

Simulated nitrogen deposition enhances the performance of an exotic grass relative to native serpentine grassland competitors

Dena M. Vallano · Paul C. Selmants ·
Erika S. Zavaleta

Received: 28 January 2012 / Accepted: 9 May 2012 / Published online: 23 May 2012
© Springer Science+Business Media B.V. 2012

Abstract Previous research suggests that atmospheric nitrogen (N) deposition may facilitate the invasion and persistence of exotic plant species in serpentine grasslands, but the relative impact of increased N availability on native and exotic competitive dynamics has yet to be clearly elucidated. In this study, we evaluated how increased N deposition affects plant performance and competitive dynamics of five native grasses and forbs (*Plantago erecta*, *Layia gaillardoides*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida*) and the most common invasive grass in Bay Area serpentine grasslands, *Lolium multiflorum*. Using a growth chamber system, we exposed *Lolium* in monoculture, and native species grown both in monoculture and in competition with the exotic *Lolium*, to all four possible combinations of

gaseous nitrogen dioxide (NO₂; a dominant atmospheric N pollutant) and soil ammonium nitrate (NH₄NO₃). In monocultures, gaseous NO₂ and soil N addition each increased shoot biomass in *Lolium* and the natives *Layia* and *Cryptantha*. *Lolium* competitive ability (mean relative yield potential—RYP) increased in response to NO₂ addition plus soil N addition against all native competitors. *Lolium* and most native species did not show differences in photosynthetic rate and stomatal conductance in response to N addition. Our findings indicate that increasing N deposition and subsequent N accumulation in the soil may confer a competitive advantage to the exotic *Lolium* over native species by stimulating greater biomass accumulation and N allocation to photosynthetic tissue in the invader.

D. M. Vallano · P. C. Selmants · E. S. Zavaleta
Environmental Studies Department, University of
California, 1156 High Street, Santa Cruz, CA 95064, USA

Present Address:

D. M. Vallano (✉)
American Association for the Advancement of Science,
1200 New York Avenue NW, Washington,
DC 20005, USA
e-mail: dena.vallano@gmail.com

Present Address:

P. C. Selmants
Department of Natural Resources and Environmental
Management, University of Hawaii at Manoa,
1910 East–West Road, Honolulu, HI 96822, USA

Keywords Nitrogen · Fertilization · Serpentine
grassland · Invasion · *Lolium multiflorum*

Introduction

Biological invasions are recognized as a threat to native biodiversity in many ecosystems and are often exacerbated by other directional anthropogenic drivers, such as atmospheric composition, climate change, land use change, and increasing nitrogen (N) deposition (D’Antonio and Vitousek 1992). Atmospheric N deposition, an increasingly pervasive global change driver, has particularly strong effects on near-urban

and historically nutrient-limited systems (Vitousek et al. 1997). Increasing levels of N deposition onto nutrient-poor systems, such as grasslands, can transform species composition by creating environmental conditions more favorable for faster-growing plants, such as exotic grasses, than native plants that are adapted to nutrient-deficient soils (Bobbink et al. 2010; Dukes and Mooney 1999). Such a shift in resource availability may be the primary mechanism controlling invasive establishment and persistence in many ecosystems (Davis and Pelsor 2001; Ochoa-Hueso et al. 2011).

In the last century, human activity has greatly increased the amount of biologically available N entering the natural environment, and this trend is predicted to continue during the coming decades (Galloway et al. 2004). In particular, the rise in atmospheric levels of N oxides and ammonia gases originating from fossil fuel combustion and fertilizer use has likely increased the input of wet and dry N deposition to typically N-limited ecosystems in the Bay Area. Regional estimates of total atmospheric N deposition in the San Francisco Bay Area, where California serpentine grasslands are concentrated, are as high as 20 kg ha⁻¹ year⁻¹ (Fenn et al. 2010). The N deposited to plants and soil is often associated with dramatic changes in the structure, function, and composition of ecosystems (Vitousek et al. 1997). Plant-available N from human-derived sources has the potential to strongly influence individual plant N status and performance, either directly by stimulating growth or indirectly by influencing competition in plant communities (Bobbink et al. 1998). Understanding how increased N deposition affects plant performance and competition between native and exotic plant species is particularly important in rare and unique ecosystems of high conservation value such as serpentine grasslands.

Researchers have investigated the effects of N availability on competition between native and exotic species in a wide variety of systems (Abraham et al. 2009; Bobbink et al. 2010; Grime 1973; Pennings et al. 2005; Pfeifer-Meister et al. 2008). Increased soil N availability decreased the competitive ability of native *Hordeum* seedlings against the annual exotic *Lolium multiflorum* (hereafter *Lolium*) in a greenhouse experiment (Kolb et al. 2002), suggesting that increased N availability confers a competitive advantage to fast-growing invasive species. In a field experiment,

Huenneke et al. (1990) found that *Lolium*, the most common invasive species in Bay Area serpentine grasslands, increased in overall abundance in response to soil N fertilization. Weiss (1999) also observed the increase in abundance of non-native grasses and decreased abundance of native forbs and grasses in Bay Area serpentine grasslands. However, both the role of N deposition in and the mechanisms underlying the successful invasion of exotic species in serpentine grasslands remain unclear.

California grasslands have been severely altered by exotic species introduced from Eurasia during the last 400 years and retain few areas dominated by native species (Corbin and D'Antonio 2004). A notable exception to this pattern has historically been serpentine grasslands that still harbor native-dominated plant communities, high native richness, and a relatively large proportion of endemic, restricted, and threatened species (Harrison and Viers 2007). Serpentine grasslands appear to have resisted invasion because of their unusual edaphic conditions that limit invasibility, including poorly developed soils, low calcium to magnesium ratios, low macronutrient availability, low moisture availability, and high concentrations of certain metals, such as nickel and cadmium (Thomsen et al. 2011).

In recent decades, researchers have recorded the presence of, and in some cases dominance by, exotic annual plant species in California serpentine grasslands (Harrison et al. 2001; Hobbs et al. 2007). These shifts in species composition suggest that exotic annual grasses may have a competitive advantage over native species (Corbin et al. 2007). A leading hypothesis implicates N fertilization from increasing fossil fuel emissions in central and coastal California (Weiss 1999) in the ongoing invasion of serpentine grasslands by exotic annual grasses that are displacing rare and endemic serpentine species (USFWS 2009). Increasing N deposition may therefore play a major role in enhancing the susceptibility of serpentine ecosystems to invasion and could lead to shifts in species composition and biodiversity (Cleland and Harpole 2010).

