

One-time seed addition promotes long-term recovery of plant diversity and productivity in a previously fertilized old field

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March 07, 2024

Abstract

Unassisted recovery of plant diversity after reductions in nutrient inputs can be slow and incomplete. Increased nutrient availability, light limitation and recruitment limitation are thought to be primary barriers to diversity recovery. In a full-factorial experiment, we tested whether removing these obstacles promoted recovery of plant diversity in a previously cultivated and fertilized old-field. Results immediately following manipulations demonstrated that these factors increased diversity to varying degrees, but it was unknown whether these restorative processes would continue or diminish over time. Here, we examine long-term responses 13 years after these manipulations and find that seed addition was the only treatment that continued to increase plant diversity and productivity. Seed addition also reduced the biomass of two invasive species that become co-dominant in this grassland after sufficient nutrient inputs. Our results suggest that alleviating recruitment limitation can accelerate the recovery of plant diversity, and ecosystem functions that depend on plant diversity.

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Data accessibility statement: Upon acceptance, all data will be archived and the data DOI will be included at the end of the article.

Running Title: Low recruitment prevents diversity recovery

Keywords: recruitment limitation, diversity, exotic species, seed addition, grassland, recovery, restoration, nutrient enrichment

Type of Article: Letters

Number of Words: Abstract: 150, Main text: 3,668, Text Box: NA

Number of References: 63

Number of Figures: 4

Number of Tables: 1

Number of Text Boxes: 0

Statement of authorship: CMC designed and set up the experiment, CPR and CE collected data, CPR and FI analyzed the data, CPR wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

1 Abstract

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3 incomplete. Increased nutrient availability, light limitation and recruitment limitation are
4 thought to be primary barriers to diversity recovery. In a full-factorial experiment, we tested
5 whether removing these obstacles promoted recovery of plant diversity in a previously cul-
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8 restorative processes would continue or diminish over time. Here, we examine long-term
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12 nutrient inputs. Our results suggest that alleviating recruitment limitation can accelerate
13 the recovery of plant diversity, and ecosystem functions that depend on plant diversity.

2 Introduction

Human activities are resulting in an accelerated loss of species from ecosystems. Food and energy demands have led to the conversion of large extents of land into cropland and pasture, and increased the use and need for fertilizer (Galloway *et al.*, 2008; Lambin & Meyfroidt, 2011). While increased agricultural and industrial activities can bring many benefits to society, they also exert pressures on ecosystems that can drastically reduce terrestrial species diversity (Bobbink *et al.*, 2010; Newbold *et al.*, 2015). In grasslands, nutrient enrichment often reduces plant diversity, causing non-random species losses (Stevens *et al.*, 2004; Suding *et al.*, 2005; Clark *et al.*, 2007; Hillebrand *et al.*, 2007; Borer *et al.*, 2014).

Reductions in anthropogenic perturbations alone do not always result in rapid recovery of species diversity. Ecosystems recovering from human disturbance may maintain lower levels of diversity for several decades, and can fail to reach diversity levels observed in less disturbed ecosystems (Jones & Schmitz, 2009; Moreno-Mateos *et al.*, 2017; Isbell *et al.*, 2019). In grasslands, nutrient enrichment results in species losses that are not always readily reversible by reducing nutrient inputs (Suding *et al.*, 2004; Isbell *et al.*, 2013; Stevens, 2016; Koshida & Katayama, 2018). For example, when nutrient inputs are high, these reductions in species diversity can persist for decades after nutrient inputs are reduced (Isbell *et al.*, 2013), unless reductions in nutrient inputs are combined with other interventions (Storkey *et al.*, 2015; Tilman & Isbell, 2015; Clark & Tilman, 2010), or if low rates of nutrient inputs had occurred for a short time (Clark & Tilman, 2008). Active management can increase diversity in areas where passive recovery is not expected (Jones *et al.*, 2018), but may still be insufficient to fully restore diversity to levels comparable to those of reference sites (Benayas *et al.*, 2009).

In grasslands, interventions to reduce nutrient levels, increase light availability, and over-

39 come recruitment limitation have been proposed as strategies for accelerating the recovery
40 of biodiversity after agricultural abandonment and reductions in nutrient inputs. Nutrient
41 enrichment can reduce biodiversity by stimulating plant growth, which can result in litter
42 accumulation and increased competition for light (Clark *et al.*, 2007; Hautier *et al.*, 2009).
43 Haying and litter removal can increase light availability, reduce nutrient levels, and increase
44 propagule establishment (Foster & Gross, 1998; Maron & Jefferies, 2001; Clark & Tilman,
45 2010). Seed addition can alleviate low dispersal and recruitment limitation (Turnbull *et al.*,
46 2000), as species can face both dispersal and environmental filters for establishment (Grman
47 *et al.*, 2015). Previous studies at our site found that species richness can be limited by re-
48 cruitment (Tilman, 1997). Soil fertility and N availability can be temporarily reduced by C
49 amendments in the form of sugar or saw dust (Burke *et al.*, 2013). Removing N from plant-
50 available pools can help increase diversity in grasslands that have experienced nutrient enrich-
51 ment (Prober *et al.*, 2005; Chisholm *et al.*, 2015). These carbon inputs can increase microbial
52 activity, which helps immobilize N and other nutrients from plant-available pools (Jonasson
53 *et al.*, 1996), and can suppress exotic species that have high N requirements (Blumenthal
54 *et al.*, 2003). However, these reductions of soil fertility can be temporary and insufficient to
55 increase plant diversity when the abundance of exotic species is high (Morghen & Seastedt,
56 1999; Prober *et al.*, 2005).

