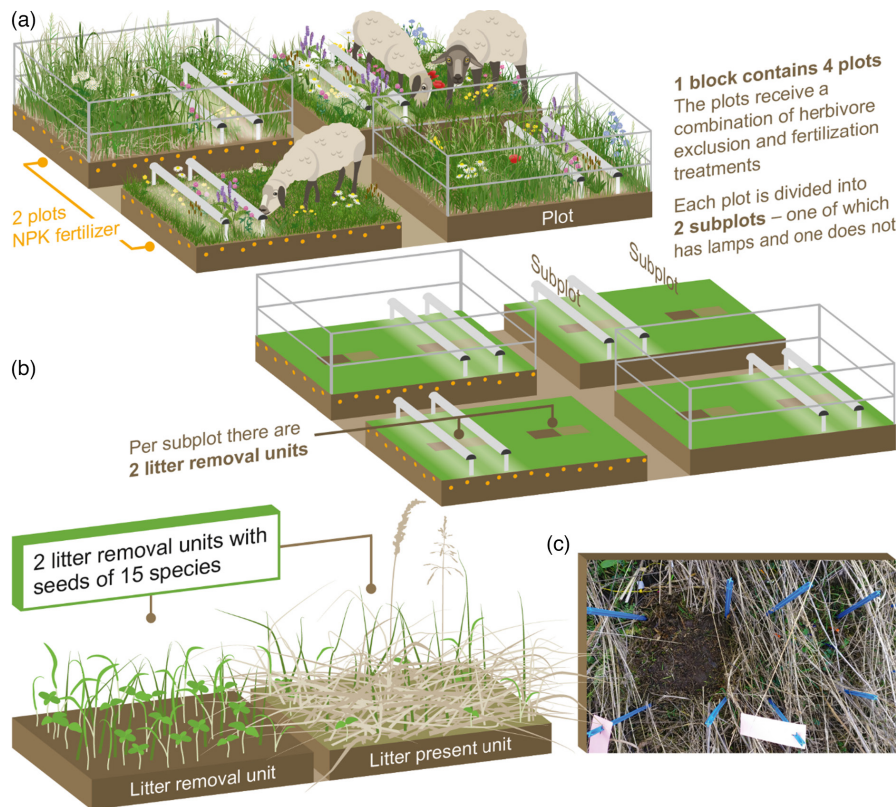
One mechanism explaining diversity loss under nutrient enrichment is unequal light availability between adult plants and seedlings, that is, diminished amount of light in dense vegetation caused by shading (DeMalach et al., 2017; Goldberg et al., 2017). This is suggested to lead to reduced species gains by interfering with colonization and recruitment dynamics (Tilman, 1993). While competition for light has been shown to be an important mechanism driving diversity loss under fertilized conditions (Eskelinen et al., 2022; Hautier et al., 2009), the importance of competition for light in early recruitment, that is, controlling species arrival, is still untested. Although many studies have addressed the impact of light on recruitment indirectly by for example, correlating unmanipulated light levels with recruitment success (Kahmen & Poschlod, 2008; Kleijn, 2003), or using litter and/or biomass removals or tiebacks of surrounding vegetation to increase light availability for seeds and seedlings (Dickson & Foster, 2011; Eskelinen & Virtanen, 2005; Foster & Gross, 1998; Grman, 2013; Gross et al., 2005; Loydi et al., 2013; Xiong et al., 2003), these do not reflect causality or may produce confounded results due to methodological reasons. For example, biomass removal could release nutrients from roots, causing a fertilization effect (Gross et al., 2005), and alter temperature (Dickson & Foster, 2011; Eskelinen et al., 2022) and humidity (Eskelinen et al., 2022), and may not reflect light as a causal factor. Therefore, testing the causal role of light in affecting recruitment requires direct experimental addition of light into the understorey where recruitment takes place.

In general, light can be an important cue for breaking seed dormancy (Seo et al., 2009; Smith, 1982) and for seedling development such as de-etiolation and cotyledon unfolding (Chen et al., 2004). Seedlings that emerge and develop early, that is, before vegetation becomes dense, have better access to light and thus a competitive advantage over those that emerge later (Miller, 1987). On the other hand, light can also be a stress factor for seedlings if, for example, irradiation is too high (Demmig-Adams & Adams 3rd, 2006).

Colonization and plant recruitment could also be negatively affected by a physical barrier of dead biomass that can prevent seeds from reaching the soil surface and germinating (Facelli & Pickett, 1991; Ruprecht et al., 2012). Dead biomass can also reduce light penetration to the ground (Foster & Gross, 1998) which can impair germination and seedling establishment (Zhang et al., 2019) or produce leachates that inhibit seedling growth (Bonanomi et al., 2011). Such negative effects of litter accumulation on species colonization and recruitment should be especially strong in nutrient-enriched conditions where more litter should accumulate due to greater productivity (Foster & Gross, 1998; Loydi et al., 2013; Stevens, Bunker, et al., 2004). To date, while many studies have manipulated litter and/or live biomass to test their roles for plant recruitment and colonization under nutrient enrichment, no attempts have been made to separate the effects of litter accumulation and reduced light levels through shading from the surrounding vegetation.

Mammalian herbivory can relax light limitation via consumption of plant biomass (Borer, Seabloom, et al., 2014; Olff & Ritchie, 1998), often selectively consuming nutrient-rich and tall-statured species (Diaz et al., 2007; Evju et al., 2009). Herbivores can thereby alleviate competition for light and maintain plant diversity (Eskelinen et al., 2022). These positive effects on diversity through preventing species extinctions can be especially pronounced in nutrient-enriched conditions (Bakker et al., 2006; Kaarlejärvi et al., 2017). Herbivores can also facilitate colonization and recruitment from seed via reducing the amount of litter by trampling, and increasing litter decomposition rates (Olofsson & Oksanen, 2002; Wang et al., 2018), which can create favourable recruitment gaps for seedlings (Vandvik & Goldberg, 2006). In the absence of herbivores, especially in nutrient-enriched conditions, both increased light limitation caused by shading of surrounding vegetation and increased amount of litter could therefore hamper species recruitment, and lead to reduced diversity.

