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California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence

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Keywords

Abstract

Early emergence of plant seedlings can offer strong competitive advantages over later-germinating neighbors through the preemption of limiting resources. This phenomenon may have contributed to the persistent dominance of European annual grasses over native perennial grasses in California grasslands, since the former species typically germinate earlier in the growing season than the latter and grow rapidly after establishing. Recently, European perennial grasses have been spreading into both non-native annual and native perennial coastal grass stands in California. These exotic perennials appear to be less affected by the priority effects arising from earlier germination by European annual grasses. In addition, these species interactions in California grasslands may be mediated by increasing anthropogenic or natural soil nitrogen inputs. We conducted a greenhouse experiment to test the effects of order of emergence and annual grass seedling density on native and exotic perennial grass seedling performance across different levels of nitrogen availability. We manipulated the order of emergence and density of an exotic annual grass (*Bromus diandrus*) grown with either *Nassella pulchra* (a native perennial grass), *Festuca rubra (*native perennial) or *Holcus lanatus* (exotic perennial), with and without added nitrogen. Earlier *B. diandrus* emergence and higher *B. diandrus* density resulted in greater reduction in the aboveground productivity of the perennial grasses. However, *B. diandrus* suppressed both native perennials to a greater extent than it did *H. lanatus*. Nitrogen addition had no effect on the productivity of native perennials, but greatly increased growth of the exotic perennial *H. lanatus,* grown with *B. diandrus*. These results suggest that the order of emergence of exotic annual versus native perennial grass seedlings could play an important role in the continued dominance of exotic annual grasses in California. The expansion of the exotic perennial grass *H. lanatus* in coastal California may be linked to its higher tolerance of earlier-emerging annual grasses and its ability to access soil resources amidst high densities of annual grasses.

Introduction

The order of emergence among competitors affects the growth, survival, and fecundity of plants across a wide range of species, especially in situations with well-defined growth windows and high densities of competitors (Miller et al. 1994; Verdù and Traveset 2005). Early emergence in relation to competitors can allow for preemption of available resources by the early emerging species, yielding disproportionate advantages (Ross and Harper 1972). Once established, the early-emerging species may maintain dominance even if it is a relatively poor competitor during later life-stages.

Priority effects have been well documented in grasses (Harper 1961; Ross and Harper 1972; Miller 1987; D'Antonio et al. 2001), and may be of particular importance in maintaining the current structure of California grasslands. These grasslands have experienced extensive invasion by exotic annual grasses and steep declines in native perennial bunchgrasses over the last two centuries (Heady 1988; D'Antonio et al. 2007). The continued success of exotic annual grasses suggests some competitive advantage over native perennial grasses (Dyer and Rice 1997; Hamilton et al. 1999; Corbin et al. 2007), although the two groups coexist in some coastal prairie sites (Heady *et al.* 1988; Stromberg et al. 2001). Several studies have shown that mature native perennial grasses can successfully compete with exotic annuals (e.g. Peart 1989b; Seabloom et al. 2003; Corbin and D'Antonio 2004b; Lulow 2006), but the natives may be sensitive to competition with annual plants at the seedling stage particularly because exotic annual grasses tend to emerge at much higher densities and earlier than native perennial grasses (Dyer and Rice 1997; Hamilton et al. 1999; Humphrey and Schupp 2004; DiVittorio et al. 2007). Exotic annual grasses germinate days after the first fall or winter rains while native perennial grasses are typically slower to germinate (e.g. Reynolds et al. 2001). Subsequent aboveground

110 production of exotic annual grasses outpaces that of native perennial grasses,

111 potentially reducing light, water, and nutrients available for emerging perennial

112 seedlings (Jackson and Roy 1986; Dyer and Rice 1999). While some native

113 bunchgrass adults are thought to be long-lived (Hamilton et al. 1999), they can

114 eventually succumb to drought, burrowing animals or other mortality sources. If they

115 cannot successfully recruit due to seedling competition from exotic annual grasses,

116 then populations should eventually decline.

117 Efforts to reintroduce native perennial bunchgrasses have had limited success

118 when annuals are present at their typical high densities (Wilson and Gerry 1995; Dyer

119 and Rice 1999; Clausnitzer et al. 1999; Brown and Rice 2000). Management

120 strategies that negate priority effects and reduce the strength of annual grass

121 competition via reduced performance or density should improve the survival of

122 perennial grass seedlings and increase the success of restoration efforts (Corbin et al.

123 2004). For example, supplementing native seed supply (Seabloom et al. 2003),

124 killing annual grasses or their seeds (Stromberg and Kephart 1996; Moyes et al.

125 2005), or the planting of native perennial grasses as seedling plugs rather than seeds

126 (Dyer et al. 1996; Buisson et al. 2006) increases native grass establishment in

127 restoration sites, demonstrating the strong role exotic annual seedling density plays in

128 the suppression of native perennial grass establishment.

129 Beyond the competitive advantage gained by exotic annual grasses via earlier

130 and abundant germination, grassland restoration efforts in California also need to deal

131 with large increases in soil nitrogen availability due to atmospheric nitrogen

132 deposition and colonization by nitrogen-fixing shrubs (Maron and Connors 1996;

133 Vitousek et al. 1997; Weiss 1999, 2006; Fenn et al. 2003; Haubensak et al. 2004; Dise

134 and Stevens 2005). Increases in soil nitrogen availability are hypothesized to confer a

135 competitive advantage to fast-growing exotic species (Vitousek et al. 1987; Huenneke

4

136   et al. 1990; Burke and Grime 1996), and appear to disproportionately benefit exotic

137   annual grasses in California (e.g. Huenneke et al. 1990; Maron and Connors 1996;

138   Kolb et al. 2002; Lowe et al. 2003). For example, encroachment by non-native

139   annual grasses onto serpentine-derived soils, long a refuge from exotic annual grass

140   competition for many California native grassland species, occur when soil nitrogen

141   levels become elevated (Huenneke et al. 1990; Weiss 1999). Continued increases in

142   nitrogen deposition predicted through the next century (Phoenix et al. 2006; Weiss

143   2006) could have widespread consequences for patterns of exotic species invasion

144   and spread. Restoration strategies in California coastal grasslands will need to

145   include the direct and indirect effects of soil nitrogen enrichment on native perennial

146   grass versus exotic annual grass performance.