Despite the anticipated rise in global N deposition (Gruber and Galloway 2008), few studies have examined differences in both growth and competitive responses to N deposition among native and exotic species in grasslands (Bobbink et al. 1998; Burke and Grime 1996; Clark and Tilman 2008). This knowledge

gap is especially acute in low-resource environments such as serpentine grasslands, which support native species that often have life history traits associated with resource conservation, resulting in low growth rates but high resource-use efficiency (Funk and Vitousek 2007). Conversely, invasive species may demonstrate higher phenotypic plasticity (Davidson et al. 2011), high growth rates, greater N allocation to foliage, and shorter N-residence time in foliage than native species, which explains why high-resource environments are more likely to be invaded by exotic species (Blumenthal et al. 2005). Serpentine grassland invaders, especially *Lolium*, appear to fit the common high growth rate, low resource-use efficiency pattern of exotic invasive species. Thus, increased N deposition may be a key factor allowing *Lolium* to invade and persist in a formerly low-resource environment that has resisted invasion for centuries (Harrison and Viers 2007).

To examine the role of N deposition in influencing invasion in this system, we compared the responses of native and exotic species to N additions in monoculture and in direct competition with each other. We explored how N deposition, from gaseous nitrogen dioxide (NO₂; a dominant N pollutant) and the resulting accumulation of soil inorganic N, affects the relative performance of native and exotic species, including both intrinsic growth responses and competitive dynamics. We were also interested in how species responses varied when exposed to different sources of N addition (soil vs. gaseous N) and if gaseous N addition alone, potentially incorporated directly through leaf uptake and indirectly through the soil via root uptake, has differing impacts on plant performance. We evaluated competition through *Lolium*'s competitive ability against its native neighbors to determine whether this species has a strong competitive effect, and subsequently, potential to establish and persist among native-dominated vegetation. We addressed the hypotheses that increased N availability will (1) increase biomass accumulation in the invasive *Lolium* than in native species, (2) stimulate leaf gas exchange and photosynthetic capacity in *Lolium* to a greater extent than in natives based on the life history traits of each group, and (3) increase the competitive ability of *Lolium* against native species. Overall, we expected that *Lolium* would have a stronger response to increasing N availability than the native species.

Materials and methods

Study site and species

We collected seeds of all species and native soil at Coyote Ridge Preserve, located on a 3,000 ha serpentine outcrop in Santa Clara County (37°12.133' N, 121°40.762' W), approximately 25 km southeast of San Jose, California. The vegetation is dominated by native annual forbs, with native annual grasses, native perennial forbs, and native perennial grasses also present. There are also large areas dominated by exotic annual grasses in the genera *Bromus*, *Avena*, and *Lolium* (Alexander et al. 2007). Several federally endangered plant and animal species occur at the site, most notably the Bay checkerspot butterfly and more than ten listed plant taxa (USFWS 2009). Total atmospheric N deposition for Coyote Ridge Preserve may be as high as 16 kg ha⁻¹ year⁻¹, indicating that N inputs are significant in this area (Fenn et al. 2010). Nitrogen oxides (NO_x) represent the bulk of emissions, whereas atmospheric ammonia (NH₃) comprises 20–30 % of total N emissions, with both likely originating from Bay Area fossil fuel emissions (Fenn et al. 2003).

We selected *Plantago erecta*, *Layia gaillardoides*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida* as the target annual native species and *Lolium multiflorum* as the annual exotic competitor. These six species represent four plant families; Asteraceae (*Lasthenia* and *Layia*), Boraginaceae (*Cryptantha*), Plantaginaceae (*Plantago*), and Poaceae (*Vulpia* and *Lolium*). *Plantago* is an abundant annual forb and the primary host plant for the threatened Bay checkerspot butterfly (*Euphydryas editha bayensis*) (USFWS 2009). *Layia* and *Lasthenia* are annual forbs endemic to California and nectar plants for the Bay checkerspot butterfly (Weiss et al. 2007). *Vulpia*, an annual grass, and *Cryptantha*, an annual forb, are characteristic native species found in serpentine grasslands (Alexander et al. 2007). *Lolium* is an exotic annual grass that is a strong competitor and is observed to be rapidly invading California serpentine grasslands (Evens and San 2004), particularly in sites experiencing high rates of N deposition. The California Invasive Plant Council has highlighted *Lolium* as a species of conservation concern (DiTomaso and Healy 2006) and it is currently thought to be displacing several rare and endemic species, including

host plants for Bay checkerspot butterfly larvae (*Plantago* and *Castilleja* sp.) and adult butterfly nectar sources (Weiss 2006). We used seeds collected as part of a separate Coyote Ridge study (Selmants et al. 2012); seeds were collected from hundreds of individuals of each species across a ~2 ha area at Coyote Ridge. The seeds were well mixed during processing and likely came from different individuals, although we could not determine the provenance of a particular seed with certainty.

Experimental design

We conducted the experiment at the University of California, Santa Cruz from May 3, 2010 to August 13, 2010 within two identical Conviron growth chambers (Model #S10H, Pembina, ND), one of which was fumigated with gaseous NO₂ (a dominant atmospheric N pollutant in the Bay Area) and the other with ambient air; the NO₂ treatment is described in detail in the following. We set day and night temperatures to 18 and 5 °C, respectively, with a 14-h photoperiod and 65 % relative humidity to simulate natural conditions at Coyote Ridge based on available data from a nearby weather station. Three to five seeds of each species were germinated in 164 ml Ray Leach Cone-tainers™, hereafter referred to as pots (Steuwe and Sons, Corvallis, WA, USA), filled with native serpentine soil collected from Coyote Ridge. A 3-cm layer of fine sand and a mesh cotton plug were placed at the bottom of each pot to allow for adequate drainage. After germination, we thinned each species to one individual per pot in monoculture treatments and two individuals (one of each species) per pot in competition treatments, by selecting the largest individual of each target species and weeding out smaller individuals. We watered plants to soil saturation four to five times per week with hand-held sprinklers to maintain adequate soil moisture. We arranged pots randomly and rotated them within each growth chamber every week to avoid any positional effects associated with environmental conditions. Because of logistical constraints of existing chambers, we did not rotate plants between multiple chambers. Temperature, humidity, and NO₂ levels within each growth chamber were maintained and recorded daily.

