# Title page

## Title

**Seed limitation interacts with biotic and abiotic causes to constrain novel species’ impact on community biomass and richness**

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## Running title

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AE and EK designed and established the overall experiment, AE conceptualized the study and collected the data. NAP analysed the data and wrote the first draft of the manuscript. All authors contributed substantially to revisions.

## Data accessibility statement

Should the manuscript be accepted, the data supporting the results will be archived on Dryad and the data DOI will be included at the end of the article.

# Abstract

Seed limitation can narrow down the number of coexisting plant species and limit plant community productivity. It is also likely to constrain community responses to changing environmental and biotic conditions. In a 10-year full-factorial experiment of seed addition, fertilisation, warming and herbivore exclusion, we tested how seed addition alters community richness and biomass, and how its effects depend on seed origin and environmental and biotic context. We found that seed addition increased richness in all treatments, and increased community biomass depending on nutrient addition and warming. Novel seeded species, originally absent from the communities, increased biomass the most, especially in fertilised plots and in the absence of herbivores, while adding seeds of local species did not affect biomass. Our results show that dispersal limitation can constrain the invasion of novel species and their effects on community biomass, and demonstrate that these relationships are contingent on trophic interactions and environmental conditions.

# Introduction

Understanding the mechanisms by which plant communities assemble is crucial to predict species range shifts and future communities’ diversity and functioning, following global changes (Grytnes et al., 2014; Wallingford et al., 2020). One important factor affecting plant community assembly is seed limitation which can constrain species richness and productivity (Tilman, 1997; Stein et al., 2008). However, although seed limitation is common in communities (Tilman, 1997; Turnbull et al., 2000; Zobel et al., 2000; Myers & Harms, 2009), not much is known about whether and how its effects are modulated by abiotic and biotic factors (Ladouceur et al., 2020). To quantify the potential for new species to establish in future communities and their effects on community richness and productivity, it is critical to understand the mechanistic context-dependency (Catford et al., 2022) of seed limitation.

Seed limitation usually restrains species richness (Tilman, 1997; Zobel et al., 2000; Myers & Harms, 2009; Germain et al., 2017), but its effect on productivity is rarely studied due to the destructive nature of biomass harvesting (Ladouceur et al., 2020). Seed limitation should constrain species richness and biomass most in conditions which maintain high niche diversity or dimensionality and allow species to complement each other in the uptake of available resource pool (Tilman, 1982; Harpole & Tilman, 2007), when species facilitate each other or benefit from biotic feedback (Barry et al., 2019). According to the “shifting limitations hypothesis”, community richness should shift from being seed limited where nutrient-scarcity or other growth-limiting factors maintain available niches by reducing biomass and competition for light, to being limited by competitive interactions in high-productivity systems (Foster et al., 2004). Similarly, complementarity effects on biomass should be lowest in high productivity because saturating richness-productivity relationships could lead to negligible impact of seed limitation on biomass (Tilman et al., 2014). Therefore, the effect of seed limitation on richness and biomass might only be observable in lower productivity systems, where nutrients or other factors limit growth and alleviate competition for light.

The presence of seed limitation is often tested with seed addition experiments (Tilman, 1997; Münzbergová & Herben, 2005), which have shown that seed addition increases community richness less in nutrient-rich or fertilised systems (Foster et al., 2004; Dickson & Foster, 2008). Similarly, in cold ecosystems such as tundra, seed addition can increase richness less in warmed conditions where temperature-limitation is removed and biomass is greater (Olsen & Klanderud, 2014; Eskelinen et al., 2017). Among biotic factors, herbivory can reduce competition for light by decreasing the biomass and cover of dominant species (Olff & Ritchie, 1998; Borer et al., 2014); seed addition should therefore increase richness more in the presence of herbivores (Eskelinen et al., 2016). Theory predicts lower potential for complementarity effects when multiple factors limiting biomass are simultaneously relaxed (Harpole et al., 2016; Barry et al., 2019). However, little is known about the effects of seed limitation on community richness and productivity under multiple constraints. In addition, most studies report only short-term (one to three years), possibly transient effects of seed addition on communities (Ladouceur et al., 2020), while longer-term establishment, driven by competition with neighbouring species, can be more constrained than germination (Turnbull et al., 2000; Eskelinen et al., 2016; Leverkus & Crawley, 2020).

Seed limitation can result from dispersal limitation, i.e., limited ability of species to disperse in a landscape, leading to the absence of certain, regionally existing species from local communities. It can also result from limited seed availability within a local community, due to low seed production and viability, or high flower herbivory of local species already present in the community (Tilman et al., 1997). Relaxing seed limitation of novel and local species may differently affect richness and biomass. Release from dispersal limitation of novel species, present in the region but absent from a local community, could result in invasion of highly competitive species, as predicted by competition-colonisation trade-off (Tilman, 1994; Ehrlén & van Groenendael, 1998; Turnbull et al., 1999), leading to decreased overall richness (Leibold et al., 2017). Further, since novel species are likely to possess novel traits (Petermann et al., 2010), they may occupy previously unoccupied niches, thereby increasing complementarity and community biomass. On the other hand, novel species’ invasions could depend on abiotic and biotic conditions under which they are the most competitive, for example, herbivory or climatic conditions (Alexander et al., 2015; Eskelinen et al., 2017; Leverkus & Crawley, 2020). Therefore, the effect of seed addition on local communities should depend on species origin and biotic and abiotic context.

We examined the context-dependency of seed limitation effects on richness and biomass of vascular plant communities in a tundra grassland. We experimentally manipulated seed limitation by adding seeds of local and novel species under full-factorial combination of nutrient enrichment, warming and herbivore exclusion. We recorded richness and species-specific biomass ten years after the seed addition and asked: 1) To what extent are community biomass and richness limited by seed availability and to what extent these effects are contingent on nutrients, temperature, and herbivory? 2) Do local and novel species differ in their responses? We hypothesised: 1) Seed limitation constrains community richness and biomass most in ambient conditions (control plots), and less under nutrient enrichment, warming, and herbivore exclusion, due to multiple limiting factors that maintain high niche diversity or dimensionality in ambient conditions. 2) Due to the competition-colonisation trade-off, seed limitation constrains the richness and biomass of novel species more than that of local species, especially in conditions where novel species are the most competitive.

# Material and methods

## Experimental site and design

Our experimental site is located in Kilpisjärvi, north-western Finland (69.055°N, 20.887°E, 750 m a.s.l.). It is a species-rich (on average 16 species per control plot), grass- and forb-dominated, low-statured tundra meadow above treeline. The growing season is short (June to September), with a mean annual temperature of -2°C. The main large herbivore in the study area is semi-domesticated reindeer (*Rangifer tarandus tarandus*), which grazes in the area from the end of June until August-September. Small mammalian herbivores encountered on the site are Norwegian lemmings (*Lemmus lemmus* L.), grey-sided voles (*Clethrionomus* *rufocanus* Sund.), and mountain hares (*Lepus timidus* L.).