147   Even as restoration efforts to reestablish native perennial grasses continue,

148   exotic perennial grasses such as *Holcus lanatus*, *Festuca arundinaceae,* and *Phalaris*

149   *aquatica* have begun a second transformation of some areas in western grasslands

150   (Elliott and Wehausen 1974; Foin and Hektner 1986; Peart 1989a; Ewing 2002). The

151   ability of exotic perennial grasses to colonize the same annual-dominated stands that

152   have excluded native perennial reestablishment (Foin and Hektner 1986; Peart 1989c)

153   suggests that at least some European perennial grass species are less susceptible to

154   priority effects and competition from annual grasses. Given the shared life-history of

155   California and European perennial bunchgrasses, the differential success of exotic

156   versus native perennials may be linked to subtle differences in reproductive output,

157   germination timing or seedling growth traits, or responses to soil nitrogen enrichment.

158   For example, *Holcus lanatus*, a common exotic perennial invader in mesic coastal

159   grassland, produces copious seed (Peart 1989a), has higher relative growth rates than

160   some California native perennial grass species (Thomsen et al. 2006a; Corbin and

161   D'Antonio unpublished data), and appears to be highly responsive to nitrogen

162 enrichment (Schippers et al. 1999; Thomsen et al. 2006a). This species may thus be

163 less hindered than native perennial grasses by rapidly germinating exotic annuals or

164 high soil nitrogen availability at the seedling stage. Comparisons of the performance

165 of native vs. exotic perennial grass seedlings when faced with soil nitrogen

166 enrichment or competition with exotic annual grasses may help explain the disparity

167 in their ability to colonize annual dominated grasslands.

168   Our objective in this study was to determine the importance of annual grass

169 emergence time and density to the growth of native and exotic perennial grass

170 seedlings with and without nitrogen addition. We hypothesized that:

171 1) Reversing the typical "annual-followed-by-perennial grass" order of

172   emergence should increase perennial (native and exotic) grass seedling growth

173   by negating the priority effect.

174 2) Performance of the native perennial grass seedlings should be reduced to a

175   greater extent than performance of exotic perennial grass seedlings when

176   grown with high densities of exotic annual grasses.

177 3) The negative impact of exotic annual grasses on native perennial grasses

178   should be greater with added nitrogen because of the greater ability of annuals

179   to respond rapidly to increased resource availability. By contrast, N addition

180   should either have no effect or benefit exotic perennials grown with exotic

181   annuals.

182   We tested these hypotheses in a greenhouse using grass seedlings grown from

183 seed. We experimentally manipulated annual grass emergence time, annual grass

184 density, and soil nitrogen availability and tracked performance of native and non-

185 native perennial grasses.

186

Materials and Methods

Study Species and Source Population Description

We chose *Nassella pulchra* A. Hitchcock (Barkworth), *Festuca rubra* L., and *Holcus lanatus* L. as the target perennial species, and *Bromus diandrus* Roth as the annual competitor. Henceforward, the grasses will be referred to by their generic names; all nomenclature follows Hickman (1993). These grass species co-occur in their ranges in California (Hickman 1993). *Nassella* is a native perennial grass that was historically abundant throughout California (Heady 1988), is among the most common native bunchgrasses today in the California Floristic Province (Bartolome and Gemmill 1981), and is a widely used species in grassland restoration. *Festuca* is a coastally restricted species that remains abundant in some remnant prairies (Stromberg et al. 2001). *Holcus* is an exotic perennial invader in mesic coastal and inland California grasslands, encroaching on both non-native annual dominated and remnant native perennial patches (Peart 1989a; Corbin et al. 2007). *Bromus* is a widespread Eurasian species that appears to be a strong competitor in California annual grasslands (Wilken and Painter 1993), particularly in more nitrogen-rich sites (Maron and Jefferies 1999; Rice and Nagy 2000; Hoopes and Hall 2002).

We collected seeds of all of the species at Tom's Point Preserve (38°13'N, 122°57'W), a private nature reserve near Tomales, CA, USA. The site has been the location for a number of field studies focused on competition and resource use of native perennial, exotic perennial, and exotic annual grasses (e.g. Corbin and D'Antonio 2004a,b; Corbin et al. 2005; DiVittorio et al. 2007). The vegetation is a mosaic of exotic annual, native perennial or exotic perennial species with large patches of *Holcus* and *Bromus*.

Experimental Design

212

213   We conducted the experiment in the Oxford Tract Greenhouse at the University of

214   California, Berkeley from March 8, 2002 to June 4, 2002 under ambient temperature

215   conditions. We arranged the experiment in a randomized full factorial design, with

216   the following fixed factors: focal perennial species (*Nassella*, *Festuca*, *Holcus*),

217   *Bromus* density (low, high), nitrogen availability (low, high), and *Bromus* emergence

218   time (simultaneous, delayed). All pots were mixed species in that they had *Bromus*

219   with three individuals of one of the perennial grass species. In addition to the mixed

220   species pots, we planted perennial grasses in monoculture (three individuals) and

221   crossed the monoculture treatments with nitrogen availability to measure perennial

222   grass response without interspecific competitors. The monoculture pots with ambient

223   nitrogen treatment for each perennial species served as a baseline of growth for that

224   species (see below). We replicated each treatment eight times.