We added seeds of 15 grassland species and investigated the roles of light limitation and litter accumulation, independently and in combination, on seed germination and early seedling establishment under nutrient enrichment and mammalian herbivory. Our experiment was carried out in an experimental grassland in Central Germany that was grazed by sheep. To reduce light limitation caused by shading of the surrounding vegetation, we provided light to the small-statured understorey plants using modern LED lamps that mimicked the spectrum of natural sun light. These lamps did not cause heating or change humidity (Figure 1; Eskelinen et al., 2022). To manipulate litter, we removed undecomposed dead plant material from previous years, in a full factorial combination with light addition. These two treatments were further performed in a full factorial combination of fertilization and exclusion of sheep. We specifically asked (1) what are the roles of light availability and litter for



**FIGURE 1** Illustration of (a) the basic experimental design with fertilization, exclusion of sheep grazing and light addition, (b) the additional litter removal treatment (brown rectangles) embedded in the basic experiment and close up illustration of two litter removal units, one with litter present, the other with litter removed and (c) photo of litter removal and litter present units. The photo has been taken right after the implementation of the litter removal treatment. Photo credit M.-T. Jessen. Images were created by G. Rada (iDiv, Media and Communications).

recruitment of grassland plants from seed? (2) Does the relative importance of light availability and litter for recruitment change under nutrient enrichment, herbivory and their interaction? We predicted that both (1) litter removal and (2) light addition would increase recruitment and that (3) litter removal and light addition would enhance recruitment more under fertilized and fenced conditions compared to unfertilized and grazed conditions. Furthermore, we analysed how light quantity (measured as light intensity) and quality (red to far red ratio (R:FR)), soil moisture, temperature and C:N ratio and litter depth were associated with recruitment.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The experiment was located in Germany, at Bad Lauchstädt Field Research Station (51°22,060N, 11°50,060E). The long-term mean annual precipitation in this area is 489 mm and the mean annual temperature is 8.9°C (Schädler et al., 2019). The soils are fertile Haplic Chernozems (Altermann et al., 2005; Schädler et al., 2019). The experiment was conducted within the Global Change Experimental Facility (GCEF) which combines land use type and climate change research (Schädler et al., 2019). We used the five extensively used pastures under ambient climatic conditions of the GCEF in our experiment. These five pastures are exposed to sheep grazing by a herd of about 20 individuals of German black-headed mutton sheep two to three times year, depending on how much vegetation biomass

is present. Each pasture (16×24 m) is grazed for a short period of high intensity for 24 h at a time (Schädler et al., 2019). Both 2018 and 2019 were significant drought years in Central Europe (Hari et al., 2020) that were characterized by severe water deficit during the summers and a strong decline in green vegetation in Central European pastures (Buras et al., 2020); during these years the pastures were grazed twice. The vegetation in the extensively used pastures resembles species-rich grassland vegetation in dryer regions of Central Germany (Schädler et al., 2019). The entire experimental area is fenced to keep out naturally occurring deer, while smaller herbivores like European hare *Lepus europaeus* can pass the fences.

### 2.2 | Basic experimental design

In 2017, we established a full factorial experiment of fertilization and herbivore exclusion; four plots sized 1.4×1.4 m, separated by a 1-m buffer zone, were established in each of the five pastures resulting in a total of 20 plots (Eskelinen et al., 2022). The four plots within each pasture were randomly assigned to one of the treatment combinations (grazed, fertilized+grazed, excluded, fertilized+excluded). The five pastures were thus considered as the block factor.

To fertilize, we applied slow-release granular NPK fertilizer mixture (Haifa Multicote 2M 40-0-0; 40% N, Super Triple Phosphate TPS (45% P<sub>2</sub>O<sub>3</sub>)), potassium sulphate fertilizer (50% K<sub>2</sub>O, 45% SO<sub>3</sub>) to fertilizer addition plots twice per growing season (later March–May and June), resulting in a yearly addition of 10gN, 10g P and 10gK per m<sup>2</sup> (see the protocol of Borer, Harpole, et al., 2014 for

grasslands around the globe). The fertilization treatment was started at the end of May 2017 when we applied the first fertilizer round. However, the herbivore exclusion treatment was started at the end of August 2017, that is, 3 months later (Eskelinen et al., 2022).

To implement the herbivore exclusion treatment, we fenced plots receiving herbivore exclusion treatment with 1.8 m × 1.8 m, 82 cm high, 10 cm mesh rectangular portable metal fences (Eskelinen et al., 2022). The fences did not exclude mice and voles (mainly *Apodemus sylvaticus* and *Microtus arvalis*) which frequently occur in the plots (Jessen & Eskelinen, personal observation). In 2019, one plot originally intended for herbivore exclusion only was accidentally fertilized and one plot originally intended for herbivore exclusion and fertilization treatment was not fertilized. We therefore treated both as fertilized in data analyses. However, excluding these plots from the analyses did not change the results qualitatively.

For the light addition treatment, the 1.4 × 1.4 m plots were further divided into two smaller subplots, 0.7 m × 1.4 m each, one of which was randomly assigned to light addition, resulting in a total of 40 subplots according to a split-plot design (Figure 1 and Eskelinen et al., 2022). To add light, we installed two 120 cm long and 3.5 cm wide, high-intensity LED lamps (C65, Valoya, Finland) designed for growing plants in growth chambers, vertical farming applications, etc.; <https://www.valoya.com/c-series-led-grow-lights/>. The light addition treatment started at the same time as the fertilization treatment, that is, at the end of May 2017 (Eskelinen et al., 2022). Our light addition treatment mimicked adding natural sunlight to a gap in vegetation in a real grassland; the spectrum was therefore designed to include all wave lengths of sunlight, including small amounts of UV and IR. The lamps did not alter top soil temperature (measured approximately 3 cm deep; Figure S1) or air temperature (Eskelinen et al., 2022).

The lamps were installed horizontally to the ground and parallel to each other at a distance of 28 cm in an area of 0.7 × 1.4 m (Figure 1 and Eskelinen et al., 2022). Each year we placed the lamps on the subplots in early spring when temperatures were above zero and there was low risk of frost damage (February–April) and took them down in the fall (October–November) when temperatures dropped close to zero, thus adding light during the active growing season (Eskelinen et al., 2022). In 2019, we switched on the lamps on 4 April, and not earlier, because temperatures still dropped below zero. By the time of our seedling survey, the lamps had been on for 46 days. The lamps in grazed subplots were removed for the period of grazing due to security reasons and switched off in the ungrazed subplots for the same time period to make sure all lighted subplots received similar light conditions. We intended to provide light for small-statured understorey plants and seedlings that were likeliest to suffer from competition for light (Eskelinen et al., 2022). Therefore, the lamps were installed 10 cm above the smallest plants and above litter that lay on the ground, except for in litter removal plots where lamps provided light to the bare ground. Our light addition treatment increased light intensity in the vegetation understorey, that is, reduced shading by the surrounding (adult) vegetation; for example, it increased light levels inside fertilized and unfertilized enclosures