We grew plants under four N treatments: (1) control with ambient soil N (low soil N) and no gaseous NO₂, (2) ambient soil N and gaseous NO₂, (3) high soil N

and no gaseous NO₂, and (4) high soil N and gaseous NO₂. Gaseous NO₂ from Scott-Marrin Specialty Gas (Riverside, CA) was diluted into the filtered air of the fumigated growth chamber continuously throughout the experiment using a high-precision rotometer (Models 03216-34, Cole-Parmer, Vernon Hills, IL, USA) at a fixed partial pressure of 30 ppb. The 30-ppb NO₂ treatment was selected to simulate a realistic deposition rate (~11 kg N ha⁻¹ year⁻¹) and ambient atmospheric NO₂ concentrations at Coyote Ridge based on available data (Weiss 1999). We monitored NO₂ and nitric oxide (NO) concentrations using a chemiluminescence NO–NO₂–NO_x analyzer (TECO Model 42, Thermo Environmental Instruments, Inc., Franklin, MA, USA) to ensure concentrations remained stable throughout the experiment. Exhaust air from the growth chamber exited the system through an output line extending outside the greenhouse. We added 6 g m⁻² of N in the form of NH₄NO₃ to our “high soil N availability” treatment to simulate 5 years of N accumulation from atmospheric deposition based on the current estimated rate of N deposition at Coyote Ridge. The four N treatments allowed us to estimate the respective influence of accumulated soil N versus atmospheric N inputs. Our N treatments included native (*Plantago*, *Layia*, *Lasthenia*, *Vulpia*, *Cryptantha*) and exotic (*Lolium*) plants grown in monoculture and every native species grown in competition with *Lolium* (two plants per pot, one of each species). We replicated each N treatment six times.

In late July and early August 2010 before harvest, we conducted gas exchange measurements on young fully expanded leaves ($n = 3/\text{treatment}$) using a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Maximum photosynthetic rate (A_{max}) and stomatal conductance (g_s) measurements were taken on species at midday between 12:30 pm and 2:30 pm, with the exception of *Layia* and *Lasthenia* whose leaves were unable to be accurately measured within the cuvette. Each leaf was acclimated for 3–5 min to 1,200 μmol photon m⁻² s⁻¹ of photosynthetic photon flux density (PPFD) before each gas exchange measurement. During measurements, cuvette ambient CO₂ concentration was maintained at 400 ppb, relative humidity within a range of 50 and 80 %, and leaf temperature between 22 and 24 °C.

After 16 weeks of growth, we harvested plants when most species had initiated flowering or were just

about to set seed. We destructively sampled all plants by cutting shoot biomass at the soil surface and separated material by species and N treatment. Root biomass was separated from soil by wet sieving and hand sorting with forceps. We included fine roots that had become detached from root systems in total root biomass when possible. We were unable to separate roots of plants grown in competition because roots were intertwined and indistinguishable from one another. We dried root and shoot biomass at 65 °C for 72 h and weighed samples to determine individual root and shoot biomass of each species across all treatments.

Data analysis

For monocultures, we compared absolute shoot and root biomass, root:shoot ratios, and photosynthesis and stomatal conductance among the four N treatments for each species. We used these measures of plant performance to examine species differences in growth strategies and to inform interpretation of plant competition dynamics (Thomsen et al. 2006). For plants grown in competition with *Lolium*, we converted shoot biomass measurements into a competition index of relative shoot yield per plant (RYP), calculated as the shoot biomass of an individual grown in competition divided by the mean biomass of the same species grown in monoculture for each N treatment (Weigelt and Jolliffe 2003): $RYP = \text{Biomass}_{\text{competition}} / \text{Mean Biomass}_{\text{monoculture}}$. This established competitive index allowed us to evaluate the relative effects of the four N treatments on growth by controlling for species differences in both size and intrinsic N response (Abraham et al. 2009; Pfeifer-Meister et al. 2008; Thomsen et al. 2006).

We examined differences in morphological and physiological parameters among plants grown in monoculture and on RYP values of plants grown in competition using one-way analyses of variance (ANOVA). We conducted two sets of analyses, one to determine differential species responses to a given level of N addition and one to determine the response of a particular species to the different levels of N addition. Data were ln-transformed where necessary to satisfy assumptions of normality, linearity, and homoscedasticity. We used Tukey's HSD post hoc tests to make pairwise comparisons among levels of N

treatment. We set $\alpha = 0.05$ for all statistical tests. Statistical analyses were performed using SAS JMP software (Version 8.0.1, 2009).

