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Ecological Impacts of Nitrogen Deposition, Drought and Nonnative Plant Invasion on Coastal Sage Scrub of the Santa Monica Mountains

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by

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ABSTRACT OF THE DISSERTATION

Ecological Impacts of Nitrogen Deposition, Drought and Nonnative Plant Invasion on Coastal Sage Scrub of the Santa Monica Mountains

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Multiple drivers of global environmental change increasingly threaten native ecosystems, including atmospheric pollution and resulting changes in climate and nutrient cycling, and the globalization of species. These factors may also have complex and interactive ecological effects. Nitrogen (N) deposition, the input of reactive N from the atmosphere to the earth's surface, is increasing dramatically worldwide due to anthropogenic air pollution, with the potential to negatively impact terrestrial plant diversity. Elevated N deposition may also interact with other drivers of environmental change, for example by promoting the invasion of nonnative plant species, or increasing plant susceptibility to drought or other secondary stressors. Perhaps nowhere in the U.S. is this of more immediate environmental concern than in southern California, which is a global hotspot of biodiversity and one of the most air-polluted and populous parts of the country. High levels of N deposition have been implicated in the widespread conversion of coastal sage scrub (CSS) to annual grasslands dominated by nonnative grasses and forbs. The Santa

Monica Mountains National Recreation Area of southern California protects a substantial area of remaining CSS, but due to the park's proximity to the City of Los Angeles, stands of CSS nearest urban areas are subject to high levels of N deposition. The state of California is also in the midst of a record-breaking drought, beginning in 2011, and this may exacerbate the negative impacts of N deposition and nonnative plant species. The objective of this work is to explore the effects of N deposition, drought and nonnative plant invasion on CSS of the Santa Monica Mountains at multiple ecologically relevant scales. I explored relationships of atmospheric N pollution and N deposition with native plant richness and cover of nonnative species at the landscape level, finding N deposition reduces richness of native herbaceous species and is associated with higher nonnative cover. I also investigated the impact of multiple realistic levels of N addition on CSS in a field fertilization experiment on the low end of the N deposition gradient during a period that coincided with the California drought. Through this experiment, I demonstrated increased N availability may reduce water-use efficiency and drought tolerance of native shrubs, resulting in increased dieback, while concomitantly favoring nonnative annual species. Finally, I explored the role of the soil microbial community in mediating impacts of these factors on native and nonnative plant species, finding that N-impacted soil communities may provide less protection against drought in native shrub seedlings and increase growth of invasive plant species. Collectively, these results illustrate the significant ecological threat of increased N deposition on the severely threated CSS of southern California, and potential interactions with other drivers of global change such as extreme drought and nonnative plant invasion.

Table of Contents

List of Tables

List of Figures

Introduction

While nitrogen (N) is highly abundant in the earth's atmosphere in the form of N_2 gas, biologically available nitrogen is limiting in most terrestrial systems (Aerts and Chapin 2000). Prior to human history, the sole routes of N fixation were lightning strikes and specific groups of N-fixing bacteria. However, since the development of modern agriculture, industry and transportation, global inputs of reactive N have more than doubled, and human alteration of the global N cycle is a significant component of global environmental change (Vitousek et al. 1997, Galloway et al. 2008). Anthropogenic N deposition due to agricultural, industrial and vehicular emissions poses a growing threat to terrestrial plant diversity worldwide (Stevens et al. 2006, Bobbink et al. 2010, Simkin et al. 2016). Elevated N deposition may also interact with other drivers of global change, such as nonnative plant invasion or extreme drought, further impacting native ecosystems. For example, increased soil N due to anthropogenic deposition may benefit nonnative species more than natives, enhancing the invasion of native ecosystems (Davis et al. 2000, Suding et al. 2005). Increased N availability may also increase plant-susceptibility to secondary stressors, such as water-stress (Jones et al. 2004, Southon et al. 2012, Meyer-Grünefeldt et al. 2015). With N deposition, global change-type drought and the spread of nonnative species expected to increase dramatically in the foreseeable future, understanding the potentially complex and interactive effects on ecosystems is of the utmost importance.

In southern California, which is notorious for its air pollution, N deposition has led to the eutrophication of soils due to both direct fertilization and alteration of N cycling (Sirulnik et al. 2007, Vourlitis et al. 2007). Large expanses of coastal sage scrub (CSS) have been heavily invaded by exotic Mediterranean species including annual grasses and forbs (Barbour et al. 2007). The CSS of southern California is one of the most threatened plant communities in the United States, now occupying a fraction, perhaps as low as ten percent, of its historic range (Westman 1981). This highly impacted ecosystem is home to an estimated 200 rare, endangered and sensitive species, including the federally endangered California Gnatcatcher, *Polioptila californa* (O'Leary 1989). Invaded areas may be resistant to re-colonization by CSS shrubs (Freudenberger et al. 1987), and N deposition is likely a contributing factor (Allen et al. 1998, Talluto and Suding 2008, Cox et al. 2014). Gaseous N pollution from the heavily populated Los Angeles and Ventura Counties, primarily from automobiles and industry, currently exceeds critical N loads that have been determined to be 10 kg N ha^{-1} yr^{-1} for CSS, based on diversity loss along an N gradient in the Riverside-Perris Plain (Fenn et al. 2010), but there are no published studies regarding the effects of N addition on Venturan CSS, which consists of a unique suite of species and experiences a generally wetter and cooler climate than more inland areas of southern California (Westman 1981).

Beginning in 2011, the state of California experienced a multi-year, extreme drought due to below-average precipitation and record-high temperatures (Griffin and Anchukaitis 2014, Coates et al. 2015, Fahrenkamp-Uppenbrink 2015). This has resulted in increased plant water-loss at the landscape level, with impacts on CSS particularly

severe (Asner et al. 2016), and N deposition may exacerbate negative impacts on plants. Increased productivity stimulated by N deposition could increase plant susceptibility to drought, resulting in higher dieback and mortality (Jones et al. 2004, Friedrich et al. 2012). Kimball et al. (2014) found N addition slowed post-fire succession of CSS, leading to a system dominated by exotic annual grasses, with this effect more pronounced under drought conditions. More arid sites may also experience higher invasion success under N deposition (Minnich and Dezzani 1998, Cox et al. 2014), further suggesting the current California drought may have interactive effects on CSS in conjunction with N deposition.

The soil microbial community often mediates N responses in plants, and previous studies have found natives to respond both negatively (Sigüenza et al. 2006a) and positively (Bozzolo and Lipson 2013) to N addition when inoculated with native soil microbial communities. Many native CSS species form associations with arbuscular mycorrhizal fungi (AMF), and increased soil N has also been shown to alter AMF diversity and community structure, negatively impacting native shrubs (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2001, Sigüenza et al. 2006a, Sigüenza et al. 2006b). Nutrient limitation may also be a driver of local adaptation in mycorrhizae (Johnson et al. 2010), and increased soil N may select for less beneficial fungi (Johnson et al. 1997). Changes in the soil microbial community, specifically AMF, may be one potential mechanism for reduced performance of natives relative to nonnatives under N deposition in natural systems.

The objective of this dissertation was to explore the ecological impacts of N deposition on CSS vegetation of the Santa Monica Mountains of southern California at multiple scales, including interactions with other drivers of global change: drought and nonnative plant invasion. My approach included both observational and manipulative field studies, as well as greenhouse experiments. I also explored the response of a number of ecologically relevant parameters to N deposition, from landscape- and community-level measures, to plant traits and feedbacks with soil microbial communities. Overall, I hypothesized that N deposition would negatively impact native CSS species while concomitantly favoring nonnative annuals.

In chapter one, I investigated relationships between atmospheric N pollution, N deposition and soil N and impacts on plant diversity and community composition of CSS across the Santa Monica Mountains, finding increased atmospheric N deposition was negatively correlated with native plant richness and positively correlated with increased cover of nonnatives. In my second chapter, I explored the impacts of experimental N deposition on CSS during four years of record-breaking drought, monitoring community- and trait-level responses. Results from this chapter indicate N addition may reduce drought tolerance and water-use efficiency of native shrub species, promoting increased dieback during extended drought. Increased N availability also increased cover and biomass of herbaceous species, especially nonnatives.

Chapters three and four examined the role of plant-soil feedbacks in mediating the response of native and nonnative CSS species to N deposition. In chapter three, I grew seedlings of the dominant native CSS shrub, *Artemisia californica*, in soils from high and

low N deposition sites or in sterile soil, and under altered N and water availability. My results demonstrated that native soil microbial communities may promote increased allocation to roots and provide protection against drought, but that N deposition may diminish this effect. In the final chapter, I examined feedbacks between three invasive plant species of CSS and N-impacted soil communities, finding N addition may promote the growth of these species due to both increased N availability, and through effects on soil biota. Together, this highlights the important role of soil microbial communities in shaping plant responses to global change drivers such as N deposition and drought.

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Relationships between atmospheric nitrogen deposition, plant diversity and invasion in coastal sage scrub of the Santa Monica Mountains

Abstract

Human activities have contributed to increased nitrogen (N) availability in terrestrial ecosystems worldwide due to atmospheric pollution and resulting N deposition and soil N enrichment may result in declines of native plant diversity and increased invasion by nonnative species. Observational and experimental studies in coastal sage scrub (CSS) of southern California have established a clear link between atmospheric N pollutants and negative ecological impacts on vegetation, such as reduced diversity and increased invasion, but impacts on Venturan CSS of the Santa Monica Mountains have yet to be fully explored. In this study, I measured levels of N pollutants in the atmosphere and determined rates of N deposition across the landscape based on atmospheric concentrations, deposition velocities of different reactive N forms, leaf area index and plant stomatal uptake. I also measured soil N availability and plant community composition of CSS at thirty sites across the Santa Monica Mountains in an effort to explore relationships between plant richness and cover with atmospheric N inputs, hypothesizing higher rates of N deposition would be associated with lower plant diversity and higher cover of nonnative species. I demonstrate N deposition is associated with reduced plant richness and increased cover of nonnative plants. As human activities continue to alter global N cycling, conservation of native ecosystems must consider the growing influence of atmospheric N inputs.

Introduction

Human alteration of the nitrogen (N) cycle has dramatically increased rates of atmospheric N deposition originating from agricultural activities and the burning of fossil fuels worldwide, and N inputs are expected to continue to increase in the future (Galloway et al. 2008). As most terrestrial ecosystems are N-limited, elevated levels of reactive N due to anthropogenic N deposition may exert a strong influence on plant communities (Vitousek et al. 2002). Nitrogen enrichment resulting from atmospheric deposition, for example, may negatively impact plant diversity (Bobbink et al. 2010, Simkin et al. 2016). Theory also predicts that increased soil N availability will promote the invasion of nonnative plant species (Davis et al. 2000), and N deposition may increase the invasibility of some ecosystems (Burke and Grime 1996, Dukes and Mooney 1999, Weiss 1999, Davis and Pelsor 2001). While ecological impacts of N deposition on plant communities of temperate regions have been the focus of much previous work (e.g. Bobbink et al. 1998, Stevens et al. 2006, Clark and Tilman 2008), effects on arid and semi-arid ecosystems, where dry vs. wet deposition predominates, are less well understood (Fenn et al. 2003, Phoenix et al. 2006).

The severity of ecosystem impacts due to anthropogenic N deposition will depend on a number of biotic and abiotic factors, including the traits and characteristics of the resident plant species (Diekmann and Falkengren‐Grerup 2002, Suding et al. 2005), climatic (Minnich and Dezzani 1998, Cox et al. 2014, Kimball et al. 2014) and edaphic factors (Allen et al. 2009). Nitrogen deposition may influence plant community

composition and diversity through a number of mechanisms, including direct toxicity (Pearson and Stewart 1993, Bobbink et al. 1998, Sheppard et al. 2011), altered competitive interactions in response to improved N nutrition and increased productivity in some species (Tilman 1982, Gilliam 2006) and increased susceptibility to secondary stressors such as herbivory or drought (Jones et al. 2004, Friedrich et al. 2012). The addition of excess N to native ecosystems may also alter ecological feedback loops, such as by promoting increased fire frequency through invasive annual grasses (Fenn et al. 2003, Rao et al. 2010), or negatively impacting plant-soil feedbacks in native, but not nonnative, plant species (Sigüenza et al. 2006a, Sigüenza et al. 2006b, Valliere and Allen 2016). The cumulative effect of these factors could result in simplified and invaded plant communities depauperate of native species.

Southern California, with its dense population and high number of automobiles, experiences high values of N deposition, particularly in the greater Los Angeles area (Padgett et al. 1999, Bytnerowicz et al. 2001, Fenn et al. 2003). Previous research on the ecological impacts of elevated N on southern Californian plant communities, such as coastal sage scrub (CSS), has indicated a potential for increased invasibility, shifts in species composition, and changes to ecosystem function (Allen et al. 1998, Fenn et al. 2003, Vourlitis et al. 2007, Cox et al. 2014, Kimball et al. 2014). The remaining CSS of southern California is one of the most threatened vegetation types in the United States, with an estimated 70-90 percent lost to development, grazing and disturbance (Westman 1981b, Cleland et al. 2016). What remains is often highly fragmented and heavily invaded by nonnatives, the most dominant of which are annual grasses and forbs from the

Mediterranean (Barbour et al. 2007, Funk et al. 2016). Coastal sage scrub is home to many low-growing, drought-deciduous, and often aromatic shrubs, with a diverse flora of annual and perennial forbs found in shrub interspaces (Kirkpatrick and Hutchinson 1977). This highly impacted ecosystem is home to an estimated two hundred sensitive plant species as and several dozen state and federally listed animal species (O'Leary 1989). Future ecological impacts of anthropogenic N deposition are expected to be more severe in mediterranean-type ecosystems, such as CSS (Sala et al. 2000, Phoenix et al. 2006, Ochoa et al. 2011), and this growing driver of global change poses a significant threat to the conservation of biodiversity within this unique plant community.

The Santa Monica Mountains National Recreation Area (SMMNRA) was established as a unit of the United States National Park Service as part of the National Parks and Recreation Act of 1978 (Public Law 95-625), with the explicit purpose of serving as an air-shed, an area free of high levels of air pollutants, for the City of Los Angeles. Despite this goal of the enacting legislation, an empirical survey of nitrogenous air pollutants due to anthropogenic emissions, and resultant impacts on native plant communities, has yet to be undertaken. Early work in the region pioneered by Westman focused on the direct impact of reactive N gases and other pollutants on plants (Westman 1979, 1981a, 1985), and while plant injury resulting from oxidized forms of N may play an important role shaping plant communities in highly polluted areas, the most severe impact of excess atmospheric reactive N is likely due to the effects of N accumulation in the soil. Westman (1981a, b) did identify a negative correlation between native plant diversity of CSS and soil N availability throughout southern California, but this study did

not explicitly consider impacts of anthropogenic N deposition. Further, much of the work exploring impacts of N deposition have been conducted in Riversidian or Diegan CSS (Allen et al. 1998, Padgett and Allen 1999, Vourlitis et al. 2007, Vourlitis and Pasquini 2009, Fenn et al. 2010) and no investigations into the effects of N deposition have been conducted in Venturan CSS, which has a unique species composition, experiences a wetter and cooler climate, and exhibits a more closed-canopy physiognomy than the CSS of more arid regions (Axelrod 1978). Given the substantial area of wildlands the Santa Monica Mountains NRA protects, over 63,000 ha, including large expanses of CSS, a better understanding of the ecological impacts of N deposition will be important for long-term conservation.

In this study, I asked: (1) How do nitrogenous atmospheric pollutants, N deposition and extractable soil N vary across the Santa Monica Mountains, and how are these related; (2) How do richness and cover of native and nonnative CSS species vary at sites receiving different levels of atmospheric N deposition across the Santa Monica Mountains; and (3) What role do environmental and soil characteristics play in mediating plant community response to N deposition. I hypothesized levels of atmospheric N pollution, N deposition and soil N would exhibit a steep east-to-west gradient of increasing concentrations, with areas closest to Los Angeles – the source of much of the region's atmospheric pollutants— being the most N enriched. I also predicted that increased N deposition would negatively impact native CSS vegetation, resulting in reduced plant diversity and increased invasion of nonnative annuals. I addressed these questions and hypotheses through a landscape-level estimation of N deposition rates

across the Santa Monica Mountains based on empirical concentrations of nitrogenous N pollutants in the atmosphere and through an accompanying survey of CSS vegetation composition and soils across the identified N deposition gradient. Given the negative impacts of anthropogenic-sourced N inputs on plant community diversity previously documented in a number of ecosystems (Stevens et al. 2006, Bobbink et al. 2010, Simkin et al. 2016), results of this study have important implications for the conservation of biodiversity in this severely threatened ecosystem and land management within the Santa Monica Mountains NRA.

Materials and Methods

Study area

The Santa Monica Mountains National Recreational Area (NRA) is a multi-agency network of parks and open space administered by the U.S. National Park Service, located in the Santa Monica Mountains, northwest of Los Angeles, CA. The region experiences a hot, dry summer and a cool winter growing season characteristic of a mediterranean-type climate. Most precipitation occurs during the winter months, with high variability within and between years. Vegetation consists of a mosaic of oak woodlands, chaparral, grasslands, and Venturan CSS, the ecosystem of interest for this research. High levels of N deposition have been previously modeled at 2-20 kg N ha^{-1} yr^{-1}, west to east, across the Santa Monica Mountains NRA (Tonnesen et al. 2007, Fenn et al. 2010), but these estimates have yet to be validated empirically.

Atmospheric sampling

In October 2011, ten atmospheric sampling sites (Table 1) were selected across the SMMNRA for monitoring concentrations of atmospheric N pollutants. Sites were chosen based on accessibility and vegetation, and distributed across the modeled N deposition gradient of the Santa Monica Mountains. The distribution also includes both coastal and inland sites. Passive atmospheric samplers were installed in October 2011 on wooden T-posts approximately 2 m off the ground to monitor atmospheric N pollutants at each site. Concentrations of N gases, nitrogen oxides (NO_x), nitrogen dioxide (NO_2) and ammonia (NH_3) were monitored with Ogawa passive samplers and concentrations of nitric acid vapor (HNO_3) with the US FS nylon passive samplers (Bytnerowicz et al. 2005). Samplers were in place for monthly exposures for one year (12 exposures total). These air-quality values were used to calculate N deposition.

Estimation of N deposition

I estimated total N deposition based on atmospheric concentrations of N deposition, land cover, leaf area and stomatal uptake of N. Our calculation of N deposition for the study area largely follows the GIS-based empirical inferential method described in Bytnerowicz et al. 2015, but with some modification. In that study, N deposition was estimated as the sum of dry surface deposition and plant stomatal uptake of N. Values were calculated for two-week periods with input from four gaseous forms of reactive N (HNO_3, NH_3, NO, and NO_2). This was done over a period of five years but only during the summer months when deposition rates peak. All vegetative cover types

were considered to have extant foliage so both surface and stomatal deposition were calculated for every period. The study setting was predominately rural and it focused on landscape scale distributions of N deposition. For the current study, I calculated dry surface deposition and stomatal uptake of N at twelve monthly intervals spanning an uninterrupted one-year period using input from three forms of reactive atmospheric N (HNO_3, NH_3, and NO). Stomatal uptake calculations ignore seasonally deciduous cover types during their respective periods of senescence. While this study provides an estimate of landscape scale N deposition, it also requires an estimate of deposition at study plot locations.

Dry surface deposition and stomatal uptake were calculated for each patch of land cover from the 62,173 ha study area, the Santa Monica Mountains NRA, during each monthly passive sampler data collection period. Values calculated from this approach are the product of surface area, cover type-specific surface deposition or stomatal uptake conductance velocity (K), ambient gas concentration, N mass, and period of exposure to each calculated form of gaseous reactive nitrogen. Surface area values are based on cover type, LAI, and land surface area values, K values are available in the literature (Bytnerowicz et al. 2015), gas concentration values are field measures collected for this study, and N mass is each studied pollutant's nitrogen proportion of molecular mass. The period of exposure for surface deposition values is calculated as continuous but stomatal uptake is calculated only for the average daylight hours of each monthly interval. The cover type and land surface area are provided by the Existing Vegetation spatial data (USFS, 2007) at a scale of 1:24,000. Type classifications are generalized from Society of

American Foresters and Society for Range Management classes for the 18 used in this study. Leaf area is obtained from satellite imagery (MODIS) with a spatial resolution of 1 kilometer and temporal resolution of 8 days and temporally weighted to match monthly study intervals. Gas concentration is a predicted value from Inverse Distance Weighting using the monthly average measures recorded by passive samplers used for the study.