225   Using a 50% fine sand, 50% peat moss soil mix (UC mix) we planted seeds of

226   a given focal species (*Nassella, Festuca* or *Holcus)* in each perennial treatment pot

227   (10 L, 20 cm diameter). After emergence, we thinned the germinated plants in each

228   pot to three seedlings spaced a minimum of 5 cm from each other and the side of the

229   pot. We transplanted additional seedlings to pots with fewer than three emerged

230   perennials.

231   We planted *Bromus* seeds either concurrently with the planting of the

232   perennial seeds ("simultaneous") or 14 days afterwards ("delayed"). This design

233   allowed us to evaluate how variation in early seedling growth timing interacts with

234   exotic annual density and identity of the competitor (perennial species).

235   We planted *Bromus* at two densities: "high" (1592 seeds m^{-2}, 50 seeds/pot)

236   and "low" (637 seeds m^{-2}, 20 seeds/pot). We based our high-density treatment on the

237   density of annual seeds found in an equivalent area of *Bromus* dominated stands at

238   Tom's Point preserve (DiVittorio et al. unpublished data) and elsewhere in the state

239   (Young et al. 1981). The low-density treatment was meant to approximate annual

240   grass seed densities reduced by management, but is not based on field measurements.

241   To create the "high" soil nitrogen treatment, we added 0.93 g of blood meal

242   (Green All 100% Organic Blood Meal; 13-0-0) to the wet soil surface in three doses

243   (0, 30, 60 days after perennial planting). This "high" level of nitrogen addition (10 g

244   N m^{-2} over the course of the experiment) is consistent with estimates of soil nitrogen

245   input by nitrogen fixing shrubs in some coastal grasslands (Maron and Jefferies 1999;

246   Haubensak et al. 2004) and falls within the range of nitrogen fertilization used in

247   previous grassland studies (e.g. Inouye and Tilman 1988). No nitrogen was added to

248   the "low" soil nitrogen treatment.

249   We positioned pots randomly with respect to treatment in the greenhouse, and

250   rotated within and between benches every two weeks. We watered plants to soil

251   saturation three or four times per week. At the first appearance of a developing

252   *Bromus* flowering culm, we destructively sampled all plants by cutting at the soil

253   surface and separated material by species. After drying the aboveground plant

254   material in an oven at 70°C for two days, we recorded the mean individual dry

255   biomass of each species by pot.

256

257   **Statistical Analysis**

258   We converted biomass measurements into an index of relative shoot yield per plant

259   (RYP), calculated as the perennial biomass in an experimental treatment divided by

260   the mean biomass of the conspecific monoculture treatment plants in ambient

261   nitrogen (hereafter referred to as control): $RYP = Biomass_{experimental} / Mean$

262   $Biomass_{control}$. This index allowed us to compare across the perennial grasses in

263 evaluating the relative effect of the treatments on their growth (Johansson and Keddy

264 1991).

265   We performed all statistical tests with SYSTAT 11.0 (Systat Software, Inc.).

266 Our analysis began with a general assessment of the impact of *Bromus* on perennial

267 grass performance using a series of one-sample t-tests. We conducted one t-test for

268 each species to compare actual growth in competition with *Bromus* to growth in a

269 hypothetical null model (RYP = 1). Competitive treatments were pooled across

270 *Bromus* density, *Bromus* emergence time, and nitrogen addition. We then determined

271 the effect of nitrogen availability on perennial species growth in monoculture with a

272 two-factor ANOVA, using species identity and nitrogen availability as independent

273 fixed factors. We evaluated the independent and interactive effects of *Bromus*

274 density, emergence timing, nitrogen availability, and perennial species identity with a

275 separate four-factor analysis of variance (ANOVA). We used Tukey HSD post-hoc

276 tests ($\alpha = 0.05$) to make any appropriate pair-wise comparisons in the four and two–

277 factor ANOVA tests.

278   The RYP data used in the t-tests were log transformed to improve normality

279 before analysis. Prior to application of the ANOVA tests we applied Cochran's test

280 for unequal variances to the raw data to assess its heteroscedacity. The subset of

281 biomass data used in the two-way ANOVA passed Cochran's test, so was left

282 untransformed. The RYP data used in the four-factor ANOVA failed Cochran's test.

283 We again performed a log transformation to both normalize the data and reduce

284 heteroscedacity. The transformed RYP data did not pass the test, but was closer to

285 the given critical value. Because a balanced design ANOVA, such as that employed

286 in this study, is robust to minor departures from homogeneity of variances

287 (Underwood 1997; McGuinness 2002), we proceeded with the four-factor ANOVA

288 on the transformed data. We present back-transformed data and errors in figures

289 when data were transformed for analysis.

290

Results

292 **Perennial response to *Bromus* presence**

293 Growth of all three perennial species was suppressed in the presence of *Bromus*.

294 When compared to the hypothetical control RYP value of 1, the aboveground

295 production of *Holcus* ($t = -9.983$, $df = 58$, $p < 0.0001$), *Nassella* ($t = -17.381$, $df = 63$,

296 $p < 0.0001$), and *Festuca* ($t = -23.150$, $df = 63$, $p < 0.0001$) was significantly reduced

297 when grown with *Bromus*. However, the degree to which *Bromus* affected perennial

298 growth differed among species. Competitive treatments reduced *Holcus* growth by

299 nearly 75%. The two native perennials experienced even greater suppression:

300 *Nassella* growth was reduced by 85% while *Festuca* growth was reduced by 95%

301 (Figure 1).