We started our experiment in August 2009 by establishing 56 plots of 0.8 x 0.7 m. Each plot was randomly assigned to a treatment combination of, crossing fertilisation, warming, and herbivore exclusion, with a total of 8 treatment combinations and 7 replicates per combination. Fertilised plots received 9.6 g N, 5.4 g P and 13.2 g K m-2 annually in the form of NPK fertiliser (16-9-22) dissolved in water, split in two doses (once mid-June and once at the end of July). Unfertilised plots received the same amount of water without fertiliser. Our nutrient addition treatment was designed to alleviate all nutrient limitation to test the role of nutrients as an abiotic constraint on invasion. It also mimics natural variation in nutrients in tundra areas (Eskelinen et al., 2009), and anthropogenic nutrient deposition which can be high especially in some alpine areas in Central Europe (Galloway et al., 2004). Herbivore exclusion treatment was implemented using cages of 160 cm diameter, 100 cm height and with a 10 mm mesh size, buried 15 cm into the soil. The warming treatment was implemented using ITEX (International Tundra Experiment) open-top Plexiglas chambers with a maximum basal diameter of 146 cm (Kaarlejärvi et al., 2013). Voles and lemmings could move in and out of the open-top chambers (Kaarlejärvi pers. Obs.), but the chambers could have affected reindeer grazing patterns (Moise & Henry, 2010). Therefore, the chambers were removed when reindeer were grazing in the area, typically in July-August. The entire experimental site was fenced during the rest of the year. Our warming treatment therefore simulated warmer spring and autumn when plant growth is most limited by temperature, which corresponds to local predictions of climate warming (Kivinen et al., 2012). Warming increased mean air temperature by 1.92°C in June and 1.23°C in August, while it did not affect soil temperature (measured in 2011). Our treatment also increased growing degree-days on average by 20% in June (2012-2014) and 11% in August and September (2012–2013, base daily mean temperature of + 5 °C). See Kaarlejärvi et al. (2013 and 2017) for more details about the experimental design.

Even though natural colonisation could, in principle, have been limited by the sides of the OTC chambers, this potential effect is unlikely to affect our conclusions. The chambers are only ca 40 cm tall and open at the top, and are removed for July-August, allowing some seed arrival into the plots. Further, if there was a detectable effect, we should have observed a consistent decrease in richness with warming; however, warming did not affect total richness (Table S1a), or the richness of local and resident richness (Table S1c). Lastly, our main findings relate to interactions among seed addition, herbivory and fertilisation, and are not affected by warming. It is therefore unlikely that our conclusions would be affected by the open-top chambers preventing natural seed rain.

Within each plot of 0.8 x 0.7 m, we marked two subplots of 25 × 25 cm. To test the impacts of seed limitation on species richness and productivity, we applied a seed addition treatment to one of these subplots and the other was left without. We selected 25 regionally common species, of which 11 were present in the study site at the start of the experiment (hereafter called “local seeded species”), and 14 were novel species to the study site (hereafter called “novel seeded species”). Five novel species never established, see details in Table S2. The local seeded species mainly occurred above treeline, while novel seeded species were a mixture of species occurring above and below treeline. However, novel seeded species that established were especially abundant below the treeline and novel seeded species that did not establish were abundant above treeline. The seed addition subplots were sown with 50 seeds per species in September 2009 and 2010. See Eskelinen et al., 2017 for additional details on the seed addition treatment.

## Data collection

In late July - early August 2019, ten years after seed addition and at peak biomass, we collected aboveground biomass from each seeded and unseeded subplot of 25 x 25 cm. Biomass was sorted into species for vascular plants. We excluded mosses, lichens, and litter from these analyses. The dry weight of the biomass was recorded after drying at 60°C for 72 hours.

To assess how seed addition and other treatments affected trait composition in communities, we collected trait data for species occurring in our experimental plots and in the seed mixture. We measured specific leaf area (SLA; m2 kg‑1 dry mass), leaf dry matter content (LDMC; mg g‑1), plant height (cm), condensed tannins (mg g‑1), total phenolics (mg g‑1), foliar carbon to nitrogen, carbon to phosphorus, and nitrogen to phosphorus ratios (C:N, C:P, and N:P ratios respectively; based on % C, N and P) using standard protocols (Pérez-Harguindeguy et al., 2013). These traits are commonly used as they reflect resource-use strategies: slow/conservative resource-use (high LDMC, high C:N and C:P ratios, high defence compounds) vs. fast/acquisitive resource-use (Wright et al., 2004; Reich, 2014; Züst & Agrawal, 2017). Traits were collected during summers 2010 and 2014 in the study region, on ten naturally occurring individuals per species outside the experimental plots. The data was completed using traits from the TRY database (Kattge et al., 2011) for four species (see Table S3). Foliar nitrogen and carbon content were analysed by combustion in automated elemental analyser EA1110 CHNS‑O (CE instruments) and P content was analysed by colorimetry after ascorbic acid digestion (John, 1970). Condensed tannins and total phenolics were extracted in 50% acetone (Price & Butler, 1977; Broadhurst & Jones, 1978, see Table S3). We then calculated community weighted means of traits (CWM traits; Garnier et al., 2004) by multiplying each species’ relative abundance (i.e. biomass) by that species’ trait value, and summing up all values per subplot.

## Statistical analyses

We conducted all analyses in R Statistical environment (R Core Team, 2021). We first tested whether seed addition affected richness and biomass and whether its effects depended on warming, fertilisation, and herbivore exclusion. For this we fitted two separate linear mixed effects models (nlme package; Pinheiro et al., 2021) with single and interactive effects of seed addition and the three experimental treatments as explanatory variables. We also tested the effect of the same treatments on CWM traits (one model per trait, and per PCA axis, Table S3). In all models, subplots were nested within plots as a random factor. Some variables were log or square-root transformed to meet assumptions of normal error distribution and homogeneity of variance (see Table S3 and S1).

Second, we tested the importance of seed limitation in controlling richness and biomass of local seeded species, novel seeded species, and resident unseeded species (i.e., all unseeded species occurring in the plots) depending on fertilisation, warming and herbivore exclusion. For these analyses, we calculated change from unseeded to seeded subplots, and used this as a response variable with warming, fertilisation, herbivore exclusion and their interactions as explanatory variables. We calculated change from unseeded to seeded plots, i.e., responsiveness to seed addition, as especially novel seeded species were practically absent from unseeded subplots. We fit separate models for novel, local and resident species, and for richness and biomass, resulting in six models. As the ‘change’ data for novels species had strongly skewed distribution and large heteroscedasticity in variances, we fit generalised linear models with gamma distribution for biomass change. The other five models were linear models (see details in Table S4).

# Results

## Species richness

Seed addition increased total species richness by three species on average (Figure 1a), independently of the other treatments (Figure 1b, Table S1a). Both local and novel species richness increased in response to seeding (Figure 1a, Table S4a-b). Increase of local seeded species richness did not depend on any treatments, while the increase of novel seeded species richness was lowest when plots were grazed and warmed (significant exclosure x warming interaction, Figure 2a, Table S4b). Seed addition also decreased unseeded resident species richness in fertilised and fenced plots (Table S4c).

## Biomass

Seed addition increased total community biomass and this effect depended on treatments (Figure 1c‑d, Table S1b). Seed addition increased biomass in plots with ambient nutrient and temperature levels, i.e., in unfertilised and unwarmed plots, where biomass was in general the lowest. It also increased biomass in the joint treatment of fertilisation and warming. However, seed addition had no effect when plots received only one of these treatments, i.e., when plots were fertilised or warmed only (Figure 3).