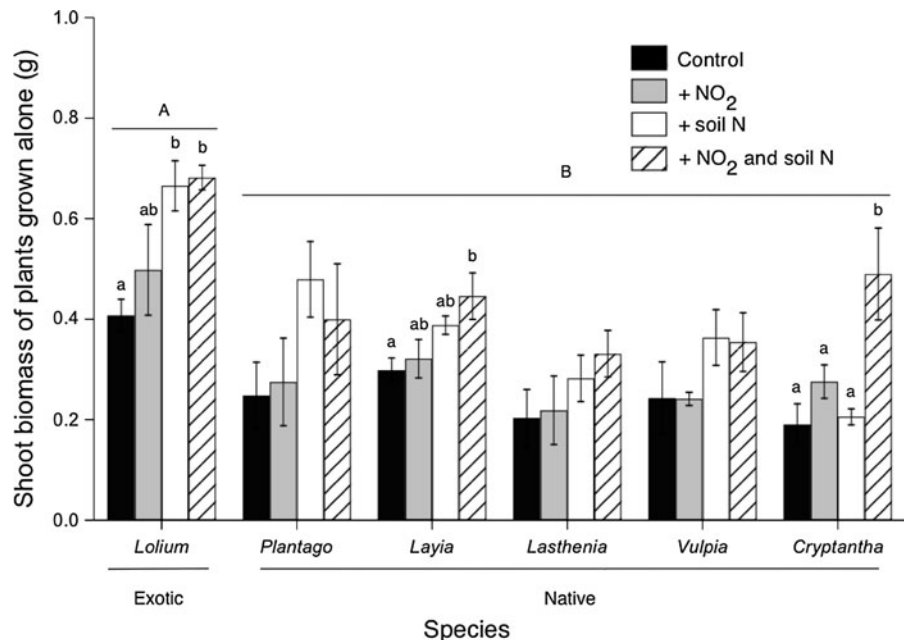
Results

Biomass and allocation effects in monoculture

Shoot biomass accumulation increased in response to N addition in *Lolium* ($F_{3,20} = 5.45, P = 0.007$), *Layia* ($F_{3,20} = 3.67, P = 0.031$), and *Cryptantha* ($F_{3,20} = 6.86, P = 0.003$), but did not differ among N treatments for *Plantago*, *Lasthenia*, and *Vulpia* ($P > 0.1$; Fig. 1). Plants fertilized with NO₂ plus soil N accumulated 46 (*Vulpia*) to 156 % (*Cryptantha*) more shoot biomass than control plants across all species. Overall, shoot biomass accumulation differed across species for the control ($F_{3,20} = 3.58, P = 0.014$), NO₂ ($F_{3,20} = 2.71, P = 0.039$), soil N ($F_{3,20} = 10.51, P < 0.0001$), and combined NO₂ plus soil N ($F_{3,20} = 3.76, P = 0.011$) treatments, with *Lolium* accumulating more shoot biomass in all treatments than any of the native species ($P < 0.05$; Fig. 1).

Root biomass did not differ in response to N fertilization within any of the species (Fig. 2a). Root biomass accumulation differed across species under added soil N ($F_{3,20} = 4.62, P = 0.004$) and combined NO₂ plus soil N treatments ($F_{3,20} = 5.26, P = 0.003$), with *Lolium* accumulating the most root biomass under added soil N, and *Cryptantha* accumulating the most root biomass under the NO₂ plus soil N treatment ($P < 0.05$; Fig. 2a). *Lasthenia* ($F_{3,20} = 3.56, P = 0.045$) was the only species that showed a change in root:shoot ratios in response to N addition (Fig. 2b), which decreased in response to the NO₂ plus soil N addition compared to control. The differences in root:shoot biomass allocation shown within *Plantago* and *Lasthenia* in response to different N treatments were primarily because of concurrent reductions in root biomass and increases in shoot biomass. Overall, root:shoot ratio differed across species under NO₂ ($F_{3,20} = 6.32, P = 0.0006$), soil N ($F_{3,20} = 7.56, P = 0.0002$), and NO₂ plus soil N ($F_{3,20} = 3.86, P = 0.012$) treatments, with *Cryptantha* having the highest root to shoot ratios in response to N addition ($P < 0.05$; Fig. 2b).

Fig. 1 Shoot biomass of exotic (*Lolium multiflorum*) and native (*Plantago erecta*, *Layia gaillardiodes*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida*) species grown alone, under control (black), added gaseous NO₂ (gray), added soil N (white), and added gaseous NO₂ plus soil N (solid lines) treatments. Different letters represent significant differences among treatments within species and between *Lolium* and native species ($P = 0.05$ significance level, Tukey HSD post hoc). Bars denote means ± 1 SE ($n = 6$)



Physiological effects

There were marked differences in photosynthetic rates across species in response to various N amendments (Fig. 3a) but within each species, photosynthesis did not differ in response to treatments. Stomatal conductance generally did not differ in response to N addition across all species (Fig. 3b), although *Vulpia* showed decreased stomatal conductance in response to N addition ($F_{3,20} = 4.34$, $P = 0.03$). *Lolium* showed the greatest increase in stomatal conductance, although not significant, under combined NO₂ plus soil N addition (87 % increase on average). *Plantago* showed a 77 % increase in stomatal conductance when grown under soil N and 54 % increase when grown under combined NO₂ plus soil N addition. *Cryptantha* showed the greatest decrease (−73%) in stomatal conductance when grown under combined NO₂ plus soil N addition.

Competitive effects

Competitive ability of *Lolium* (mean RYP) increased in response to N addition when grown in competition with each native species (Fig. 4a; *Plantago*: $F_{3,20} = 3.98$, $P = 0.02$; *Layia*: $F_{3,20} = 10.69$, $P = 0.0002$; *Lasthenia*: $F_{3,20} = 5.35$, $P = 0.01$, *Vulpia*: $F_{3,20} = 4.83$, $P = 0.01$; *Cryptantha*: $F_{3,20} = 11.29$, $P =$