302

303 **Perennial response to nitrogen addition in monoculture**

304 Nitrogen availability significantly interacted with perennial grass species identity in

305 the monoculture treatments (Table 1a). *Holcus* and *Festuca*, but not *Nassella*,

306 responded to nitrogen addition with increased aboveground growth (Figure 2).

307 *Holcus* grew significantly larger than the other two perennial species: in both the

308 ambient and added nitrogen treatments *Holcus* growth surpassed *Festuca* and

309 *Nassella*, while the aboveground production of the two native grasses was statistically

310 indistinguishable (Figure 2).

311

312 Perennial response to varying *Bromus* density, nitrogen addition, and
313 order of emergence

314 There were two significant three-way interactions in the four-factor ANOVA: 1)

315 Perennial sensitivity to *Bromus* density varied with nitrogen addition and order of

316 emergence, and 2) Perennial species identity interacted with *Bromus* density and

317 nitrogen addition (Table 1b). These interactions are discussed in detail below.

318

319 *Bromus density x nitrogen addition x order of emergence*

320 Delayed *Bromus* emergence significantly increased productivity of the perennial

321 grasses (Table 1b). Positive production responses by the perennials in delayed

322 *Bromus* emergence treatments occurred at both *Bromus* densities although the

323 'release' was stronger at low *Bromus* density (Figure 3).   Nitrogen addition had no

324 effect on this pattern. High *Bromus* density generally resulted in lower perennial

325 grass growth, although the effect was negated in the simultaneous *Bromus* emergence

326 pots with added nitrogen (Figure 3).

327

328   *Perennial species identity x Bromus density x nitrogen addition*

329   The three perennials differed greatly in their responses to reduced *Bromus* density and

330   increased nitrogen availability (Table 1b). In the high nitrogen treatment, *Holcus*

331   RYP was significantly greater than that of *Nassella* or *Festuca* at both high and low

332   *Bromus* density (Figure 4). *Nassella* and *Festuca*, the two native species, responded

333   similarly to each other in the high nitrogen treatment.   By contrast, in the low

334   nitrogen treatment *Nassella* outperformed *Festuca* and, at the high *Bromus* density,

335   outperformed *Holcus*. In the low *Bromus* density treatment, *Holcus* and *Nassella*

336   RYP were similar while *Festuca* RYP was significantly lower (Figure 4).

337   Overall, *Holcus* experienced the least growth in the high *Bromus* density, low

338   nitrogen level treatment (Figure 4). Reduced *Bromus* density and increased soil

339   nitrogen availability tended to increase *Holcus* performance (Figure 4). Like *Holcus*,

340   *Festuca* exhibited the least growth in the high *Bromus* density treatment at the lower

341   nitrogen level. Although *Festuca* growth increased in the low *Bromus* density

342   treatment, overall, *Festuca* experienced the most consistent suppression by *Bromus* of

343   the three perennial grasses (Figure 4). In contrast to *Holcus* and *Festuca, Nassella*

344   experienced its lowest level of growth in high *Bromus* density with high soil nitrogen.

345   *Nassella* RYP was significantly increased with reductions in either nitrogen

346   availability or *Bromus* density.

347

Discussion

349   This study gauged the importance of density and timing of emergence of an abundant

350   annual grass on potentially coexisting perennial grasses under different levels of soil

351   fertility. The annual grass competitor *Bromus* suppressed growth of all three

352   perennial species relative to their growth in monoculture. As predicted, the level of

353   suppression tended to increase with *Bromus* density and decrease when perennial

354   grasses emerged before *Bromus*. Nitrogen addition increased the suppression of one

355   native perennial by *Bromus*, while the other native perennial showed no response.

356   Suppression of the exotic perennial by *Bromus* was decreased with nitrogen addition.

357

358 **Order of emergence and density impacts on native perennial grass**
359 **seedling growth**

360   Both the native and exotic perennial grasses responded strongly to delays in *Bromus*

361   emergence, increasing relative yield per plant (RYP) up to eight-fold (Figure 3). The

362   reduced *Bromus* density treatment similarly increased perennial grass RYP. The

363   strength of these responses demonstrates the potential importance of timing of

364   emergence for perennial grass seedlings and supports findings of previous studies in

365   California grasslands and other semi-arid grassland systems that highlight the

366   sensitivity of the seedling stage in perennial grasses (Bartolome and Gemmill 1981;

367   Dyer et al. 1996; Dyer and Rice 1997; Clausnitzer et al. 1999; Hamilton et al. 1999;

368   Brown and Rice 2000; Humphrey and Schupp 2004; Lenz and Facelli 2005).

369   California native perennial grasses must survive the summer drought, and accordingly

370   invest less in their aboveground growth or reproductive efforts in the first year than

371   do exotic annual grasses (Jackson and Roy 1986; Holmes and Rice 1996). Annual

372   grasses, on the other hand, must complete their life cycle before California's annual

373   summer drought and so are among the earliest plants to germinate following the onset

374   of the rainy season. The high seedling densities and rapid aboveground growth

375   exhibited by exotic annual grasses creates an intense competitive environment for

376   later emerging seedlings (Jackson and Roy 1986; Corbin et al. 2007).