The need to estimate deposition for CSS vegetation sampling sites at specific locations required that patch cover type values at those locations be changed to CSS for small patches smaller than 1 km resolution. All study site locations were already classified as some type of shrub cover but only seven were specifically CSS. There were no LAI values from satellite imagery for some parts of the study area. At these locations, values from adjoining areas with similar cover characteristics were substituted. Three of the study plots were near the edge of developed areas where LAI values were not available from satellite imagery or were lower than plots further from development. Deposition for these locations was calculated using monthly LAI values more representative of nearby areas where CSS cover predominates; the average LAI from plot locations with the most CSS cover (top 25%) in the immediate vicinity.

Vegetation sampling

Using aerial imagery and vegetation maps of the Santa Monica Mountains NRA, I identified thirty sites (Table 2) of mature CSS that spanned the east to west N deposition gradient indicated by previous modeling attempts (Fenn et al. 2003, Tonnesen et al. 2007) and our own estimations based on empirically determined atmospheric N concentrations.

I selected sites that had last burned greater than 10 years ago and had at least ~50% native CSS shrub cover across the site. Vegetation sampling was conducted April to May in 2015 during the peak of the spring growing season. At each site, over an area of approximately one hectare, I randomly located ten 5 × 5 m vegetation sampling plots. For each plot I recorded species present and estimated percent cover to the nearest 1% in a gridded frame. From this data I calculated total plant richness for each site (i.e. the total number of species found at each vegetation sampling location), mean plot richness (per 25 m^2 plot) and mean percent cover of each plant species.

Soil sampling

I collected soil samples for soil extractable N analysis in summer 2015, when soil extractable N concentrations in this system are typically at their highest (Padgett et al. 1999). At each site, I collected ten replicate samples, each consisting of two composited 10 cm deep cores, one from underneath the shrub canopy and one from the adjacent shrub interspace. Soil samples were transported to the lab where they were allowed to air-dry before sieving them through a 2 mm sieve and extracting N using 2M KCl. Total extractable soil N was analyzed at the University of California, Riverside Environmental Science Research Laboratory using an AQ2 Discrete Autoanalyzer (Seal Analytical, Mequon, Wisconsin). I also analyzed soils from each site for particle size (i.e. texture), phosphorus (P) availability (Olsen-P) and exchangeable cation concentrations (K, Ca, Na and Mg) at the University of California, Davis Analytical Laboratory.

Statistical analysis

I used regression analyses to compare calculated N deposition with total soil N and forms of extractable soil N across vegetation sampling sites (n = 30). To understand the influence of environmental drivers, including N deposition, on plant community data, I utilized multiple stepwise regression to estimate how plant community richness and cover (analyzed separately by native and nonnative functional group) were related to environmental variables. This method quantifies the amount of variation in a dependent variable (here species plant richness or cover) explained by one or more statistically independent covariates by building a model from those variables that together explain the most variability in the dependent variable. Data were tested for significant violations of normality and log-transformed as needed. I also performed simple linear and non-linear regressions of plant richness and cover (by native and nonnative functional group) with N deposition.

To determine the effects of site characteristics (including N deposition), climate and soil variables on plant community diversity and composition, I performed principal components analysis (PCA) on site characteristics and soil variables from all vegetation sampling locations (n = 30) in order to aggregate environmental factors for further data analysis. PCA reduces data dimensionality through covariance analysis between multiple factors, thereby summarizing the factors in a reduced number of axes, or principal components (Nichols 1977, Tabachnick et al. 2001). Prior to analysis, data were square-root transformed. I performed PCA separately on data matrices of site characteristics and soil chemistry and texture data, and from each analysis extracted the axes explaining the

most total variance. I evaluated axes based on eigenvalues and the cumulative variance explained. I then performed pair-wise correlations among the principal components retained and all environmental variables.

I next investigated compositional relationships among plant communities at each of the vegetation sampling sites (n = 30) using non-metric multidimensional scaling (NMDS) of weighted averages of plant species cover, using Bray-Curtis coefficients as a distance measure. This ordination method is a robust tool for explorations of changes in plant community composition (Kenkel and Orlóci 1986, Clarke 1993). I limited then number of species included in the NMDS ordination analysis to those that occurred in at least ten percent of all sampling locations to reduce the impact of rare species on results, with a total of 80 species included in the final ordination (total number of species = 113). I used a random starting point, and considered a maximum number of six dimensions. I retained axes based on final stress and instability.

The scores of the axes extracted from NMDS ordination of sites (n = 30) by species' cover (n = 80) were then used as vegetation composition parameters and compared to all environmental variables individually, as well as with principal components derived from our PCAs of site characteristics and soil variables using regression in order to understand relationships between vegetation composition and environmental factors. Next I used regression techniques to compare measures of plant richness and cover (analyzed separately by native and nonnative functional group) with aggregated environmental variables and NMDS axis scores across vegetation sampling sites (n = 30) to explore how suites of environmental variables and vegetation

composition influence these metrics. Finally, I performed correlations of percent cover of the dominant plant species across vegetation sampling sites with N deposition and soil extractable N, as well as with principal components derived from PCAs of site and soil variables and the scores of major axes extracted from NMDS ordination.

Descriptive statistics and regression analyses were performed using SAS (version 2013; SAS Institute Inc., Cary, North Carolina, USA). PCAs of site and soil variables were completed in RStudio (Version 0.98.57, RStudio, Inc.) using the function *prcomp* in the "vegan" R package. NMDS ordination was applied to the species' cover matrix using the program PC-ORD (Version 5.0, MJM Software; McCune and Mefford 1999).

Results

Atmospheric N concentrations

Atmospheric concentrations of reactive N varied across sites (Table 1), due largely to differences in atmospheric NO_2. Monthly atmospheric N from HNO_3 (ANOVA, $F = 1.50$, $P = 0.2233$) and NH_4 ($F = 0.27$, $P = 0.6024$) did not differ significantly across sites, while N from NO_2 showed a significant increase across sampling locations from west to east ($F = 5.37$, $P = 0.0222$).

Estimates of N deposition

Across the Santa Monica Mountains NRA, estimates of N deposition ranged from less than 1 kg ha^{-1} yr^{-1}, to greater than 75 kg ha^{-1} yr^{-1} at some higher elevation locations

with high LAI (Fig. 1). While geographic patterns of deposition are complex, estimated values tend to be higher closer to urban Los Angles to the east. Across CSS vegetation sampling sites, estimates of N deposition ranged from 3.3 to 17.7 kg ha^{-1} yr^{-1}, with the highest deposition rates observed at the more urban vegetation sampling sites to the east (Fig. 2).

N deposition and soil N

Total soil extractable N showed a significant positive correlation with calculated values of N deposition across CSS vegetation sampling locations (Fig. 3a; $R^2 = 0.14$; $P = 0.0433$). However, soil N from ammonium (NH_4^+) showed no pattern with respect to N deposition (Fig 3b; $R^2 = 0.06$; $P = 0.9468$), and this relationship was driven by soil N from nitrate (NO_3^-) increases (Fig 3c; $R^2 = 0.18$; $P = 0.0181$).

Influence of N deposition on plant richness

I explored correlations between N deposition and two measures of plant richness: total plant richness per site, and mean richness per plot (25 m^2), analyzed separately by native and nonnative functional groups. Total plant richness per site was negatively correlated with N deposition (Fig 4a; $R^2 = 0.21$, $P = 0.0113$), as was total native richness (Fig 4b; $R^2 = 0.27$, $P = 0.0032$). This was due largely to the strong negative relationship with native herbaceous species and N deposition (Fig 4c; $R^2 = 0.41$, $P = 0.0001$) and not native shrub richness (Fig 4d; $R^2 = 0.01$, $P = 0.9116$). The total number of nonnative species per site tended to be higher at high N deposition sites, but this was not

statistically significant (Fig 4e; R^2 = 0.12, P = 0.0640). However, percent native richness at each site was negatively correlated with N deposition (Fig 4f; R^2 = 0.22, P = 0.0097).

The mean number of plant species per vegetation sampling plot also declined significantly with N deposition (Fig 5a; R^2 = 0.23, P = 0.0079). Similarly to pattern of site totals, the mean number of native species per plot was negatively correlated with N deposition (Fig 5b; R^2 = 0.25, P = 0.0048), and this was again due to native herbaceous species (Fig 5c; R^2 = 0.36, P = 0.0005) and not numbers of native CSS shrub species (Fig 4d; R^2 = 0.05, P = 0.6967). The mean number of nonnative species per plot was not significantly correlated with N deposition (Fig 5e; R^2 = 0.05, P = 0.2393), but the percent of native species occurring in each plot exhibited a significant negative relationship (Fig 5f; R^2 = 0.14, P = 0.0393).

Influence of N deposition on plant cover

I also examined relationships of percent cover of native and nonnative plant functional groups with rates of N deposition across vegetation sampling sites. Neither total percent cover (Fig 6a; R^2 = 0.03, P = 0.4254) nor percent cover of native shrubs (Fig 6b; R^2 = 0.01, P = 0.6430) was correlated with rates of N deposition. Percent cover of native herbaceous species, however, showed a slight negative relationship (Fig 6c; R^2 = 0.13, P = 0.0492). Mean percent cover of nonnative species showed the opposite pattern, with total nonnative cover (Fig 6d; R^2 = 0.15, P = 0.0379) increasing significantly with N deposition. This relationship was not driven by cover of nonnative annual grasses (Fig 6e;

R^2 = 0.02, P = 0.5209), but by increasing percent cover of nonnative forbs (Fig 6f; R^2 = 0.16, P = 0.0277).

Relationships between nonnative cover and plant richness

Regression analysis of plant cover and richness data revealed a strong negative relationship between mean percent cover of nonnative species and both the total number of native species found at each sampling location (Fig 7a; R^2 = 0.47, P < 0.0001) and the mean number of species per plot (Fig 7b; R^2 = 0.48, P < 0.0001).

Role of environmental variables

Results from multiple stepwise regressions indicate that plant richness and cover are influenced by multiple environmental factors including N deposition (Table 2) and soil variables (Table 3). A number of site and soil variables were identified as significant model effects in analyses of measurements of total plant richness (Table 4), mean plant richness per plots (Table 5). Nitrogen deposition and soil N were identified as significantly negatively correlated with total plant richness, total native richness, total richness of native herbaceous species, mean total richness, mean native richness and mean richness of herbaceous species. Longitude (°W) showed the opposite effect and was positively correlated with these metrics, as was soil sodium. Mean maximum temperature and VPD were significantly negatively correlated with measures of native richness.

Stepwise regression also identified a number of environmental factors in addition to N deposition influencing the percent cover of native and nonnative plant functional

groups (Table 6). Latitude (°N) was negatively correlated with total native percent cover, and native shrub cover, but positively correlated with nonnative cover, specifically of nonnative forbs. Conversely, longitude (°W) was positively correlated with total native cover and negatively correlated with total percent cover of nonnative plant species. Native herbaceous cover was positively correlated with elevation and mean precipitation but showed a negative relationship with mean minimum temperature. Soil texture and calcium (Ca) also influenced percent cover of functional groups, with native shrub cover positively associated with more loamy soils high in Ca, and nonnative cover, especially of nonnative grasses, negatively correlated with soil clay content.

Aggregation of site and soil characteristics

I retained three principle components from PCA of site characteristics (Site PC1, Site PC2, Site PC3) that showed an eigenvalue >1 and explained a cumulative variance of 78.2% (Table 7). Site PC1 was positively associated with latitude, elevation, and a number of climate variables including precipitation, maximum temperature and minimum and maximum VPD and negatively associated with minimum temperature (Table 8). Site PC2 was positively associated with minimum temperature, N deposition and time since fire, and negatively associated with longitude and fire frequency. Site PC3 was positively associated with minimum VPD, N deposition and fire frequency and negatively associated with time since fire.

From the PCA of soil variables, I retained three principle components (Soil PC1, Soil PC2, Soil PC3) that showed eigenvalues >1 and explained a cumulative variance of

77.4% (Table 9). Soil PC1 was positively associated with soil pH, P, K, Ca and percent silt and clay, and negatively associated with sand content. Soil PC2 was positively associated with soil N, P and K, and negatively associated with soil pH and Ca. Soil PC3 was positively associated with the soil exchangeable cations Na and Mg, and negatively associated with soil N availability.

Ordination of plant community composition

Results of NMDS ordination showed three axes were sufficient to explain vegetation composition. The final stress level for a three-dimensional solution was 3.75, with a final instability of 0.0001, indicating good test performance (McCune et al. 2002). These three axes (NMDS1, NMDS2, NMDS3) explained a cumulative variance of 0.18, 0.43 and 0.54, respectively. Axes were also highly orthogonal (>90%). From correlations of environmental and soil factors and aggregated variables from PCA with the three NMDS axes, I found NMDS1 was positively correlated with latitude and negatively correlated with N deposition, as well as mean minimum temperature and Site PC2 (Table 10). NMDS2 was positively correlated with N deposition and soil N, as well as mean minimum temperature, and negatively correlated with Site PC2 and Soil PC3. NMDS3 was mostly explained by soil texture, showing a positive correlation with percent soil clay and a negative relationship with soil sand content.

Influence of environmental factors and community composition

Comparisons of aggregated environmental factors from PCAs and NMDS ordination axes with vegetation data revealed significant correlations between these composite environmental and composition parameters with measures of total plant richness (Table 11) and mean plant richness (Table 12) of native and nonnative plant functional groups. Total native richness across sites was negatively correlated with Site PC1 and Site PC2, both of which were positively associated with rates of N deposition, and a positive relationship with Soil PC3, NMDS1 and NMDS2 (Table 11). Total shrub richness was also positively correlated with these two NMDS axes. Total native forb richness was negatively associated with Site PC1 and Site PC2, likely driving the significant relationship between total richness and these principal components. The total number of nonnative species was unrelated to any of the extracted environmental or vegetation composition parameters. Mean native richness per vegetation sampling plot (Table 12) showed similar relationships with aggregated environmental and vegetation variables as measures of total richness (Table 11), with a few exceptions. Here, the number of native shrub species was unrelated to any of the PC or NMDS axes, while the number of nonnative species was positively correlated with Site PC3.

Percent cover of native and nonnative plant functional groups was also related to a number of our aggregated variables from PCA and NMDS (Table 13). Total native cover was positively correlated with Soil PC1, an axis largely explained by soil texture, but negatively correlated with Soil PC2, an axis positively associated with soil nutrients N, P and K and negatively associated with pH and Ca. These relationships were driven largely by patterns of native shrub cover. Percent cover of native herbaceous species

exhibited a positive relationship with NMDS1 and NMDS2, and a negative relationship with Site PC3. Total percent cover of nonnative species was unrelated to these aggregated environmental and vegetation variables, but when analyzed separately by functional group, nonnative annual grasses showed a positive correlation with Site PC2, and nonnative forb cover showed a negative relationship with both Soil PC3 and NMDS3.

Effects on plant species

I also analyzed vegetation data at the species level, exploring correlations of dominant plant species with rates of N deposition, soil N and aggregated environmental factors from PCA and axes from NMDS ordination of vegetation sampling sites in species space (Table 14). Percent cover of several species exhibited statistically significant relationships with N deposition and/or soil extractable N. Two nonnative species, the annual grass *Bromus diandrus* and annual/biennial forb *Hirschfeldia incana*, were positively correlated with N deposition and soil extractable N. Several native species, *Stipa pulchra*, *Dichelostemma capitatum*, *Mirabilis laevis* and *Hesperoyucca whipplei* showed the opposite pattern. Two native shrubs, *Encelia californica* and *Malosma laurina,* however, showed increasing percent cover with N deposition and/or N availability. Many species also exhibited significant relationships with principal components from PCAs of site and soil variables. Site PC2 was positively correlated with native and nonnative species that showed a positive response to N. Soil PC3 was positively correlated with a number of native shrub and herbaceous species, but negatively correlated with the invasive annual grass *Bromus rubens*. Another common

invader of CSS, *Erodium cicutarium,* was positively correlated with soil PC2, a component associated with higher levels of soil N.

Scores from NMDS ordination axes were also strongly correlated with the percent cover of numerous plant species (Table 14). NMDS1 showed a negative relationship with the native shrubs *Artemisia californica* and *Malosma laurina*, but a positive relationship with several other native forbs and shrubs. NMDS2 was positively correlated with the nonnatives *Brassica nigra, Bromus diandrus*, *Centaurea melitensis*, and *Hirschfeldia incana* and the native shrubs *Encelia californica* and *Malosma laurina* – mostly species that responded positively to N deposition and/or soil N. NMDS3 was positively correlated with some native species, but exhibited a negative relationship with other natives.

Discussion

Results of this study support a growing body of evidence showing that atmospheric inputs of N due to anthropogenic activities exert a strong influence on plant community diversity and composition (Fenn et al. 2003, Stevens et al. 2006, Bobbink et al. 2010, Simkin et al. 2016). Specifically, I observed a significant decline in the number and percent cover of native forb species across the established N deposition gradient of the Santa Monica Mountains. Further, I found soil N eutrophication resulting from anthropogenic N emissions appears to favor the growth of nonnative species, consistent with ecological theory (Davis et al. 2000) and both observational (Talluto and Suding

2008, Cox et al. 2014) and experimental studies in CSS (Allen et al. 1998, 2016, Kimball et al. 2014). The success of these nonnative plant invaders may also be a significant driver of reduced native diversity, as illustrated by the strong negative correlation between native richness and nonnative cover. Our results also highlight the important role of other environmental factors, such as geographic, climatic or soil variables, in shaping plant communities, including community and species-level responses to N deposition.

Atmospheric N pollutants

Throughout the United States, and particularly in southern California, oxidized forms of reactive N, mainly NO_3^- resulting from vehicular and industrial air pollution, represent the primary nitrogenous pollutants in the atmosphere (Padgett et al. 1999, Bytnerowicz et al. 2001, Fenn et al. 2010). I observed a steep gradient of atmospheric concentrations of nitrogen oxides increasing eastward across the Santa Monica Mountains, likely resulting from the high levels of air pollution originating from the Los Angeles Basin (Haagen-Smit 1952, Bytnerowicz et al. 1987, Su et al. 2009, Bytnerowicz et al. 2015). This steep east-to-west gradient of nitrogenous air pollutant concentrations strongly influenced patterns of N deposition across the Santa Monica Mountains, and these results have important implications for native vegetation in the region (Allen et al. 1998, Fenn et al. 2003, Fenn et al. 2010, Cox et al. 2014)

Estimation of N Deposition

Consistent with initial hypotheses and previous attempts at modeling N deposition (Tonnesen et al. 2007, Fenn et al. 2010) across the Santa Monica Mountains NRA, our calculations of N deposition show a steep increase in N inputs from east-to-west similar to the observed patterns of atmospheric N pollution, with higher rates of N deposition in areas closer to urban Los Angeles. A number of factors will influence rates of N deposition, including climate, ambient N concentrations, physiochemical properties of different N forms and vegetation characteristics (Lovett 1994, Hertel et al. 2011). Due to their high reactivity and water solubility, NH_3 and HNO_3 possess high deposition velocities and are readily deposited to plant surfaces (Hanson and Lindberg 1991), and thus, while oxidized forms of N dominate in the atmosphere, concentrations of these molecules are the major determinant of deposition in our system and others (Bytnerowicz et al. 2015). Leaf area may have also played an important role in shaping observed patterns of N deposition, with higher elevation vegetation consisting of closed canopy chaparral intercepting higher levels of N deposition than more open grassland and scrubland ecosystems (Weathers et al. 2001, Bytnerowicz et al. 2015). Nevertheless, our comparison of N deposition rates across CSS sampling sites revealed a strong gradient of deposition, even in more open canopy, low elevation vegetation.