57

58 While there is evidence that carbon amendments, litter removal, and seed addition can
59 help overcome obstacles for the recovery of diversity following nutrient enrichment and inva-
60 sion by exotic species, there is variation in experimental outcomes and most studies consider
61 only the initial establishment phase, rarely revisiting sites to test whether initial effects
62 persisted. Early results from our study showed that increasing light by removing litter
63 increased species richness, particularly when paired with seed addition (Clark & Tilman,
64 2010). In other studies, exotic grasses reduced diversity despite biomass and litter being re-

65 moved through grazing or prescribed burning (Gabbard & Fowler, 2007). In restored prairie
66 communities, Grman *et al.* (2015) found species specific filters for establishment, with some
67 species being more limited by dispersal and others by site conditions. Here we have the
68 opportunity to test the long-term outcomes of eliminating these three barriers for diversity
69 recovery (light limitation, dispersal limitation, and elevated nutrient levels) in a previously
70 cultivated field that has experienced nutrient addition and cessation in a fully-crossed ex-
71 perimental design.

72

73 We experimentally alleviated these obstacles for diversity recovery (recruitment limita-
74 tion, reduced light availability, and elevated nutrient levels), individually and in combination,
75 to test their long-term effects on grassland plant diversity in a previously cultivated and fer-
76 tilized old field. Specifically, the field was (1) cultivated in row crops until 1934, then allowed
77 to passively recover after being abandoned from agriculture; (2) experimentally fertilized for
78 about a decade from 1982 to 1994, during which time plant diversity was substantially re-
79 duced; (3) allowed to passively recover after fertilization stopped in 1994; (4) subdivided into
80 experimental plots in 2004 to test which intervention(s) could help restore plant diversity, and
81 sampled in 2004 and 2005 as previously reported (Clark & Tilman, 2010); and (5) resampled
82 in 2017, the results of which are reported herein (Figure 1). Since levels of plant diversity
83 were still low 10 years after cessation of nutrient enrichment, we tested the effectiveness
84 of litter removal (to increase light), organic carbon amendments (to reduce N availability),
85 and seed addition (to overcome potential dispersal limitation) for increasing diversity in a
86 full-factorial experiment. Grassland responses immediately following the experimental ma-
87 nipulation were presented by Clark and Tilman (2010), showing that alleviating recruitment
88 limitation by adding seeds along with increasing light availability by removing litter was the
89 most effective strategy to initially increase diversity. Here we present plant diversity and
90 aboveground biomass responses 13 years after the one-time interventions and ask: which

91 strategies, or combination of strategies, had long-lasting effects on species diversity, above-
92 ground biomass, and the relative abundance of native species. Our study provides a unique
93 opportunity to evaluate the long-term effectiveness of these management strategies in the
94 broader context of system recovery dynamics following relaxation of perturbations.

95 **3 Materials and methods**

96 **3.1 Site Description and Initial Manipulations**

97 The experiment is located at Cedar Creek Ecosystem Science Reserve, MN within a field
98 that was cultivated until 1934, when it was abandoned and received no further management
99 until 1982. At that time, a section of the field was divided into six large “macroplots” (20
100 m x 50 m each), in order to study the effects of nutrient enrichment on plant community
101 dynamics (CDR LTER E004 at Field C). In 1982, plots started receiving differing rates of
102 nitrogen (N) fertilization, which continued until 1994. Four of the plots were fertilized with
103 N at two rates ($54 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ or $170 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and with non-N nutrients (P, K,
104 Ca, Mg, S, and trace metals) to ensure that N was the only limiting nutrient. The other two
105 plots were not fertilized with N or other nutrients. Additional details on these experimental
106 treatments have been previously published (Tilman, 1987). During the experiment, plant
107 diversity decreased due to fertilization, with two C3 grass species (*Elymus repens* and *Poa*
108 *pratensis*) becoming dominant. These two species have also become co-dominant in other
109 experimentally fertilized plots in this and other fields within the site (Clark & Tilman,
110 2010; Isbell *et al.*, 2013). Diversity remained low even a decade after experimental nutrient
111 enrichment stopped (Clark & Tilman, 2010).

3.2 Experimental Design (2004-present)

To test which mechanisms might be preventing the recovery of biodiversity in this grassland after fertilization stopped, Clark and Tilman (2010) set up a full-factorial experiment to test whether litter accumulation, elevated nutrient levels, or recruitment limitation could explain the lack of recovery of biodiversity. They removed litter to reduce light limitation, added carbon to reduce plant available N, added seeds to reduce recruitment limitation, or left plots untreated. This resulted in 8 treatment combinations: untreated control, seed addition, litter removal, C addition, seed addition + litter removal, seed addition + C addition, C addition + litter removal, and all treatments combined. The treatments were applied to 56 2 m x 2 m plots within one of the “macroplots” that was previously fertilized at a rate of 54 kg N ha⁻¹ yr⁻¹ (8 treatment combinations x 7 replicates = 56 plots). The plots are directly adjacent to each other, but separated by aluminum flashing buried 40 cm into the ground.