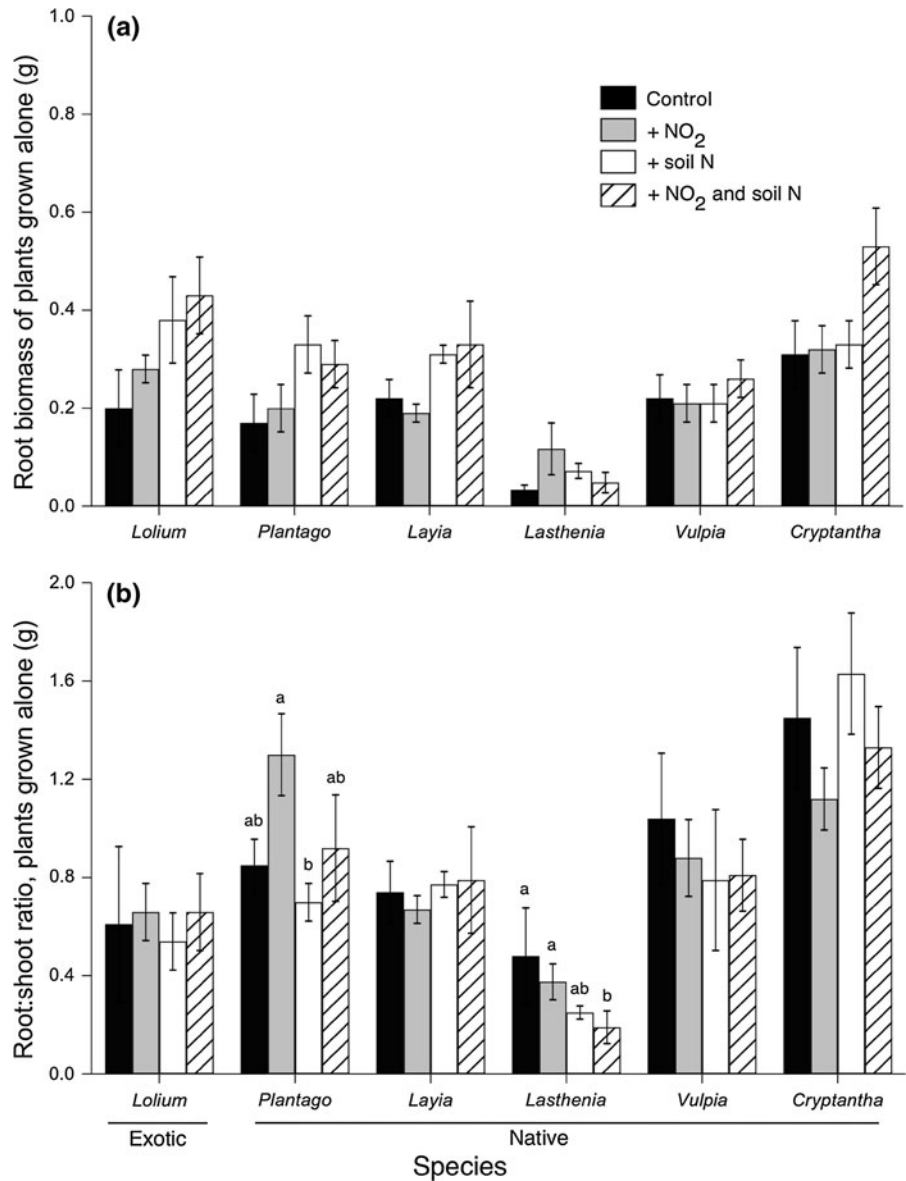
0.0001). In general, *Lolium* RYP was lowest in the control (no added N) treatment across all native species and highest in the soil N and the NO₂ plus soil N addition treatments. *Lolium* RYP increased in response to NO₂ relative to control only when competing with *Lasthenia* ($P = < 0.05$), while no effect of NO₂ alone on *Lolium* RYP was observed when grown in competition with other native species. In contrast, mean RYP of native species grown in competition with *Lolium* generally did not change in response to N addition, with the exception of and *Cryptantha* (Fig. 4b; *Cryptantha* $F_{3,20} = 4.84$, $P = 0.01$). *Cryptantha* RYP increased in response to the combined NO₂ plus soil N addition treatment.

Discussion

Biomass and allocation effects

Our findings support the hypotheses that *Lolium* increases its intrinsic growth rate and competitive ability against a variety of native species under increased N availability, particularly in response to soil N addition alone and the combination of NO₂ plus soil N addition (Figs. 1 and 4a). The observed increases in *Lolium* shoot biomass accumulation (Fig. 1), a correlate of relative competitive ability

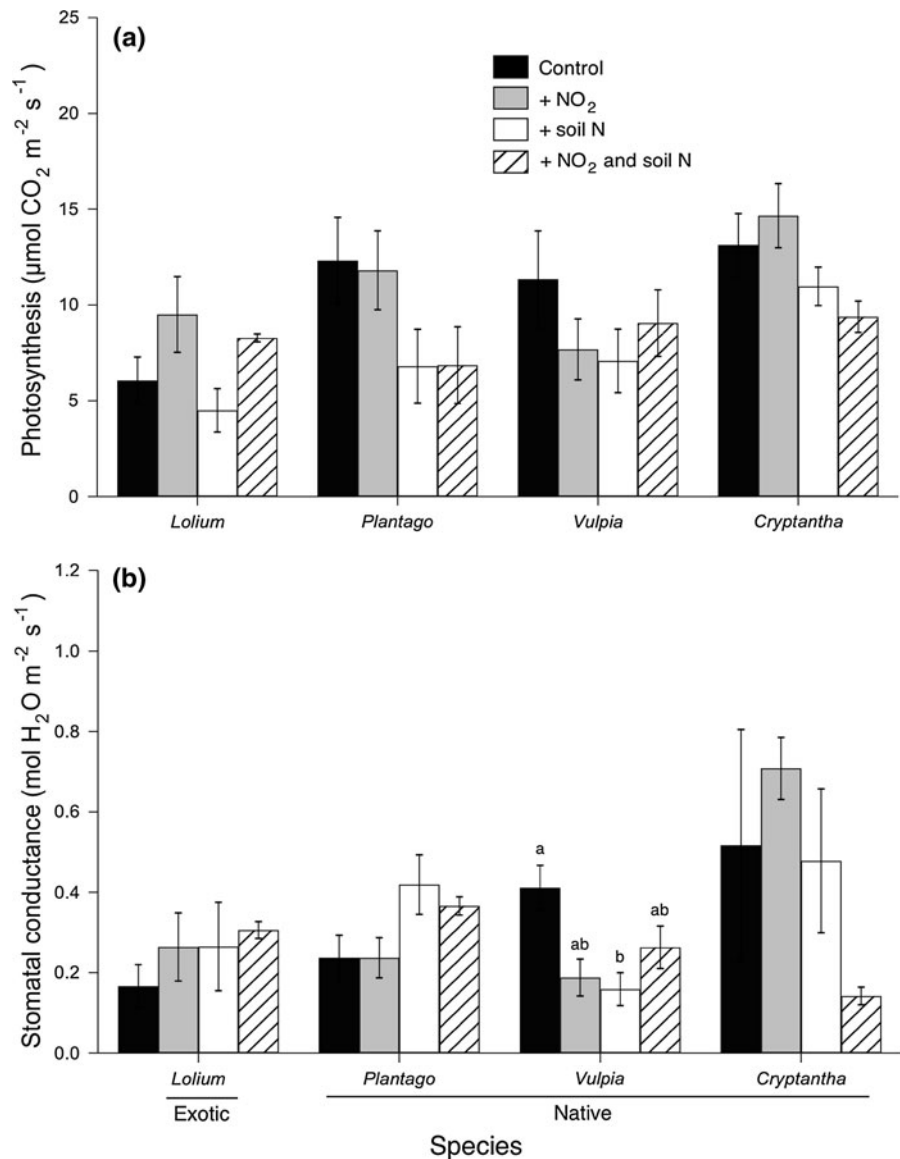
Fig. 2 Root biomass (a) and root to shoot ratio (b) of exotic (*Lolium multiflorum*) and native (*Plantago erecta*, *Layia gaillardiodes*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida*) species grown alone, under control (black), added gaseous NO₂ (gray), added soil N (white), and added gaseous NO₂ plus soil N (solid lines) treatments. Different letters represent significant differences among treatments within a species ($P = 0.05$ significance level, Tukey HSD post hoc). Bars denote means ± 1 SE ($n = 6$)