Soil N availability

While gaseous forms of N may have direct negative impacts on native CSS species (Haagen-Smit et al. 1952, Westman 1979, 1985), many native species are either annual or drought deciduous, senescing during the summer dry period when atmospheric

N pollutants are at their highest (Bytnerowicz et al. 2001, Bytnerowicz et al. 2015). The most detrimental effects of N pollution in this system are therefore caused by N eutrophication of soils (Padgett et al. 1999, Fenn et al. 2003). As expected, I found a positive relationship between rates of N deposition and total extractable soil N across vegetation sampling locations, largely due to soil N in the form of nitrate (NO_3^-). This is consistent with other described gradients of atmospheric N pollutants and terrestrial N accumulation in the region (Padgett et al. 1999, Fenn et al. 2003). While direct N eutrophication resulting from atmospheric N inputs may be the primary driver of enhanced N, elevated N deposition may also increase rates of decomposition and mineralization, further contributing to higher soil N availability (Sirulnik et al. 2007, Vourlitis et al. 2007). I also found evidence of higher nonnative cover at sites subject to higher levels of N deposition, and the presence of nonnative species, such as nonnative annual grasses, may also increase rates of nitrification in soils (Ehrenfeld 2003, Hawkes et al. 2005).

Impacts of N deposition on plant communities

Ecological theory, as well as prior empirical and experimental evidence implicates N deposition as a causal factor in the decline of native plant species richness in a number of ecosystems (Stevens et al. 2006, Bobbink et al. 2010, Simkin et al. 2016). Declines in plant diversity with increasing primary productivity are frequently observed (Grime 1973, Tilman 1982, Gough et al. 2000), and as most terrestrial ecosystems are N-limited (Aerts and Chapin 2000), the addition of excess N may reduce plant diversity by

favoring those plant species that are better adapted to exploit available soil N and respond with rapid growth. Both abundance- and functional-based mechanisms may explain species loss under N fertilization; species that are rare or lower in abundance, or those that are slow growing, perennial, small-statured, form symbiotic relationships with N-fixing bacteria, or utilize C_4 photosynthetic pathway, are more likely to be excluded under high N conditions (Craine et al. 2002, Suding et al. 2005, Clark et al. 2007). Results from this study support this theory, and I observed significant declines in native CSS species, especially native forb species, with increasing levels of N deposition and accompanying enhanced soil N availability.

When I compared N deposition and soil N availability with percent cover of plants at the species-level, several native plant species representing a variety of plant functional groups, showed a significant negative correlation. The traits of these species may provide insight into the mechanisms driving species loss at sites subject to elevated N deposition. For example, *Dichelostemma capitatum* is a basal sprouting forb (from a bulb), and such species may be easily outcompeted under high productivity, low-light conditions (from taller neighbors), and are likely poor competitors with other faster growing annuals (Stevens et al. 2006). This may also explain the significant decline of the prostrate sub-shrub *Mirabilis laevis* across the N deposition gradient. Previous work has shown the perennial bunchgrass *Stipa pulchra* does not respond to N fertilization with increased productivity (Abraham et al. 2009), and this may be partially responsible for this species' decline under elevated N deposition, as I observed. Finally, *Hesperoyucca whipplei* is a C_4 succulent, representing another functional group expected

to decline under fertilized conditions (Suding et al. 2005) due to its slow growth rate and resource-conserving growth strategy. These relationships represent declines in plant cover with increasing atmospheric N inputs, and if these more vulnerable species continue to be excluded from the plant community, they may undergo local, or even regional extinction under predicted rates of future N deposition.

Increased N availability may have other indirect effects on plant species and communities by increasing susceptibility to secondary stressor and/or altering interactions with other organisms, both beneficial and antagonistic (Bobbink et al. 2010). These factors also undoubtedly influenced observed patterns of vegetation due to varying tolerances and response among plant species. Elevated N deposition may result in intensified herbivory on plants due to higher foliar N concentrations (Van Der Wal et al. 2003, Throop and Lerdau 2004), increased plant susceptibility to drought (Friedrich et al. 2012) and altered mycorrhizal functioning and diversity (Egerton-Warburton and Allen 2000, Sigüenza et al. 2006a, Sigüenza et al. 2006b). These indirect effects may also have complex and interactive effects on plant communities. For example, Jones et al. (2004) found N deposition in conjunction with a severe drought and insect outbreak resulted in increased tree mortality, and Valliere and Allen (2016) demonstrated N fertilization and N-impacted soil microbial communities reduce drought tolerance in seedlings of a native CSS shrub.

While generally native richness and cover declined with increasing N deposition, some native species may benefit from added N. In our study, cover of the native shrub species *Encelia californica* showed a positive correlation with both N deposition and soil

N availability. The percent cover of this species also explained a high amount of variance in our ordination of plots in species space, illustrating its increasing importance at more N-polluted sites to the east. Seedlings of this species are faster-growing than other CSS perennials (Valliere, unpublished data), and this rapid growth may promote its success under high N conditions. This species also flowers and sets seed early in it's life cycle and following fire, and its seedlings establish more vigorously than those of other CSS shrub species (Keeley et al. 2006), and these traits could promote its persistence and success under high N deposition. In the absence of nonnative competitors, natives, including native shrubs, may respond positively to soil N addition (Allen et al. 1998, Padgett and Allen 1999, Vourlitis and Pasquini 2009), and the negative response overall of native species, especially native forbs, is likely due to competition from more nitrophilic species, whether they be native or nonnative (Suding et al 2005)

Nonnative species and plant diversity

Our results do suggest that nonnative annual plant species may be important drivers of reduced native plant richness of CSS under chronic N deposition. Furthermore, as mean native plant richness declined with increasing N deposition, the number of nonnative species increased. Annual grasses and forbs typically represent the major nonnative invaders of mediterranean ecosystems, including CSS (Barbour et al. 2007, Funk et al. 2016). These invasive nonnative species are predicted to be the "winners" of soil N eutrophication (Grime 1973, Davis et al. 2000, Davis and Pelsor 2001), and previous N addition experiments in CSS of southern California have demonstrated

increased growth of these species (Allen et al. 1998, Kimball et al. 2014), and N deposition is a major contributor to conversion of native CSS shrublands to grasslands dominated by nonnative species (Talluto and Suding 2008, Cox et al. 2014). The success of these species under high N conditions is likely a function of a number of advantageous functional traits: early phenology, high germination rates, annual lifecycle and rapid growth rate (Chiariello 1989, Davis et al. 2000, Diekmann and Falkengren‐Grerup 2002, Suding et al. 2005, Wainwright and Cleland 2013).

Interestingly, the higher percent cover of nonnative plant species with increasing N deposition was due to increased growth of nonnative forbs and not annual grasses, such as *Centaurea melitensis, Erodium cicutarium* and *Hirschfeldia incana.* Each of these species is known to respond to N enrichment with increased growth (Padgett and Allen 1999, Brooks 2003, Bozzolo and Lipson 2013), though typically nonnative annual grasses are expected to dominate under high N conditions. Annual herbaceous communities of CSS may vary greatly year-to-year due to precipitation (Talbot et al. 1939, Heady 1958, Keeley et al. 1981), including the composition of nonnative species (Cox and Allen 2008). I only sampled vegetation during one growing season, and it is quite possible that in addition to N deposition, the timing and amount of precipitation favored nonnative forb species during this particular sampling year.

Role of site characteristics

A number of environmental variables in addition to N deposition and soil N availability were identified as important factors influencing plant richness and

community composition. For example, both mean maximum temperature and maximum VPD were negatively correlated with richness of native herbaceous species, suggesting more arid CSS sites are less species-rich. Cover of these species was also higher with increasing mean precipitation and elevation, and negatively correlated with mean minimum temperature. These climate variables may interact with N deposition as well, as illustrated by multivariate analysis of vegetation and environmental factors. It has been suggested that Riverisidian CSS of the more xeric interior of southern California is more susceptible to the negative impacts of N deposition (Westman 1985, Minnich and Dezzani 1998), and Kimball et al. (2014) found reduced water inputs increased nonnative cover in recently burned CSS subject to N fertilization. It follows that drier and warmer conditions at some sites may favor nonnatives, especially at high N deposition sites.

Longitude, or distance west, was positively associated with native forb richness and negatively correlated with nonnative richness, indicating that nonnative grasses and forbs may be replacing natives at more urban sites to the east. This pattern is likely the result of N deposition in combination with other potentially confounding factors. For example, vegetation is more fragmented and patch sizes smaller on the more developed, eastern part of the mountain range, and both these factors are known to be major determinants of diversity (Bender et al. 1998, Fahrig 2003). More urban sites are often closer to highways, roads and trails, and this could have also contributed to the number and cover of native and nonnative species at these sites.

Role of soil nutrients and texture

Coastal sage scrub is known to exist on a variety of soil types and parent materials differing in texture and chemistry (Wells 1962, Kirkpatrick and Hutchinson 1980). Other soil nutrients beyond extractable N showed important relationships with plat community diversity and composition. Soil Na, for example, was positively correlated with measures of native plant richness, mostly native herbaceous species at both the site and plot level. This plant community is generally not found in highly saline soils (Kirkpatrick and Hutchinson 1980). I also observed increasing percent cover of native shrub species at sites with higher concentrations of soil calcium. This is consistent with the findings of (Westman 1981a,d), who observed similar positive correlations between soil calcium availability and native cover due to increasing cover of native CSS shrubs. Calcareous soils may be phosphorus-fixing and therefore low in plant- available P, and such soils are known to promote native shrubs over annual grasses in Mediterranean shrublands (Rabinovitch-Vin 1983).

Soil texture may also play an important role in mediating CSS diversity and community composition (Kirkpatrick and Hutchinson 1980, Westman 1981d, a). While percent sand, silt and clay were found to be unrelated to measures of plant diversity individually, soil texture explained the most variance in the first principal component of our PCA of soil variables, which was significantly correlated with measures of vegetation composition. Sites with soils with similar proportions of sand, silt and clay (i.e. loamy soils) tended to have higher native shrub cover and lower cover of nonnative annual grasses, as evidenced by both regression and multivariate analyses. Talluto and Suding

(2008) determined the opposite to be true, and in their landscape survey of CSS vegetation annual grass cover was positively related to percent silt and clay, but others have reported preferences of invasive species in CSS, such as *Centaurea melitensis*, for sandy substrates (Westman 1981a).

Influence of fire history

Any investigation of diversity relations in CSS would be incomplete without a consideration of fire history. As in many semi-arid ecosystems, fire is a natural and important ecological process in CSS (Westman 1981d, Malanson 1984, Keeley and Fotheringham 2001, Keeley 2002). Historically, mean fire return intervals for CSS of the Santa Monica Mountains averaged approximately 20 years (O'Leary and Westman 1988), but modern fire frequencies have increased due to human activities (Zedler 1995). Surprisingly, I found little influence of fire history parameters, specifically mean fire return interval and time since last fire, on plant species richness and cover, although multivariate analyses revealed a positive association between N deposition, fire frequency and reduced native richness, as would be expected. All sites with known fire history were at least 10 years old, which is sufficient time for recovery (Zedler 1995). However, incomplete fire records for several of our higher N deposition sites complicate analysis of fire history parameters.

Previous work has established a clear link between increased rates of N deposition and fire frequency and severity, to the detriment of native CSS species (Fenn et al. 2003, Cox et al. 2014). This process is largely driven by a positive feedback loop where

elevated N availability promotes nonnative annuals, resulting in higher fine fuel loads and increased fire risk (Fenn et al. 2003, Rao et al. 2010) and contributing to vegetation-type conversion (Talluto and Suding 2008, Cox et al. 2014). The Santa Monica Mountains NRA is the most urban national park in the country, with parks and open spaces embedded in an urban-suburban matrix, especially eastward towards the City of Los Angeles. As such, wildland fires are often actively combatted, and fire suppression efforts have important implications for CSS fire regimes and long-term vegetation dynamics (Minnich 1983, Keeley et al. 1999). While increased N deposition may indeed be increasing growth of nonnative grasses and forbs in the Santa Monica Mountains, potentially heightening fire-risk, due to human intervention and active fire management, many of our more polluted sites burned *less* recently and frequently than low deposition sites on the western end of the mountain range. In fact, it is conceivable that very short fire return intervals may negatively impact diversity of this fire-adapted ecosystem (Petraitis et al. 1989).

Conclusions

The Santa Monica Mountains NRA protects a substantial area of wildlands and open spaces in the greater Los Angeles area, including a large amount of remaining intact CSS in southern California. Due to its proximity to the City of Los Angeles, these lands are inevitably impacted by human activities, and our results implicate atmospheric N deposition as a significant ecological threat to CSS. The effects of N deposition and other human disturbances may also harm a number of important, rare, threatened and

endangered plant and animal species that inhabit CSS (McCaull 1994, Bowler 2000, Rubinoff 2001, Burger et al. 2003), and have negative impacts on ecosystem processes and services (Jefferies and Maron 1997, Fenn et al. 2003, Bobbink et al. 2010, Fenn et al. 2010). N deposition may also limit restoration of this important ecosystem (Allen et al. 2000, Cox and Allen 2008). While our results strongly suggest atmospheric N pollution negatively impacts native plant diversity, the relationships I describe here are correlative, not necessarily causal, and further experimentation is required to identify underlying mechanisms. However, the steep gradient of N pollution I observed indicates profound alteration of regional N cycling, and will inevitably influence local ecosystems. As human activities continue to contribute to higher rates of N deposition, conservation of remaining native ecosystems must consider the growing influence of atmospheric N inputs on vegetation patterns.

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Table 1.1. Location of atmospheric monitoring sites (n = 10) and average monthly atmospheric gaseous N concentrations from October 2011 to November 2012 and standard errors.

			Atmospheric N Concentrations (μg N m^{-3})					
	Latitude	Longitude	HNO_3		NH_4		NO_x	
Atmospheric Sampling Sites	(° N)	(° W)	Mean	SE	Mean	SE	Mean	SE
1. Deer Creek	34.0828	118.9843	1.43	±0.58	0.78	±0.07	1.42	±0.16
2. Rancho Sierra Vista	34.1555	118.9660	0.74	±0.13	1.11	±0.20	1.85	±0.12
3. Zuma Canyon	34.0370	118.8187	0.91	±0.23	0.96	±0.07	2.37	±0.26
4. Rocky Oaks	34.0999	118.8160	1.07	±0.37	1.10	±0.12	2.09	±0.22
5. Paramount Ranch	34.1155	118.7563	1.39	±0.42	1.07	±0.10	2.64	±0.21
6. Solstice Canyon	34.0415	118.7501	1.52	±0.64	1.06	±0.12	2.87	±0.38
7. Palo Comado	34.1754	118.7507	1.62	±0.57	0.90	±0.11	2.20	±0.11
8. Mulholland	34.1359	118.5784	1.07	±0.29	1.09	±0.08	3.14	±0.21
9. Paseo Miramar	34.0566	118.5589	1.01	±0.34	1.04	±0.10	3.90	±0.37
10. Franklin Canyon	34.1269	118.4092	1.83	±0.63	1.24	±0.17	5.99	±0.46

Table 1.2. General site characteristics for vegetation sampling locations (n = 30), including geographic location, elevation, mean precipitation (30 yr. normals), average minimum and maximum temperature, minimum and maximum vapor pressure deficit (VPD), calculated N deposition values based on atmospheric N, and fire history information including time of last fire and fire frequency (since 1925). nd = no data available.

Vegetation Sampling Sites	Latitude (° N)	Longitude (° W)	Elev. (m)	Mean Prec. (mm)	Min. Temp. (C°)	Max. Temp. (C°)	Min. VPD	Max. VPD	Nitrogen Deposition (kg ha^{-1} yr^{-1})	Last Fire	Fire Freq.
1. Deer Creek	34.0828	118.9843	214	379	12.4	23.8	3.8	19.4	6.2	1993	4
2. Rancho Sierra Vista	34.1555	118.9660	258	433	11.1	22.7	2.8	17.5	3.8	1993	3
3. Leo Carillo	34.0584	118.9367	88	331	11.8	22.3	2.5	17.1	6.7	1993	4
4. West Mulholland	34.0869	118.9165	332	449	11.0	22.7	2.9	18.0	6.1	1993	3
5. Decker Canyon	34.0462	118.8946	147	358	11.8	23.3	2.6	18.7	5.5	2003	5
6. Encinal Canyon	34.0621	118.8783	147	358	12.4	23.6	3.7	19.2	6.4	1985	3
7. Lake Eleanor	34.1324	118.8552	370	490	11.5	22.4	3.7	17.1	6.0	1985	2
8. Triunfo Creek	34.1309	118.8243	362	483	11.3	23.3	3.1	18.5	3.3	1978	2
9. Zuma Canyon	34.0370	118.8187	185	380	12.3	21.7	2.7	15.6	5.1	1978	3
10. Kanan Dume	34.0386	118.7996	235	409	12.2	22.6	3.0	16.9	9.1	1985	5
11. Solstice Canyon	34.0415	118.7501	185	398	12.4	21.6	3.0	15.2	16.6	2007	6
12. Palo Comado	34.1754	118.7507	338	397	11.4	24.4	3.8	21.6	6.5	2005	4
13. Century Lake	34.1100	118.7378	304	418	11.6	23.9	3.6	19.8	9.7	1996	6
14. Corral Canyon	34.0520	118.7371	185	398	12.2	22.4	2.9	16.4	13.3	2007	7
15. Cheeseboro Canyon	34.1465	118.7332	338	397	11.5	24.3	3.6	20.9	5.8	2005	4
16. Malibu Creek	34.0946	118.7010	252	480	11.9	23.1	3.4	17.8	10.0	1993	6
17. Malibu Canyon	34.0512	118.6934	153	380	12.6	21.9	2.9	15.5	13.5	2007	4
18. Big Rock	34.0472	118.6289	158	390	12.5	24.0	3.8	19.7	11.1	1993	2
19. Old Topanga	34.0947	118.6090	515	507	12.1	24.8	3.7	21.8	14.4	1993	2
20. Lower Topanga	34.0462	118.5815	92	386	12.8	21.9	3.1	15.2	15.1	1993	3
21. East Mulholland Drive	34.1359	118.5784	314	507	12.2	24.8	4.0	22.0	6.2	1984	1
22. Paseo Miramar	34.0566	118.5589	90	372	12.6	22.6	3.2	16.5	13.1	1973	2
23. Will Rogers	34.0536	118.5150	87	372	12.8	22.1	2.5	15.4	12.6	1978	2
24. Westridge	34.1260	118.5013	417	512	12.1	24.6	3.5	21.1	13.0	1978	1
25. Getty View	34.0919	118.4713	212	452	12.3	25.0	3.0	21.4	11.0	1961	1
26. Stone Canyon	34.1419	118.4581	337	495	11.6	25.0	2.9	21.5	9.0	nd	nd
27. Lower Franklin Canyon	34.1046	118.4097	302	453	12.3	25.2	2.8	21.9	14.8	nd	nd
28. Upper Franklin Canyon	34.1269	118.4092	302	453	12.1	25.5	2.8	22.7	17.2	nd	nd
29. Fryman Canyon	34.1234	118.3819	292	445	12.1	25.0	2.7	21.3	17.2	nd	nd
30. Runyon Canyon	34.1077	118.3530	262	429	12.3	23.9	2.7	18.7	14.4	1984	2

Table 1.3. Soil characteristics for each vegetation sampling site (n = 30) including soil pH, extractable soil N from NO_3^-, extractable soil N from NH_4^+, total KCl extractable N, soil phosphorus (Olsen-P), concentrations of exchangeable cations (K = potassium, Na = sodium, Ca = calcium, Mg = magnesium) and soil texture (percent composition of sand, silt and clay).