In contrast to species richness, local and novel seeded species differed in their biomass responses. Seed addition increased the biomass of novel seeded species that established but did not affect the biomass of local seeded species (Figure 1c, Table S4d-e). The positive effect of seed addition on the biomass of novel species was also greater in fertilised and fenced plots (Figure 1d and 2b, Table S4e). Furthermore, warming interacted with exclosure and fertilization (Table S4e): it reduced the difference in novel species biomass between fenced and unfenced plots, i.e., alleviated the negative impact of herbivory and slightly reduced the benefit of herbivore exclusion, and amplified it between unfertilised and fertilised plots, i.e., enhanced the positive impact of fertilization (Figure 2 and S1). Biomass of unseeded resident species, in contrast, decreased with seed addition in fertilized plots, indicating a shift in species composition from dominance by resident species to dominance by novel species (Figure 1d, Table S4f). Therefore, an increase in novel species biomass did not necessarily translate into an increase in the total amount of biomass produced.

## Trait composition

In addition to richness and biomass, seed addition also affected CWM traits: SLA increased, while C:N ratio, C:P ratio and LDMC decreased with seed addition in fertilised plots (seed addition × fertilisation interaction, Figure S2, Table S3). Seed addition therefore shifted functional composition towards faster traits in fertilised plots, while it had no effect on trait composition in unfertilised plots. Seed addition also increased height and decreased N:P ratio, independently from other treatments. Community means of tannin and phenol content did not respond to seed addition (Table S3).

# Discussion

Our study resulted in four major discoveries. First, reducing seed limitation had positive long-term effects on community richness, and these effects persisted even under varying environmental and biotic conditions that have previously been considered as an obstacle for longer-term establishment (Foster, 2001; Foster et al., 2004). Second, alleviating seed limitation led to a concomitant increase in richness and biomass, contrasting some earlier findings (Ladouceur et al., 2020). Third, introducing novel species to the community affected biomass, while adding seeds of species already present locally did not – a novel finding that we have not seen elsewhere. Fourth, the impacts of relaxing seed limitation of novel species were contingent on herbivory and soil nutrients: novel species invasions produced more biomass when protected from herbivores and when nutrient limitation was also relaxed. These findings highlight the importance of seed limitation for community diversity and productivity, and the conditionality of these effects on species origin, trophic interactions and nutrient limitation.

Ten years after seed addition, species richness was on average three species higher in seeded compared to unseeded plots. This result shows that relaxing seed limitation can have long-term effects on community richness. Increasing richness with seed addition is a common finding from relatively short-term experiments (one to three years) (Tilman, 1997; Turnbull et al., 2000; Zobel et al., 2000; Cadotte et al., 2006; Myers & Harms, 2009; Ladouceur et al., 2020), while long-term effects on richness are less common or not often addressed (Turnbull et al., 2000; Ladouceur et al., 2020). In general, initial gain of species by seed addition can decrease with time (Olsen & Klanderud, 2014; Leverkus & Crawley, 2020), reflecting, for example, greater role of competitive interactions limiting long-term establishment than germination (Olsen & Klanderud, 2014). A few other studies report long-term positive effects of seed addition on richness (Eskelinen et al., 2016; Leverkus & Crawley, 2020); however, in these studies, richness increased with seed addition only in the presence of herbivores. We found that seed addition enhanced richness under various conditions, also in the absence of herbivores and in fertilised plots, where biomass of vegetation was considerably higher and light limitation greater (Kaarlejärvi et al., 2017). This finding is counter to ecological theory and empirical studies showing lower chances for niche partitioning with resource addition and exclusion of herbivory (Harpole & Tilman, 2007; Dickson & Foster, 2008; Olsen & Klanderud, 2014; Harpole et al., 2016; Eskelinen et al., 2017; Klaus et al., 2017), which should make establishment harder. However, because of the relatively low initial productivity of the site, biomass may not have been high enough to induce strong competition for light and prevent new species’ establishment. In addition, our seed mixture included novel species from more nutrient-rich and productive conditions, possessing traits that confer greater competitive ability under fertilisation and herbivore exclusion (Eskelinen et al., 2017). These traits enable invading nutrient-rich and fenced plots and could explain the consistent increase in richness.

We found that seed addition also increased community biomass, demonstrating that seed limitation is an important factor constraining community productivity and ecosystem functioning. Our result contradicts findings from a recent meta-analysis (Ladouceur et al., 2020), which reported no effects of seed addition on biomass. However, this meta-analysis did not consider different limiting factors that could constrain biomass response to seed addition. We found that warming and fertilisation modified the increase of total biomass with seed addition, unlike the increase in richness which did not depend on the treatments. This suggests that local abiotic conditions may modify relationship between richness and productivity, being in line with findings that richness and productivity are not necessarily linked at the local scale (Leibold et al., 2017; Hagan et al., 2021), for example, due to saturating diversity-productivity relationship (Tilman et al., 2014; Ladouceur et al., 2020). In our study, community biomass increased with seed addition when nutrients and temperature were limiting, but not when plots were fertilised or warmed. This finding is consistent with theoretical work suggesting greater potential to observe complementarity effects and greater effect of seed addition on biomass when multiple factors limit biomass and competition for light (Harpole & Tilman, 2007; Harpole et al., 2016). Surprisingly, we also found that seed addition increased biomass in plots with joint nutrient addition and warming. This result is counterintuitive based on above theory; however, it may reflect a shift in competitive interactions between the resident unseeded and seeded species. In fertilised plots, the biomass of unseeded resident species was lower in plots with seed addition than in plots without seed addition (Table S4f), possibly because resident species were competitively disfavoured under fertilisation, while seeded species benefitted from these conditions. Relaxing growth-limiting factors therefore reduced possibilities for complementarity in species’ biomass production. At the same time, it likely triggered a shift in competitive interactions, favouring establishment of novel competitive species at the expense of resident species. Our results suggest that the effects of seed limitation on biomass are highly dependent on growth-limiting factors and competitive interactions among residents and novel species.

Our seed mixture included species from the local species pool, initially present in the local community, and from the regional species pool, initially absent from the community. Seed origin is rarely discussed in seed addition experiments. Some studies found that seed addition effects were mostly due to the addition of local species (Turnbull et al., 2000; Gross et al., 2005). Here we show that although local seeded species affected richness, the impact of seed addition on community biomass was mostly due to the establishment of novel seeded species (Figure 1c). Our novel seeded species pool contained tall, high SLA species common in the region but mainly occurring below treeline. In contrast, local seeded species pool was a mixture of relatively low-statured but both high and low SLA species that occur at our experimental site above the treeline. The positive impact of novel species on biomass could be due to their complementary set of traits compared to the existing communities (Petermann et al., 2010; Cadotte, 2017; Li et al., 2022). Novel seeded species could therefore fill previously unoccupied niches, leading to complementarity in biomass production between species. Our results are also in line with competition-colonisation trade-off theory (Tilman, 1994; Ehrlén & van Groenendael, 1998; Turnbull et al., 1999), which predicts that species’ competitive ability in resource-rich conditions should correlate negatively with ability to disperse far. When relaxing dispersal limitation, species having higher competitive ability should be able to germinate and establish (Tilman, 1997; Leibold et al., 2017), leading to an enhanced biomass. Overall, our results highlight that the origin of the added species and the traits of the novel species matter for long-term effect of seed addition on community biomass.