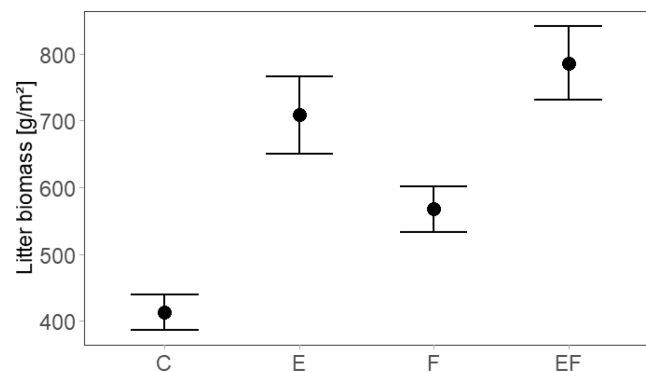
where the light limitation was greatest, on average by 57% (Eskelinen et al., 2022). Our design was not intended to address the mechanisms by which litter affects seedlings and therefore we did not attempt to provide light under litter. Furthermore, trying to install lamps under litter would have caused considerable disturbance, confounding possible light effects with mechanical disturbance. In many plots, our lamps would also not have fitted under litter, which can form a thin, yet functionally effective layer. Nevertheless, the light addition treatment, on average, did increase light intensity in plots with litter present (Figure S2) and increasing litter depth led to lower light intensity (Figure S3). When the plants grew during the course of the summer, the lamps were gradually raised to follow the growth of the smallest plants (Eskelinen et al., 2022). All lamps were set to the same height in all experimental plots to ensure equal conditions. We set the lamps to switch on 2 h after sunrise and switch off 2 h before sunset, and they automatically switched off when the air temperature exceeded 28°C to prevent overheating (Eskelinen et al., 2022).

### 2.3 | Litter removal experiment

In January 2019, we established two 20 × 20 cm litter removal units, separated by 10 cm, within each of the 40 subplots, resulting in 80 L removal units and representing the sub-subplot level according to a split-split-plot design (Figure 1). One of these units was randomly assigned to litter removal, and the other was left intact. The purpose of our litter removal treatment was to target dead biomass that had accumulated during the previous years. We implemented the treatment in January when most of the biomass was from previous years and dead, either lying on the ground as litter or standing dead and about to soon fall down. However, there was little green biomass mixed with the live biomass, mostly short grass leaves which were not possible to disentangle from the dead biomass, and were therefore removed with the dead biomass. On average we removed about 400 g m<sup>-2</sup> in control plots, 550 g m<sup>-2</sup> in fertilized plots, 700 g m<sup>-2</sup> in enclosed plots and 775 g m<sup>-2</sup> in enclosed and fertilized plots (Figure 2). These are medium to high litter amounts (Loydi et al., 2013). As no live biomass was removed when the growing season and growth of most plants started, our treatment can functionally be called 'litter removal'.

### 2.4 | Seed addition

To study seedling recruitment response to the treatments, we selected 15 common Central European grassland species (Table S1), abundant in the experimental plots, and added 50 seeds per species to all 80 L removal units. Based on a preseed addition seedling survey, seedling number was low under grazed conditions (data not shown), possibly because grazers also consumed flowers and affected seed production. Therefore, by adding a fixed amount of seeds independent of the species we ensured that there was a sufficient amount of seeds in each unit, all species had the same opportunity to respond



**FIGURE 2** Litter biomass (mean  $\pm$  SE) in relation to fertilization, herbivore exclusion and their interaction. The dead biomass was collected from 20  $\times$  20 cm litter removal units when establishing the litter removal treatment in January 2019, oven dried and weighed. The biomass values on the y-axis were scaled up to 1 m<sup>2</sup>. Herbivory exclusion increased litter biomass ( $F_{1,12} = 22.91$ ,  $p < 0.001$ ) and there was a trend that this effect was magnified by fertilization (E  $\times$  F interaction,  $F_{1,12} = 3.22$ ,  $p = 0.098$ ). C, control; F, fertilization; E, herbivore exclusion; EF, fertilization and herbivore exclusion.

to the treatments, and seed availability would not prevent examining treatment effects on recruitment for any species (for a similar approach see Aicher et al., 2011; Eskelinen et al., 2016; Zobel et al., 2000). Species were chosen to encompass a broad range of size (e.g. seed size and adult plant stature) as indicators of germination success and relative competitive ability (Keddy et al., 2002). The seeds originated from a local wild collection from 2018 (Saale-Saaten Stolle, Germany) but represented the same accessions used for the initial establishment of the GCEF in 2014. Before sowing the seeds in the field, we tested their germination rate, ensuring the viability of the seed material (Table S1), and to make sure we were able to identify all species even from their first true leaves. Seeds of all 15 species were mixed together and manually sprinkled over the units. We used a cardboard frame to prevent seeds from spreading outside the experimental units. Vegetation was gently shaken to ensure that seeds settled to the ground. Sowing was done in January to make sure that all seeds experienced natural cold stratification.

## 2.5 | Seedling survey

In winter/spring 2019, the units were monitored frequently starting from the beginning of February to capture all emerging seedlings. However, due to cold late winter and early spring of 2019, with frequent ground frost from February to mid-April (UFZ, 2019, Table S2), no seedlings were detected before April. The first seedlings appeared in late April when the occurrence of below zero temperatures started to diminish, and growing season and vegetation growth properly started. Seedlings were counted and marked at the end of April and May. We identified all fully germinated seedlings (cotyledons and at least one true leaf out) into species and recorded their number. We counted seeded and naturally occurring species. Since we did not mark the seeds, it was not possible to distinguish

naturally occurring seedlings from sown seedlings. Plants were counted as seedlings as long as their cotyledons were present. In this study we report data from the second sampling occasion (21–23 May), since this represents the peak number of seedlings. We also checked the plots in June and July for seeds germinating later; however, most seedlings had matured (i.e. no longer had cotyledons) and could no longer be counted as seedlings. Furthermore, as both 2018 and 2019 were extreme drought years with high temperatures and very little rain in June and July (56% of precipitation in relation to long-term observations from 1961 to 1990; DWD, 2018, 2019) conditions were not favourable for any further germination. The 2018 summer drought may have changed the composition of naturally occurring seedlings through shifts in abundance in adult plant communities, but it should not have affected the treatment effects on early recruitment observed at the end of May 2019. Precipitation levels in March–April in 2019 were in line with 10-year average values (Table S2).