Organic carbon (C) amendments consisted of commercial sucrose applied in 2004 and 2005 every two weeks during the growing season (April-September, total 10 applications) at a rate of 2000 g C m⁻² yr⁻¹. While this level of C addition is high, Blumenthal et al. (2003) showed that adding less than 1000 g C m⁻² yr⁻¹ might not increase native species biomass after seeding in our region. They chose this level to ensure they saw an effect of this factor, as they were more interested in the interactions among factors rather than differences between levels of one factor. Litter was removed by manual raking before the first sucrose addition of the season in 2004 and 2005.

Seeds were added in April and October 2004 at a rate of 0.5 g m⁻² for each of the following 10 species; *Lespedeza capitata*, *Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Achillea millefolium*, *Artemesia ludoviciana*, *Asclepias tuberosa*, *Liatris aspera*, *Sol-*

138 *idago nemoralis*, and *Solidago rigida* (Table S1). All seeds were sourced from a local nursery
139 (Prairie Restorations, Princeton, MN). While we did not measure seed rain directly in our
140 field, this level of seed addition represents about twice the seed rain observed in other old
141 fields, and about 25% of the seed rain observed in remnant tall grass prairies (Schott &
142 Hamburg, 1997). Sampling one year after the experimental manipulations suggested that
143 the seed addition increased plant species diversity, and that these effects were amplified by
144 litter removal, which increased light penetration. Carbon addition successfully reduced in-
145 organic N levels (measured with ion exchange resins), but did not increase the number of
146 species and even reduced the positive effects of litter removal on diversity. See Clark and
147 Tilman (2010) for additional methodological details and initial results from the first year
148 after treatments were applied.

149 **3.3 Measurements**

150 In 2017, thirteen years after the experimental manipulations, we re-sampled the experiment
151 to test for long-term effects of these manipulations on plant diversity and productivity. We
152 estimated plant diversity and community responses to our treatments by estimating percent
153 cover and doing a destructive harvest. All measurements were done in late July, which
154 corresponds to peak biomass of the growing season. Percent cover was visually estimated
155 for each species, plant litter, and bare ground in a 1x1 m area at the center of each plot.
156 For the destructive harvest, we clipped a 0.1 by 1.5 m strip just above the soil surface at the
157 center of each plot using hand clippers. Clipped aboveground biomass was sorted into live
158 biomass by species, the previous year's standing dead biomass and plant litter. All plant
159 samples were dried to constant mass for two weeks in an oven at 60° C and then weighed.

3.4 Statistical Analysis

We conducted all statistical analyses using R version 3.6.0 (R Core Team, 2019). From the biomass and cover data, we calculated inverse Simpson's diversity and Simpson's evenness using the `vegan` package (`vegan: Community Ecology Package`. R package version 2.5-2). We used analysis of variance to test for differences in response to experimental manipulations (litter removal, C amendment, and seed addition, as well as all factorial combinations) on species richness, inverse Simpson's diversity, evenness, and aboveground live biomass. We first fit the full model (considering all higher order interactions) and then removed the highest-order interactions that were not significant ($p < 0.05$) until only the main effects or significant interactions remained. For all but one of our tests, we did not find any significant interactions. Given that we know that invasion by two C3 grasses (*Elymus repens* and *Poa pratensis*) follows nutrient addition (Clark & Tilman, 2010; Isbell *et al.*, 2013), we tested for the effect of our treatments on the relative biomass of these two species, and on the relative biomass of sown species, all of which were native. We calculated relative biomass by dividing the aboveground live biomass of the species (or group of species) of interest over the community aboveground live biomass (sum across all species) for every plot. We then used generalized linear models to test whether the relative biomass of these two species responded to our manipulations. We used a quasibinomial distribution given that relative biomass is bounded (0-1).

4 Results

Seed addition was the only treatment that resulted in a lasting increase diversity and continued to increase aboveground biomass in the experiment 13 years after our short-term experimental manipulation. Seed addition significantly increased inverse Simpson's diversity ($p = 0.002$, Figure 2-a), and increased total aboveground biomass, by 32% (80 g m^{-2} , p

184 <0.001, Figure 2-b). Seed addition increased species richness, on average, by 16% (1.25 spp.
185 per 0.15 m²) across all treatments that included seed addition (p = 0.014, Figure 2-c). No
186 treatment had any significant long-term effect on Simpson's evenness (Figure 2-d, Table 1).
187 We found similar results from our percent cover survey (Figure S1, Table S2), where we
188 found that seed addition caused a long-lasting increase in total plant cover by 10% (p =
189 0.005), and increased species richness by 13% (p = 0.036).