The impact of novel species invasion on community biomass was modulated by herbivores, nutrients, and warming. In general, herbivores are strong regulators of diversity, biomass and ecosystem functions (Olff & Ritchie, 1998; Knapp et al., 1999; Wardle et al., 2004; Borer et al., 2014). They can also modulate the effects of global changes on vegetation by mitigating species loss under warmer temperature (Post & Pedersen, 2008; Kaarlejärvi et al., 2017). We found that seed addition increased novel species biomass especially in fenced plots, demonstrating that herbivores can modify the impact of novel species on community biomass once their dispersal limitation is relaxed. Although the presence of herbivores can favour colonization and establishment of novel species, it can also limit community biomass (Olff & Ritchie, 1998; Myers & Harms, 2009; Eskelinen et al., 2016). Many of the novel species that established in our study were relatively tall herbs having high foliar N and SLA (Eskelinen et al., 2017), which are traits that can increase not only competitive ability for light but also susceptibility to herbivores (Fargione & Tilman, 2002; Díaz et al., 2007; Evju et al., 2009; Eskelinen et al., 2012; Allbee et al., in press). Biomass of such species can therefore increase in the absence of herbivores (Eskelinen et al., 2017). In addition, warming slightly reduced the negative effect of herbivores on novel species biomass especially when combined with fertilization, possibly because warmer temperature favoured compensatory growth of novel species after being grazed (Ramula et al., 2019). However, in the absence of herbivores warming slightly reduced the benefit from herbivore exclusion, demonstrating that warming can have complex and sometimes ambiguous interactions with herbivory. Furthermore, we also found that the effects of relaxing dispersal limitation on novel species’ biomass were greater in fertilised plots, and in particular under warmer conditions, suggesting that temperature and nutrients colimit novel species’ impact on biomass. Nutrient addition should disproportionally favour fast-growing and tall species (Laliberté & Tylianakis, 2012), such as many of our novel species that established to the study site, and lead to extinction of short‑statured, slow-growing species (Hautier et al., 2009; Borer et al., 2014; Kaarlejärvi et al., 2017). In our study, the biomass increment of novel seeded species under fertilisation was also reflected in the shift of community traits as CWM SLA increased, and LDMC, C:N ratio and C:P ratio decreased in fertilised seed addition plots. These all are traits associated with resource-acquisitive strategy (Wright et al., 2004; Reich, 2014). Our findings suggest that both seed limitation and nutrient availability can constrain community productivity and prevent shifts in community composition towards resource-acquisitive functional traits, in particular in the context of increasing temperatures (Fridley et al., 2016). Taken together, our results highlight the importance of herbivores, soil nutrient availability, and temperature in controlling how the impacts of dispersal limitation and novel species invasions become manifested in plant community productivity.

# Conclusions

We found that one time addition of seeds had strong and persistent effects on community richness, biomass, and composition ten years after seed addition, highlighting the fundamental role of dispersal limitation and seed availability in controlling plant communities. Our finding that simultaneously relaxing dispersal and nutrient limitations and herbivore pressure favoured novel competitive species is in line with theory and recent conceptual work (Harpole & Tilman, 2007; Barry et al., 2019), and demonstrates that complementarity effects on biomass can raise when novel species with complementary traits are introduced to the system via relaxing dispersal limitation. Therefore, anthropogenic nutrient enrichment and reduction of native or domestic herbivore populations may assist range expansions of highly competitive novel species, and climate warming may modify these responses. These changes in community biomass and composition will also likely be reflected in the overall ecosystem by affecting other functions such as litter productivity, carbon and nutrient cycling, or soil properties (Belovsky & Slade, 2000; Wardle et al., 2004; Charles et al., 2022). We conclude by emphasizing that to properly understand the effects of seed limitation on communities, it is crucial to consider complex interdependencies among species origin, trophic interactions, and abiotic environment. Future studies should examine how increases in richness and biomass due to novel species invasions translate to other ecosystem functions, such as nutrient and carbon cycling.

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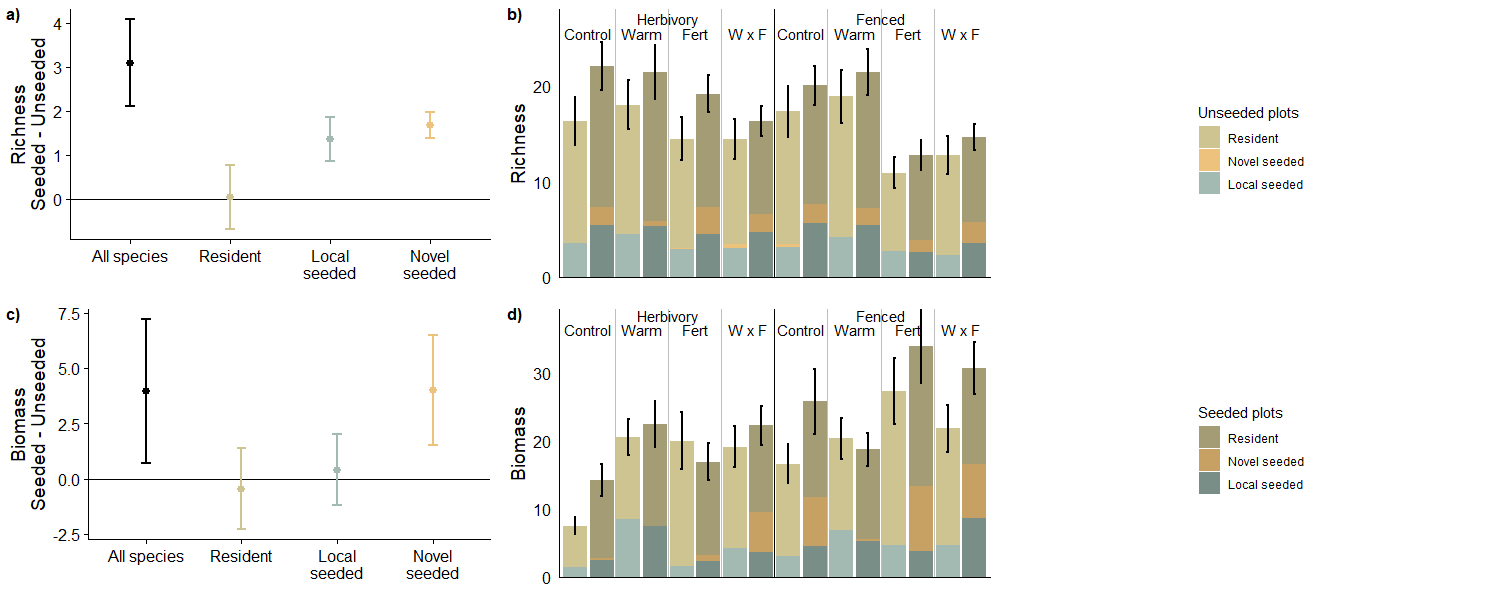
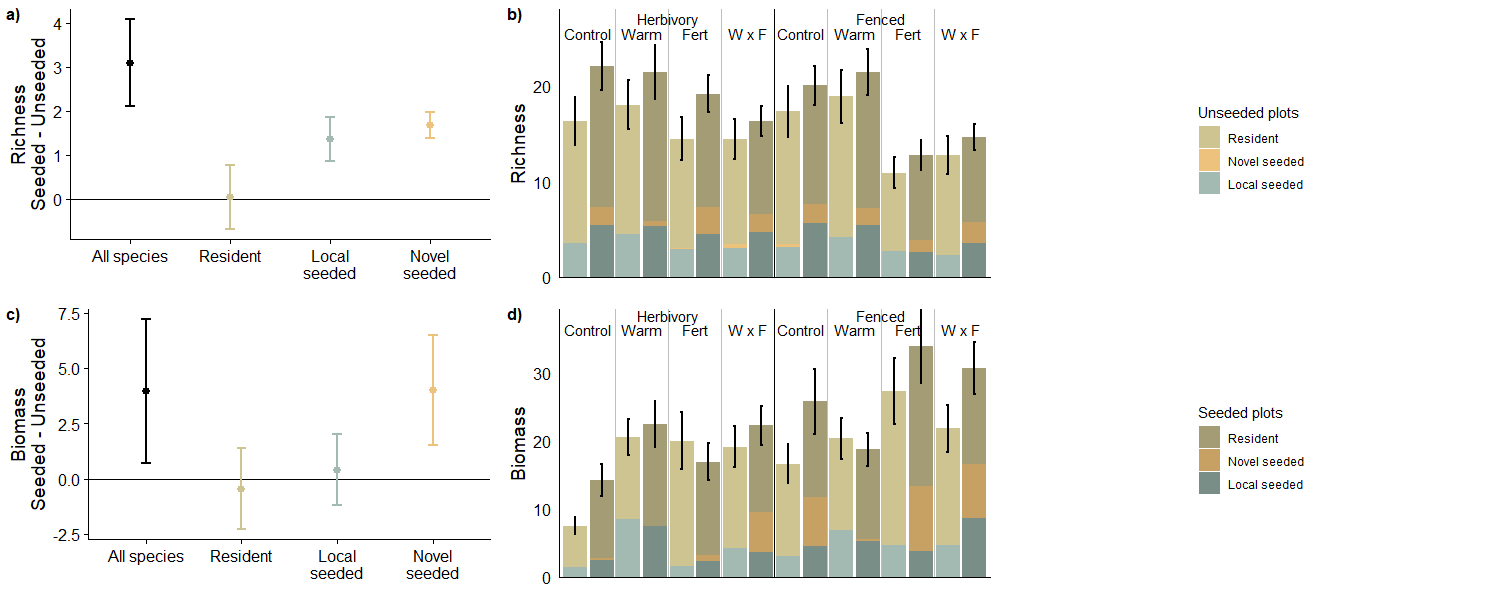
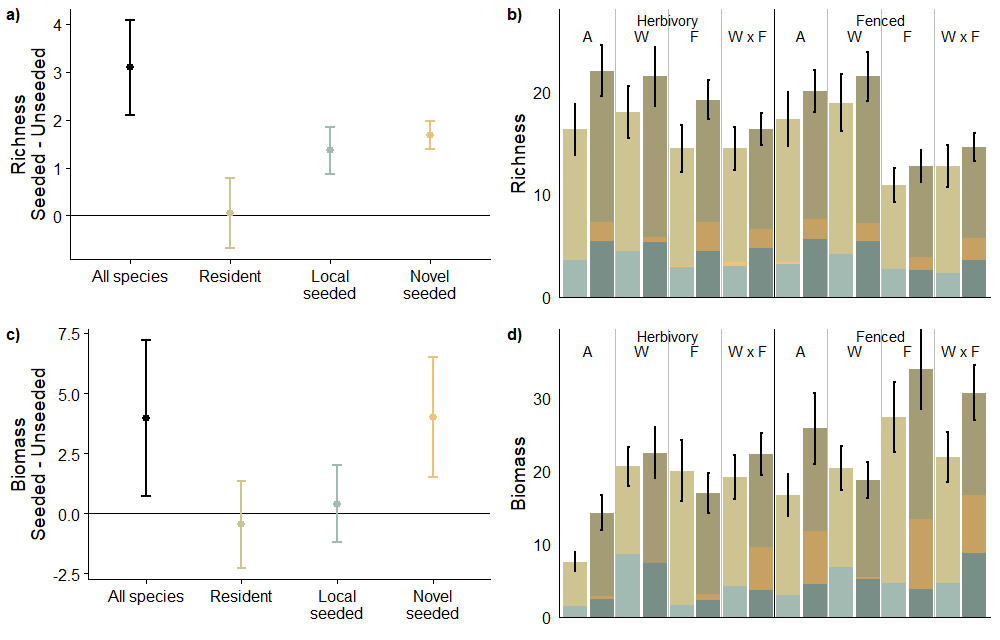
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# Figures