## 2.6 | Abiotic measurements

We recorded litter depth (cm) at the centre of each unit from which litter was not removed ( $n = 40$ ). We measured volumetric water content (VWC) and soil temperature at the centre of each unit ( $n = 80$ ) at a depth of 3.81 cm with a soil moisture probe (TDR 100, Spectrum Technologies). The measurements were taken at the end of May all on the same day 1 week after the latest rain event (7.5 mm). We collected two composite soil samples from each unit that were later pooled, dried in room temperature, and analysed for C:N ratio (Vario EL CNHS analyser, Elementar Analysensysteme GmbH). We measured light intensity at each unit using a light metre (LI-250A, LI-190R, LI-COR). Light measurements were taken on a cloudless day around noon at ground level. The measurements were taken under live vegetation and litter layer when present to quantify light conditions at the ground layer at the time of seedling sampling. However, in many plots litter layer was too thin for our light sensor to fit under it and the light measurements do not reflect the effect of litter on light. Light quality was measured at the same time with a handheld spectrometer (GL Spectis 1.0 touch, GL Optic, Poland). We focused on red to far red ratio (R:FR), an important indicator of vegetation density and composition (Skalova et al., 1999). The live and dead biomass collected at the time of implementing the litter removal treatment was oven-dried at 60°C for 48 h and weighed. No permit was needed to conduct the field work of this study.

## 2.7 | Statistical analysis

We used linear mixed effects models to assess the interactive effects of herbivore exclusion, fertilization, light addition and litter removal (all fixed factors) on seedling number and richness. To account for the split-split-plot experimental design, that is, that litter removal units were sub-subplots nested within subplots that in turn were nested

within plots that were organized in blocks, our random effects included subplot nested within plot and plots nested within block. All models had the same random structure. We limited the amount of interactions to three-way interactions, since four-way interactions were not significant and did not change the results otherwise. Significance of the factors was tested with F-tests. We used model diagnostic plots to check the homogeneity of variances and the normality of errors (Crawley, 2007) and both seedling number and richness were square-root transformed to meet the assumptions. We also tried running the same models as GLMMs with a Poisson error structure, but these models failed to converge. To additionally test for variation in effect sizes between light addition and litter removal we calculated their effect sizes on total seedling number and richness as log ratios of the treatment responses divided by the control. Data were pooled across enclosure and fertilization treatments for these analyses. We used linear mixed effect models to assess statistical differences of the effect sizes between the treatments.

We used similar linear mixed effects models with the same random structure than above to test the effects of the treatments on individual seedling species that were common enough in the plots, that is, had three or more observations in each treatment combination. Of the 36 species observed we ran separate models for six species. It was not possible to test the remaining 30 species.

We assessed the relationship between (total and sown) seedling number and richness and the environmental variables (light intensity and quality, soil moisture, temperature and C:N ratio) and litter depth using multiple linear mixed effects models with similar random structure as above. We standardized all explanatory variables to facilitate the comparison of values with different units and orders of magnitude. Collinearity of the single factors in the models was tested using the variance inflation factor (VIF) and factor reordering. Since litter depth was measured only in units where litter was present (i.e. in half of the units compared to measurements taken for the other factors), the relationship between seedling number and richness (total and sown) and litter depth was tested in separate models. In these models, plot was nested within block, which accounts for the nested sampling design.

To investigate the interactive effects of herbivore exclusion, nutrient enrichment, light addition and litter removal on the community composition of seedlings, we applied permutational multivariate analysis of variances (Anderson, 2001) with Bray–Curtis dissimilarity and 999 permutations. We included block as a random variable in the models. Total seedling and sown seedling communities were analysed separately. We used nonmetric multidimensional scaling (NMDS) to illustrate significant community responses to the treatments.

We used the `LME4` package (Bates et al., 2015) to run linear mixed effect models, the `CAR` package for assessing the significance of the treatments effects (Fox & Weisberg, 2019), the `VEGAN` package for running PERMANOVA and NMDS (Oksanen et al., 2020), the `TIDYVERSE` package for data manipulation (Wickham et al., 2019) and the `GGPLOT2` package for plotting (Wickham, 2016) in R statistical software (R Core Team, 2020 version 4.0.2).

## 3 | RESULTS

### 3.1 | Effects of litter removal, light addition and other treatments on seedling number and richness

Thirteen of 15 originally sown species were found in at least some of the experimental units. Only *Heracleum sphondyleum* and *Linaria vulgaris* did not germinate at all. The maximum number of seedlings (sown species only) in one unit was 65 and the maximum number of all seedlings (including unseeded species) in one unit was 85. The maximum seedling richness (sown species only) in one unit was 9 and the maximum seedling richness of all species (including unseeded species) in one unit was 16. In total, we found 36 different seedling species in the entire experimental set-up.

Litter removal significantly increased both total and sown seedling number and richness (Figure 3a,b, Table S3, Figure S4a,b): total seedling number was on average 83% higher, and total seedling richness 33% higher, in litter removal units compared to intact units. Sown seedling number was on average 112% higher, and sown seedling richness 33% higher, in litter removal units compared to intact units.

Light addition had no significant main or interactive effects on either (total and sown) seedling number or seedling richness (Figure 3a,b, Table S3, Figure S4a,b), but fertilization significantly decreased total seedling number and richness (Figure 3a,b, Table S3).

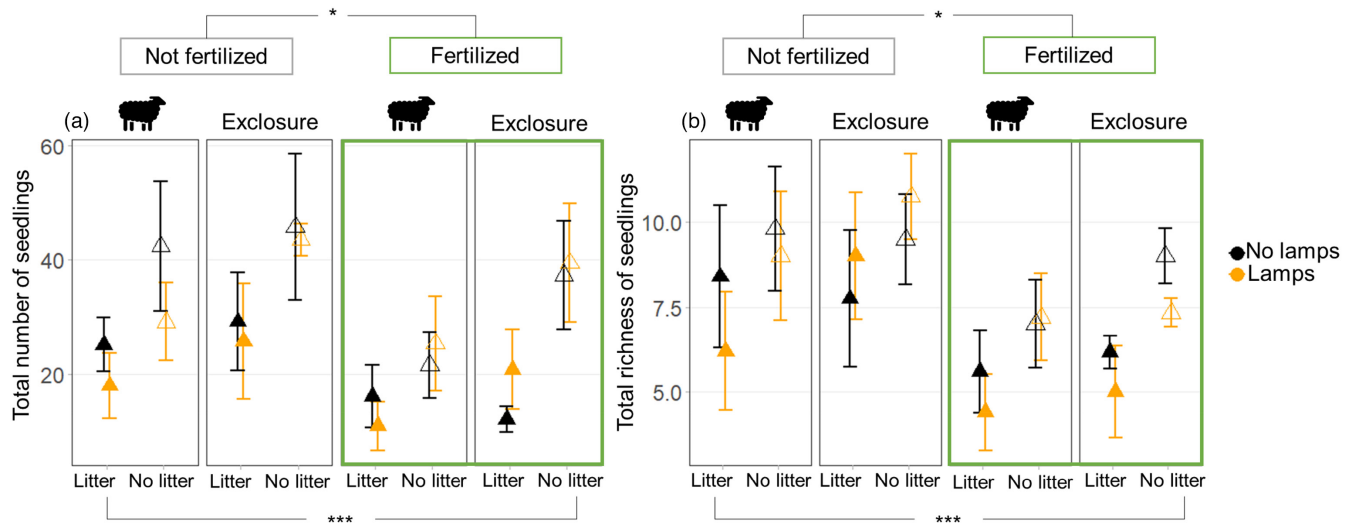
Comparing the effect sizes of litter removal and light addition, litter accumulation had a strong negative effect on both total and sown seedling number and richness regardless of light addition or unlighted conditions, whereas light addition had no effect independently or in combination with litter removal (Figure S5a–d).