190

191 Seed addition was the only treatment to significantly reduce the relative biomass of the
192 two exotic species that became co-dominant after high rates of nutrient enrichment. Seed ad-
193 dition caused a long-lasting reduction in the relative biomass of *E. repens* by 15% (p <0.001,
194 Figure 3-a), and a reduction in the relative biomass of *P. pratensis* by 11% (p <0.001,
195 Figure 3-b). The reductions in relative biomass of these two exotic species corresponds to
196 a decline in aboveground biomass of *E. repens* by half (p <0.001, Figure 3-c), but did not
197 significantly reduce the aboveground biomass of *P. pratensis* (p >0.05, Figure 3-d).

198

199 Seed addition almost tripled the aboveground biomass of sown species (p <0.001, Figure
200 S2-c), all of which were native. Sown species contributed 25% of the aboveground biomass in
201 plots that did not receive seed addition, which increased to 58% in plots that received seed
202 addition (p <0.001, Figure S2-a). The relative biomass of other, non-sown native species
203 decreased by 36% (p <0.001, Figure S2-b), however the aboveground biomass of non-sown
204 native species decreased only marginally (p = 0.06, Figure S2-d, Table S3). This means that
205 the increase in aboveground biomass we measure is likely due to the presence of sown species.
206 However, this increase in biomass of sown species did not result in decreased aboveground
207 biomass of non-sown native species.

208

209 Interventions that included seed addition, either alone or in combination with other in-

210 terventions, caused long-lasting increases in both richness and aboveground biomass (Figure
211 4). On average, interventions that included seed addition increased aboveground biomass
212 by 32% when compared to interventions that did not include seed addition. Seed addition
213 alone, when not combined with other interventions, increased aboveground biomass by 43%
214 when compared to controls.

215 5 Discussion

216 We found that reducing recruitment limitation, in the form of seed addition, caused a sub-
217 stantial and long-lasting increase in biodiversity and biomass production. Although theoret-
218 ical and empirical findings suggest that further reducing soil nutrient levels after cessation
219 of external nutrient inputs can help increase diversity (Prober *et al.*, 2005; Chisholm *et al.*,
220 2015), our study shows that recruitment limitation is also a major barrier for diversity re-
221 covery. Our results are consistent with previous studies that found that the benefits of seed
222 addition on richness can outweigh the benefits of reducing soil fertility (Kardol *et al.*, 2008),
223 and that seed availability is the most important predictor of site species richness during
224 restoration (Xiong *et al.*, 2003). A recent data-synthesis on seed addition experiments found
225 that seed addition increased richness by the same magnitude we report (1-2 species at local
226 scales), but did not result in increased aboveground biomass (Ladouceur *et al.*, 2020). The
227 discrepancy in biomass responses between our study and Ladouceur *et al.* could result from
228 the comparatively short-term responses considered in many of the studies they draw from
229 (9 of 12 studies included were shorter than 5 years, compared to our 13-year experiment),
230 as ours and other long-term seed addition experiments find a long-lasting increase on both
231 diversity and productivity (Bullock *et al.*, 2007). Our findings add to the growing number of
232 long-term studies that find recruitment limitation to be an important mechanism preventing
233 the recovery of plant diversity in abandoned agricultural lands and successional grasslands,

234 and uniquely show that these effects of seed addition are not significantly altered, in the
235 long-term, by other simultaneous interventions.

236

237 The effectiveness of our seed addition treatment at increasing diversity could be attributed
238 to having high seeding density and evenness. Short term studies have found that seed mixes
239 with high diversity and density are more effective at increasing plant diversity shortly after
240 restoration (Carter & Blair, 2012). Previous studies have also found that increasing forb
241 seed density relative to grass seed density during restoration can increase diversity and the
242 establishment of forb species (Dickson & Busby, 2009; Grman *et al.*, 2015). Although we did
243 not manipulate the proportions of species in our seed mix, our experiment shows that the
244 diversity-enhancing effects of seed mixes with high density of seeds, and high richness and
245 evenness of species, could be long-lasting. However, this is not to say that more seeds and
246 species will always lead to linear increases in plant diversity or biomass production. Other
247 short-term empirical studies have reported saturating or diminishing returns of increased
248 seed density and diversity on restoration outcomes (Burton *et al.*, 2006; Wilkerson *et al.*,
249 2014).

250

251 The lasting increase in richness that we observe in our experiment could be due to seed
252 addition slowing down species losses rather than from further species recruitment. Com-
253 paring our results from one and 13 years after experimental manipulations, we find similar
254 species richness in plots that received seed addition both immediately following manipulation
255 and more than a decade later (Figure S3). In contrast, for plots where we removed litter
256 and initially observed the greatest increase in richness (Clark & Tilman, 2010), the effects
257 on richness were not persistent and richness has now declined to levels comparable to those
258 of untreated plots (Figure S3). While we do not have sufficient evidence to support or refute
259 a self-sustaining increase in diversity due to seed addition in our experiment, it is important

260 to consider that richness is declining in our site (Isbell *et al.*, 2013, 2019). The increase in
261 richness caused by seed addition in our experiment is at least strong enough to counteract
262 the general pattern of diversity decline in our site.