## **Figure 1**

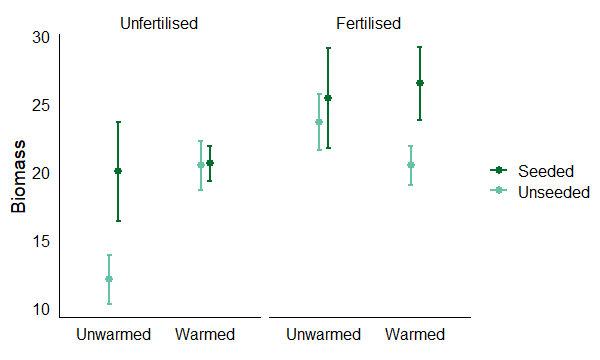
(a-b) Species richness and (c-d) biomass (g per 25 × 25 cm plot) of resident, novel seeded, and local seeded species. Difference in (a) species richness and (c) biomass between seeded and unseeded plots. Positive values indicate an increase in (a) richness and (c) biomass with seed addition; mean ± 95% confidence interval, N = 56. (b) Species richness and (d) biomass in unseeded and seeded plots, in different treatment combinations: A, ambient environment; W, warming; F, fertilisation; W × F, warming and fertilisation; mean ± 95% confidence interval, N = 112. For model outputs, see Table S1 and S4.

Chart, box and whisker chart

Description automatically generated

## **Figure 2**

Difference in (a) species richness and (b) biomass (g per 25 × 25 cm plot) of novel seeded species between seeded and unseeded plots in different treatment combinations. Positive values indicate an increase in (a) richness and (b) biomass with seed addition. Mean ± 95% confidence interval, N = 56. For model outputs, see Table S4b and e.



## **Figure 3**

Community biomass (g per 25 × 25 cm plot) depending on seed addition, warming, fertilisation and their joint treatment. Mean ± 95% standard error, N = 112. For model output, see Table S1b.

# Supplementary

## Table S1

**Linear mixed effects models**

1. **Community species richness**

Results of the linear mixed effects model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), seed addition and their interactions on community species richness.

*lme(Community richness ~ Fertilisation x Warming x Exclosure x Seeding, random = ~ 1|Plot)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Community richness | numDF | denDF | F-value | p-value |
| (Intercept) | 1 | 48 | 2177.947 | <.0001 |
| Fertilisation | 1 | 48 | 47.991 | <.0001 |
| Warming | 1 | 48 | 0.745 | 0.393 |
| Exclosure | 1 | 48 | 5.485 | 0.023 |
| Seeding | 1 | 48 | 37.821 | <.0001 |
| F x W | 1 | 48 | 0.322 | 0.573 |
| F x E | 1 | 48 | 5.257 | 0.026 |
| W x E | 1 | 48 | 2.116 | 0.152 |
| F x S | 1 | 48 | 1.063 | 0.308 |
| W x S | 1 | 48 | 1.730 | 0.195 |
| E x S | 1 | 48 | 2.792 | 0.101 |
| F x W x E | 1 | 48 | 0.662 | 0.420 |
| F x W x S | 1 | 48 | 0.011 | 0.916 |
| F x E x S | 1 | 48 | 0.062 | 0.805 |
| W x E x S | 1 | 48 | 1.548 | 0.220 |
| F x W x E x S | 1 | 48 | 0.032 | 0.860 |

1. **Community biomass**

Results of the linear mixed effects model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), seed addition and their interactions on community biomass.