Vegetation Sampling Sites	pH	NO_3^- (μg N g^{-1})	NH_4^+ (μg N g^{-1})	Total N (μg N g^{-1})	P (ppm)	X-K (ppm)	X-Na (ppm)	X-Ca (meq 100g)	X-Mg (meq 100g)	Soil Texture		
										% Sand	% Silt	% Clay
1. Deer Creek	6.6	0.6	7.1	7.7	17.5	320.8	160.8	15.4	12.2	41	30	29
2. Rancho Sierra Vista	6.6	5.8	7.8	13.7	18.8	305.2	44.8	11.6	5.2	64	23	13
3. Leo Carillo	5.9	2.2	10.5	12.6	6.9	181.7	43.7	18.1	7.5	55	24	21
4. West Mulholland	5.9	1.1	5.2	6.3	12.3	70.0	30.0	12.5	5.2	71	17	12
5. Decker Canyon	6.2	4.0	7.7	11.6	11.8	125.3	52.7	9.8	3.1	69	17	14
6. Encinal Canyon	6.4	3.5	16.2	19.7	39.1	330.3	56.7	19.5	11.4	37	31	32
7. Lake Eleanor	6.6	9.1	13.1	22.2	9.4	457.3	186.0	11.7	11.3	48	25	27
8. Triunfo Creek	6.2	3.2	21.7	24.9	42.7	351.3	22.3	13.6	3.4	43	39	18
9. Zuma Canyon	7.9	8.0	9.9	17.9	15.8	447.0	74.0	36.6	6.8	22	28	51
10. Kanan Dume	7.7	0.8	20.8	21.6	12.8	275.0	75.7	28.2	5.7	31	34	35
11. Solstice Canyon	7.1	0.4	16.3	16.7	10.7	231.6	26.0	18.9	3.2	44	30	27
12. Palo Comado	7.8	3.5	22.6	26.1	14.3	191.6	14.8	8.9	3.0	74	18	9
13. Century Lake	6.2	1.9	7.5	9.4	18.7	393.3	27.3	14.3	8.4	26	37	37
14. Corral Canyon	6.8	3.1	15.7	18.8	18.0	339.0	26.0	22.0	2.5	28	37	35
15. Cheeseboro Canyon	6.4	11.7	31.7	43.4	40.7	442.7	86.3	14.5	8.1	43	29	29
16. Malibu Creek	7.0	3.5	10.5	14.0	6.9	77.0	49.7	26.7	18.4	73	19	8
17. Malibu Canyon	7.9	2.2	13.7	15.9	5.8	119.3	31.7	24.4	4.6	65	19	16
18. Big Rock	6.2	2.5	20.9	23.4	8.9	105.7	49.7	15.8	3.9	68	18	14
19. Old Topanga	6.6	6.6	10.2	16.8	20.5	367.0	12.0	11.9	5.1	53	23	24
20. Lower Topanga	6.4	6.1	50.1	56.2	22.6	496.0	95.3	16.1	3.8	54	28	18
21. East Mulholland Drive	6.4	9.4	36.6	46.0	23.7	386.8	32.2	19.4	3.9	34	39	27
22. Paseo Miramar	6.9	3.5	16.2	19.7	6.8	119.2	56.2	10.5	10.7	72	17	12
23. Will Rogers	5.4	18.9	29.2	48.0	40.5	364.7	91.0	11.4	10.1	36	32	32
24. Westridge	7.8	1.5	10.9	12.4	11.6	169.0	25.0	42.3	2.3	23	39	38
25. Getty View	5.7	2.7	17.1	19.8	15.1	101.0	52.7	8.4	13.7	59	27	14
26. Stone Canyon	7.7	3.0	29.6	32.6	10.9	246.7	38.0	34.9	1.4	46	35	19
27. Lower Franklin Canyon	5.2	7.7	52.2	59.9	14.3	102.3	18.0	10.8	2.4	77	14	9
28. Upper Franklin Canyon	6.3	1.7	34.6	36.3	15.5	177.8	12.2	8.6	2.6	67	21	13
29. Fryman Canyon	5.6	12.4	31.6	44.0	26.9	450.7	49.3	13.8	2.5	59	28	13
30. Runyon Canyon	5.6	2.0	36.6	38.6	14.8	176.7	25.3	15.2	5.8	82	10	8

Table 1.4. Results of step-wise multiple regressions of site and soil variables on total plant species richness, native richness, native shrub richness, native herbaceous richness and nonnative richness for each vegetation sampling location (n = 30), including the direction of the correlation and correlation coefficients. Significant model effects are bolded. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Total Richness		Total Native Richness		Native Shrub Richness		Native Herb Richness		Nonnative Richness	
Site Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Latitude (° N)	ns	0.05	ns	0.12	ns	0.10	ns	0.04	ns	0.05
Longitude (° W)	+	**0.40*** **	+	**0.51*** **	ns	0.02	+	**0.71*** **	-	**0.19***
Elevation (m)	ns	0.04	ns	0.03	ns	0.01	ns	0.04	ns	0.01
Mean Precipitation (mm)	ns	0.02	ns	0.03	ns	0.02	ns	0.05	ns	0.01
Mean Min. Temp (°C)	ns	0.03	ns	0.04	ns	0.01	ns	0.13	ns	0.02
Mean Max. Temp (°C)	-	**0.17***	-	**0.21***	ns	0.01	-	**0.28***	ns	0.08
Mean Min. VPD	ns	0.01	ns	0.01	ns	0.03	ns	0.01	ns	0.01
Mean Max. VPD	ns	0.13	-	**0.15***	ns	0.01	-	**0.10***	ns	0.04
N Deposition (kg ha^{-1} yr^{-1})	-	**0.21***	-	**0.27***	ns	0.01	-	**0.20*** **	ns	0.18
Time Since Fire (yr.)	ns	0.02	ns	0.02	ns	0.05	ns	0.04	ns	0.01
Fire Frequency	ns	0.01	ns	0.01	ns	0.03	ns	0.04	ns	0.01
pH	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.05
Soil N (μg N g^{-1})	-	**0.19***	-	**0.23***	ns	0.09	-	**0.25***	ns	0.06
Olsen- P (ppm)	ns	0.04	ns	0.02	ns	0.08	ns	0.01	ns	0.02
X-K (ppm)	ns	0.01	ns	0.01	ns	0.05	ns	0.01	ns	0.01
X-Na (ppm)	+	**0.13***	+	**0.13***	ns	0.01	+	**0.19***	ns	0.01
X-Ca (ppm)	ns	0.01	ns	0.02	ns	0.05	ns	0.02	ns	0.02
X-Mg (meq 100g)	ns	0.04	ns	0.05	ns	0.01	ns	0.11	ns	0.01
% Sand	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.03
% Silt	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.01
% Clay	ns	0.01	ns	0.02	ns	0.01	ns	0.01	ns	0.03

Table 1.5. Results of step-wise multiple regressions of site and soil variables on mean plant species richness (per 5 m^2 plot), mean native richness, mean native shrub richness, mean native herbaceous richness and mean nonnative richness for each vegetation sampling location (n = 30), including the direction of the correlation and correlation coefficients. Significant model effects are bolded. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Mean Richness		Mean Native Richness		Mean Native Shrub Richness		Mean Native Herb Richness		Mean Nonnative Richness	
Site Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Latitude (° N)	ns	0.12	ns	0.11	**-**	**0.33***	ns	0.01	ns	0.02
Longitude (° W)	+	**0.60*****	+	**0.58*****	ns	0.14	+	**0.57*****	ns	0.03
Elevation (m)	ns	0.03	ns	0.02	ns	0.03	ns	0.01	ns	0.03
Mean Precipitation (mm)	ns	0.04	ns	0.05	ns	0.04	ns	0.01	ns	0.02
Mean Min. Temp (°C)	ns	0.03	ns	0.01	ns	0.04	**-**	**0.22***	ns	0.04
Mean Max. Temp (°C)	**-**	**0.34***	**-**	**0.31***	**-**	**0.18***	**-**	**0.17***	ns	0.07
Mean Min. VPD	ns	0.01	ns	0.01	ns	0.02	ns	0.01	ns	0.11
Mean Max. VPD	**-**	**0.25***	**-**	**0.22***	**-**	**0.14***	ns	0.11	ns	0.01
N Deposition (kg ha^{-1} yr^{-1})	**-**	**0.23***	**-**	**0.25***	ns	0.01	**-**	**0.37****	ns	0.05
Time Since Fire (yr.)	ns	0.03	ns	0.01	ns	0.01	ns	0.01	ns	0.07
Fire Frequency	ns	0.04	ns	0.02	ns	0.01	ns	0.02	+	**0.12***
pH	ns	0.03	ns	0.02	ns	0.02	ns	0.01	ns	0.04
Soil N (μg N g^{-1})	**-**	**0.30***	**-**	**0.28***	ns	0.13	**-**	**0.24***	ns	0.01
Olsen- P (ppm)	ns	0.04	ns	0.02	ns	0.09	ns	0.01	ns	0.04
X-K (ppm)	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.01
X-Na (ppm)	+	**0.17***	+	**0.17***	ns	0.01	+	**0.13***	ns	0.01
X-Ca (ppm)	ns	0.01	ns	0.01	ns	0.04	ns	0.01	ns	0.01
X-Mg (meq 100g)	ns	0.05	ns	0.01	ns	0.01	ns	0.01	ns	0.01
% Sand	ns	0.02	ns	0.01	ns	0.03	ns	0.01	ns	0.01
% Silt	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.01
% Clay	ns	0.03	ns	0.03	ns	0.06	ns	0.01	ns	0.01

Table 1.6. Results of step-wise multiple regressions of site and soil variables on mean percent cover of all natives, native shrubs, native herbaceous species, all nonnatives, nonnative grasses and nonnative forbs for each vegetation sampling location (n = 30), including the direction of the correlation and correlation coefficients. Significant model effects are bolded. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Total Native % Cover		Native Shrub % Cover		Native Herb % Cover		Total Nonnative % Cover		Nonnative Grass % Cover		Nonnative Forb % Cover	
Environmental Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Latitude (° N)	-	**0.18***	-	**0.28***	ns	0.11	+	**0.15***	ns	0.01	+	**0.15***
Longitude (° W)	+	**0.20***	ns	0.05	ns	0.08	-	**0.28***	ns	0.10	-	**0.21***
Elevation (m)	ns	0.02	ns	0.09	+	**0.14***	ns	0.01	ns	0.02	ns	0.01
Mean Precipitation (mm)	ns	0.01	ns	0.04	+	**0.20***	ns	0.01	ns	0.03	ns	0.01
Mean Min. Temp (°C)	ns	0.01	ns	0.05	-	**0.25***	ns	0.01	ns	0.03	ns	0.09
Mean Max. Temp (°C)	-	**0.15***	ns	0.10	ns	0.01	ns	0.11	ns	0.01	ns	0.01
Mean Min. VPD	ns	0.02	ns	0.03	ns	0.01	ns	0.02	ns	0.01	ns	0.06
Mean Max. VPD	ns	0.13	ns	0.08	ns	0.01	ns	0.06	ns	0.01	ns	0.05
N Deposition (kg ha^{-1} yr^{-1})	ns	0.02	ns	0.01	-	**0.13***	+	**0.15***	ns	0.04	+	**0.16***
Time Since Fire (yr.)	ns	0.01	ns	0.03	ns	0.02	ns	0.06	ns	0.13	ns	0.01
Fire Frequency	ns	0.06	ns	0.12	ns	0.01	ns	0.01	ns	0.09	ns	0.01
pH	ns	0.05	ns	0.08	ns	0.01	+	**0.16***	ns	0.02	ns	0.01
Soil N (μg N g^{-1})	-	**0.21***	ns	0.11	ns	0.03	ns	0.01	ns	0.05	+	**0.18***
Olsen- P (ppm)	ns	0.02	ns	0.05	ns	0.02	ns	0.01	ns	0.02	ns	0.01
X-K (ppm)	ns	0.01	ns	0.01	ns	0.01	ns	0.02	ns	0.01	ns	0.01
X-Na (ppm)	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.02	ns	0.03
X-Ca (ppm)	+	**0.16***	+	**0.16***	ns	0.03	ns	0.02	ns	0.01	ns	0.01
X-Mg (meq 100g)	ns	0.01	ns	0.02	ns	0.04	ns	0.01	ns	0.03	ns	0.03
% Sand	-	**0.20***	-	**0.19***	ns	0.01	ns	0.12	ns	0.07	ns	0.08
% Silt	+	**0.16***	ns	0.11	ns	0.01	ns	0.05	ns	0.01	ns	0.05
% Clay	+	**0.16***	+	**0.21***	ns	0.03	-	**0.13***	-	**0.14***	ns	0.07

Table 1.7. Eigenvalues and variance explained for the axes extracted from principal components analysis (PCA) of site characteristics and soil variables from all vegetation sampling locations (n = 30).

	Site Variables			Soil Variables		
PCA	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	4.46	2.65	1.40	3.82	2.32	1.60
Variance Explained	41.34	24.08	12.77	38.20	23.20	15.98
Cumulative Variance Explained	41.34	65.42	78.19	38.20	61.40	77.38

Table 1.8. Eigenvector coefficients (loadings) for variables included in principal components analysis (PCA) of site characteristics from all vegetation sampling locations (n = 30), and direction, correlation coefficients and *P*-values from simple regressions of variables on principal components. Significant correlations are bolded.

	Site PC1				Site PC2				Site PC3			
Site Variables	Loadings	Direction	R^2	*P*	Loadings	Direction	R^2	*P*	Loadings	Direction	R^2	*P*
Latitude (° N)	**0.39**	+	**0.70**	**<0.0001**	-0.15				-0.04			
Longitude (° W)	-0.06				**-0.55**	**-**	**0.79**	**<0.0001**	-0.15			
Elevation (m)	**0.40**	+	**0.73**	**<0.0001**	-0.08				0.12			
Mean Precipitation (mm)	**0.36**	+	**0.57**	**<0.0001**	0.11				-0.02			
Mean Min. Temp (°C)	**-0.24**	**-**	**0.26**	**<0.0001**	**0.45**	+	**0.54**	**<0.0001**	0.17			
Mean Max. Temp (°C)	**0.39**	+	**0.70**	**<0.0001**	0.14				0.16			
Mean Min. VPD	**0.31**	+	**0.43**	**0.0003**	0.02				**0.34**	+	**0.16**	**0.0396**
Mean Max. VPD	**0.41**	+	**0.76**	**<0.0001**	0.05				0.16			
N Deposition (kg ha^{-1} yr^{-1})	-0.16				**0.42**	+	**0.46**	**0.0001**	**0.45**	+	**0.28**	**0.0056**
Time Since Fire (yr.)	0.10				**0.36**	+	**0.35**	**0.0015**	**-0.59**	**-**	**0.49**	**<0.0001**
Fire Frequency	-0.21				**-0.35**	**-**	**0.33**	**0.0022**	**0.46**	+	**0.3**	**0.0038**

Table 1.9. Eigenvector coefficients (loadings) for variables included in principal components analysis (PCA) of soil variables from all vegetation sampling locations (n = 30), and direction, correlation coefficients and *P*-values from simple regressions of variables on principal components. Significant correlations are bolded.

	Soil PC1				Soil PC2				Soil PC3			
Site Variables	Loading	Direction	R^2	*P*	Loading	Direction	R^2	*P*	Loading	Direction	R^2	*P*
pH	**0.39**	+	**0.15**	**0.0345**	**-0.75**	**-**	**0.56**	**<0.0001**	-0.07			
Soil N (μg N g^{-1})	-0.07				**0.67**	+	**0.45**	**<0.0001**	**-0.41**	**-**	**0.17**	**0.0247**
Olsen- P (ppm)	**0.41**	+	**0.17**	**0.0248**	**0.75**	+	**0.56**	**<0.0001**	-0.14			
X-K (ppm)	**0.71**	+	**0.51**	**<0.0001**	**0.52**	+	**0.26**	**0.003**	0.01			
X-Na (ppm)	0.32				0.24				**0.75**	+	**0.56**	**<0.0001**
X-Ca (ppm)	**0.56**	+	**0.31**	**0.0012**	**-0.63**	**-**	**0.39**	**0.0002**	-0.18			
X-Mg (meq 100g)	0.01				0.05				**0.88**	+	**0.78**	**<0.0001**
% Sand	**-0.98**	**-**	**0.95**	**<0.0001**	0.03				0.02			
% Silt	**0.88**	+	**0.77**	**<0.0001**	0.08				-0.17			
% Clay	**0.92**	+	**0.84**	**<0.0001**	-0.11				0.08			

Table 1.10. Correlations of environmental variables and the first three axes extracted from non-metric multidimensional scaling (NMDS) ordination of weighted averages of plant species cover at all vegetation sampling locations (n = 30) and the direction of significant relationships (in bold). ns = not significant.

	NMDS1			NMDS2			NMDS3		
Environmental Variables	Direction	r^2	*P*	Direction	r^2	*P*	Direction	r^2	*P*
Site Characteristics									
Latitude (° N)	+	**0.16**	**0.0297**	ns	0.04	0.2586	ns	0.01	0.5881
Longitude (° W)	ns	0.05	0.2136	-	**0.23**	**0.0070**	ns	0.02	0.4692
Elevation (m)	ns	0.05	0.2480	ns	0.02	0.3937	ns	0.01	0.6428
Mean Precipitation (mm)	ns	0.02	0.4456	ns	0.01	0.5311	ns	0.06	0.1804
Mean Min. Temp (°C)	-	**0.18**	**0.0177**	+	**0.15**	**0.0373**	ns	0.01	0.7212
Mean Max. Temp (°C)	ns	0.01	0.7501	ns	0.01	0.6401	ns	0.03	0.4034
Mean Min. VPD	ns	0.05	0.2550	-	**0.20**	**0.0124**	ns	0.11	0.0680
Mean Max. VPD	ns	0.01	0.9079	ns	0.01	0.9659	ns	0.04	0.2790
N Deposition (kg ha^{-1} yr^{-1})	-	**0.17**	**0.0451**	+	**0.22**	**0.0094**	ns	0.02	0.5327
Time Since Fire (yr.)	ns	0.02	0.4354	ns	0.02	0.0428	ns	0.13	0.0718
Fire Frequency	ns	0.04	0.3158	ns	0.08	0.1598	ns	0.09	0.1321
Site PC1	ns	0.05	0.2623	ns	0.11	0.0987	ns	0.02	0.5098
Site PC2	-	**0.24**	**0.0109**	+	**0.20**	**0.0227**	ns	0.05	0.2524
Site PC3	ns	0.01	0.8427	ns	0.02	0.5117	-	**0.17**	**0.0358**
Soil Variables									
pH	ns	0.02	0.4988	ns	0.03	0.3828	ns	0.05	0.2346
Soil N (μg N g^{-1})	ns	0.02	0.1396	+	**0.14**	**0.0495**	ns	0.01	0.8619
Olsen- P (ppm)	ns	0.01	0.8112	ns	0.07	0.1261	ns	0.06	0.1951
X-K (ppm)	ns	0.02	0.4072	ns	0.06	0.1540	ns	0.05	0.5346
X-Na (ppm)	ns	0.01	0.8028	ns	0.02	0.3536	ns	0.04	0.3217
X-Ca (ppm)	ns	0.01	0.8162	ns	0.02	0.4212	ns	0.01	0.4536
X-Mg (meq 100g)	ns	0.01	0.7229	ns	0.10	0.0523	ns	0.05	0.2165
% Sand	ns	0.01	0.6067	ns	0.09	0.0923	-	**0.11**	**0.0476**
% Silt	ns	0.08	0.1771	ns	0.08	0.2165	ns	0.05	0.2332
% Clay	ns	0.01	0.8692	ns	0.10	0.0628	+	**0.14**	**0.0428**
Soil PC1	ns	0.02	0.5082	ns	0.10	0.0930	ns	0.09	0.1052
Soil PC2	ns	0.01	0.8155	ns	0.02	0.7742	ns	0.01	0.7804
Soil PC3	ns	0.01	0.6121	-	**0.12**	**0.0455**	ns	0.07	0.1611

Table 1.11. Correlations of aggregated variables from principal components analysis (PCA) of site and soil characteristics and the first three axes extracted from non-metric multidimensional scaling (NMDS) ordination of weighted averages of plant species cover and total richness of native and nonnative plant functional groups at all vegetation sampling locations (n = 30) and the direction of significant relationships (in bold). * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Total Richness		Total Native Richness		Native Shrub Richness		Native Herb Richness		Nonnative Richness	
Aggregated Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Site Characteristics PCA										
Site PC1	ns	0.04	ns	0.03	ns	0.01	ns	0.02	ns	0.01
Site PC2	ns	0.14	-	**0.17***	ns	0.01	-	**0.44****	ns	0.08
Site PC3	-	**0.20***	-	**0.25****	ns	0.06	-	**0.20***	ns	0.10
Soil Variables PCA										
Soil PC1	ns	0.01	ns	0.01	ns	0.01	ns	0.01	ns	0.01
Soil PC2	ns	0.02	ns	0.02	ns	0.09	ns	0.01	ns	0.01
Soil PC3	+	**0.16***	+	**0.19***	ns	0.01	+	**0.29***	ns	0.06
NMDS Species Cover										
NMDS1	ns	0.11	+	**0.16***	ns	0.01	+	**0.21***	ns	0.10
NMDS2	ns	0.01	ns	0.01	ns	0.02	ns	0.08	ns	0.01
NMDS3	ns	0.12	+	**0.13***	+	**0.17***	ns	0.01	ns	0.01