263

264 Seed addition produced a long-lasting reduction in the relative biomass of exotic species
265 in our experiment. The presence and persistence of exotic species is often thought to be a
266 major obstacle for successful restoration of degraded grasslands (Scholes *et al.*, 2018). For
267 example, in rangelands across North America, the persistence of crested wheatgrass (an
268 introduced species) has been identified as a major obstacle to increase native plant diver-
269 sity (Fansler & Mangold, 2011). Reducing soil fertility through C amendments can reduce
270 the abundance of exotic species in some sites. In grassy woodlands in New South Wales, C
271 amendments drastically reduced the growth of exotic plant species and increased the abun-
272 dance of native species (Prober *et al.*, 2005). Similarly, at our site, native C4 grasses are
273 often dominant under low soil fertility conditions, partly because they outcompete other
274 species by driving soil nutrients below the levels at which the exotic species at our site can
275 replace themselves (Dybzinski & Tilman, 2007). Fertilization favors exotic C3 grasses at
276 our site (Wedin & Tilman, 1993). Our new results here suggest that restoring the native
277 plant community may require more than managing soil fertility. That is, when a history
278 of agricultural land use and fertilization promote invasion by exotic species that thrive on
279 fertile soils, it may be necessary to not only reduce levels of soil fertility, but also to provide
280 seed inputs of native species, which may have become too rare to take advantage of restored
281 abiotic conditions.

282

283 Seed addition increased the relative biomass of the sown native species, but did not pro-
284 mote other, already present native species. The persistent presence of these sown species
285 more than a decade after being planted suggests that their presence was not transient. At

286 the same time, seed addition did not exclude already present native species from the com-
287 munity, which has also been reported in other experiments at our site (Foster & Tilman,
288 2003). The ability of these species to establish, persist, and contribute to increased biomass
289 production is consistent with the spatial insurance hypothesis (Loreau *et al.*, 2003), which
290 suggests that species can optimally sort across heterogeneous environments at intermediate
291 dispersal rates, with more productive species outcompeting less productive species at each
292 site. Low dispersal can constrain ecosystem functions like biomass production by limiting
293 the arrival of species that best match the local environment (Leibold *et al.*, 2017). Given
294 that plant communities in our site have been shown to be dispersal limited (Tilman, 1997),
295 our results suggest that our seed addition rate was intermediate: higher than the low levels
296 that prevent species sorting, but lower than the excessively high levels that could overwhelm
297 the community and exclude other native species already present in our plots.

298

299 Our results suggest that seed addition could result in a long-lasting increase in diversity,
300 aboveground biomass, and the abundance of native species under a wide range of conditions.
301 In our experiment, seed addition increased diversity, total plant cover, and aboveground
302 biomass both when seed addition treatments were applied alone or in combination with
303 other manipulations. Considering that our site has very nutrient-poor sandy soils and high
304 precipitation, it is possible that seed addition might need to be paired with other interven-
305 tions to achieve similar results in places with different soil types or land-use history. The
306 importance of other covariates like soil attributes and local weather might explain variation
307 in the degree to which seed addition can increase diversity and productivity in different
308 sites (Brudvig *et al.*, 2017). For example, Groves *et al.* (2020) found lasting legacies of plant-
309 ing year precipitation on the outcome of restoration. Adopting cost-effective strategies and
310 setting clear goals and priorities is also important for widespread land restoration, as the
311 most effective strategies to increase plant diversity do not always match the strategies that

312 will result in the greatest land area being restored (Kimball *et al.*, 2015).

313

314 Our results may be relevant in many regions of the world that are now experiencing
315 relaxation of land use, nutrient enrichment, or both. While land conversion from natural to
316 human uses is still expected as demand for food and fiber continues to grow (FAO, 2018), it
317 is estimated that an area the size of South Africa has been abandoned from agriculture in the
318 last 15 years (Poore, 2016). These abandoned lands could provide important habitat for con-
319 servation (Corlett, 2016). Land abandoned from agriculture can be heavily degraded and can
320 have lower levels of plant diversity and productivity for decades or centuries after abandon-
321 ment (Cramer *et al.*, 2008; Isbell *et al.*, 2019). Nutrient enrichment from prior management
322 or ongoing deposition can cause further species losses and prevent the recovery of biodiver-
323 sity (Tilman, 1997; Suding *et al.*, 2005; Clark & Tilman, 2008). Global trends indicate a
324 continued increase in nitrogen (N) deposition (Galloway *et al.*, 2008; Bobbink *et al.*, 2010),
325 but rates of dry N deposition are starting to decline in some regions (Jia *et al.*, 2016; Zhang
326 *et al.*, 2018; Lloret & Valiela, 2016). This highlights the need to understand the mechanisms
327 that could promote the recovery of biodiversity after changes in land use and nutrient inputs.

328

329 Our study shows that short-term interventions can promote long-term increases in diver-
330 sity and associated ecosystem functions in areas that have been cultivated and experienced
331 long-term elevated nutrient inputs. While recovery to levels of diversity similar to those of
332 less disturbed sites might be impossible or slow (Jones & Schmitz, 2009; Moreno-Mateos
333 *et al.*, 2017; Isbell *et al.*, 2019), an increase in species richness of 1-2 species within a decade
334 is comparable to reductions in richness caused by nutrient enrichment (Borer *et al.*, 2014).
335 Here, we demonstrate that alleviating recruitment limitation, in the form of seed addition,
336 during one growing season can increase diversity and aboveground biomass and that these
337 effects can be persistent more than a decade after the intervention.