*lme(log(Community biomass) ~ Fertilisation x Warming x Exclosure x Seeding, random = ~ 1|Plot)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Community biomass | numDF | denDF | F-value | p-value |
| (Intercept) | 1 | 48 | 5894.262 | <.0001 |
| Fertilisation | 1 | 48 | 15.660 | 0.000 |
| Warming | 1 | 48 | 7.071 | 0.011 |
| Exclosure | 1 | 48 | 19.012 | 0.000 |
| Seeding | 1 | 48 | 7.414 | 0.009 |
| F x W | 1 | 48 | 6.248 | 0.016 |
| F x E | 1 | 48 | 0.685 | 0.412 |
| W x E | 1 | 48 | 13.165 | 0.001 |
| F x S | 1 | 48 | 0.846 | 0.362 |
| W x S | 1 | 48 | 1.057 | 0.309 |
| E x S | 1 | 48 | 0.001 | 0.976 |
| F x W x E | 1 | 48 | 1.806 | 0.185 |
| F x W x S | 1 | 48 | 4.743 | 0.034 |
| F x E x S | 1 | 48 | 1.635 | 0.207 |
| W x E x S | 1 | 48 | 0.053 | 0.818 |
| F x W x E x S | 1 | 48 | 0.111 | 0.740 |

1. **Species richness of seeded and unseeded species present locally**

Results of the linear mixed effects model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), seed addition, and their interactions on the richness of all seeded and unseeded species present locally, i.e., the sum of local and resident species richness, excluding novel seeded species.

*lme(Sum of local & resident species richness ~ Fertilisation x Warming x Exclosure x Seeding, random=~ 1|Plot)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Community richness | numDF | denDF | F-value | p-value |
| (Intercept) | 1 | 160 | 644.855 | <.0001 |
| Fertilisation | 1 | 48 | 17.813 | 0.000 |
| Warming | 1 | 48 | 0.446 | 0.507 |
| Exclosure | 1 | 48 | 1.747 | 0.193 |
| Seeding | 1 | 160 | 1.260 | 0.263 |
| F x W | 1 | 48 | 0.276 | 0.602 |
| F x E | 1 | 48 | 0.961 | 0.332 |
| W x E | 1 | 48 | 0.373 | 0.544 |
| F x S | 1 | 160 | 0.340 | 0.561 |
| W x S | 1 | 160 | 0.126 | 0.723 |
| E x S | 1 | 160 | 0.485 | 0.487 |
| F x W x E | 1 | 48 | 0.147 | 0.703 |
| F x W x S | 1 | 160 | 0.016 | 0.898 |
| F x E x S | 1 | 160 | 0.073 | 0.788 |
| W x E x S | 1 | 160 | 0.024 | 0.876 |
| F x W x E x S | 1 | 160 | 0.000 | 0.989 |

## Table S2

**Species list**

Novel seeded species that established were present regionally especially below the treeline and were absent from our study site originally, while there were also novel seeded species that did not establish and were abundant above treeline. Local species were abundant above treeline and at our study site (Benum 1958; Oksanen & Virtanen 1995; Virtanen et al. 1999; Olofsson et al. 2002; Virtanen et al. 2010; Kaarlejärvi & Olofsson 2014).

|  |  |  |
| --- | --- | --- |
| **Local seeded species that established** | Abundant above or below the treeline | Growth form/functional group |
| *Astragalus alpinus* | Above | N-fixer |
| *Geranium sylvaticum* | Below | Forb |
| *Gnaphalium supinum* | Above | Forb |
| *Hierochloe hirta* | Above | Grass |
| *Phleum alpinum* | Above | Grass |
| *Poa alpina* | Above | Grass |
| *Sibbaldia procumbens* | Above | Forb |
| *Solidago virgaurea ssp. virgaurea* | Below | Forb |
| *Trollius europeaus* | Below | Forb |
| *Vaccinium myrtillus* | Above | Shrub |
| *Vaccinium vitis-idaea* | Above | Shrub |
|  |  |  |
| **Novel seeded species** |  |  |
| *Angelica archangelica* | Below | Forb |
| *Anthriscus sylvestris* | Below | Forb |
| *Cerastium fontanum* | Below | Forb |
| *Cicerbita alpina* | Below | Forb |
| *Cornus suecica* | Below | Shrub |
| *Epilobium angustifolium* | Below | Forb |
| *Erigeron uniflorus* | Above | Forb |
| *Polenium acutiflorum* | Below | Forb |
| *Silene dioica* | Below | Forb |
|  |  |  |
| **Novel seeded species that did not establish** | | |
| *Empetrum nigrum* | Above | Shrub |
| *Gentiana nivalis* | Above | Forb |
| *Lychnis alpina* | Above | Forb |
| *Milium effusum* | Below | Grass |
| *Minuartia stricta* | Above | Forb |

## Table S3

**Functional trait analysis**

To characterise community response to the treatments, we measured a set of functional traits following standard protocols (Pérez-Harguindeguy et al., 2013): height (cm), specific leaf area (SLA; m2 kg−1 dry mass), leaf dry matter content (LDMC; mg g-1), foliar carbon to nitrogen ratio (C:N; based on % C and N), carbon to phosphorus ratio (C:P; based on % C and P), nitrogen to phosphorus ratio (N:P; based on % N and P), condensed tannins (mg g−1) and total phenolics (mg g−1). Traits were collected during summer 2010 and 2014 in the study region, on ten naturally occurring individuals per species. The data was completed using traits from the TRY database (Kattge et al., 2011) for four species. We measured nitrogen, carbon and phosphorus content on two grams of dry leaf material. N and C were measured by combustion in automated elemental analyser EA1110 CHNS-O (CE instruments), and P by colorimetry after ascorbic acid digestion (John, 1970). We extracted tannins and phenolics in 50% acetone. Tannins were analysed by a modified version of the vanillin method (the results are expressed as catechin equivalents, Broadhurst & Jones, 1978) and phenolics following the Price and Buttler method (results are expressed as tannic acid equivalents, Price & Butler, 1977). Species with tannin concentrations under the detection limit (2 mg g−1) were assigned a value of 1 mg g−1. We then calculated community weighted means of traits (CWM traits) using the relative abundance of each species, given by the biomass proportion of each species.