377   Priority effects have been reported in numerous systems across a broad range

378   of taxonomic groups (e.g. Harper 1961; Lawler and Morin 1993; Shorrocks and

379   Bingley 1994, D'Antonio et al. 2001; Kennedy and Bruns 2005). In California

380 grasslands, established native perennial stands are generally resistant to invasion by

381 exotic annual grasses (Seabloom et al. 2003; Corbin and D'Antonio 2004b; Lulow

382 2006), representing a priority effect manifested through arrival in a previous growing

383 season. However, priority effects for exotic annual grasses competing with perennial

384 seedlings occur over much shorter time scales. Previous work has shown that

385 differences in emergence timing of even a few days can be enough to reverse

386 competitive hierarchies between similar grass types (Harper 1961; Ross and Harper

387 1972; Rice and Dyer 2001). Some grasses, including *Nassella*, may even accelerate

388 emergence in response to annual grass neighbors, presumably to minimize the

389 impacts of later emergence (Dyer et al. 2000). Here we demonstrate the strong

390 priority effects that annual grasses can have on the seedlings of potentially coexisting

391 perennial grasses; reversing the order of emergence of the grass types had a large

392 effect on perennial grass seedling productivity, even when greatly outnumbered by an

393 exotic annual grass.

394   Early emergence and high densities of exotic annual seedlings are obstacles

395 that must be overcome for the restoration and maintenance of native-dominated

396 grasslands (Stromberg et al. 2007). Successful restoration of native perennial

397 grasslands often employs at least one of three strategies which improve perennial

398 seedling performance: increasing native seed input (e.g. Seabloom et al. 2003),

399 decreasing annual grass seed input/germination (e.g. Moyes et al. 2005), or planting

400 plugs of native perennial grasses rather than starting them from seed (e.g. Dyer et al.

401 1996; Huddleston and Young 2004). The first two strategies reduce the relative

402 abundance of exotic annual grasses, while the last, in effect, reverses the order of

403 emergence of exotic annual and native perennial grasses.

404   Once established, some stands of native perennials are able to exclude annual

405 grass invasion (Corbin and D'Antonio 2004b), and some studies suggest native

406   perennial grasses are stronger per-capita competitors than exotic annual grasses (e.g.

407   Seabloom et al. 2003; DiVittorio et al. 2007). However, the high rate of soil turnover

408   in California grasslands due to gopher and feral pig disturbance will create numerous

409   opportunities for exotic annual grasses to compete with native perennial seedlings

410   within restoration projects over the long-term (Hobbs and Mooney 1985, Kotanen

411   1995, Stromberg and Griffin 1996). Given that exotic annual propagule supply

412   grossly exceeds that of native perennials, asymmetry in competition through

413   emergence timing and density will likely limit the long-term ability of native

414   perennial grasses to reestablish dominance in restored areas unless disturbance is

415   minimized.

416

417   **Nitrogen addition impacts on native perennial grass seedling growth**

418   Differential responses to nitrogen addition between the two native perennial grass

419   species point toward differences in their ability to access this resource amidst

420   abundant exotic annual grass cover. *Nassella* in the monoculture treatment did not

421   increase aboveground growth following nitrogen addition, which suggests it was not

422   nitrogen limited in this study. Alternatively, the response could have been reflected

423   by reduced investment in belowground growth (Chapin 1980), which we did not

424   measure in this study. However, *Nassella* root:shoot did not decrease with nitrogen

425   fertilization in another study (Thomsen et al. 2006a). When nitrogen was added to

426   *Nassella* grown with a high density of *Bromus*, *Nassella* experienced a relative

427   decrease in aboveground growth. *Festuca*, on the other hand, did exhibit a positive

428   growth response to nitrogen addition in monoculture, demonstrating that it was

429   nitrogen limited. However, *Festuca* showed no response to nitrogen addition when

430   grown with *Bromus*, suggesting that *Festuca* was unable to benefit from added

431   nitrogen with neighboring annuals present. The responses of both *Nassella* and

432 *Festuca* are consistent with an indirect negative relationship between soil nitrogen

433 and native perennial grass performance due to increased productivity of exotic annual

434 grasses.

435   Our findings complement a number of studies in California and other semi-

436 arid grasslands that found that exotic annual grasses benefit disproportionately from

437 soil nitrogen supplementation relative to native perennial grasses (e.g. Huenneke et al.

438 1990; Maron and Conners 1996; Claassen and Marler 1998; Prober et al. 2002; Lowe

439 et al. 2003; Abraham et al. unpublished data; but see Kolb and Alpert 2003). This

440 suggests that native perennial grass seedlings are less likely to establish when faced

441 with both exotic annual grass competition and persistent or increasing soil nitrogen

442 enrichment. While this does not bode well for grassland restoration efforts, Seabloom

443 et al. (2003) found that, with seed supplementation, perennial grass seedlings were

444 capable of establishing in stands of exotic annual grasses across a gradient of nitrate

445 availability. However, their exotic annual grass stands were not dominated by

446 *Bromus diandrus*; it is possible that the identity of the annual grass competitor plays a

447 role in determining the outcome of perennial grass seedlings. D'Antonio and Corbin

448 (2004a) showed a first year reduction in annual grass suppression of native perennial

449 seedlings after soil amendment with sawdust (to reduce available soil nitrogen), but

450 found that there was no long-term benefit to the native grasses. Active management

451 of soil nitrogen levels may prove to be less important for maintenance of native

452 perennial dominated grasslands than reducing the density of exotic annual grass

453 competitors (DiVittorio et al. 2007).

454

455 **Comparison of exotic and native perennial grass seedling response**

456 Productivity, tolerance of annual grass competition, and response to resource pulses

457 differed significantly between native and exotic perennial grasses in this study. While

458   each perennial species responded to the inclusion of *Bromus* with marked reductions

459   in growth, *Holcus* tended to be less affected than either native perennial. Relative to

460   *Festuca*, *Holcus* suffered a smaller overall reduction in growth in every treatment

461   combination. In low nitrogen conditions, *Nassella* RYP was equal to or greater than

462   *Holcus* RYP. However, *Nassella* RYP was lower than that of *Holcus* when nitrogen

463   levels. Additionally, *Holcus* productivity was much greater than that of either native

464   perennial in monoculture (Figure 2).