### 3.2 | Environmental changes in relation to seedling number and richness

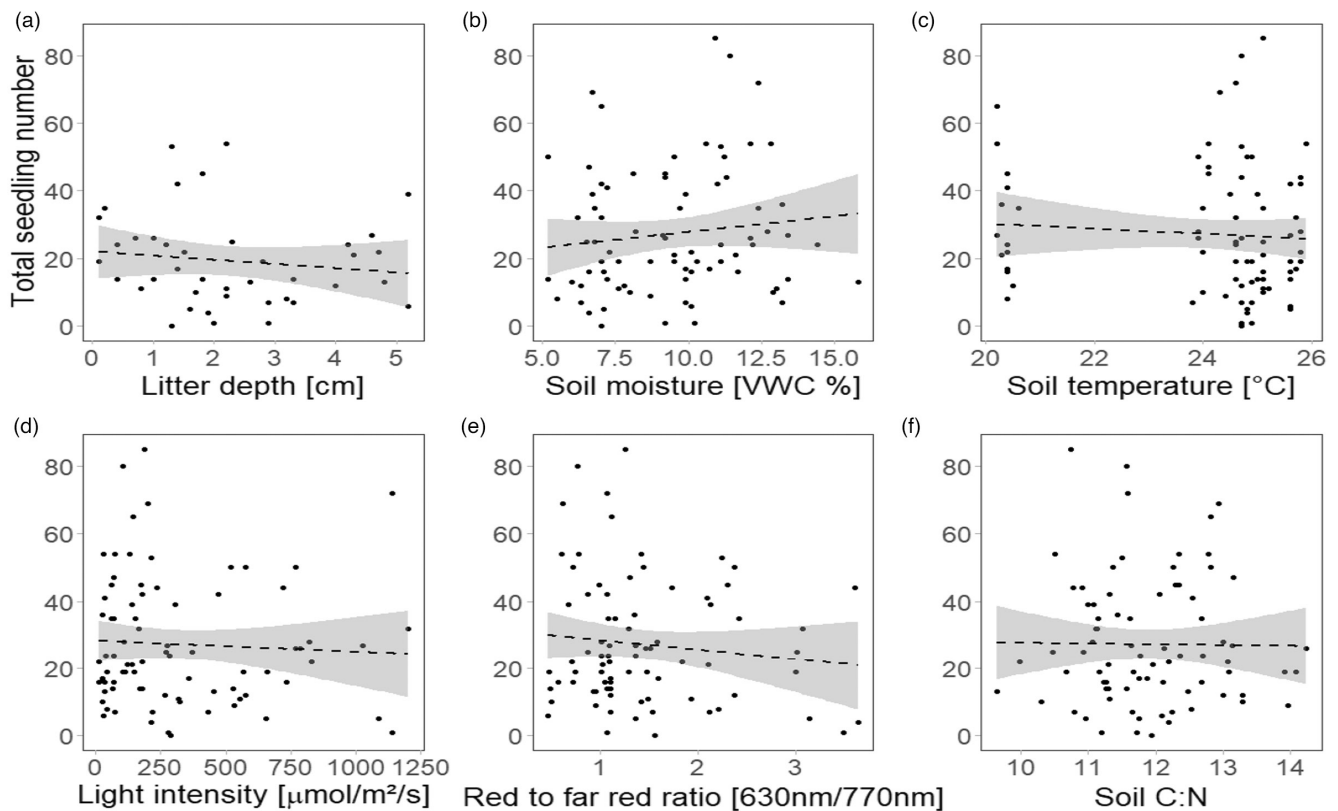
Total and sown seedling number and richness were not significantly related to light intensity or light spectrum (Figures 4d,e and 5d,e, Table S4), even though the range of measured light intensity values was high (11.54–1202.50  $\mu\text{mol}/\text{m}^2/\text{s}$ , Figures 4d and 5d). In addition, neither total and sown seedling number nor richness were significantly correlated with soil moisture, soil temperature or soil C:N ratio (Figures 4b,c,f and 5b,c,f, Table S3). Sown seedling number decreased with increasing litter depth, but this effect was only marginally significant (Table S4). Total seedling number and total and sown seedling richness showed no significant relation to litter depth (measured only from plots where litter was still present, i.e. no litter removal plots; Figures 4a and 5a, Table S4).

### 3.3 | Effects of litter removal, light addition and other treatments on individual seedling species

Consistent with total seedling number, three of the tested individual species that responded to the treatments were positively affected



**FIGURE 3** (a) Total number of seedlings (mean  $\pm$  SE) and (b) total seedling richness (mean  $\pm$  SE) under light addition, litter removal, herbivore exclusion and fertilization sampled in May 2019. The asterisks indicate statistical significance (\*\*\* $p$  < 0.001; main effect of litter removal, \* $p$  < 0.05; main effect of fertilization).