(Thomsen et al. 2006), support the competitive advantage seen in *Lolium* in response to N addition (Fig. 4a). The variation in shoot biomass accumulation in response to N addition among species may be because of intrinsic differences in functional traits and phenotypic plasticity (Funk and Vitousek 2007; Peperkorn et al. 2005; Suding et al. 2005). Native serpentine grasses and forbs are well adapted to the low-nutrient conditions characteristic of serpentine soil, likely resulting in slower growth rates, high resource-use efficiency, and lower plasticity in response to N addition. Invasive species such as

Lolium, however, tend to have faster growth rates and respond more opportunistically to nutrient (especially N) addition through higher phenotypic plasticity and larger increases in shoot and root biomass production (Davidson et al. 2011; Marschner 2002). However, there is not always a generally consistent relationship between native versus exotic species origin and N addition (Lowe et al. 2002, 2003; Padgett and Allen 1999). It is plausible that some native species such as *Layia* and *Cryptantha* may also have increased capacity to take advantage of additional N through species-specific functional traits and characteristics,

Fig. 3 Maximum photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at $1,200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (a) and stomatal conductance ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (b) of exotic (*Lolium multiflorum*) and native (*Plantago erecta*, *Layia gaillardiodes*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida*) species grown alone, under control (black), added gaseous NO_2 (gray), added soil N (white), and added gaseous NO_2 plus soil N (solid lines) treatments. Different letters represent significant differences among treatments within a species ($P = 0.05$ significance level, Tukey HSD post hoc). Bars denote means ± 1 SE ($n = 3$)



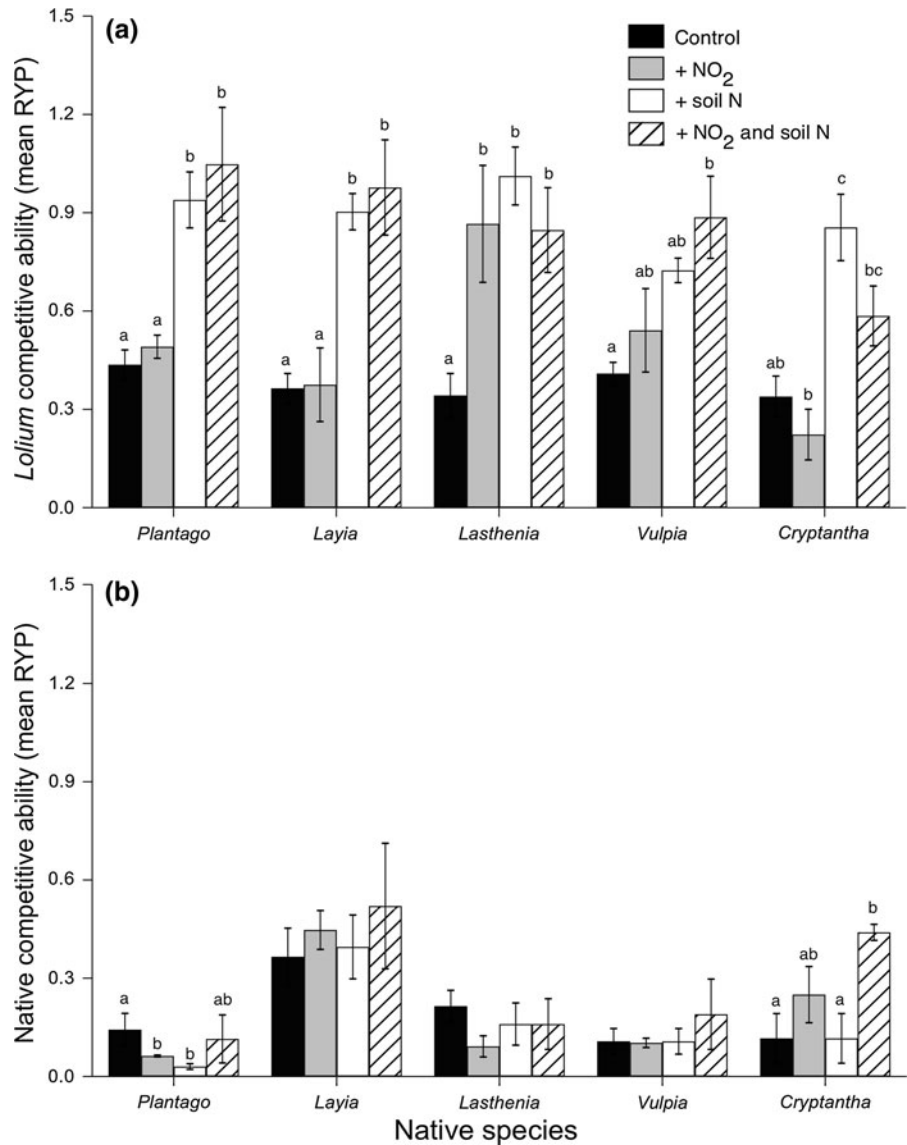
including their relative growth rates (RGRs), chemical compositions, and differences in rates of photosynthesis and respiration (Lowe et al. 2003; Poorter et al. 1995).