Table 1.12. Correlations of aggregated variables from principal components analysis (PCA) of site and soil characteristics and the first three axes extracted from non-metric multidimensional scaling (NMDS) ordination of weighted averages of plant species cover and mean richness (per $5m^2$ plot) of native and nonnative plant functional groups at all vegetation sampling locations (n = 30) and the direction of significant relationships (in bold). * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Mean Total Richness		Mean Native Richness		Mean Native Shrub Richness		Mean Native Herb Richness		Mean Nonnative Richness	
Aggregated Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Site Characteristics PCA										
Site PC1	ns	0.01	ns	0.04	ns	0.10	ns	0.01	ns	0.01
Site PC2	**-**	**0.24***	**-**	**0.25***	ns	0.01	**-**	**0.39****	ns	0.01
Site PC3	ns	0.05	ns	0.14	ns	0.01	ns	0.14	+	**0.23***
Soil Variables PCA										
Soil PC1	ns	0.02	ns	0.01	ns	0.01	ns	0.01	ns	0.01
Soil PC2	ns	0.05	ns	0.03	ns	0.11	ns	0.01	ns	0.02
Soil PC3	+	**0.22***	+	**0.17***	ns	0.02	+	**0.23***	ns	0.01
NMDS Species Cover										
NMDS1	+	**0.18***	+	**0.17***	ns	0.02	+	**0.25****	ns	0.02
NMDS2	ns	0.02	ns	0.01	ns	0.07	ns	0.04	ns	0.05
NMDS3	+	**0.15***	+	**0.16***	ns	0.07	+	**0.16***	ns	0.10

Table 1.13. Correlations of aggregated variables from principal components analysis (PCA) of site and soil characteristics and the first three axes extracted from non-metric multidimensional scaling (NMDS) ordination of weighted averages of plant species cover and mean percent cover of native and nonnative plant functional groups at all vegetation sampling locations (n = 30) and the direction of significant relationships (in bold). * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Total Native % Cover		Native Shrub % Cover		Native Herb % Cover		Total Nonnative % Cover		Nonnative Grass % Cover		Nonnative Forb % Cover	
Aggregated Variables	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2	Direction	r^2
Site Characteristics PCA												
Site PC1	ns	0.09	**-**	**0.24***	ns	0.12	ns	0.02	ns	0.01	ns	0.02
Site PC2	ns	0.06	ns	0.01	ns	0.13	ns	0.07	+	**0.16***	ns	0.03
Site PC3	ns	0.02	ns	0.01	**-**	**0.15***	ns	0.04	ns	0.02	ns	0.08
Soil Variables PCA												
Soil PC1	+	**0.16***	+	**0.13***	ns	0.01	ns	0.06	ns	0.04	ns	0.04
Soil PC2	**-**	**0.15***	**-**	**0.16***	ns	0.01	ns	0.02	ns	0.01	ns	0.02
Soil PC3	ns	0.01	ns	0.01	ns	0.04	ns	0.05	ns	0.01	**-**	**0.13***
NMDS Species Cover												
NMDS1	ns	0.07	ns	0.01	+	**0.22****	ns	0.07	ns	0.03	ns	0.06
NMDS2	ns	0.01	ns	0.02	ns	0.02	ns	0.02	ns	0.06	ns	0.01
NMDS3	ns	0.04	ns	0.01	+	**0.13***	ns	0.02	ns	0.11	**-**	**0.11***

Table 1.14. Correlations of N deposition, soil N, aggregated variables from principal components analysis (PCA) of site and soil characteristics and the first three axes extracted from non-metric multidimensional scaling (NMDS) ordination of weighted averages of plant species cover and mean percent cover of dominant native and nonnative plant species and the direction of significant relationships (in bold). NS = native shrub, NF = native forb, NSu = native succulent, NSs = native sub-shrub, NG = native grass, NNF = nonnative forb, NNG = nonnative grass. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, ns = not significant.

	Origin/	N Dep.	Soil N	Site Characteristics PCA			Soil Variables PCA			NMDS Plant Species Cover		
Plant Species	Group	(kg ha^{-1} yr^{-1})	(ug N g^{-1})	PC1	PC2	PC3	PC1	PC2	PC3	NMDS1	NMDS2	NMDS3
Acmispon glaber	NS	0.05	0.01	0.04	0.01	0.05	0.01	0.01	**0.31**** +	0.06	0.03	**0.23**** +
Artemisia californica	NS	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	**0.22**** -	0.08	**0.19*** -
Brassica nigra	NNF	0.03	0.02	0.01	0.04	0.06	0.01	0.02	0.01	0.01	**0.20*** +	**0.21*** -
Bromus diandrus	NNG	**0.15*** +	**0.23**** +	0.08	0.06	0.02	0.01	0.01	0.06	0.01	**0.16*** +	0.04
Bromus rubens	NNG	0.01	0.04	0.02	**0.11*** +	0.01	0.01	0.01	0.03	0.01	0.04	0.09
Calystegia macrostegia	NF	0.05	0.04	0.04	0.04	0.03	0.02	0.02	**0.36**** -	0.02	0.04	0.05
Centaurea melitensis	NNF	0.03	0.01	0.03	0.01	0.06	0.01	0.01	0.01	0.01	**0.20*** +	**0.19*** -
Cyptantha clevelandii	NF	0.03	0.02	0.01	0.02	0.03	0.01	0.01	**0.36**** +	0.11	0.03	**0.23*** +
Dichelostemma capitatum	NF	**0.19*** -	**0.30**** -	0.01	**0.27**** -	0.03	0.01	0.04	**0.16*** +	0.02	0.01	0.03
Emmenanthe penduliflora	NF	0.07	0.06	0.06	0.02	0.03	0.05	0.13	0.01	0.01	0.10	0.10
Encelia californica	NS	**0.15*** +	**0.13*** +	0.03	**0.14*** +	0.01	0.01	0.01	**0.19*** -	0.04	**0.56***** +	0.04
Eriogonum fasciculatum	NS	0.01	0.03	0.09	0.02	0.03	0.03	0.01	**0.34**** +	0.08	0.02	**0.38**** +
Erodium cicutarium	NNF	0.01	0.08	0.11	0.04	0.11	0.01	**0.20*** +	0.01	0.01	0.01	0.01
Eucrypta chrysanthemifolia	NF	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.02	0.03	0.08
Hazardia squarrosa	NS	0.01	0.06	0.01	0.02	**0.16*** +	0.01	**0.14*** -	0.01	0.01	0.01	0.01
Hesperoyucca whipplei	NSu	0.04	**0.20*** -	0.03	0.05	0.07	0.01	0.03	0.02	**0.17*** +	0.01	**0.20*** +
Hirschfeldia incana	NNF	**0.16*** +	**0.18*** +	0.01	**0.24*** +	0.02	0.07	0.02	0.08	0.07	**0.28*** +	0.01
Malosma laurina	NS	**0.25*** +	0.03	0.11	**0.23*** +	0.05	0.06	0.01	0.01	**0.20*** -	**0.17*** +	0.03
Marah macrocarpus	NF	0.07	0.08	0.10	0.06	0.01	0.01	0.01	0.11	0.02	0.05	0.03
Mirabilis laevis	NSs	**0.19*** -	0.02	0.01	0.07	0.12	0.05	0.01	0.02	**0.28**** +	0.01	0.02
Phacelia cicutaria	NF	0.02	0.01	0.02	0.10	0.08	0.03	0.02	0.02	0.07	0.01	0.01
Rafinesquia californica	NF	0.02	0.05	0.02	0.04	0.01	0.03	0.01	0.06	0.01	**0.21*** -	0.07
Salvia leucophylla	NS	0.05	0.05	0.04	0.11	0.14	**0.18*** +	0.01	0.01	0.01	**0.40*** -	**0.63***** -
Salvia mellifera	NS	0.01	0.03	0.01	0.04	0.01	0.01	0.01	0.01	**0.56*** +	0.05	0.19
Stipa pulchra	NG	0.06	**0.14*** -	0.03	0.02	0.01	0.01	0.01	**0.15*** +	0.01	0.01	0.01

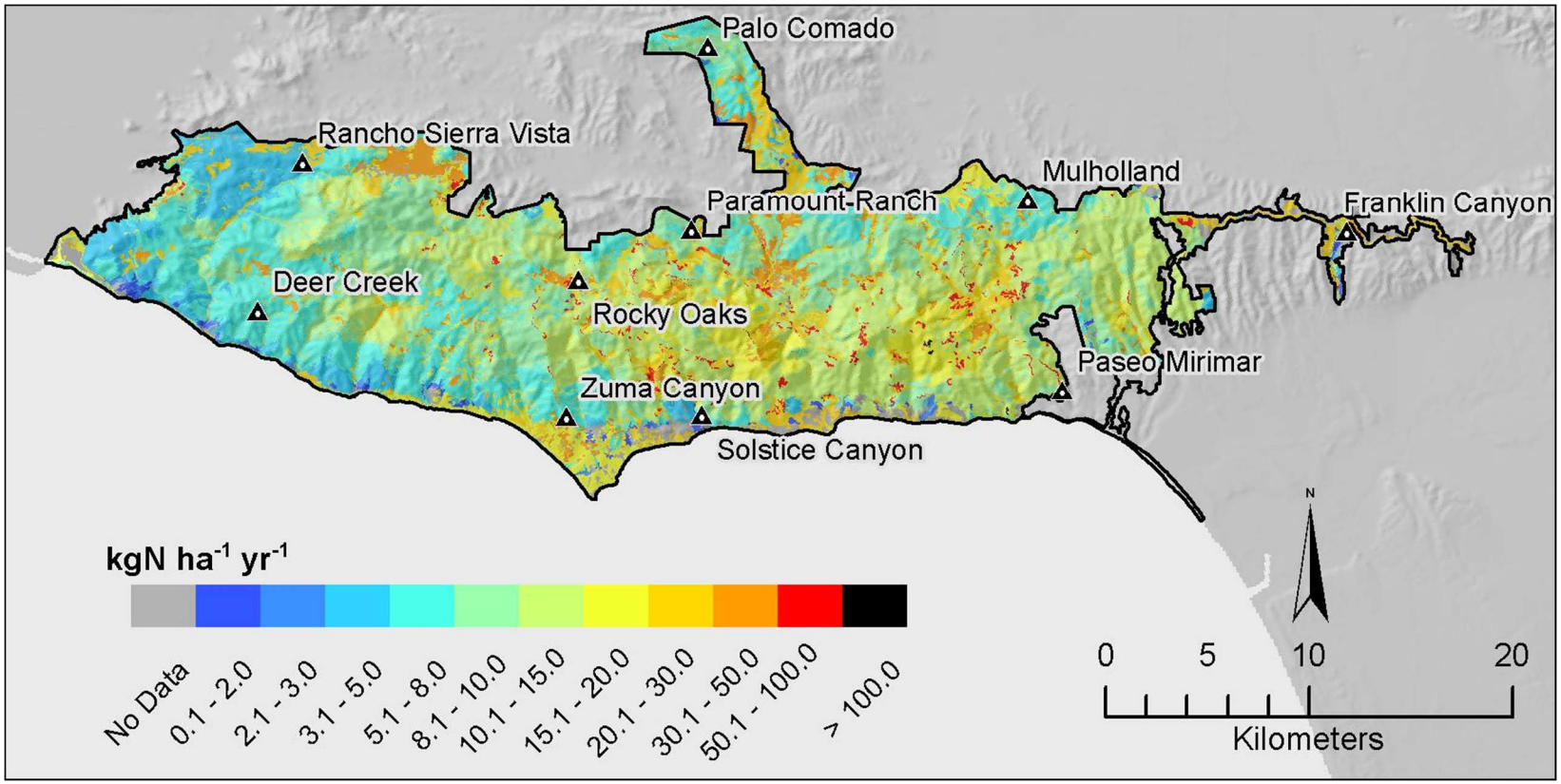

Figure 1.1. Estimation of total N deposition across the Santa Monica Mountains NRA based on atmospheric concentrations, land cover, deposition velocities and LAI. Atmospheric monitoring sites are represented by triangles and labeled with site names.

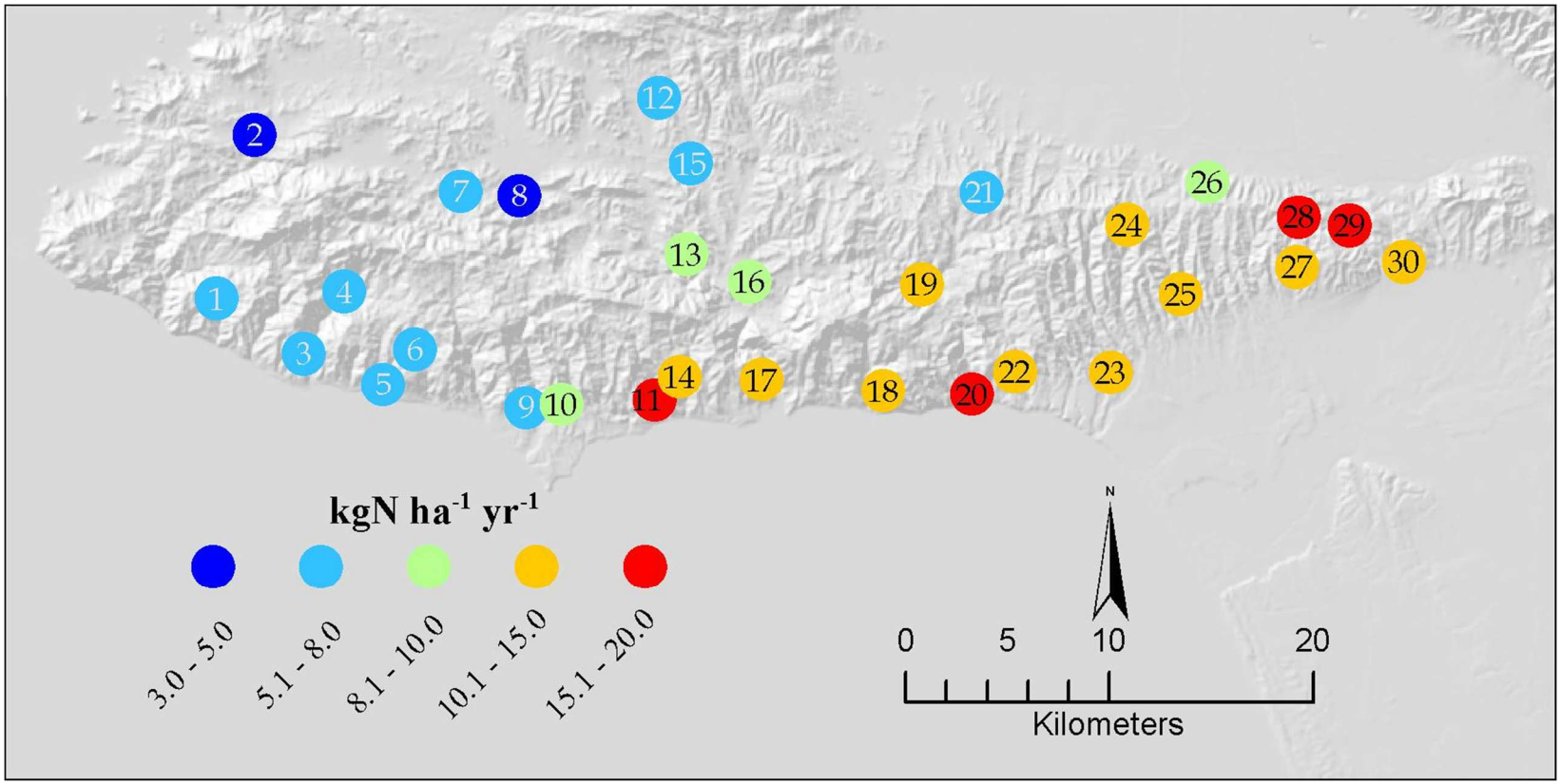

Figure 1.2. Location and estimated rates of N deposition for CSS vegetation sampling sites across the Santa Monica Mountains NRA (n = 30). Site names and numbers are shown in Table 2.

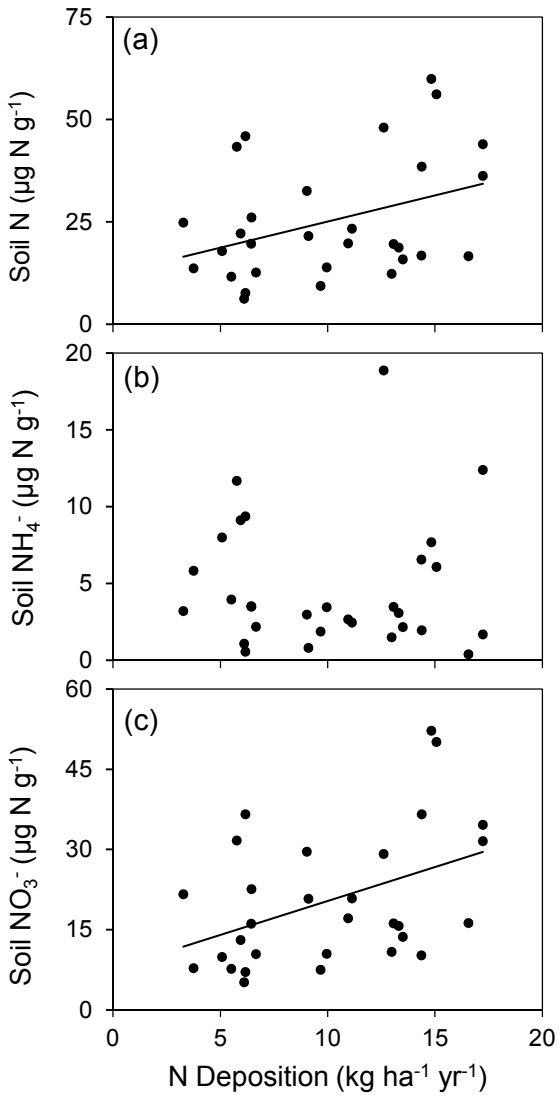

Figure 1.3. Correlations between estimated N deposition and (a) total soil extractable N, (b) soil extractable NH_4-N and (c) soil extractable NO_3-N at 30 vegetation sampling sites. Presence of a regression line indicates the correlation was significant ($\alpha = 0.05$).