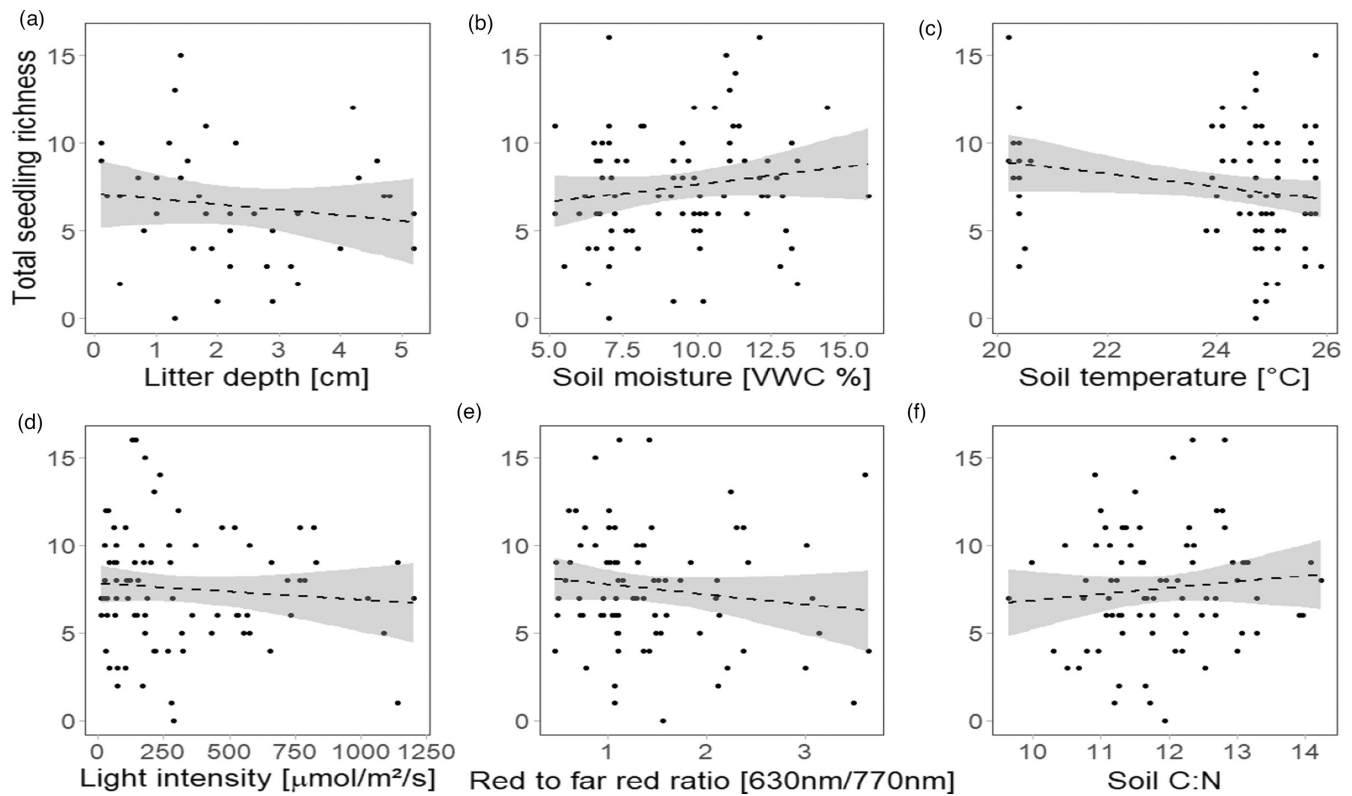


**FIGURE 4** Relationships between total seedling number and (a) litter depth, (b) soil moisture, (c) soil temperature, (d) light intensity at the ground (under litter and live vegetation when present), (e) red to far red ratio and (f) soil C:N ratio. All measurements were taken at the seedling survey units. The dashed line indicates that there is no significant relationship. Note that litter depth was only recorded in half of the experimental units (those without litter removal treatment). Figures for sown seedlings (data not shown) are qualitatively the same.

by litter removal: there was a significant positive main effect on *Achillea millefolium*, *Daucus carota* and *Plantago media* (Figure S6a). In addition, herbivore exclusion had a positive impact on *Galium album* (Figure S6b). *Pimpinella saxifraga* and *Silene latifolia* were tested but did not respond to any of the treatments.

### 3.4 | Effects of litter removal, light addition and other treatments on the seedling community

Both total and sown seedling species community compositions were altered by litter removal and herbivore exclusion (PERMANOVA results;



**FIGURE 5** Relationships between total seedling richness and (a) litter depth, (b) soil moisture, (c) soil temperature, (d) light intensity at the ground (under litter and live vegetation when present), (e) red to far red ratio and (f) soil C:N ratio. All measurements were taken at the seedling survey units. The dashed line indicates that there is no significant relationship. Note that litter depth was only recorded in half of the experimental units (those without litter removal treatment). Figures for sown seedlings (data not shown) are qualitatively the same.

Table S5, Figure S7a,b). Herbivore exclusion and litter removal also interacted to affect sown seedling community composition (PERMANOVA results; Table S5, Figure S7c). Light addition and fertilization had no effect on total and sown seedling community compositions.

## 4 | DISCUSSION

We found that recruitment success (seedling number and richness) was strongly controlled by the presence of litter, while light addition above the litter layer exhibited no significant impact on early seedling recruitment neither independently nor in combination with litter removal. Furthermore, a large range of measured light intensity values did not correlate with recruitment success, reinforcing our experimental result that light was not important for early seedling establishment. These findings were independent of nutrient enrichment and herbivory. Our results therefore suggest that light limitation hampering early recruitment through shading by surrounding adult vegetation is not the main driver of diversity loss in a wide variety of conditions, and not even under nutrient enrichment and exclusion of herbivores. Rather, a combination of factors associated with build-up of dense litter layer determines how many species and seedlings germinate. Conditions which promote accumulation of dead biomass can therefore be important filters preventing recruitment from the local species pool.

Our first prediction was supported as litter removal greatly increased seedling number and richness. Litter removal also increased seedling number of most of the individual species that we tested and altered seedling community composition. These results are in line with earlier litter removal experiments which have shown an increase in seedling number or density in response to litter removal (Jensen & Meyer, 2001; Johnson et al., 2018; Wilsey & Polley, 2003). Litter addition studies also confirm these results (Foster & Gross, 1998; Xiong et al., 2003; Zhang et al., 2019). Together these findings highlight the important role of undecomposed litter, that is, the legacy of previous years' vegetation, in suppressing recruitment from seed. Accumulated dead biomass can hamper seedling establishment by creating a mechanical barrier that prevents seeds from reaching soil surface (Donath & Eckstein, 2010; Facelli & Pickett, 1991; Ruprecht et al., 2012) and, on the other hand, can suppress seedlings that have already germinated. Litter can also inhibit germination through leachates (Ruprecht et al., 2008) or by blocking incoming light and precipitation (Facelli & Pickett, 1991). In addition, rodents may be more active under the protective cover of litter, since dead plant material can provide shelter and cover, increasing seed and seedling predation (Edwards & Crawley, 1999; Fraser & Madson, 2008; Hulme, 1996; Smith et al., 2021). In our study, recruitment success was not associated with light intensity and quality, soil moisture, soil temperature or soil C:N ratio, suggesting that litter likely acted as a mechanical barrier, rather than affected seedling recruitment via any



environmental variables. Furthermore, seedling richness and number were unrelated to litter depth (Figures 4a and 5a), even though litter depth had a negative impact on light (Figure S3), suggesting that even a thin litter layer can reduce seedling recruitment independent of its impact on light. In addition, litter-driven recruitment patterns are likely to be controlled not only by one but also by a combination of factors (Lamb, 2008) which jointly affect recruitment, and subsequently influence community diversity.