338 **6 Acknowledgements**

339 We thank Alex Portales Reyes for help with data collection in the field. The support of
340 Cedar Creek Ecosystem Science Reserve staff including Troy Mielke, Dan Bahauddin, Kally
341 Worm, Jim Krueger, Susan Barrot, Caitlin Potter, and other staff and interns was essential
342 for the completion of this research and manuscript. We thank Isbell and Dee lab members
343 Laura Dee, Jane Cowles, Clare Kazanzki, Meredith Palmer, Kaitlin Kimmel, Zhinyuan Ma,
344 Jushan Liu, Hugh Ratcliffe, Aislyn Keyes, Maggie Anderson, and Chad Zirbel for feedback
345 on early drafts of this manuscript. Sarah Hobbie, Peter Kennedy, Jess Gutknecht, Julie
346 Grossman, and Dave Tilman provided important feedback on research ideas and on this
347 manuscript. We acknowledge funding support from the US National Science Foundation's
348 Long-Term Ecological Research (LTER) programme (DEB-1831944). CPR was supported
349 by fellowships from the Interdisciplinary Center for the Study of Global Change at the
350 University of Minnesota, the Doctoral Dissertation Fellowship from the UMN Graduate
351 School, and summer fellowship support from Cedar Creek and the UMN EEB department.
352 The views expressed in this manuscript are those of the author and do not necessarily
353 represent the views or policies of the US EPA.

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Table 1: Linear model results for aboveground biomass, species richness, and inverse Simpson's diversity in response to seed addition, litter removal and carbon amendments. Generalized linear model results for the relative biomass of *E. repens* and *P. pratensis*. First and second order interactions were not significant ($P > 0.1$).

| Source | Biomass | Richness | 1/D | Evenness | <i>E. repens</i> relative biomass | <i>P. pratensis</i> relative biomass |
|-----------------|--------------------------|---------------------|-------------------------|-------------------|--------------------------------------|-----------------------------------------|
| Seed Addition | $F_{1/52} = 13.02^{***}$ | $F_{1/52} = 6.40^*$ | $F_{1/52} = 10.13^{**}$ | $F_{1/52} = 0.75$ | $t_{1/55} = -4.8^{***}$ | $t_{1/55} = -4.5^{***}$ |
| Litter Removal | $F_{1/52} = 1.72$ | $F_{1/52} = 0.04$ | $F_{1/52} = 0.11$ | $F_{1/52} = 0.32$ | $t_{1/55} = -1.40$ | $t_{1/55} = 0.118$ |
| Carbon Addition | $F_{1/52} = 0.14$ | $F_{1/52} = 1.88$ | $F_{1/52} = 0.002$ | $F_{1/52} = 1.34$ | $t_{1/55} = 1.36$ | $t_{1/55} = -0.53$ |

$p < 0.1$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

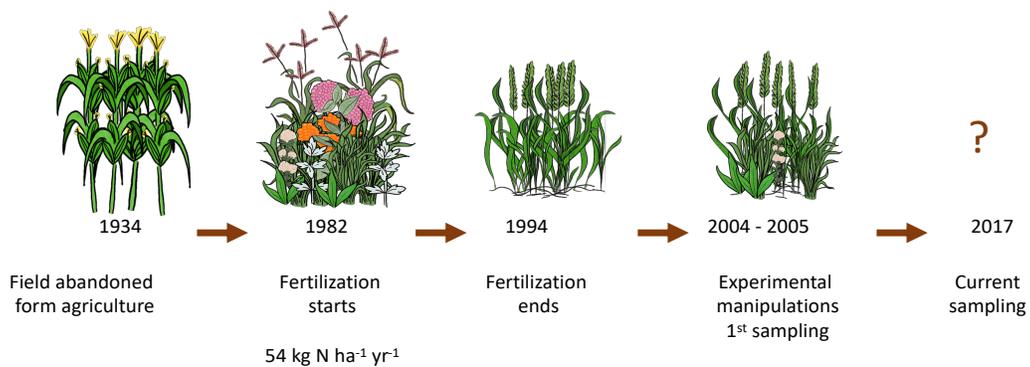


Figure 1: Field land use history and manipulation timeline. The field where our experiment is located was cultivated until 1934, after which it was abandoned. The section of the field where our experiment is located is within a 20 m x 50 m plot that was fertilized with 54 $kg N ha^{-1} yr^{-1}$ from 1982 to 1994. After fertilization stopped, no further manipulations happened until 2004, when we applied our treatments (seed addition, litter removal, carbon addition). We now present data from samples collected in 2017.