465   A field study of *Holcus* establishment in exotic annual grasslands found that

466   *Holcus* seedling growth was not affected by competition with annual grasses

467   (Thomsen et al. 2006b). Thomsen et al. (2006a) also demonstrated that *Holcus* has

468   much higher initial relative growth rates than native perennial grasses, under both

469   high and low nitrogen conditions. Individual size in grasses is often positively

470   correlated with survival likelihood and competitive ability (Davies et al. 1999; Ewing

471   2002; Page and Bork 2005). As such, even if exotic annual grasses suppressed

472   *Holcus, Festuca,* and *Nassella* equally, *Holcus* would likely be better able to compete

473   with other grassland species due to its faster growth rate and larger size. Given the

474   results of this study and Thomsen et al. (2006b), it is likely that *Holcus* seedlings

475   better establish in exotic annual grass dominated stands than do native perennials.

476   Unlike the two native perennials, *Holcus* growth increased with additions of

477   nitrogen both in monoculture and in combination with *Bromus*, suggesting that

478   *Holcus* is a relatively strong competitor for soil resources with *Bromus*, at least when

479   water is not limiting as in this study. This response was mirrored in a field study;

480   elevated light and nutrient availability due to gopher disturbance increased *Holcus*

481   colonization relative to an exotic annual, *Vulpia bromoides* (Peart 1989c). *Holcus*

482   also showed increased productivity with higher nitrogen availability while in

483   competition with native perennial grasses (Schippers et al. 1999; Ewing 2002;

484 Thomsen et al. 2006a), so appears to be not only highly responsive to nitrogen

485 enrichment, but able to access that nitrogen alongside a range of potential

486 competitors.

487

488 **Implications for management**

489 California's annual-dominated grasslands have excluded native perennial grasses for

490 decades, but in the coastal zone are often colonized by a number of exotic perennial

491 bunchgrass species; this suggests that there are critical differences between traits of

492 these native and exotic perennial grasses. In this study the native and exotic perennial

493 grasses we investigated responded differently to both soil fertilization and

494 competition with exotic annual grasses. Currently, common strategies for native

495 grass management are based on mitigating the emergence of exotic annual grasses,

496 and the associated negative effects of nitrogen enrichment that accompany their high

497 density and rapid growth. However, management tools to specifically limit the

498 encroachment of exotic perennial grass are largely undeveloped, and the efficacy of

499 current management techniques on limiting their spread is not well known (Stromberg

500 et al. 2007). Management practices that reduce annual grass seedling density could

501 inadvertently increase the abundance of *Holcus,* particularly in areas with high soil

502 nitrogen availability. As European perennial grasses become increasingly abundant

503 and widespread in California coastal grasslands, land management based on

504 generalizable life history differences among native perennial and exotic annual

505 grasses will lose efficacy. Successful management for native grassland species will

506 require continued research into the traits of a wider range of native and exotic

507 bunchgrasses and their species-specific responses to current restoration practices and

508 future environmental conditions.

Acknowledgements

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809　Table 1. Summary of two-factor and four-factor ANOVA tests. a) Two-factor
810　ANOVA. Effect of perennial species identity (PS) and nitrogen availability (N) on
811　aboveground production. b) Four-factor ANOVA. Effects of perennial species
812　identity (PS), *Bromus* emergence time (BET), *Bromus* density (BD), and nitrogen
813　availability (N) on relative shoot yield per plant. Significant results are in bold.
814

a) Two-Factor ANOVA

Treatment	df	Mean-Square	F	P
Perennial Species	2	47.808	161.19	**<0.001**
Nitrogen	1	6.695	22.573	**<0.001**
PS x N	2	1.841	6.209	0.004
Error	40	0.297		

b) Four-Factor ANOVA

Treatment	df	Mean-Square	F	P
Perennial Species	2	45.797	32.823	**<0.001**
Bromus Emergence Time	1	296.475	212.490	**<0.001**
Bromus Density	1	26.100	18.707	**<0.001**
Nitrogen	1	7.133	5.113	**0.025**
PS x BET	2	4.573	3.278	**0.040**
PS x BD	2	0.160	0.115	0.892
PS x N	2	15.665	11.228	**<0.001**
BET x N	1	0.211	0.151	0.698
BET x BD	1	0.841	0.603	0.439
BD x N	1	5.412	3.879	0.051
PS x BET x BD	2	0.467	0.335	0.716
PS x BET x N	2	3.722	2.668	0.072
PS x BD x N	2	5.012	3.592	**0.030**
BET x BD x N	1	7.240	5.189	**0.024**
PS x BET x BD x N	2	1.463	1.049	0.353
Error	162	1.395		

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821   Figure 1. Relative shoot yield of perennials (RYP) in response to competition with
822   *Bromus*, averaged across all *Bromus* density, nitrogen, and *Bromus* emergence time
823   treatments (mean ± 1 SE). An RYP below 1 suggests a reduction in growth with
824   *Bromus* competitors relative to growth in monoculture.

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826   Figure 2. Perennial aboveground growth in monoculture with and without nitrogen
827   addition (mean ± 1 SE). Vertical lines indicate non-significant differences within a
828   given nitrogen treatment. Asterisks indicate differences within a single species across
829   nitrogen treatments; 'n.s.' indicates non-significance.