Our second prediction was not supported as light addition did not significantly increase seedling number and richness. Moreover, light addition had no significant effect even when litter was removed, indicating that reduced light via shading by surrounding vegetation is not an important factor affecting seed germination and early establishment. These experimental results were confirmed by our observational results that even though our light measurements spanned a great magnitude of light intensity values (11.54 to 1202.50  $\mu\text{mol}/\text{m}^2/\text{s}$ ), light intensity was not associated with seedling number and richness, supporting our conclusion that light does not have a significant impact on early recruitment success. In earlier studies manipulating the light environment for seedlings indirectly (e.g. via cutting biomass, removing litter), positive treatment effects have been interpreted as at least partly reflecting greater light limitation and competition for light with adult plants (Foster & Gross, 1998; but see Eskelinen & Virtanen, 2005; Jutila & Grace, 2002; Loydi et al., 2015; Xiong et al., 2003). These studies, however, do not separate between the effects of light and litter, and do not control for altered nutrient, temperature and humidity levels that can be caused by biomass removal or vegetation tiebacks (Eskelinen et al., 2022; Grman, 2013; Gross et al., 2005). Our results of direct light addition, independently and in combination with litter removal, suggest that litter rather than light availability determines early recruitment success.

Our study was not designed to examine the mechanisms by which litter affects early germination and we therefore cannot say for sure why litter hampered germination. It would be difficult to experimentally disentangle the variable effects of litter on seedling emergence. For example, adding light under litter without simultaneously disturbing and increasing space, that is, affecting the physical barrier that litter forms, would be challenging. However, even though light reduction could still be one mechanism through which litter inhibits germination, our result that light addition, even in the absence of litter, did not affect total recruitment suggests that changed light availability is not the main mechanism.

It is also possible that the mechanisms maintaining grassland plant coexistence may differ between early seedlings and later plant life stages. In mature plant communities competition for light has been shown to reduce diversity both in greenhouse conditions and in the field (Eskelinen et al., 2022; Hautier et al., 2009). While adult plants compete for light to maintain a positive carbon balance (Mooney, 1972), early seedlings can tolerate low light levels; they do not compete with adult plants for light because they are morphologically inferior and have a less light demanding physiology (Kitajima & Myers, 2008). Rather, newly emerging seedlings have evolved to develop under light-limited conditions by, for example, adapting the

photosynthetic capacity of their cotyledons and first true leaves accordingly (Lawrence et al., 2020). Young seedlings can also live on seed resources for a while and therefore are not fully dependent on light for resource acquisition until photosynthesis is fully initiated (Fenner, 1987; Kennedy et al., 2004; Kitajima & Myers, 2008). Consequently, light availability, altered by surrounding vegetation, might not be as critical in early seedling establishment as previously thought but likely increases in importance with plant maturation. This is supported by findings from the same study system, that light addition mitigated or offset the negative effects of fertilization and herbivore exclusion on whole community diversity (Eskelinen et al., 2022). The impacts of light limitation might therefore act through extinctions in later life stages; however, more research is needed to disentangle the effects of light in different life stages.

We expected stronger litter- or light-driven microsite limitation for seedlings under fertilization and exclusion of herbivores. In contrast to this prediction, litter removal and light addition did not have a stronger positive effect on seedling recruitment under fertilized and fenced conditions, even though especially herbivore exclusion greatly increased the amount of dead biomass (by 53%; Figure 2). In our experiment, both litter removal and herbivore exclusion act in parallel by affecting the amount of litter, possibly explaining why we only found significant treatment effects for litter removal. Nevertheless, the strong effect of herbivore exclusion on litter accumulation suggests that herbivore exclusion is an important factor for litter build-up and thus indirectly influences seedling recruitment. The fertilization treatment alone did not significantly increase litter biomass which may explain why suppression of seedling recruitment by litter was not magnified by nutrient enrichment.

We found that fertilization alone, independent of litter removal and light addition, decreased seedling number and richness, which indicates that fertilization did not reduce recruitment via litter accumulation or light limitation, in contrast to what was suggested in previous studies (Bergholz et al., 2015; Myers & Harms, 2009; Stevens, Bunker, et al., 2004). Our result is therefore rather linked to physiological responses of seedlings to higher ammonium levels in fertilized plots (Figure S8b), which can create a toxic environment for seedlings (Britto & Kronzucker, 2002), or slightly reduced pH in fertilized plots (Figure S8a), which can also negatively affect seedlings (Bardgett et al., 1999). In general, higher ammonium levels could also favour pathogens (Lekberg et al., 2021) or affect other factors that could be harmful for seedlings but which were not tested in our experiment. Overall, our finding shows that potential direct effects of fertilization can be an additional obstacle to diversity via recruitment, but the effect is marginal compared to the effect of litter accumulation.

Seedling community responses to the treatments mostly followed the responses of seedling number and richness, with litter removal being the most influential factor. In addition, herbivore exclusion altered seedling community composition, possibly via its effects on adult plant community composition and litter (Figure 2), or via trampling. One species, *G. album*, also directly suffered from grazing, emphasizing that individual species responses can also vary.

To conclude, our results provide novel insights into the mechanisms that drive coexistence and diversity in the recruitment phase. We show that litter controlled species richness and abundance on the seedling level via reduced species recruitment, while light availability, altered by surrounding adult vegetation, did not matter for recruitment, neither independently nor in combination with litter removal. This contrasts with findings from adult plant communities where light addition has been shown to offset diversity decline in a greenhouse (Hautier et al., 2009) and in the field (Eskelinen et al., 2022). However, to our knowledge this is the first study to directly add light in the field to test its role in affecting recruitment and is the first study separating the roles of litter accumulation and light availability in the early recruitment phase. Our results contribute to the knowledge needed for planning restoration efforts by emphasizing the detrimental effect of litter accumulation for recruitment from seed. This knowledge is important for restoration practices using seed addition to maintain diversity in natural and seminatural grasslands (Hernández et al., 2021; Johnson et al., 2018; Török et al., 2021), which should benefit from additional litter removal.