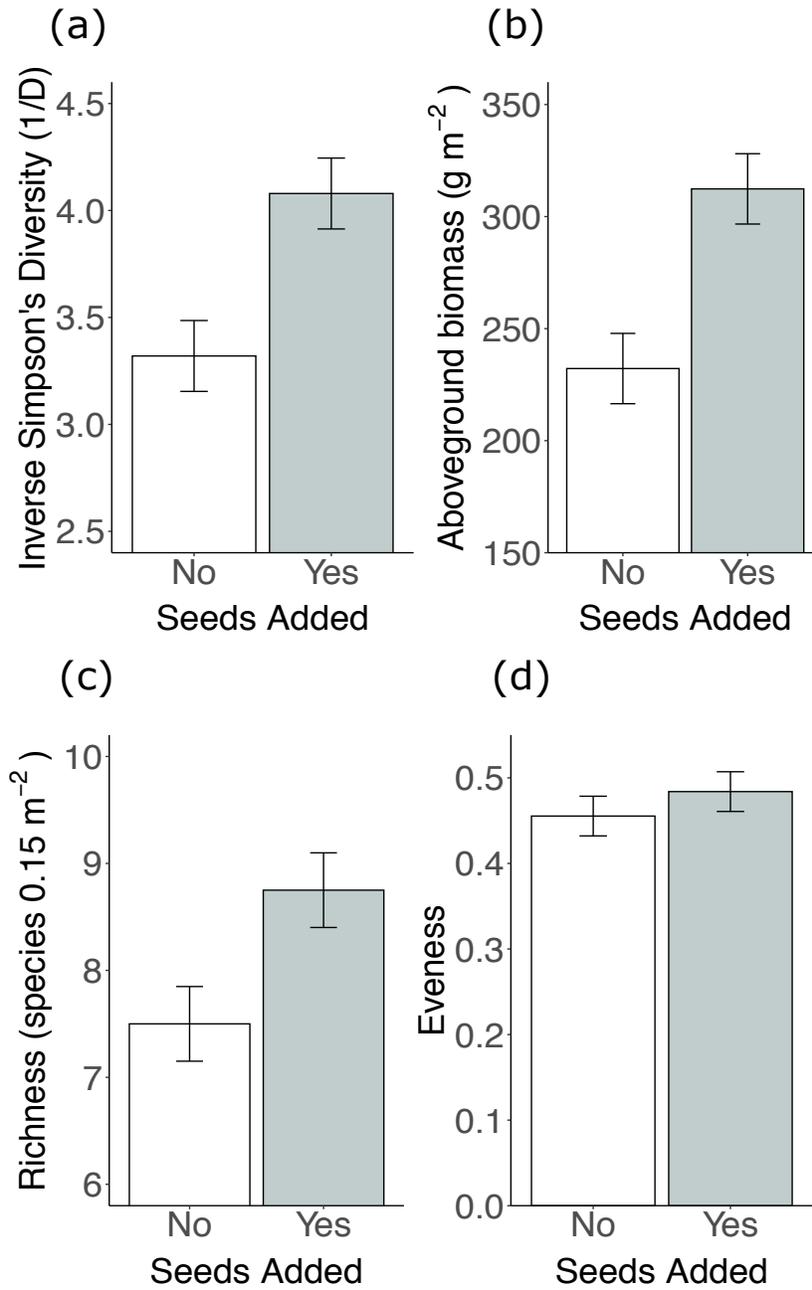


Figure 2: Seed addition increased (a) inverse Simpson's diversity, (b) aboveground biomass, and (c) species richness ($p < 0.05$). Seed addition did not have any significant effect on (d) species evenness. Error bars indicate one standard error of the mean.