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831   Figure 3. Relative shoot yield of perennials (RYP) in high and low *Bromus* density
832   treatments at simultaneous and late *Bromus* emergence times, separated by nitrogen
833   addition treatment (mean ± 1 SE). Horizontal lines indicate non-significant
834   differences within a given nitrogen and emergence time treatment combination.
835   Shared letters indicate non-significant differences within a given density of *Bromus*
836   across nitrogen addition and emergence time treatment combinations.

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838   Figure 4. Relative shoot yield of perennials (RYP) with and without nitrogen
839   addition, separated by *Bromus* density treatment (mean ± 1 SE). Horizontal lines
840   indicate non-significant differences within a given density and nitrogen treatment
841   combination. Shared letters indicate non-significant differences within a single
842   species across all density and nitrogen treatment combinations.

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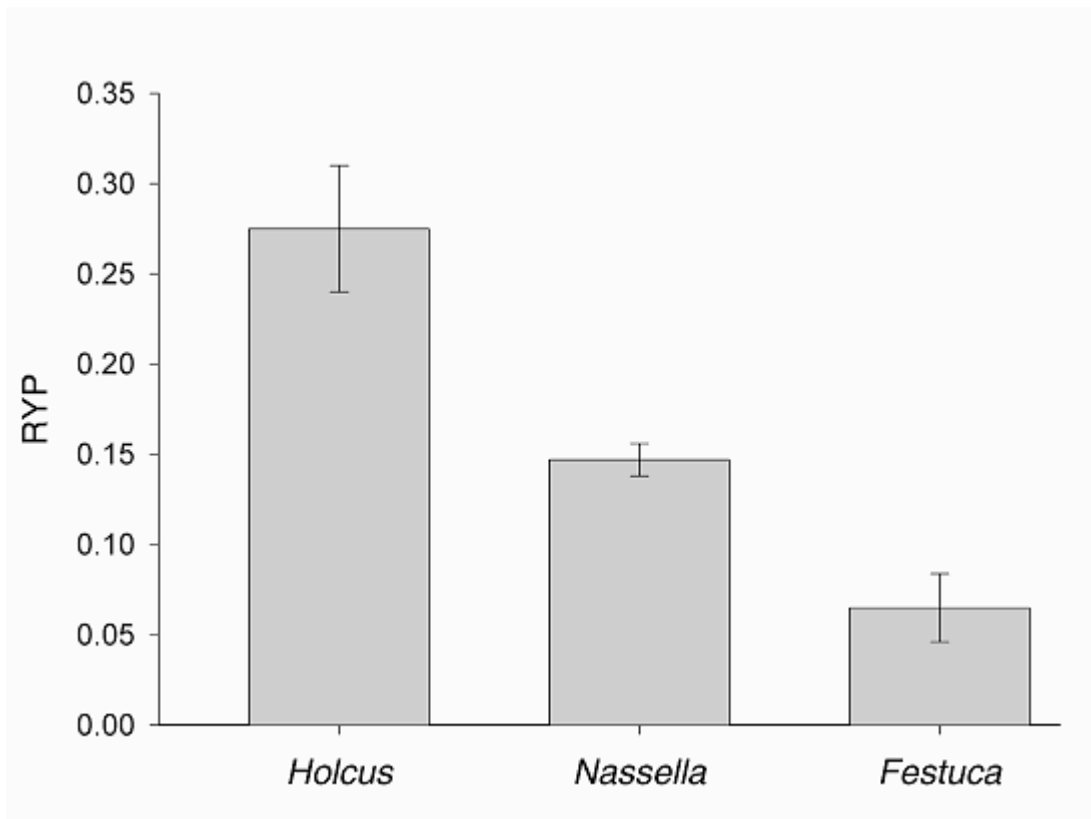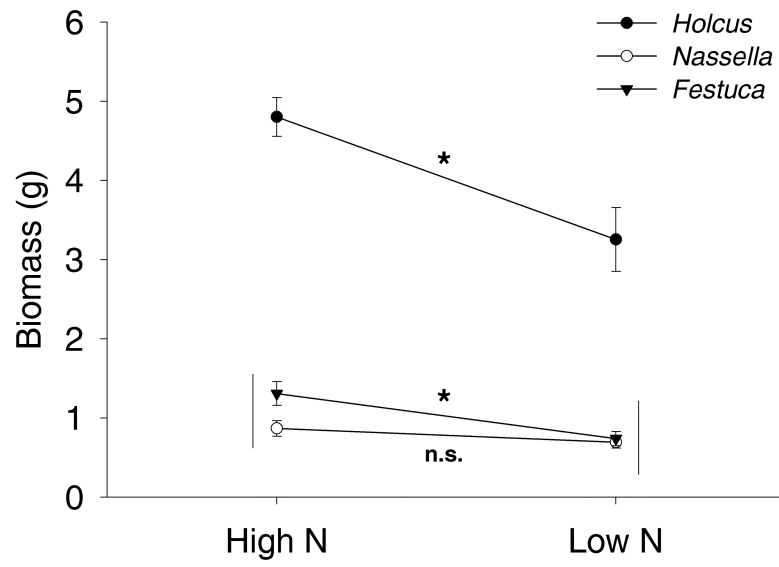854
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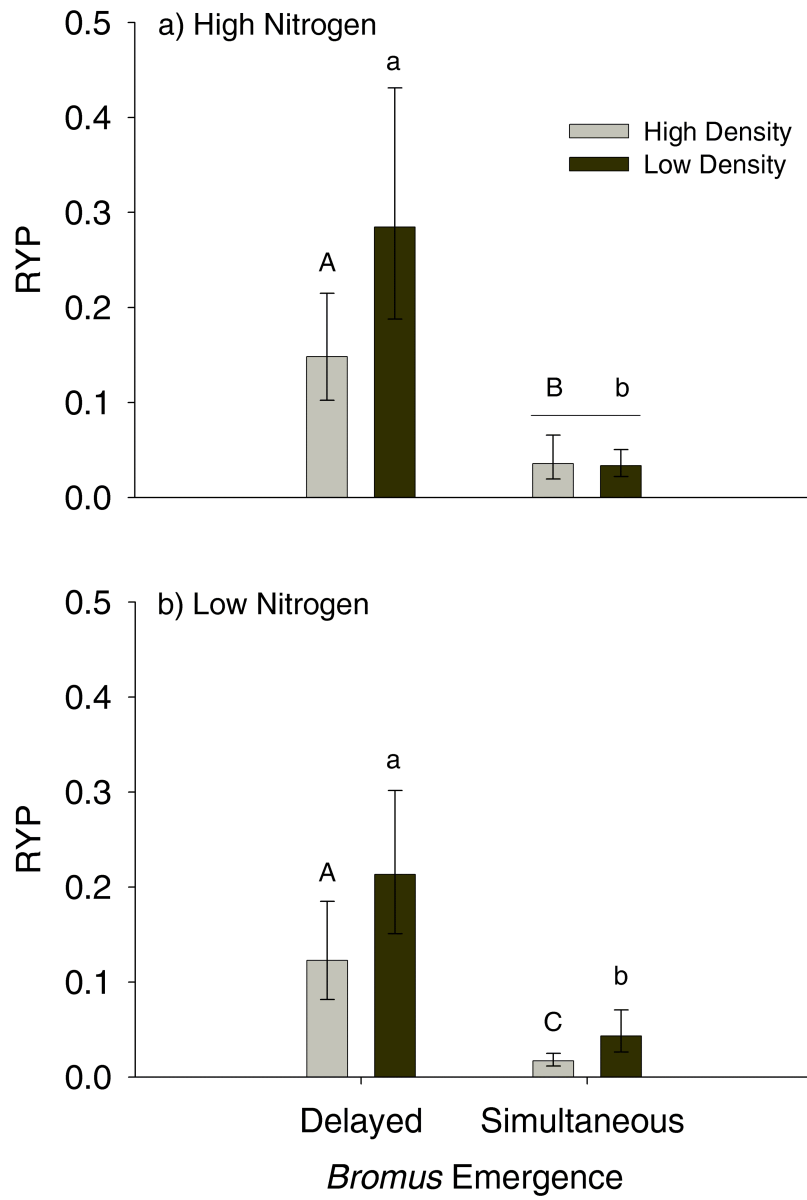860
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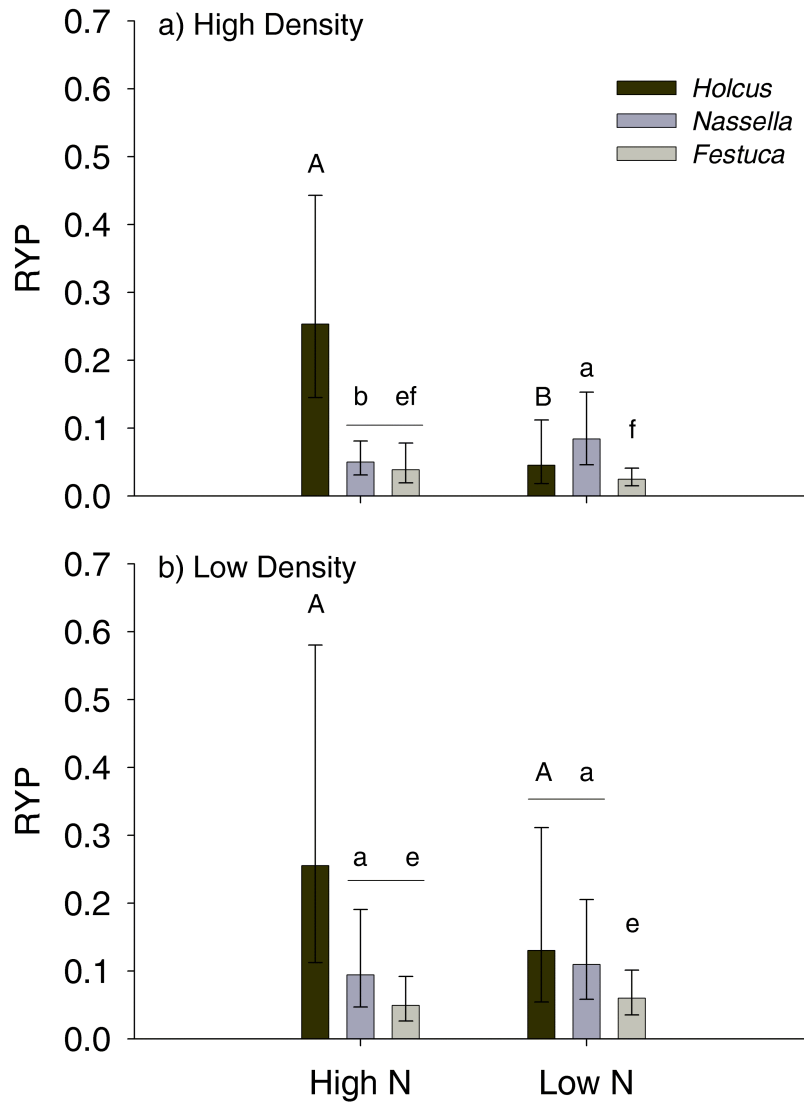884
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899   Figure 2

911   Figure 3

a) High Nitrogen

b) Low Nitrogen

Bromus Emergence

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