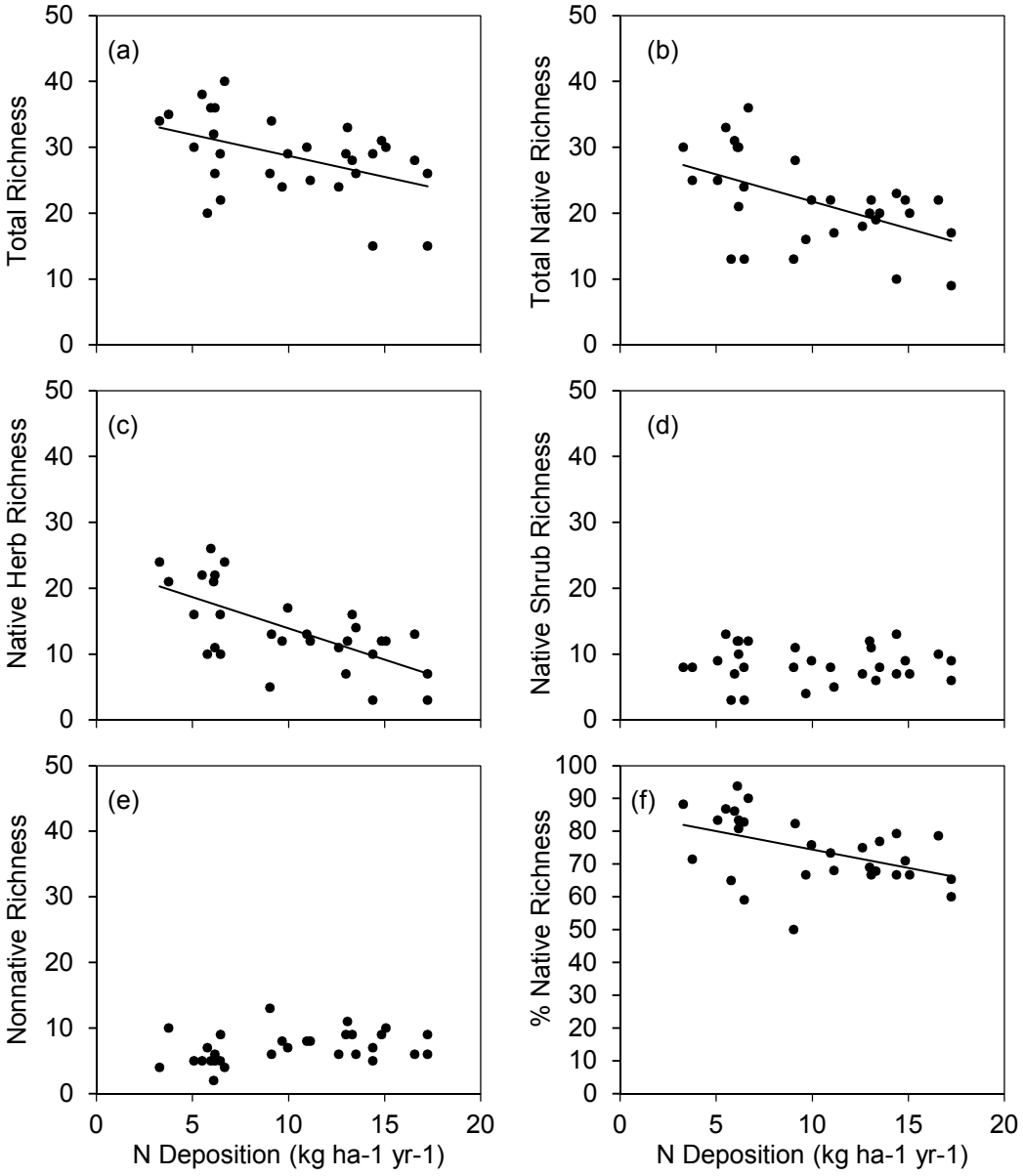

Figure 1.4. Correlations of total plant richness by native and nonnative functional group and percent native richness and calculated N deposition at each vegetation sampling site (n = 30), including (a) total plant richness, (b) total native richness, (c) total native richness of herbaceous species, (d) total native shrub richness, (e) total nonnative richness and (f) percent native richness. Presence of a regression line indicates significance (α = 0.05).

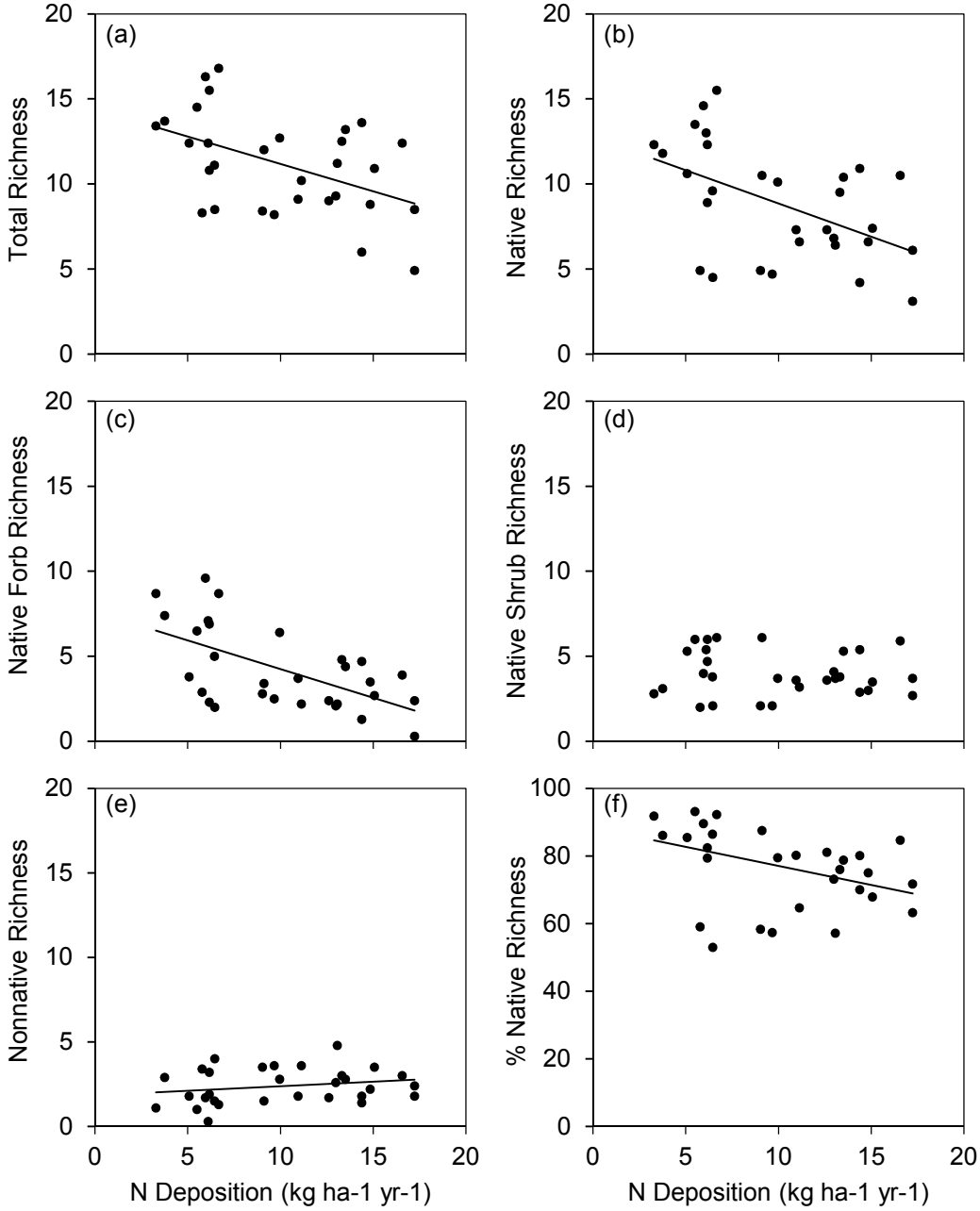

Figure 1.5. Correlations of mean plant richness per plot by native and nonnative functional group and percent native richness and calculated N deposition at each vegetation-sampling site (n = 30), including (a) total richness, (b) total native richness, (c) native forb richness, (d) native shrub richness, (e) nonnative richness and (f) mean percent native richness. Presence of a regression line indicates significance ($\alpha = 0.05$).

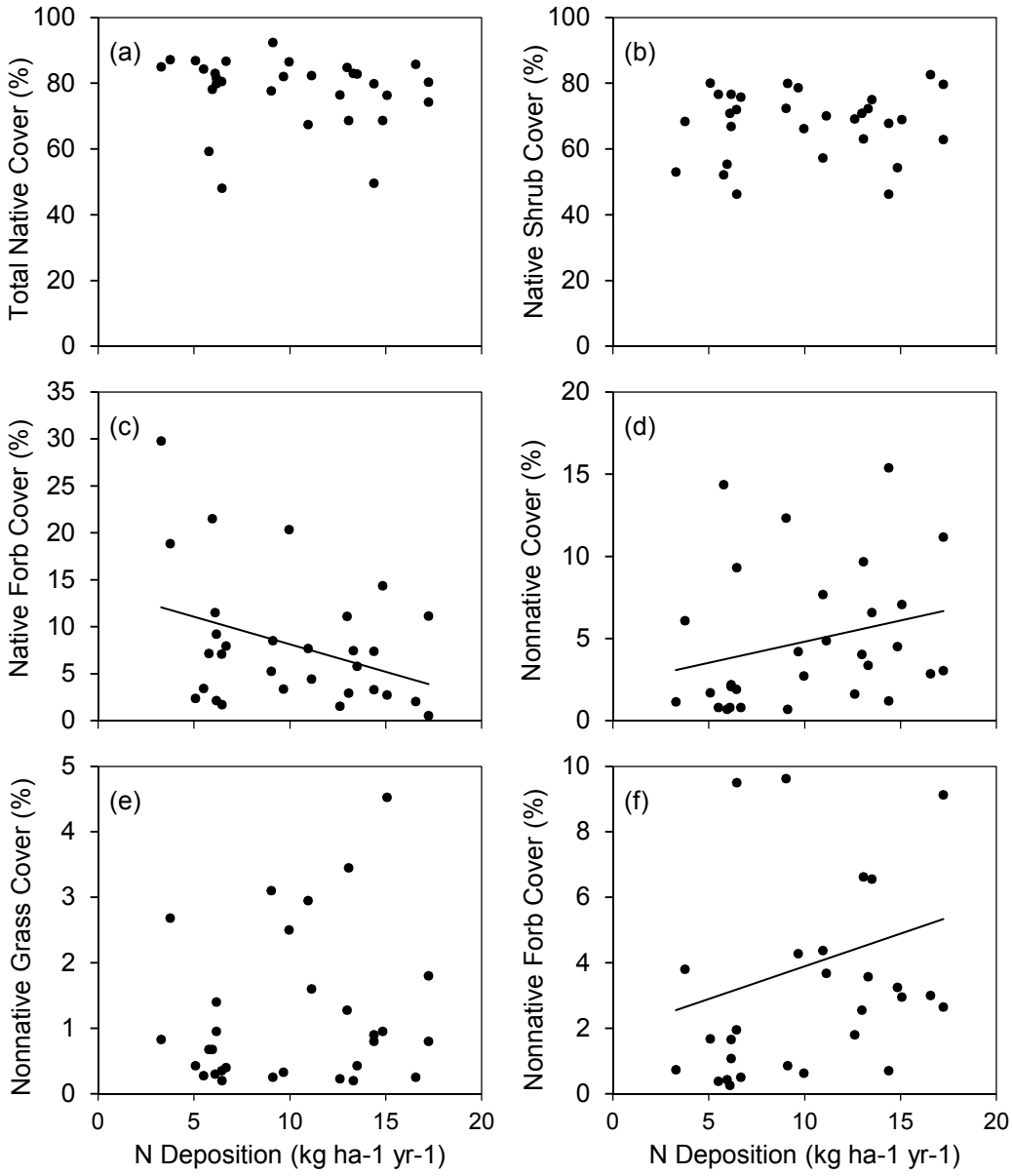

Figure 1.6. Correlations of percent cover of native and nonnative functional groups with N deposition, including (a) total native percent cover, (b) percent cover of native shrubs, (c) percent cover of native herbaceous species, (d) total nonnative cover, (e) nonnative annual grass cover and (f) nonnative forb cover. Presence of a regression line indicates significance ($\alpha = 0.05$).

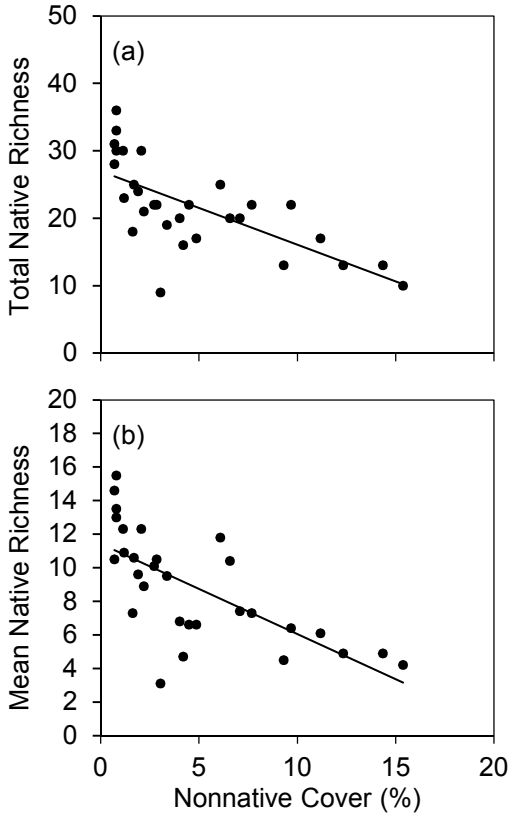

Figure 1.7. Correlations of (a) total native richness and (b) mean number of native species per $5m^2$ plot at each vegetation sampling location (n = 30) with mean percent cover of nonnative species. Note different *y*-axis labels and scale. Presence of a regression line indicates a significant relationship ($\alpha = 0.05$).

Experimental nitrogen deposition promotes shrub dieback and invasion of California coastal sage scrub during extreme drought

Abstract

Increased anthropogenic nitrogen (N) deposition may exacerbate negative ecological effects of other global change drivers, such as drought and nonnative plant invasion. Understanding these impacts will be increasingly important under predicted changes in climate, increased rates of N deposition and the growing globalization of species. In southern California, high levels of N deposition have resulted in widespread invasion of coastal sage scrub (CSS), a threatened shrubland community consisting of many drought-deciduous shrub species and a diverse array of herbaceous annuals and perennials. Significantly below-average rainfall has led to dieback of perennial CSS vegetation in the region, and N deposition could play an important role in shaping community response to prolonged drought. I investigated effects of multiple realistic levels of simulated dry N deposition on woody and herbaceous vegetation of CSS from 2011 to 2015, a period coinciding with extreme drought conditions. I hypothesized N addition would increase native shrub productivity, but that this would increase susceptibility to drought and result in increased shrub loss over time. I also predicted that N addition would favor nonnatives, particularly annual grasses, leading to higher biomass and cover of these species. Over four years, all levels of N addition resulted in increased soil N availability. I found high N availability contributed to greater dieback of native shrub cover. This was likely due to increased productivity in shrubs subject to high levels of N addition, as evidenced by

increased leaf area in high N shrubs and high leaf litter production early in the study. After four years of N addition, high N plots showed the lowest shrub cover and leaf production, but the highest amount of woody litter production, further suggesting these shrubs experienced increased dieback. Carbon isotope analysis also revealed that N addition reduced water-use efficiency of native shrubs, potentially contributing to dieback during the extended drought. Experimental N addition also resulted in increased cover and biomass of nonnative species in some years, particularly annual forbs. Together, these results suggest the impacts of drought on this ecosystem may be more severe under elevated N deposition, potentially contributing to vegetation type-conversion.

Introduction

Terrestrial plant communities are increasingly threatened by multiple components of global environmental change, including anthropogenic nitrogen (N) deposition (Vitousek et al. 1997, Galloway 2005, Bobbink et al. 2010), climate change and associated extreme weather events (Meehl et al. 2000, Walther et al. 2002, Bell et al. 2004) and the invasion of nonnative species (Vitousek et al. 1996, Levine and D'Antonio 2003). These factors may have complex and interactive effects on ecosystems, making it difficult to predict ecological impacts under future global change (Dukes and Mooney 1999, Tylianakis et al. 2008). In southern California, high rates of N deposition due to atmospheric pollution have been implicated in the widespread conversion of native coastal sage scrub (CSS) shrublands to grasslands dominated by nonnative annuals (Allen et al. 1998, Fenn et al. 2010). The region has also experienced a multi-year, record-breaking drought, beginning in 2011 (Griffin and Anchukaitis 2014, Coates et al. 2015), which may exacerbate the negative impacts of elevated N deposition and nonnative plant invasion on this ecosystem (Kimball et al. 2014, Valliere and Allen 2016).

Along with other mediterranean-climate ecosystems worldwide, such as the Chilean mattoral, Spanish maquia, South African fynbos and Australian kwongan, California's mediterranean-type vegetation has been identified as a biodiversity "hotspot," constituting a major conservation priority (Mittermeier et al. 1999, Myers et al. 2000). These mediterranean eco-regions may be particularly vulnerable to changes in climate (Hannah et al. 2002, Midgley et al. 2002, Loarie et al. 2008) and increased levels of N deposition (Sala et al. 2000, Phoenix et al. 2006, Ochoa-Hueso et al. 2013), but the

impacts of these often co-occurring drivers of global change simultaneously on CSS is poorly understood. This severely threatened, semi-arid plant community consists of a number of drought-deciduous shrub species and a diverse array of herbaceous annuals and perennials (Barbour et al. 2007). Highly impacted by development and other human-caused disturbances, CSS has been reduced to as little as ten percent of its historic range (Westman 1981), and what remains is increasingly subject to elevated N deposition and invasion of nonnatives (Allen et al. 2005, Talluto and Suding 2008, Cox et al. 2014).

Annual grasses and forbs represent the major plant invaders of mediterranean-climate ecosystems worldwide, including CSS (Fox 1990, Funk et al. 2016). Under some conditions, native shrublands may undergo conversion to systems dominated by a few nonnative invaders, such as the conversion of CSS to nonnative annual grasslands dominated by Eurasian species (Minnich and Dezzani 1998, Stylinski and Allen 1999, Gaertner et al. 2009). Fire often plays a key role in the invasion of these species, and annual grasses, such as *Avena* and *Bromus* species typically dominate in CSS following fire (O'Leary and Westman 1988, Callaway and Davis 1993, Keeley 2002, Kimball et al. 2014). Once established, these annual grasses increase fine fuel loads, resulting in larger and more frequent fires -- a positive feedback loop that further facilitates invasion (D'Antonio and Vitousek 1992, Vilà et al. 2001, Brooks et al. 2004). However, invasion and vegetation-type conversion may also occur in the absence of fire, possibly in response to other disturbances, such as N deposition and drought (Cox et al. 2014).

Southern California experiences some of the highest levels of N deposition in the U.S., with levels ranging from 20 – 45 kg ha^{-1} yr^{-1} or greater found in urban areas (Fenn

et al. 2003). As in other dryland ecosystems, this falls largely as dry N deposition during the summer dry period, entering the system as a sudden pulse of N with the first rains of the winter growing season (Allen et al. 1998). The resulting increase in soil N availability appears to favor nonnative annual species, particularly grasses, over natives (Allen et al. 1998, Wood et al. 2006, Kimball et al. 2014), though this has not been observed in all cases (Vourlitis and Pasquini 2009).

As in other mediterranean-type ecosystems, California's CSS experiences highly variable precipitation from year to year, as well as within years (Haston and Michaelsen 1997, Jones 2000, Lana et al. 2008), with rainfall variability and extreme drought events expected to increase under predicted global climate change (Jones 2000, Bell et al. 2004). Native CSS species are adapted to withstand short-term seasonal drought through avoidance: most shrub species are drought deciduous; herbaceous perennials survive belowground as roots, bulbs or tubers; and annuals persist in the seed bank. However, prolonged drought may result in canopy dieback and mortality of perennial species due to hydraulic failure, depletion of carbohydrate reserves or increased susceptibility to herbivory and other stressors (McDowell et al. 2008, McDowell et al. 2013) and drought has been cited as a possible cause of conversion of CSS to nonnative annual grasslands (Minnich and Dezzani 1998, Steers 2011, Kimball et al. 2014). Kimball et al. (2014) for example, found post-fire recovery was lower under experimentally reduced precipitation, which supports previous correlative studies documenting reduced CSS shrub cover with increasing aridity (Keeley et al. 2005), and a positive relationship between shrub recruitment and precipitation (Keeley et al. 2006). While native CSS species may also

exhibit positive growth responses under elevated N deposition in the absence of competition from nonnatives (Padgett and Allen 1999), this could increase susceptibility to drought (Bobbink and Lamers 2002, Valliere and Allen 2016). Work in other ecosystems has found higher plant productivity due to N deposition may decrease plant drought tolerance, resulting in increased dieback and mortality (Bobbink and Lamers 2002, Friedrich et al. 2012, Meyer-Grünefeldt et al. 2015), but this has not been previously documented in CSS.