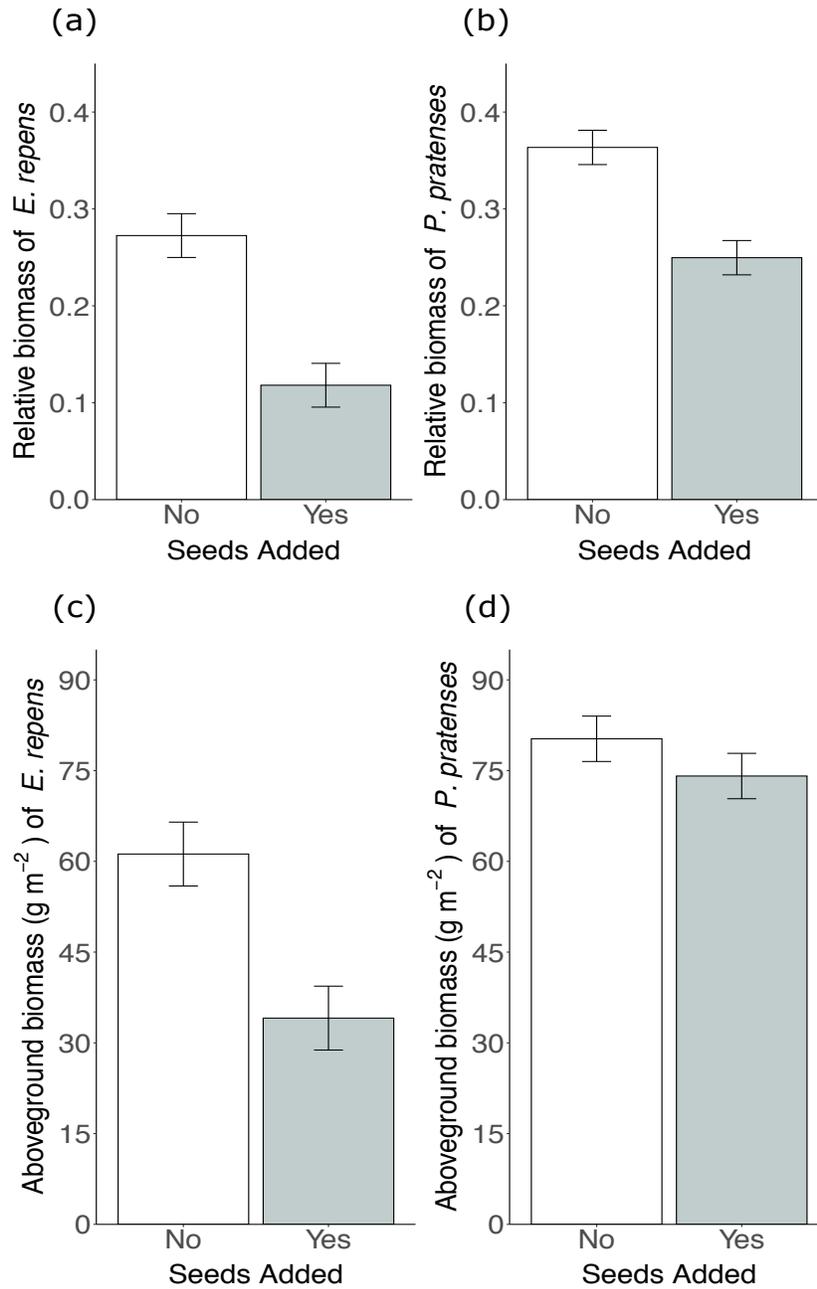


Figure 3: Seed addition reduced the (a) relative biomass of *Elymus repens*, (b) relative biomass of *Poa pratensis* and (c) aboveground biomass of *Elymus repens* ($p < 0.05$). Seed addition did not reduce the (d) aboveground biomass of *Poa pratensis*. These two exotic species become co-dominant after sufficient nutrient inputs. Error bars indicate one standard error of the mean.

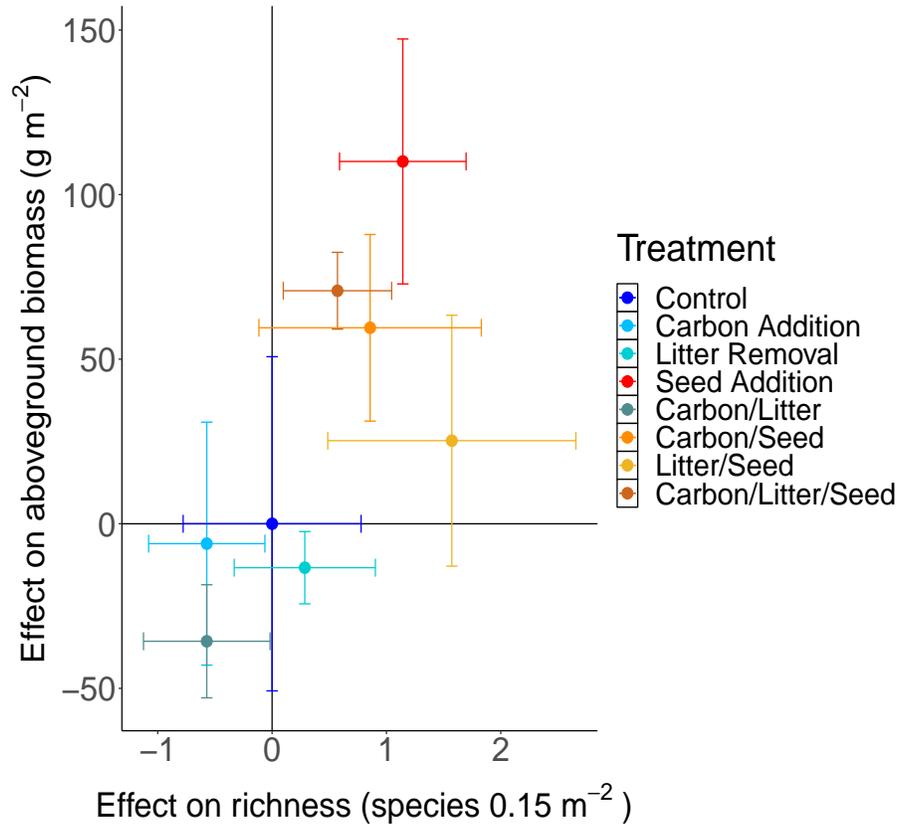


Figure 4: Seed addition increased plant species richness and aboveground biomass. While seed addition was the only significant effect among the different treatments (and there were no interactions among treatments), it is important to note that all interventions that included seed addition tended to increase diversity and aboveground biomass (displayed here in warm colors). We calculated the effect on aboveground biomass and richness as the difference between treatment and control groups. Error bars indicate one standard error of the mean.