#### AUTHOR CONTRIBUTIONS

Maria-Theresa Jessen collected and analysed the data and wrote the manuscript with substantial contributions from Anu Eskelinen. Harald Auge and W. Stanley Harpole contributed in editing the manuscript. Anu Eskelinen developed and established the experiment with contributions from W. Stanley Harpole and Harald Auge.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

#### PEER REVIEW

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#### DATA AVAILABILITY STATEMENT

All data associated with this study are publicly available at Figshare <https://doi.org/10.6084/m9.figshare.22285528.v1> (Jessen et al., 2023).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** Seeded species used in this experiment, their functional group and germination percentage in the laboratory. The germination percentage in the laboratory was determined by germinating 100 seeds of each species in a Petri dish with regular irrigation regime, and counting all germinating seeds. *Linaria vulgaris*, *Pimpinella saxifraga* and *Ranunculus acris* were cold stratified in a refrigerator at 4°C for 2 weeks before germinating their seeds. The seeds for *Heracleum sphondylium* were not available at the time of the test period. We grew the seeds in the lab prior to seeding in the field to ensure that the seed material was viable and to be able to identify germinating species in the field.

**Table S2.** Air temperature, precipitation and amount of days with below 0°C at ground level data for Bad Lauchstädt area from January to May, i.e., from when seeds were sown (January) until when seedlings were surveyed. Shown are the year 2019, when this study was conducted and the longer-term average (2009–2019). The data are monthly averages and taken from the publicly available weather reports of the Research Station Bad Lauchstädt (<https://www.ufz.de/index.php?de=46100>) using data of the German Meteorological Service (Deutscher Wetterdienst). Air temperature was recorded 2 m above-ground level and minimum temperature, that was used to calculate the number of days when temperature was below 0°C, was measured 5 cm above ground level.

**Table S3.** Results of the linear mixed effects models testing the effects of litter removal, light addition, herbivore exclusion, fertilization and their interactions on total seedling number (sown and naturally occurring seedlings), sown seedling number, total species richness (richness of sown and naturally occurring seedlings), and sown species richness. In the models, subplot was nested within plot which was nested within block as a random factor. Significant results ( $p < 0.05$ ) are in bold and marginally significant results ( $p < 0.1$ ) are in italics. \*Data are square-root transformed.

**Table S4.** Results of linear mixed effects models assessing the relationships between (total and sown) seedling number and richness and light intensity at the ground level (below litter layer where present), light quality (R:FR), soil moisture, soil surface temperature and soil C:N ratio across species. In the models, subplot was nested within plot which was nested within block as a random effect. All predictors were standardized to facilitate comparison of

values with different units and orders of magnitude. The relationship between litter depth and seedling number and richness was tested in a separate model, because data on litter depth were only recorded in half of the plots (those with litter present). Significant results are printed in bold ( $p < 0.05$ ), tendencies are printed in italics ( $p < 0.1$ ). \*Data are log transformed.

**Table S5.** Results of permutational multivariate analysis of variance (PERMANOVA) for the total and sown seedling community in 2019. The analyses were based on Bray-Curtis dissimilarities. Only the significant effects ( $p < 0.05$ ) are shown.

**Figure S1.** Soil temperature (means  $\pm$  SE) with respect to litter removal and light addition. Soil surface temperature was measured from all experimental units using a soil moisture probe (TDR 100, Spectrum Technologies, USA) in May 2019. Soil temperature was not significantly affected by light addition ( $F_{1,16} = 4$ ,  $p = 0.063$ ) and or litter removal ( $F_{1,33} < 0.01$ ,  $p = 0.930$ ).

**Figure S2.** Light intensity (means  $\pm$  SE) with respect to litter removal and light addition. Light intensity was measured from all experimental units at ground level using a light meter (LI-250A, LI-190R, LI-COR, USA) in May 2019. Light addition  $F_{1,57} = 7.38$ ,  $p = 0.009$ .

**Figure S3.** Litter depth in relation to light intensity in plots without and with additional LED illumination. The line is a regression line with the 95% confidence intervals shown as shaded area. Litter depth  $F_{1,26} = 4.13$ ,  $p = 0.052$ .

**Figure S4.** Number of (a) sown seedlings (mean  $\pm$  SE) and (b) richness of sown seedlings (mean  $\pm$  SE) with respect to light addition, litter removal, herbivore exclusion and fertilization sampled in May 2019. Open triangles denote units from where litter has been removed, while filled triangles denote units where litter is present. Statistics are shown in **Table S3**.

**Figure S5.** Effect sizes (mean  $\pm$  SE) of light addition (lamps), litter removal (no litter) and light addition plus litter removal (no litter + lamps) on (a) total seedling number, (b) total seedling richness, (c) sown seedling number and (d) sown seedling richness. Effect sizes were calculated as log ratios of the treatment responses divided by the control (i.e. litter present and no lamps). Total seedling number: main effect of litter removal  $F_{1,38} = 25.47$ ,  $p < 0.001$ , total seedling richness: main effect of litter removal  $F_{1,38} = 17.06$ ,  $p < 0.001$ , sown seedling number: main effect of litter removal  $F_{1,34} = 20.73$ ,  $p < 0.001$ , sown seedling richness: main effect of litter removal  $F_{1,34} = 12.26$ ,  $p = 0.001$ . There were no main or interactive effects of light addition on seedlings.

**Figure S6.** The effects of (a) litter removal and (b) herbivore exclusion on the seedling number of individual species. Data are means  $\pm$  SE. *Achillea millefolium* (Achmil; main effect of litter removal,  $F_{1,33} = 24.03$ ,  $p < 0.001$ ), *Daucus carota* (Daucar; main effect of litter removal,  $F_{1,33} = 15.49$ ,  $p < 0.001$ ), *Plantago media* (Plamed; main effect of litter,  $F_{1,33} = 10.45$ ,  $p = 0.003$ ), *Galium album* (Galalb; main effect of herbivore exclusion,  $F_{1,12} = 9.25$ ,  $p = 0.010$ ).

**Figure S7.** Non-metric multidimensional scaling (NMDS) of the total seedling community and its relationship with (a) litter removal and (b) herbivory exclusion. Non-metric multidimensional scaling (NMDS) of the sown seedling community and its relationship with (c)

litter removal and herbivory exclusion. The ordinations were based on Bray-Curtis dissimilarity. We only show the treatments which were significant according to the permutational analysis of variance (PERMANOVA). The statistics are shown in [Table S5](#).

**Figure S8.** pH (a) and NH<sub>4</sub> (b) in response to combinations of herbivore exclusion, fertilization and light addition. Data are means  $\pm$  SE. pH: main effect of fertilization  $F_{1,12}=11.71$ ,  $p=0.005$ , NH<sub>4</sub>: main effect of fertilization  $F_{1,12}=5.59$ ,  $p=0.035$ . Data for soil pH and NH<sub>4</sub> concentration were collected in 2020 on the plot level, and analyzed at the University of Manchester, UK.

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