Between 2011 and 2015, California experienced an exceptionally severe drought event, driven by below-average precipitation combined with record high temperatures (Griffin and Anchukaitis 2014, Diffenbaugh et al. 2015, Fahrenkamp-Uppenbrink 2015). This resulted in widespread increases in plant water-stress at the landscape level (Asner et al. 2016) and dieback and mortality of woody perennial vegetation, including CSS (Coates et al. 2015). This has the potential to impact long-term vegetation dynamics, and loss of native CSS shrub species and the resultant canopy opening could create a window of opportunity for the spread of nonnative plant species. Furthermore, N deposition could intensify this effect by contributing to higher mortality of native CSS perennial species while simultaneously increasing growth and performance of nonnative annuals, but the impacts of N inputs on CSS during this period have yet to be investigated.

Both N deposition and drought may favor conversion of CSS to nonnative annual grasslands (Minnich and Dezzani 1998, Allen et al. 2005, Cox et al. 2014, Kimball et al. 2014), and these factors may also have interactive effects. When reduced water-inputs were coupled with N addition, for example, Kimball et al. (2014) found post-fire

recovery of native species was lower than with either of these factors alone. This has the potential to exert a strong influence on ecological feedback loops in this ecosystem. For example, if both drought and N deposition increase the growth of nonnative annuals, this is likely to promote the occurrence and spread of wildfire, especially during prolonged dry conditions, exacerbating the grass-fire cycle (D'Antonio and Vitousek 1992, Vilà et al. 2001) and further altering historic fire regimes (Zedler et al. 1983, Malanson 1984, Minnich and Dezzani 1998, Keeley 2002). As human activities continue to contribute to elevated atmospheric N inputs (Vitousek et al. 1997, Galloway 2005), changes in climate (Meehl et al. 2000, Bell et al. 2004, Loarie et al. 2008) and the global homogenization of species (Vitousek et al. 1996, Hobbs 2000), understanding the ecological consequences of these factors in concert will be increasingly important.

In this study I explored the impacts of multiple realistic levels of simulated N deposition on CSS vegetation of the Santa Monica Mountains, southern California, for four years during an extreme, multi-year drought. I asked: (1) How do different levels of N addition influence native CSS shrub cover and productivity during extended drought? (2) How do N inputs influence the CSS understory herbaceous community? (3) How does N addition influence cover and biomass of nonnative annuals? I hypothesized that N addition would initially increase native shrub productivity, but that this would increase susceptibility to drought and result in reduced shrub cover and growth over time. I also predicted N addition would result in increased cover and biomass of nonnative annual species. Our results have important implications for the conservation of mediterranean-type ecosystems in a changing world.

Materials and Methods

Study Site

I conducted field experiments at Rancho Sierra Vista (34.15° N, 118.96° W), a unit of the National Park Service located in the foothills of the Santa Monica Mountains, southern California, USA. The region experiences a typical mediterranean-type climate, with hot dry summers, and variable precipitation occurring during the cooler winter months. Mean annual precipitation at the site is 420 mm, however rainfall has been below average since the study was initiated in 2011 (Fig. 1). Modeled rates of N deposition are approximately 8.8 kg N ha^{-1} yr^{-1} (Tonnesen et al. 2007, Fenn et al. 2010). The site last burned in 1993.

Precipitation data

While drought was not initially considered as a factor in the experimental design, implementation of the study coincided with the onset of the extreme drought in California in 2011. I obtained daily precipitation data for the study site from the PRISM Climate Group at Oregon State University, USA (http://prism.oregonstate.edu, September 2015). From this data, I calculated annual and monthly rainfall totals during the study period, as well as 30-year averages (Fig. 1).

Nitrogen Addition Plots

I installed experimental N addition plots in November 2011, prior to the first rains of the growing season. Plots measured 6 × 6 m and were located in mature CSS

dominated by *Artemisia californica* Less. (Asteraceae), a drought-deciduous shrub that is the foundation species of CSS. Plots were subsequently fertilized annually in the fall to simulate the accumulation of dry N deposition during summer months. Fertilizer was applied in dry form at the time of the first rainfall each fall (November or December). Plots received one of four rates of N addition from calcium nitrate (CaN_2O_6) and urea (CH_4N_2O): control = 0 kg N ha^{-1}; low = 5 kg N ha^{-1}; medium = 15 kg N ha^{-1}; high = 30 kg N ha^{-1}. I included ten replicate plots at each N addition level in a randomized block design. These rates correspond to realistic ranges of N deposition found within the Santa Monica Mountains (Tonnesen et al. 2007).

Soil Nitrogen

Between 2011 and 2015, I collected soils for extractable N analysis seasonally, sampling during the winter growing season and again during the summer dry period once vegetation had senesced. Within each plot, I took three 10 cm deep soil cores at the drip-line of mature *A. californica* shrubs. Soil N was extracted using potassium chloride (KCl) extraction and analyzed using an AQ2 Discrete Analyzer (SEAL Analytical, Inc., Southampton UK) at the University of California, Riverside.

Vegetation Sampling

Vegetation sampling was done in the interior 5 × 5 m of plots to limit edge effects. I measured shrub cover along 15 m of transects within N addition plots each year in the spring between 2012 and 2015 at the peak of the growing season. I also collected

fresh leaf tissue for determination of leaf N and C content and measured shrub volume of marked *A. californica* shrubs within plots to detect possible changes in biomass. Biomass of mature *A. californica* shrubs was estimated using regressions of volume and biomass for this species developed by Vourlitis and Pasquini (2009). I also sampled cover of herbaceous vegetation by species in shrub interspaces using four replicate 1.0 × 0.5 m quadrats per plot, beginning in 2012. Cover of each species was estimated to the nearest 1% in gridded frames. In a subset of quadrats, vegetation was clipped to determine plant biomass. Herbaceous biomass was then estimated using regression equations from ln-transformed cover and dry mass data. Finally, each year I tracked species richness within plots.

Leaf Traits

In the spring of 2013 and 2014, I collected terminal branches representing the new season's growth of *A. californica* shrubs for measurement of leaf-level traits including leaf area (LA), specific leaf area (SLA) and leaf:sapwood area ratio (LA:SA). I collected three branches from one shrub per plot in each of the N addition treatments. Samples were transported to the laboratory where leaf area was measured using a LI-3100C Area Meter (LI-COR, Inc., Lincoln, Nebraska USA). Sapwood area was estimated by taking the diameter of the stem, and converting this value to the area of a circle. Leaf tissue from *A. californica* shrubs was dried for 48 hours at 60° C, ground and analyzed for percent C and N using a Thermo-Finnigan FlashEA 1112 Nitrogen and Carbon Analyzer (Thermo

Fisher Scientific, Waltham, Massachusetts, USA) at the University of California, Riverside, Environmental Science Research Laboratory.

Leaf $\delta^{13}C$

I also analyzed four years of leaf tissue samples from A. californica shrubs in control and high N addition plots for leaf carbon isotopic composition ($\delta^{13}C$) for the assessment of carbon gain versus water loss (intrinsic water-use efficiency; WUE). The carbon isotopic composition of C_3 plant material is largely regulated by the CO_2 concentration at the site of carboxylation (intercellular CO_2 concentration, ci), which determines the degree of carbon isotope fractionation during CO_2 assimilation (Farquhar et al. 1982). Because ci represents the balance between supply through stomata and uptake by photosynthesis, it is also related to the ratio of carbon gain to water loss and therefore is a reliable proxy for photosynthetic WUE within a species (Farquhar and Richards 1984). Values for $\delta^{13}C$ were determined with an elemental analyzer (ECS 4010, Costech Inc., Valencia, CA) interfaced with an isotope ratio mass spectrometer (Delta V Advantage; Thermo Scientific, Bremen, Germany) at the University of California Facility for Isotope Ratio Mass Spectrometry (FIRMS), Riverside, California, USA.

Productivity

To measure potential changes in litter production and shrub productivity, at the end of the 2012 growing season, I installed litter traps below mature *A. californica* shrubs in plots receiving three levels of N addition (0, 1.5 and 3 g N m^{-2}). Litter traps consisted

of a 20 × 25 cm rectangular wooden frame 5 cm in height with a stainless steel mesh bottom. Litter samples were collected regularly until summer 2015 (every 2-4 months depending on litter fall), dried at 60° C, sorted into leaf and woody litter and weighed.

Decomposition

For three growing seasons, from 2012 to 2015, I also deployed litter bags in N addition plots to better understand effects on N cycling and litter decomposition. Each summer, freshly senesced leaf litter was collected from *A. californica* shrubs within each N treatment. I then dried samples at 60° C, and filled 10 cm^2 nylon mesh bags with 1 g samples of leaf litter. I placed two replicate litter bags in each plot and collected half after 3 months of exposure, and the remainder after 6 months. I dried collected litter at 60° C and weighed samples to calculate mass lost. I also analyzed percent C and N of fresh leaves and litter at each stage of decomposition using a Thermo-Finnigan FlashEA 1112 Nitrogen and Carbon Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the University of California, Riverside.

Data Analysis

I used repeated measures analysis of variance (ANOVA) to assess whether N addition caused significant variation in soil N, leaf tissue N, leaf tissue $\delta^{13}C$, shrub biomass, cover and productivity, native and nonnative herbaceous cover and biomass, and species richness. I interpreted a significant direct effect of N treatment or N × time interaction as evidence that N addition had resulted in significant changes to the plant

community. Prior to analysis, I tested data for variance-covariance homogeneity and sphericity using Box's *M* and Mauchly's tests respectively. Data that violated these assumptions was analyzed using Geisser-Greenhouse corrections. Leaf level traits and results of the decomposition study were analyzed within year using ANOVA. I also used ANOVA to test the effects of N addition on soil N, leaf tissue N, leaf tissue $\delta^{13}C$, shrub biomass, cover and productivity, native and nonnative herbaceous cover and biomass, and species richness within years. Statistical analyses were conducted using SAS (version 2013; SAS Institute Inc., Cary, North Carolina, USA).

Results

Precipitation

Precipitation was below average for the duration of the study (Fig. 1), with rainfall totals during the winter growing season, November to May, ranging from 35% to 44% of 30-year averages. Monthly rainfall totals were below 30-year averages 40 of the 49 months of the study.

Soil Nitrogen

Nitrogen addition significantly enhanced soil N availability over time (Fig. 2), as evidenced by a significant effect of N ($F_{3,308} = 19.14$, $P < 0.0001$), time ($F_{1,308} = 64.73$, $P < 0.0001$) and the interaction of N × time ($F_{3,308} = 8.08$, $P < 0.0001$). Early in the study, soil N did not differ among treatments, but after two years N availability was incrementally higher in each of the N addition treatments.

Shrub Cover and Biomass

The dominant shrub species within plots was *A. californica*, accounting for over 95% of shrub cover each year. Native shrub cover was significantly influenced by N addition ($F_{3,148} = 3.12$, $P = 0.0280$), time ($F_{1,148} = 142.17$, $P < 0.0001$) and the interaction of N × time ($F_{3,148} = 2.77$, $P = 0.0437$; Fig. 3a). Shrub cover was similar among N treatments from 2012 to 2013; however in 2014, cover was greatly reduced in all treatments, with High N plots having lowest mean cover. In the final year of the study, shrub cover remained the lowest in High N and Medium N plots, while Control plots had the highest mean percent cover. Mean biomass of *A. californica* shrubs varied through time ($F_{1,148} = 10.06$, $P = 0.0018$), but was not significantly affected by N addition (N, $F_{3,148} = 0.20$, $P = 0.8954$; N × Time, $F_{3,148} = 0.08$, $P = 0.9715$; Fig. 3b).

Shrub Productivity

Leaf litter production of *A. californica* shrubs was significantly influenced by time ($F_{1,81} = 69.94$, $P < 0.0001$) and the interaction of N × time ($F_{2,81} = 6.24$, $P = 0.0300$; Fig. 4a). After one year of N addition, shrubs within High N plots produced the highest amount of leaf litter. However, in subsequent years, leaf litter production was reduced in all treatments, and in the final year shrubs within Control plots produced higher leaf litter than those in N addition plots. Production of woody litter was significantly influenced by N ($F_{2,81} = 7.98$, $P = 0.0007$), with shrubs within High N plots dropping the highest amount of woody litter in two out of the three years measured (Fig. 4b). Time ($F_{1,81} =$

3.05, $P = 0.0843$) and the interaction of N × time ($F_{2,81} = 0.13$, $P = 0.8825$) had no significant effect (Fig. 4b).

Leaf Traits

N addition resulted in significant increases in leaf area of *A. californica* shrubs in both years measured (Table 1). In 2014, shrubs within Medium and High N addition plots also exhibited significantly higher sapwood area of terminal branches (Table 1). There were no significant effects of N addition on specific leaf area or the ratio of leaf area to sapwood area. I also observed significant effects of simulated N deposition on C and N content of *A. californica* leaves, with N addition leading to higher percent N, and reduced C:N ratios in three of the four years measured (Table 2). In 2014 and 2015, this increase in N content was also accompanied by a significant reduction in percent leaf C (Table 2).

Decomposition

Results from the litter bag decomposition differed in the two years the study was conducted. During the 2013-14 growing season, I observed no significant effect of N addition on mass loss or decomposition constants (Table 3). In 2015, litter in N addition plots had significantly less mass remaining at 180 days and exhibited higher decomposition rates (Table 3).

Leaf $\delta^{13}C$

Carbon stable isotope ratios of leaf tissue from *A. californica* shrubs varied by year (Time, $F_{1,38}$ = 3.48, P = 0.023) and N addition (N, $F_{1,38}$ = 6.79, P = 0.0141), with shrubs from control plots exhibiting significantly higher (less negative) $\delta^{13}C$ values in two years, 2013 and 2014, compared to shrubs from high N addition plots (Fig. 5). The lower (more negative) $\delta^{13}C$ of leaves from high N shrubs indicates reduced intrinsic WUE relative to control plots (Farquhar et al. 1989).

Herbaceous Vegetation

The cover and biomass of herbaceous vegetation varied by year (Time, P < 0.0001 for all analyses), with higher cover and biomass observed during the 2013 and 2015 growing seasons (Fig. 6a-f). Increased N availability had no effect on total native herbaceous cover (N, $F_{3,148}$ = 0.82, P = 0.48; N × Time, $F_{1,148}$ = 0.88, P = 0.4548; Fig. 6a), but lead to significant increases in nonnative cover in some years (N, $F_{3,148}$ = 5.22, P = 0.0019; Fig. 6b). However, when analyzed by functional group, only nonnative forb cover responded significantly to N addition (N, $F_{3,148}$ = 7.29, P = 0.0001; N × Time, $F_{1,148}$ = 2.85, P = 0.0393; Fig. 6c), not nonnative annual grasses (N, $F_{3,148}$ = 2.21, P = 0.0895; N × Time, $F_{1,148}$ = 0.48, P = 0.6965; Fig. 6d).

Nitrogen addition significantly increased native herbaceous biomass (N, $F_{3,148}$ = 2.72, P = 0.0453; N × Time, $F_{1,148}$ = 3.37, P = 0.0201; Fig. 6e), due largely to increased biomass in High N plots in 2015. Nonnative biomass was generally higher and also responded positively to N addition (N, $F_{3,148}$ = 15.96, P < 0.0001; N × Time, $F_{1,148}$ = 5.70,

P = 0.0010; Fig. 6f), with increased biomass observed in Medium and High N addition plots in 2013 and 2015.

Species Richness

Native species richness increased over time (Time, $F_{1,148}$ = 183.11, P < 0.0001), with the highest number of species occurring in 2015. This was due to higher occurrences of native annual and herbaceous perennial species within plots with time. There was no significant effect of N addition (N, $F_{3,148}$ = 0.53, P = 0.6620; N × Time $F_{3,148}$ = 0.58, P = 0.6620) on the number of native species. Nonnative species richness was generally lower than native richness, but showed a similar pattern increasing over time (Time, $F_{1,148}$ = 96.27, P < 0.0001), with no significant effect of N addition (N, $F_{3,148}$ = 2.48, P = 0.6420; N × Time $F_{3,148}$ = 0.72, P = 0.5437).

While *A. californica* was the dominant shrub species at our site, other woody perennials, such as *Acmispon glaber* and *Baccharis pilularis,* were also present in much smaller numbers. The most frequently observed native herbaceous species were *Chenopodium californicum*, *Crassula connata*, *Cryptantha intermedia*, *Deinandra fasciculata* and *Dichelostemma capitatum*. Nonnative species included forbs, mainly *Centaurea melitensis*, *Erodium cicutarium* and *Hirschfeldia incana*, and the annual grasses *Bromus diandrus*, *Bromus rubens*, *Lamarckia aurea*, and *Schismus barbatus.*

Discussion

My results demonstrate N addition contributes to dieback of native CSS shrubs and concomitant increases in nonnative annual plant species during an extreme drought event in southern California. This work supports the hypothesis that N deposition may increase the severity of the negative ecological consequences of other global change factors, such as changes in climate and nonnative plant invasions (Dukes and Mooney 1999, Bobbink et al. 2010). Previous work in this ecosystem has shown N addition and reduced precipitation results in lower shrub recovery and increased nonnative cover following fire (Kimball et al. 2014), while similar studies conducted in mature CSS did not produce such effects (Vourlitis and Pasquini 2009). However, this study suggests that these vegetation shifts may occur in mature shrublands subject to N deposition during periods of severe drought, even in the absence of fire.

Dry-season N addition resulted in a dramatic increase in soil N availability over time in all plots receiving supplemental N, with levels as high as 100 ug N g^{-1} in plots receiving 3 g N m^{-2} yr^{-1}, five times those typical of sites receiving ambient levels of N deposition (Padgett et al. 1999, Fenn et al. 2010). As in other dryland ecosystems, reactive N inputs resulting from atmospheric pollution typically accumulate on soil and plant surfaces as dry deposition during the summer dry period, later becoming available for uptake with the first winter rains. Thus, soil N availability at high deposition sites peaks prior to the first rains of the growing season, after which N decreases to levels comparable with low deposition sites as N is immobilized and leached from the soil (Padgett et al. 1999, Fenn et al. 2003). However, my results show that during extended

periods of low rainfall, N continues to accumulate in the soil. This suggests that during prolonged drought, even relatively low chronic inputs of N could result in significantly elevated soil N availability.

The accumulation of soil N I observed with N addition is likely due to low plant activity and uptake and reduced leaching during drought. I observed higher foliar N concentrations in native shrubs and increased litter-fall in N additions plots, which could have also contributed to soil N pools. I also observed increased rates of decomposition in one year of the study. Others have found increased rates of mineralization with N addition (Sirulnik et al. 2007, Vourlitis et al. 2007), and this may have had an effect in my soils. Nonnative species can also strongly influence nutrient cycling (Ehrenfeld 2003), and there is evidence that nonnative annual grasses may increase pools of N and rates of mineralization and nitrification (Hawkes et al. 2005, Parker and Schimel 2010). Thus, higher nonnative cover and biomass in N addition plots could have also contributed to observed levels of N availability.

Over four years of below-average rainfall, native shrub cover was greatly reduced due to dieback of branches and entire shrubs, mostly *A. californica*. This is consistent with previous reports of shrub mortality during extended drought (Minnich and Dezzani 1998) and recent documentation of CSS dieback in southern California during the same study period using hyperspectral and infrared aerial imagery (Coates et al. 2015). Similar shrub loss was widely documented in Spanish shrublands during a period of severe drought (Peñuelas et al. 2001). I found shrub dieback was particularly pronounced in plots receiving high levels of N addition (3 g N m^{-2} yr^{-1}), which exhibited the greatest

shrub loss, largely between 2013 and 2014, and had the lowest shrub cover in the final years of the study. I also observed increased woody litter production in shrubs subject to the highest level of N addition, which further reflects the increased shrub dieback indicated by shrub cover data. This strongly suggests the potential for N deposition to exacerbate shrub loss during extended drought.