Physiological effects

We did not find differences in photosynthetic capacity and stomatal conductance in *Lolium* and most native species in response to N addition, whereas stomatal conductance decreased in only *Vulpia* in response to added soil N (Fig. 3). With a larger sampling size, the observed patterns might have yielded significant

effects of N addition on photosynthetic capacity. However, the trends are consistent with *Lolium*'s enhanced competitive ability shown under increased N availability and suggest a tentative physiological mechanism for the differential biomass accumulation responses of *Lolium* and native species to N addition observed. Although there are no other studies that have explicitly measured these traits in *Lolium* grown under increased N availability, our physiological results are consistent with similar responses to N addition shown in other fast-growing annual grasses and support the hypothesis that exotic species can outperform more slow growing natives on shorter, more seasonal time

Fig. 4 Competitive ability (mean RYP) of *Lolium multiflorum* grown with native (*Plantago erecta*, *Layia gaillardiodes*, *Lasthenia californica*, *Vulpia microstachys*, and *Cryptantha flaccida*) species (a) and mean RYP of native species grown in competition with *L. multiflorum* (b), under control (black), added gaseous NO₂ (gray), added soil N (white), and added gaseous NO₂ plus soil N (solid lines) treatments. Different letters represent significant differences among treatments within a species ($P = 0.05$ significance level, Tukey HSD post hoc). Bars denote means ± 1 SE ($n = 6$)



scales of increased N availability (Hull and Mooney 1990; Poorter et al. 1995). A decrease in native species stomatal conductance, as seen in *Vulpia*'s response to soil N addition, may limit photosynthetic capacity and decrease drought tolerance, subsequently reducing competitive ability of natives against *Lolium* under these conditions. A potential increase in competition may be especially acute during periods of water stress, which are typical during the growing season for annual species in serpentine grasslands. Several intrinsic species characteristics not measured in this study, including carbon economy and nitrate reductase activity, may also confer a short-term advantage to

Lolium under high N availability (Poorter et al. 1995; Taub 2002).

Competitive effects

The strength of the absolute and relative responses of *Lolium* to increased N availability, matched with its larger size and competitive advantage when paired with native species even under lower levels of N availability, suggests that the primary mechanism whereby *Lolium* might reduce native diversity in serpentine grasslands is by increased competition for light. Hautier et al. (2009) demonstrated that increased

competition for light was the mechanism driving plant diversity loss in a grassland microcosm when excess N was added. Our results indicate that accumulation of N deposition in the soil at Coyote Ridge is likely to enhance aboveground biomass accumulation of *Lolium* compared with the native species included in this study and could lead to declines of this subset of natives and potentially other species with similar traits through light competition with the increasingly productive *Lolium*. Conversely, at control levels of low N availability, the competitive ability of *Lolium* is at its lowest levels against all native species (Fig. 4a). *Lolium* competitive ability did not significantly increase against native species in response to NO₂, with the exception of *Lasthenia*, suggesting that N deposited from the atmosphere must accumulate in the soil over time before a disproportionate competitive advantage is conferred to *Lolium* in this system. Overall, the competitive ability of most native species as indicated by mean RYP was smaller than the mean RYP of *Lolium* across treatments and did not increase in response to increased N availability (Fig. 4b), suggesting that native species would not compete well against *Lolium* under a field scenario of increased N deposition. However, *Cryptantha* showed higher mean RYP under conditions of combined NO₂ plus soil N addition, which suggests that species-level traits may confer some resistance to competition from *Lolium*.

Although our study includes only a small subset of native species and one exotic annual grass, our competition results suggest that generally native annual grass and forb seedlings may be less likely to establish and successfully persist when exposed to the combination of exotic annual grass competition and increasing N availability. These findings support existing studies that have shown comparable increases in competitive success of fast-growing invasive species in response to natural and human-derived increases in N availability (Burke and Grime 1996; Huenneke et al. 1990; Kolb et al. 2002; Lowe et al. 2003; Thomsen et al. 2006). The fact that our design assesses competition only on a 1:1 basis suggests our results are conservative with respect to the impact of *Lolium* on native species under increasing N deposition, because we do not account for seed rain or the high germination rate of *Lolium* compared with natives (P. Selmants, personal observation). However, disturbance may modify the effects of N inputs; recent

work by Pasari (2011) found that chronic low-level N additions combined with moderate grazing disturbance increased native species diversity in experimental plots at Coyote Ridge.

Implications

Although the short time frame and growth chamber conditions of our study may limit our ability to extrapolate our results to field conditions, our work provides empirical support for the existing hypothesis that increased N deposition facilitates invasion of *Lolium* in Bay Area serpentine grasslands, with potential negative effects on native species richness in one of the few remaining native-dominated grassland ecosystems in California. The strong positive growth and competitive response of *Lolium* to short-term N addition in our study suggest that decreasing N inputs to biodiverse serpentine grasslands would facilitate successful conservation of serpentine grassland habitat and native species of conservation concern. Although we did not find strong species responses to NO₂ alone, the observed additive effects of NO₂ plus soil N on plant performance and competitive dynamics indicate that gaseous N inputs, and in particular direct leaf uptake of N, may play an important role in species responses to increasing N deposition. Trends in physiological responses to N addition highlight the necessity for greater understanding of the mechanisms controlling the interactions among invasive and native serpentine grassland species as a crucial step to developing successful management and restoration strategies.

More broadly, our unique approach and findings provide valuable insight into variation in competitive dynamics between native and exotic species in response to increasing N deposition across a wide range of historically nutrient-poor grasslands, including calcareous grasslands, neutral-acid grasslands, and montane subalpine grasslands. It is clear that competitive relations, species composition, and biodiversity have already been affected in such systems. Much more data are needed on how plant species differentially respond to increased N deposition and the how the resulting competition leads to shifts in species composition and native biodiversity. A greater understanding of how gaseous versus soil N deposition and uptake can differentially affect performance and competitive dynamics will be necessary to determine

if and what management intervention is necessary to mitigate excessive N inputs. Successful management of N-sensitive species and ecosystems and mitigation of increasing N impacts will require a coordinated effort emphasizing broader investigation of N sources, species traits and responses to different restoration strategies, and anticipated changes in environmental conditions.

Acknowledgments This work was supported by a University of California Faculty Research Grant and Kearney Foundation Grant to E.S.Z. The authors would like to thank the Zavaleta lab group for feedback, Jen Funk for Li-Cor assistance, Jim Velzey for greenhouse logistical support, and Matt Meckel, Margot Fair, Alex Schrock, Megan Gehrke, and Tim Lipovsky for field and laboratory assistance during seed collection and harvesting.