Results of linear mixed effects models

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | All traits | | SLA | | LDMC | | C:N ratio | |
|  | numDF | denDF | F-value | p-value | F-value | p-value | F-value | p-value |
| (Intercept) | 1 | 48 | 19053 | <.0001 | 6837 | <.0001 | 6100 | <.0001 |
| Fertilisation | 1 | 48 | 5.710 | 0.021 | 14.752 | 0.000 | 12.094 | 0.001 |
| Warming | 1 | 48 | 0.002 | 0.964 | 0.577 | 0.451 | 1.680 | 0.201 |
| Exclosure | 1 | 48 | 0.447 | 0.507 | 17.397 | 0.000 | 5.180 | 0.027 |
| Seeding | 1 | 48 | 4.727 | 0.035 | 10.704 | 0.002 | 6.013 | 0.018 |
| F x W | 1 | 48 | 0.126 | 0.724 | 0.841 | 0.364 | 0.483 | 0.490 |
| F x E | 1 | 48 | 0.263 | 0.611 | 0.246 | 0.622 | 0.003 | 0.955 |
| W x E | 1 | 48 | 2.176 | 0.147 | 0.531 | 0.470 | 0.148 | 0.702 |
| F x S | 1 | 48 | 5.832 | 0.020 | 11.364 | 0.002 | 5.063 | 0.029 |
| W x S | 1 | 48 | 0.033 | 0.856 | 0.059 | 0.809 | 0.167 | 0.685 |
| E x S | 1 | 48 | 0.494 | 0.486 | 0.784 | 0.380 | 1.079 | 0.304 |
| F x W x E | 1 | 48 | 0.545 | 0.464 | 0.131 | 0.719 | 0.149 | 0.701 |
| F x W x S | 1 | 48 | 2.235 | 0.142 | 0.681 | 0.413 | 1.757 | 0.191 |
| F x E x S | 1 | 48 | 0.870 | 0.356 | 0.000 | 0.995 | 0.000 | 0.998 |
| W x E x S | 1 | 48 | 0.000 | 0.992 | 0.472 | 0.496 | 0.120 | 0.731 |
| F x W x E x S | 1 | 48 | 0.330 | 0.568 | 0.883 | 0.352 | 0.010 | 0.920 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | C:P ratio |  | Height |  | N:P ratio |
|  | F-value | p-value | F-value | p-value | F-value | p-value |
| (Intercept) | 7019.5 | <.0001 | 6982 | <.0001 | 11390 | <.0001 |
| Fertilisation | 11.318 | 0.002 | 31.815 | <.0001 | 0.540 | 0.466 |
| Warming | 0.582 | 0.449 | 2.236 | 0.141 | 1.925 | 0.172 |
| Exclosure | 18.097 | 0.000 | 13.882 | 0.001 | 7.473 | 0.009 |
| Seeding | 19.142 | 0.000 | 16.064 | 0.000 | 14.065 | 0.001 |
| F x W | 0.433 | 0.514 | 4.170 | 0.047 | 0.263 | 0.611 |
| F x E | 0.203 | 0.655 | 0.051 | 0.822 | 0.809 | 0.373 |
| W x E | 0.443 | 0.509 | 0.773 | 0.384 | 0.173 | 0.680 |
| F x S | 4.664 | 0.036 | 1.645 | 0.206 | 0.722 | 0.400 |
| W x S | 0.174 | 0.679 | 0.360 | 0.551 | 1.656 | 0.204 |
| E x S | 0.722 | 0.400 | 0.463 | 0.500 | 0.124 | 0.726 |
| F x W x E | 0.484 | 0.490 | 0.628 | 0.432 | 0.212 | 0.647 |
| F x W x S | 2.599 | 0.114 | 1.194 | 0.280 | 0.172 | 0.680 |
| F x E x S | 0.000 | 1.000 | 1.228 | 0.273 | 0.321 | 0.573 |
| W x E x S | 0.303 | 0.585 | 2.368 | 0.130 | 2.131 | 0.151 |
| F x W x E x S | 0.975 | 0.328 | 0.233 | 0.632 | 0.754 | 0.389 |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Tanins | |  | Phenols |  | PCA axis 1 | | PCA axis 2 | |
|  | F-value | p-value | | F-value | p-value | F-value | p-value | F-value | p-value |
| (Intercept) | 514 | <.0001 | | 6180 | <.0001 | 0.000 | 1.000 | 0.000 | 1.000 |
| Fertilisation | 24.580 | <.0001 | | 1.433 | 0.237 | 19.508 | 0.000 | 3.893 | 0.054 |
| Warming | 3.401 | 0.071 | | 6.470 | 0.014 | 1.395 | 0.243 | 3.228 | 0.079 |
| Exclosure | 1.014 | 0.319 | | 2.377 | 0.130 | 13.994 | 0.001 | 0.155 | 0.695 |
| Seeding | 0.584 | 0.448 | | 0.068 | 0.795 | 13.341 | 0.001 | 0.068 | 0.796 |
| F x W | 1.776 | 0.189 | | 0.259 | 0.613 | 1.435 | 0.237 | 1.261 | 0.267 |
| F x E | 0.406 | 0.527 | | 0.928 | 0.340 | 0.002 | 0.965 | 0.240 | 0.626 |
| W x E | 3.090 | 0.085 | | 0.578 | 0.451 | 1.187 | 0.281 | 0.659 | 0.421 |
| F x S | 0.033 | 0.857 | | 2.020 | 0.162 | 7.948 | 0.007 | 2.418 | 0.127 |
| W x S | 0.137 | 0.713 | | 0.352 | 0.556 | 0.009 | 0.926 | 0.006 | 0.940 |
| E x S | 0.294 | 0.590 | | 2.709 | 0.106 | 0.689 | 0.411 | 2.217 | 0.143 |
| F x W x E | 1.344 | 0.252 | | 3.577 | 0.065 | 0.297 | 0.589 | 3.040 | 0.088 |
| F x W x S | 0.041 | 0.840 | | 1.244 | 0.270 | 1.933 | 0.171 | 1.178 | 0.283 |
| F x E x S | 0.196 | 0.660 | | 0.012 | 0.914 | 0.002 | 0.965 | 0.240 | 0.626 |
| W x E x S | 1.755 | 0.192 | | 1.881 | 0.177 | 0.217 | 0.643 | 3.432 | 0.070 |
| F x W x E x S | 0.169 | 0.683 | | 0.473 | 0.495 | 0.344 | 0.560 | 0.022 | 0.883 |

## Table S4

**Linear models**

1. **Local seeded species richness**

Results of the linear model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), and their interactions on the difference in local seeded species richness between seeded and unseeded plots.

*lm(Richness difference ~ Fertilisation x Warming x Exclosure)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Local seeded species richness | Df | Sum Sq | Mean Sq | F value | Pr(>F) |  |
| Fertilisation | 1 | 3.500 | 3.500 | 1.010 | 0.320 |  |
| Warming | 1 | 0.286 | 0.286 | 0.083 | 0.775 |  |
| Exclosure | 1 | 1.143 | 1.143 | 0.330 | 0.568 |  |
| Fertilisation x Warming | 1 | 12.071 | 12.071 | 3.485 | 0.068 | . |
| Fertilisation x Exclosure | 1 | 8.643 | 8.643 | 2.495 | 0.121 |  |
| Warming x Exclosure | 1 | 1.143 | 1.143 | 0.330 | 0.568 |  |
| Fertilisation x Warming x Exclosure | 1 | 1.786 | 1.786 | 0.516 | 0.476 |  |

1. **Novel seeded species richness**

Results of the generalised linear model, using Poisson distribution, testing the effect of fertilisation, warming, herbivore exclusion (exclosure), and their interactions on the difference in novel seeded species richness between seeded and unseeded plots.

*lm(Richness difference ~ Fertilisation x Warming x Exclosure,)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Novel seeded species richness | Df | Sum Sq | Mean Sq | F value | Pr(>F) |  |
| Fertilisation | 1 | 2.571 | 2.571 | 2.427 | 0.126 |  |
| Warming | 1 | 2.571 | 2.571 | 2.427 | 0.126 |  |
| Exclosure | 1 | 0.071 | 0.071 | 0.067 | 0.796 |  |
| Fertilisation x Warming | 1 | 0.643 | 0.643 | 0.607 | 0.440 |  |
| Fertilisation x Exclosure | 1 | 2.571 | 2.571 | 2.427 | 0.126 |  |
| Warming x Exclosure | 1 | 10.286 | 10.286 | 9.708 | 0.003 | \*\* |
| Fertilisation x Warming x Exclosure | 1 | 0.643 | 0.643 | 0.607 | 0.440 |  |

1. **Resident species richness**

Results of the linear model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), and their interactions on the difference in resident unseeded species richness between seeded and unseeded plots.