References

- Abraham JK, Corbin JD, D'Antonio CM (2009) California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. *Plant Ecol* 201:445–456
- Alexander EB, Coleman RG, Keeler-Wolf T, Harrison S (2007) Serpentine geoeology of western North America: geology, soils, and vegetation. Oxford University Press, New York
- Blumenthal DM, Jordan NR, Svenson EL (2005) Effects of prairie restoration on weed invasions. *Agr Ecosyst Environ* 107:221–230
- Bobbink R, Hornung M, Roelofs JGM (1998) The effects of airborne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *J Ecol* 86:717–738
- Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erismann JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* 20:30–59
- Burke MJW, Grime JP (1996) An experimental study of plant community invasibility. *Ecology* 77:776–790
- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712–715
- Cleland EE, Harpole WS (2010) Nitrogen enrichment and plant communities. *Ann N Y Acad Sci* 1195:46–61
- Corbin JD, D'Antonio CM (2004) Competition between native perennial and exotic annual grasses: implications for an historical invasion. *Ecology* 85:1273–1283
- Corbin JD, Dyer AR, Seabloom EW (2007) Competitive interactions. In: Stromberg MR, Corbin JD (eds) *California grasslands: ecology and management*. University of California Press, Berkeley, pp 156–168
- D'Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass fire cycle, and global change. *Annu Rev Ecol Syst* 23:63–87
- Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol Lett* 14:419–431
- Davis MA, Pelsor M (2001) Experimental support for a resource-based mechanistic model of invasibility. *Ecol Lett* 4:421–428
- DiTomaso JM, Healy EA (2006) *Weeds of California and other western states*. University of California, DANR, Davis, CA
- Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? *Trends Ecol Evol* 14:135–139
- Evens J, San S (2004) Vegetation associations of a serpentine area: Coyote Ridge, Santa Clara County, California. California Native Plant Society, Sacramento, CA
- Fenn ME, Haeuber R, Tonnesen GS, Baron JS, Grossman-Clarke S, Hope D, Jaffe DA, Copeland S, Geiser L, Rueth HM, Sickman JO (2003) Nitrogen emissions, deposition, and monitoring in the western United States. *Bioscience* 53:391–403
- Fenn ME, Allen EB, Weiss SB, Jovan S, Geiser LH, Tonnesen GS, Johnson RF, Rao LE, Gimeno BS, Yuan F, Meixner T, Bytnerowicz A (2010) Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *J Environ Manage* 91:2404–2423
- Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature* 446:1079–1081
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vorosmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226
- Grime JP (1973) Competitive exclusion in herbaceous vegetation. *Nature* 242:344–347
- Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* 451:293–296
- Harrison SP, Viers JH (2007) Serpentine grasslands. In: Stromberg MR, Corbin JD, D'Antonio CM (eds) *California grasslands: ecology and management*. University of California Press, Berkeley, pp 145–155
- Harrison S, Rice K, Maron J (2001) Habitat patchiness promotes invasion by alien grasses on serpentine soil. *Biol Conserv* 100:45–53
- Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science* 324:636–638
- Hobbs RJ, Yates S, Mooney HA (2007) Long-term data reveal complex dynamics in grassland in relation to climate and disturbance. *Ecol Monogr* 77:545–568
- Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71:478–491
- Hull JC, Mooney HA (1990) Effects of nitrogen on photosynthesis and growth rates of four California annual grasses. *Acta Oecol* 11:453–468
- Kolb A, Alpert P, Enters D, Holzapfel C (2002) Patterns of invasion within a grassland community. *J Ecol* 90:871–881
- Lowe PN, Lauenroth WK, Burke IC (2002) Effects of nitrogen availability on the growth of native grasses exotic weeds. *J Range Manage* 55:94–98
- Lowe PN, Lauenroth WK, Burke IC (2003) Effects of nitrogen availability on competition between *Bromus tectorum* and *Bouteloua gracilis*. *Plant Ecol* 167:247–254

- Marschner H (2002) Mineral nutrition of higher plants. Academic Press, London
- Ochoa-Hueso R, Allen EB, Branquinho C, Cruz C, Dias T, Fenn ME, Manrique E, Perez-Corona ME, Sheppard LJ, Stock WD (2011) Nitrogen deposition effects on Mediterranean-type ecosystems: an ecological assessment. *Environ Pollut* 159:2265–2279
- Padgett PE, Allen EB (1999) Differential responses to nitrogen fertilization in native shrubs and exotic annuals common to Mediterranean coastal sage scrub of California. *Plant Ecol* 144:93–101
- Pasari J (2011) Grassland invasion, management, and multifunctionality. PhD dissertation. University of California, Santa Cruz
- Pennings SC, Clark CM, Cleland EE, Collins SL, Gough L, Gross KL, Milchunas DG, Suding KN (2005) Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos* 110:547–555
- Peperkorn R, Werner C, Beyschlag W (2005) Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. *Funct Plant Biol* 32:933–944
- Pfeifer-Meister L, Cole EM, Roy BA, Bridgman SD (2008) Abiotic constraints on the competitive ability of exotic and native grasses in a Pacific Northwest prairie. *Oecologia* 155:357–366
- Poorter H, Vande Vijver C, Boot RGA, Lambers H (1995) Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on nitrate supply. *Plant Soil* 171:217–227
- Selmants PC, Zavaleta ES, Pasari JR, Hernandez DL (2012) Realistic plant species losses reduce invasion resistance in a California serpentine grassland. *J Ecol* 100:723–731
- Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc Natl Acad Sci USA* 102:4387–4392
- Taub DR (2002) Analysis of interspecific variation in plant growth responses to nitrogen. *Can J Bot* 80:34–41
- Thomsen MA, Corbin JD, D'Antonio CM (2006) The effect of soil nitrogen on competition between native and exotic perennial grasses from northern coastal California. *Plant Ecol* 186:23–35
- Thomsen D, Baythavong BS, Rice KJ (2011) Invasions and the evolution of range limits. In: Harrison S, Rajakaruna N (eds) *Serpentine: the evolution and ecology of a model system*. University of California Press, Berkeley, CA, pp 201–219
- USFWS (2009) Bay checkerspot butterfly five year review summary and evaluation. USFWS, Portland, OR
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750
- Weigelt A, Jolliffe P (2003) Indices of plant competition. *J Ecol* 91:707–720
- Weiss SB (1999) Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. *Conserv Biol* 13:1476–1486
- Weiss SB (2006) Impacts of nitrogen deposition on California ecosystems and biodiversity. California Energy Commission, Sacramento, CA
- Weiss SB, Wright DH, Niederer C (2007) Serpentine vegetation management project. Creekside Center for Earth Observation, Menlo Park, CA