*lm(Richness difference ~ Fertilisation x Warming x Exclosure)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Resident species richness | Df | Sum Sq | Mean Sq | F value | Pr(>F) |  |
| Fertilisation | 1 | 13.018 | 13.018 | 1.979 | 0.166 |  |
| Warming | 1 | 7.875 | 7.875 | 1.197 | 0.279 |  |
| Exclosure | 1 | 30.018 | 30.018 | 4.564 | 0.038 | \* |
| Fertilisation x Warming | 1 | 21.875 | 21.875 | 3.326 | 0.074 | . |
| Fertilisation x Exclosure | 1 | 30.018 | 30.018 | 4.564 | 0.038 | \* |
| Warming x Exclosure | 1 | 0.161 | 0.161 | 0.024 | 0.876 |  |
| Fertilisation x Warming x Exclosure | 1 | 2.161 | 2.161 | 0.329 | 0.569 |  |

1. **Local seeded species biomass**

Results of the linear model testing the effect of fertilisation, warming, and herbivore exclusion (exclosure), and their interactions on the difference in local seeded species biomass between seeded and unseeded plots.

*lm(Biomass difference ~ Fertilisation x Warming x Exclosure)*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Local seeded species biomass | Df | Sum Sq | Mean Sq | F value | Pr(>F) |  |
| Fertilisation | 1 | 11.460 | 11.455 | 0.289 | 0.593 |  |
| Warming | 1 | 2.600 | 2.601 | 0.066 | 0.799 |  |
| Exclosure | 1 | 8.080 | 8.083 | 0.204 | 0.654 |  |
| Fertilisation x Warming | 1 | 70.590 | 70.588 | 1.782 | 0.188 |  |
| Fertilisation x Exclosure | 1 | 8.290 | 8.290 | 0.209 | 0.649 |  |
| Warming x Exclosure | 1 | 24.150 | 24.150 | 0.610 | 0.439 |  |
| Fertilisation x Warming x Exclosure | 1 | 44.780 | 44.780 | 1.131 | 0.293 |  |

1. **Novel seeded species biomass**

Results of the generalised linear model, using Gamma distribution, testing the effect of fertilisation, warming, herbivore exclusion (exclosure), and their interactions on the difference in novel seeded species biomass between seeded and unseeded plots.

*glm((Biomass difference +1) ~ Fertilisation x Warming x Exclosure, family = Gamma)*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Novel seeded species biomass | Df | Deviance | Resid. Df | Resid. Dev | F | Pr(>F) |  |
| Null |  |  | 55 | 93.225 |  |  |  |
| Fertilisation | 1 | 10.448 | 54 | 82.777 | 8.459 | 0.005 | \*\* |
| Warming | 1 | 0.434 | 53 | 82.343 | 0.351 | 0.556 |  |
| Exclosure | 1 | 10.837 | 52 | 71.506 | 8.774 | 0.005 | \*\* |
| Fertilisation x Warming | 1 | 12.581 | 51 | 58.924 | 10.186 | 0.002 | \*\* |
| Fertilisation x Exclosure | 1 | 3.939 | 50 | 54.986 | 3.189 | 0.080 | . |
| Warming x Exclosure | 1 | 5.902 | 49 | 49.084 | 4.778 | 0.034 | \* |
| Fertilisation x Warming x Exclosure | 1 | 0.000 | 48 | 49.083 | 0.000 | 0.986 |  |

1. **Resident species biomass**

Results of the linear model testing the effect of fertilisation, warming, herbivore exclusion (exclosure), and their interactions on the difference in resident unseeded species biomass between seeded and unseeded plots.

*lm(Biomass difference ~ Fertilisation x Warming x Exclosure)*

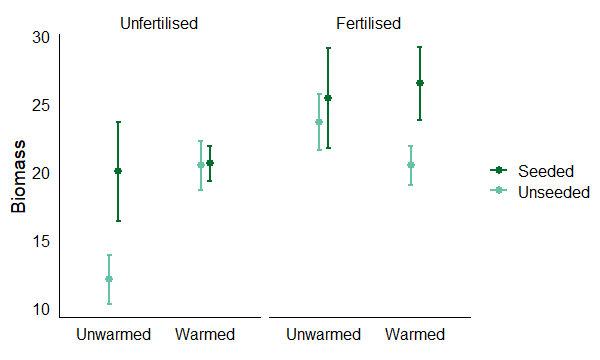
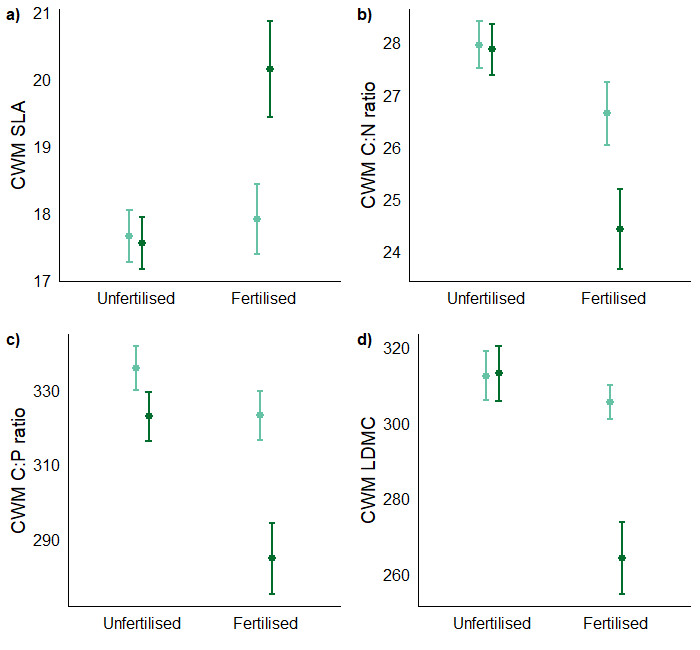
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Resident species biomass | Df | Sum Sq | Mean Sq | F value | Pr(>F) |  |
| Fertilisation | 1 | 374.80 | 374.80 | 8.625 | 0.005 | \*\* |
| Warming | 1 | 2.170 | 2.170 | 0.050 | 0.824 |  |
| Exclosure | 1 | 39.380 | 39.380 | 0.906 | 0.346 |  |
| Fertilisation x Warming | 1 | 17.090 | 17.090 | 0.393 | 0.534 |  |
| Fertilisation x Exclosure | 1 | 80.520 | 80.520 | 1.853 | 0.180 |  |
| Warming x Exclosure | 1 | 3.230 | 3.230 | 0.074 | 0.786 |  |
| Fertilisation x Warming x Exclosure | 1 | 22.580 | 22.580 | 0.520 | 0.475 |  |

**Chart, bubble chart, box and whisker chart

Description automatically generated**

## Figure S1

Difference in biomass (g per 25 × 25 cm plot) of novel seeded species between seeded and unseeded plots with respect to (a) warming and exclosure and (b) warming and fertilisation. See Figure 2b for all treatments effects. Positive values indicate an increase in biomass with seed addition. Mean ± 95% standard error, N = 56. For model outputs, see Table S4e.



## Figure S2

Community weighted mean (a) specific leaf area (SLA, m2 kg-1), (b) carbon to nitrogen ratio (C:N ratio), (c) carbon to phosphorus ratio (C:P ratio), (d) leaf dry matter content (LDMC, mg g-1) depending on seed addition, fertilisation, and their interaction. Other traits did not respond to seed addition, see details in Table S3. Mean ± 95% standard error, N = 112.