Nitrogen deposition may increase plant susceptibility to drought by stimulating higher rates of growth and productivity (Bobbink et al. 2010, Friedrich et al. 2012, Meyer-Grünefeldt et al. 2015). In European heathlands, for example, increases in shrub productivity under elevated N deposition has been found to increase plant susceptibility to other secondary factors, such as drought (Bobbink and Lamers 2002). My data suggest this may also occur in semi-arid shrublands subject to N deposition. Many native CSS species, such as *A. californica* are adapted to seasonal drought, losing their leaves during the summer dry season to in response to water deficit (Harrison et al. 1971, Kirkpatrick and Hutchinson 1980). However, with sufficient soil moisture these species grow rapidly and exhibit high rates of gas exchange, which may result in increased susceptibility to xylem embolism (Harrison et al. 1971, Kolb and Davis 1994, Jacobsen et al. 2007), and this could be especially true under high N conditions. Pivovaroff (2016) found *A. californica* to be very physiologically responsive to N addition in the field, increasing rates gas exchange and water use, which could increase susceptibility to cavitation and reduce drought tolerance. The initial higher leaf litter production and increased leaf area I observed in shrubs from high N plots demonstrate shrubs responded to N addition with increased growth productivity. Higher leaf area in shrubs subject to high levels of N

addition also likely increased evaporative loss (Pivovaroff et al. 2016), which could have depleted soil moisture more quickly in these plots, hastening the onset of water-stress. The higher foliar N that I observed in these shrubs further suggests these shrubs attained greater concentrations of photosynthetic enzymes in leaves, enhancing their physiological capacity. Thus, it appears increased productivity and growth at the leaf-level contributed to higher dieback of this species under N addition.

This hypothesis of increased susceptibility to drought with increased productivity under high N conditions (Bobbink et al. 2010) is further supported by $\delta^{13}C$ data representing intrinsic WUE of native shrubs within N addition plots over four years. In 2013 and 2014, shrubs in control plots exhibited increased WUE, as evidenced by more enriched $\delta^{13}C$ vales (Farquhar et al. 1989), relative to shrubs from high N addition plots. By not increasing WUE during extended drought, shrubs subjected to high N availability likely increased evaporative water loss over control plants and elevated water-stress, resulting in the more severe dieback that I observed.

Shrub biomass was highest in 2011, the first year of the study, when my estimations of biomass based on shrub volume matched values previously recorded for mature CSS (Gray and Schlesinger 1981). From 2011 to 2015, shrub biomass generally declined over time across N treatments, similar to shrub cover. However, while shrub cover and litter production were significantly influenced by N treatment, I found no significant effects on N addition on mean shrub biomass of mature *A. californica* individuals. This is likely due to the way I calculated shrub biomass – using measurements of height and width – as this method assumes shrubs are spherical

(Vourlitis and Pasquini 2009), which was increasingly untrue during the uneven dieback of shrub branches. Furthermore, the higher rates of woody litter production and greatly diminished leaf litter production over time in shrubs subject to high N addition supports results from shrub cover transects showing increased dieback and shrub loss.

In addition to increased susceptibility to drought-stress through higher productivity, elevated soil N could have also had other belowground effects that influence the ability of shrubs to withstand prolonged drought. Nitrogen addition has been found to reduce root:shoot ratios in seedlings of *A. californica* (Valliere and Allen 2016), and increased N availability could have also altered biomass allocation and reduced root allocation in mature shrubs. Grulke et al. (1998), for example, documented reduced root biomass in pinyon pine, a tree species of southern California, under chronic N deposition. Diversity and function of arbuscular mycorrhizae is also negatively affected by N deposition (Egerton-Warburton and Allen 2000, Sigüenza et al. 2006a). Mycorrhizae can play an important role in plant drought tolerance (Augé 2001) and N-impacted soil communities may offer less protection against under drought (Valliere and Allen 2016). It is also possible that elevated soil N had a direct negative impact on native shrubs, resulting in increased dieback (Bobbink and Lamers 2002). Both *A. californica* and *Encelia californica,* another drought-deciduous CSS shrub species, exhibited increased mortality when grown in soils with > 80 ug N g^{-1} for several months (Allen et al. 2005), and I observed levels of N availability exceeding this amount in high N addition plots, though it is unlikely this high concentration extended beyond the soil surface (> 10 cm).

Competitive interactions often play an important role in mediating community response to N deposition (Bobbink and Lamers 2002), and competition with nonnative annual species may also have played a role in facilitating shrub loss. Shallow-rooted annuals may be more competitive for water, depleting soil moisture in the upper soil profile to the detriment of deeper-rooted perennials (Davis and Mooney 1985, Eliason and Allen 1997, Wood et al. 2006). This effect could be even more pronounced under high N due to increased growth of nonnatives, as shown in this study. Wood et al. (2006), for example, found that annual grasses at high N deposition sites reduced water percolation, depriving co-occurring natives of water and concentrating available soil N in the upper profile for ready uptake by nonnatives. This altered ecohydrological response could be especially severe during periods of extended drought, and be an important contributor to shrub loss.

Resource availability often plays a key role in mediating invasion of nonnative plant species (Davis et al. 2000), and N addition has been found to result in increased invasion in multiple ecosystems (Brooks 2003, Schwinning et al. 2005, Ochoa-Hueso et al. 2013), including CSS (Allen et al. 2005, Kimball et al. 2014). Here I demonstrate that drought, in conjunction with N addition, may create a window of opportunity for invasion success through the resultant accumulation of soil N and opening of the shrub canopy. Nonnative annual species were particularly successful and increased over time in all plots, regardless of N addition, likely due in part to increased light availability with shrub canopy loss (Zedler et al. 1983, Keeley et al. 2005). Similar to previous work in this system, I found N addition led to higher growth of nonnative annuals (Padgett and Allen

1999, Wainwright and Cleland 2013, Kimball et al. 2014), and this is consistent with correlative studies showing increased conversion of CSS to nonnative annual grasslands under elevated N deposition (Talluto and Suding 2008, Cox et al. 2014).

Nonnative annual grasses and forbs may be successful under high N conditions and drought for a number of reasons. These species germinate earlier than natives and before most shrubs leaf out in this system (Wainwright and Cleland 2013), which provides first access to soil moisture, as well as soil N when it is at its highest. In addition to priority effects, nonnative annuals may also be superior competitors for water (Eliason and Allen 1997, Wood et al. 2006). Further, competition for N and other nutrients is directly mediated by soil moisture, and this may reinforce the dominance of annuals under dry conditions (Everard et al. 2010), as these annual species may be more competitive under high N conditions (Sharma et al. 2010). Nonnatives may also succeed while native CSS perennials suffer due to their annual life-cycle. While rainfall was significantly below average each year, there was sufficient soil moisture for some species to grow and produce seed, and these annuals avoid the worst of dry conditions as seeds in the soil seedbank. Finally, mycorrhizal species that dominate in N-impacted soils may disproportionately benefit annual nonnatives (Sigüenza et al. 2006a, b, Allen et al. 2016).

Conclusions

The results of this study indicate that chronic N addition may facilitate native CSS shrub loss and invasion of nonnative annuals during periods of extended drought. The addition of N may negatively impact native shrubs through increased productivity,

resulting in reduced WUE and increased susceptibility to drought. Furthermore, the resulting shrub mortality and opening of the canopy may create a window of opportunity for the invasion of nonnative plant species, potentially contributing to vegetation-type conversion. Anthropogenic N deposition, drought and the invasion of nonnative species are major drivers of global change expected to increase in the future (Galloway 2005, Giorgi and Lionello 2008, Bradley et al. 2010), and CSS and other mediterranean-type ecosystems are predicted to be particularly vulnerable (Sala et al. 2000, Moreno and Oechel 2012). This work highlights the potential for multiple components of global change to negatively impact these sensitive ecosystems, and has important implications for ecosystem services and long-term conservation.

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Table 2.1. Leaf-level traits of *A. californica* including mean leaf area (LA), specific leaf area (SLA), sapwood area (SA) and the ratio of leaf area to sapwood area (LA:SA) from terminal branches representing new growth collected in spring 2013 and 2014 from N addition plots (C = 0 g N m^{-2}; L = 0.5 g N m^{-2}; M = 1.5 g N m^{-2}; H = 3.0 g N m^{-2}). Values in the same row, for each year, followed by different letters are significantly different based on ANOVA.

	2013						2014					
Plant Trait	C	L	M	H	*F*	*P*	C	L	M	H	*F*	*P*
LA (cm^2)	**11.6b**	**14.5ab**	**16.1b**	**16.3b**	**2.96**	**0.048**	**8.2b**	**8.3b**	**13.9a**	**15.8a**	**11.36**	**<0.0001**
SLA (cm g^{-1})	129.7	135.5	134.9	138.8	0.14	0.934	132.5	128.8	134.7	128.2	0.23	0.875
SA (mm^2)	5.2	6.5	6.5	6.4	1.98	0.135	**3.9b**	**4.2b**	**7.4a**	**7.8a**	**6.49**	**0.001**
LA:SA	2.2	2.3	2.5	2.6	1.53	0.223	2.3	2.3	2.2	2.6	0.49	0.698

Table 2.2. Mean foliar percent N, percent C and C:N ratio of *A. californica* shrubs within N addition plots (C = 0 g N m^{-2}; L = 0.5 g N m^{-2}; M = 1.5 g N m^{-2}; H = 3.0 g N m^{-2}) from spring 2012 to spring 2015. Values for each parameter with different letters within the same row are significantly different based on ANOVA ($\alpha = 0.05$).

	Leaf tissue N (%)						Leaf tissue C (%)						Leaf tissue C:N					
Year	C	L	M	H	*F*	*P*	C	L	M	H	*F*	*P*	C	L	M	H	*F*	*P*
2012	**2.2b**	**2.1b**	**2.4a**	**2.4a**	**6.79**	**0.001**	47.3	46.6	47.9	47.0	0.59	0.6271	**20.0a**	**21.9a**	**19.7b**	**19.7b**	**7.01**	**0.0008**
2013	2.4	2.6	2.4	2.6	2.09	0.1182	47.6	47.9	47.7	47.6	0.53	0.6651	20.5	20.2	18.5	18.6	2.16	0.1092
2014	**2.9ab**	**2.6b**	**2.9a**	**3.2a**	**4.90**	**0.0059**	**47.2a**	**46.9ab**	**46.9ab**	**46.1b**	**3.03**	**0.0420**	**16.6a**	**17.9a**	**16.4a**	**14.4b**	**6.27**	**0.0015**
2015	**2.9b**	**3.0b**	**3.5a**	**3.6a**	**10.11**	**<0.0001**	**47.9a**	**47.9a**	**48.4a**	**46.8b**	**14.39**	**<0.0001**	**16.3a**	**16.2a**	**14.0b**	**13.1b**	**9.27**	**<0.0001**

Table 2.3. Results from decomposition experiments for two growing seasons (2013-2014 and 2014-2015) using litter bags deployed in N addition plots (C = 0 g N m^{-2}; L = 0.5 g N m^{-2}; M = 1.5 g N m^{-2}; H = 3.0 g N m^{-2}) for 90 and 180 day exposures, including comparisons of percent mass loss for each exposure and decomposition rate constants and ANOVA statistics. Values within columns for each year followed by different letters are significantly different ($\alpha = 0.05$).

	% Mass Remaining						Decomposition Rate Constant				
Year	90 days	*F*	*P*	180 days	*F*	*P*	*k*-value	R^2	*p*	*F*	*P*
2014											
C	82.7	2.01	0.1300	72.8	2.27	0.0967	0.0018	0.91	<0.0001	2.42	0.0815
L	85.1	-	-	73.1	-	-	0.0018	0.93	<0.0001	-	-
M	80.0	-	-	70.6	-	-	0.0019	0.91	<0.0001	-	-
H	83.0	-	-	67.7	-	-	0.0022	0.84	<0.0001	-	-
2015											
C	72.4	2.10	0.1173	**72.0a**	**4.49**	**0.0089**	**0.0018b**	0.89	<0.0001	**4.38**	**0.0100**
L	67.6	-	-	**66.3b**	-	-	**0.0023a**	0.75	<0.0001	-	-
M	69.3	-	-	**65.6b**	-	-	**0.0024a**	0.80	<0.0001	-	-
H	69.3	-	-	**65.7b**	-	-	**0.0023a**	0.80	<0.0001	-	-

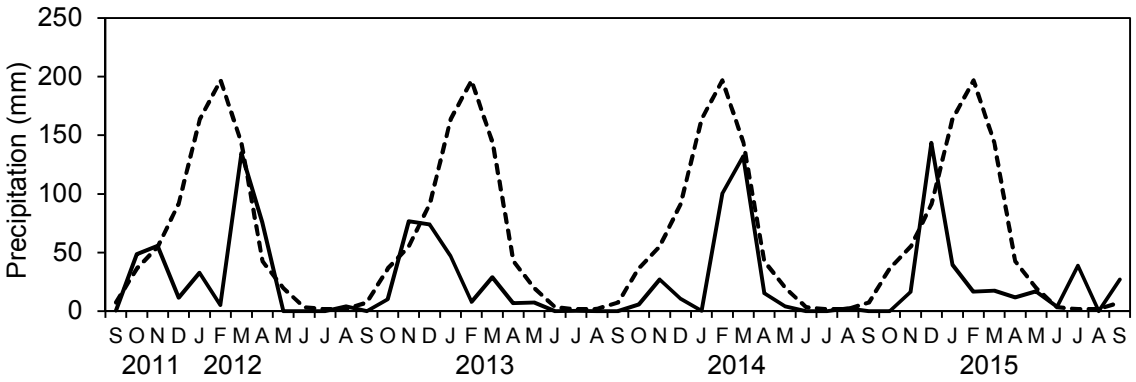

Figure 2.1. Monthly total precipitation (solid line) and monthly 30 year averages (dashed line) for the study site from September 2011 to September 2015.

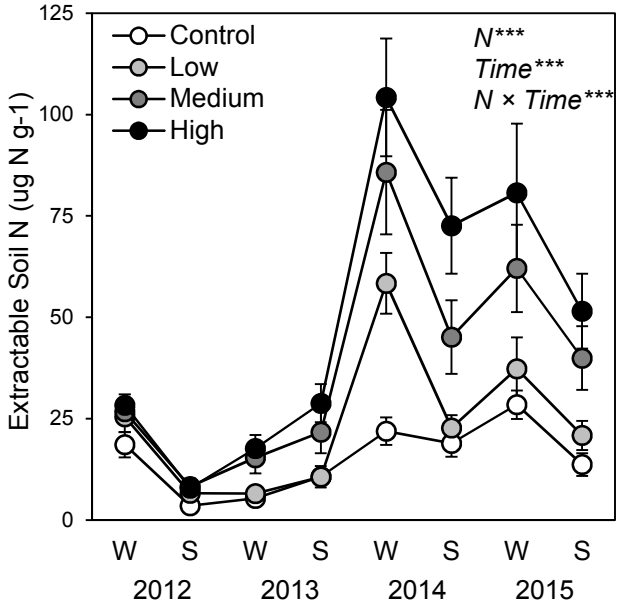

Fig 2.2. Total KCl extractable N of soils from N addition plots (Control = 0 g N m^{-2}; Low = 0.5 g N m^{-2}; Medium = 1.5 g N m^{-2}; High = 3.0 g N m^{-2}) through time, from winter (W) 2012 to Summer (S) 2015. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Nonsignificant factors are not listed.

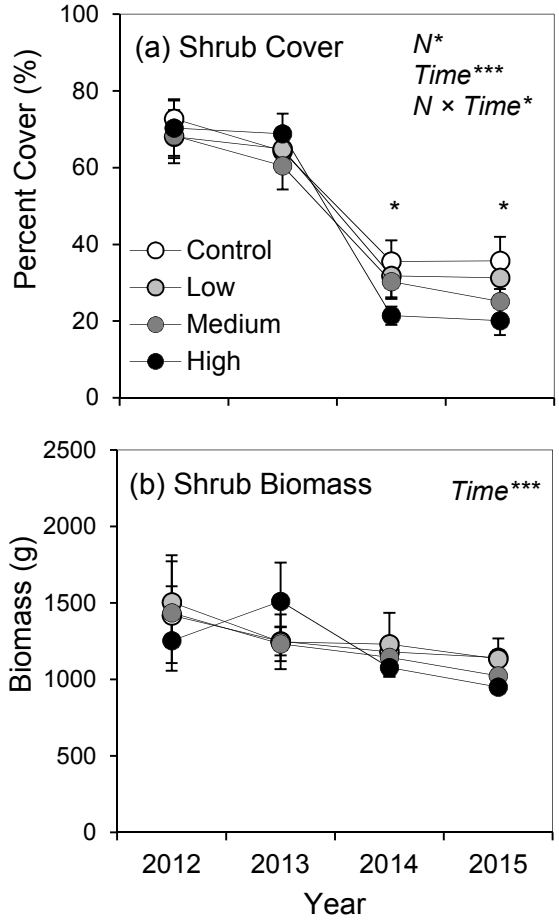

Fig 2.3. Native shrub cover (a) and *A. californica* shrub biomass (b) within N addition plots (Control = 0 g N m^{-2}; Low = 0.5 g N m^{-2}; Medium = 1.5 g N m^{-2}; High = 3.0 g N m^{-2}) through time from 2012 to 2015. Values represent means ± SE. Asterisks above data points indicate significant effect of N when analyzed with ANOVA by year. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Nonsignificant factors are not listed.

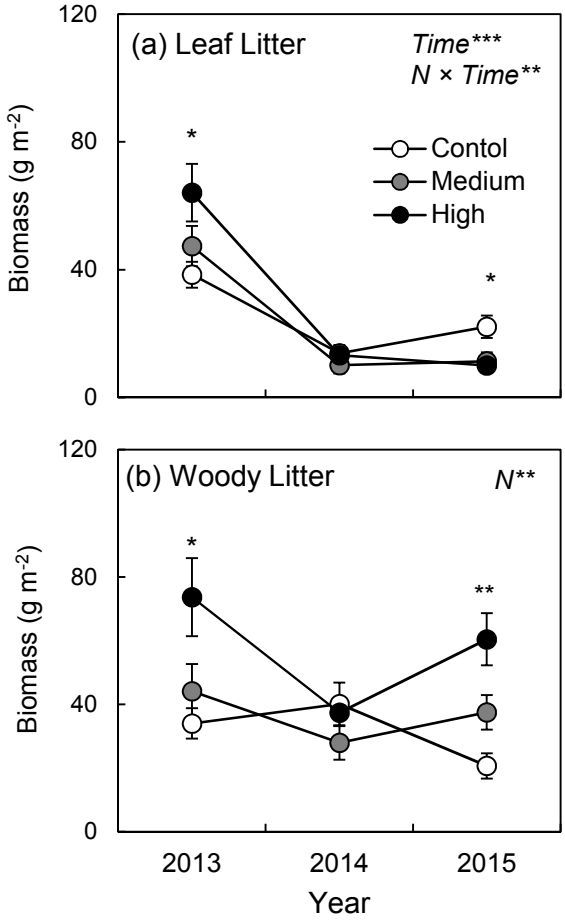

Fig 2.4. Leaf litter (a) and woody litter (b) biomass production of *A. californica* shrubs from litter traps within N addition plots (Control = 0 g N m^{-2}; Medium = 1.5 g N m^{-2}; High = 3.0 g N m^{-2}) through time from 2013 to 2015. Values represent means ± SE. Asterisks above data points indicate significant effect of N when analyzed with ANOVA by year. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Nonsignificant factors are not listed.

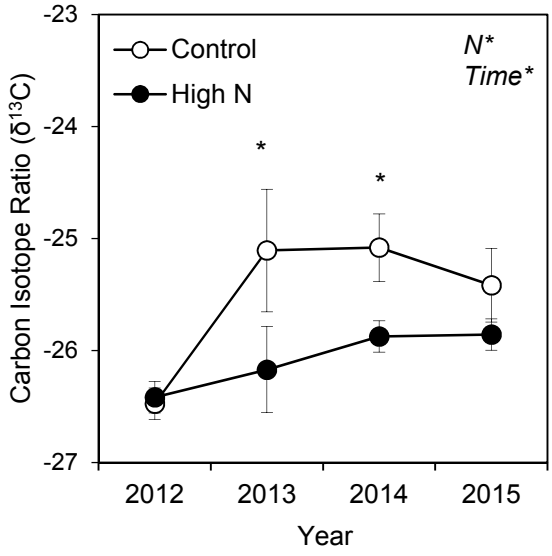

Figure 2.5. Carbon isotope ratios leaf tissue from *A. californica* shrubs from within N addition plots (Control = 0 g N m^{-2}; High = 3.0 g N m^{-2}) through time from 2012 to 2015. Values represent means ± SE. Asterisks above data points indicate significant effect of N when analyzed with ANOVA by year. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Nonsignificant factors are not listed.

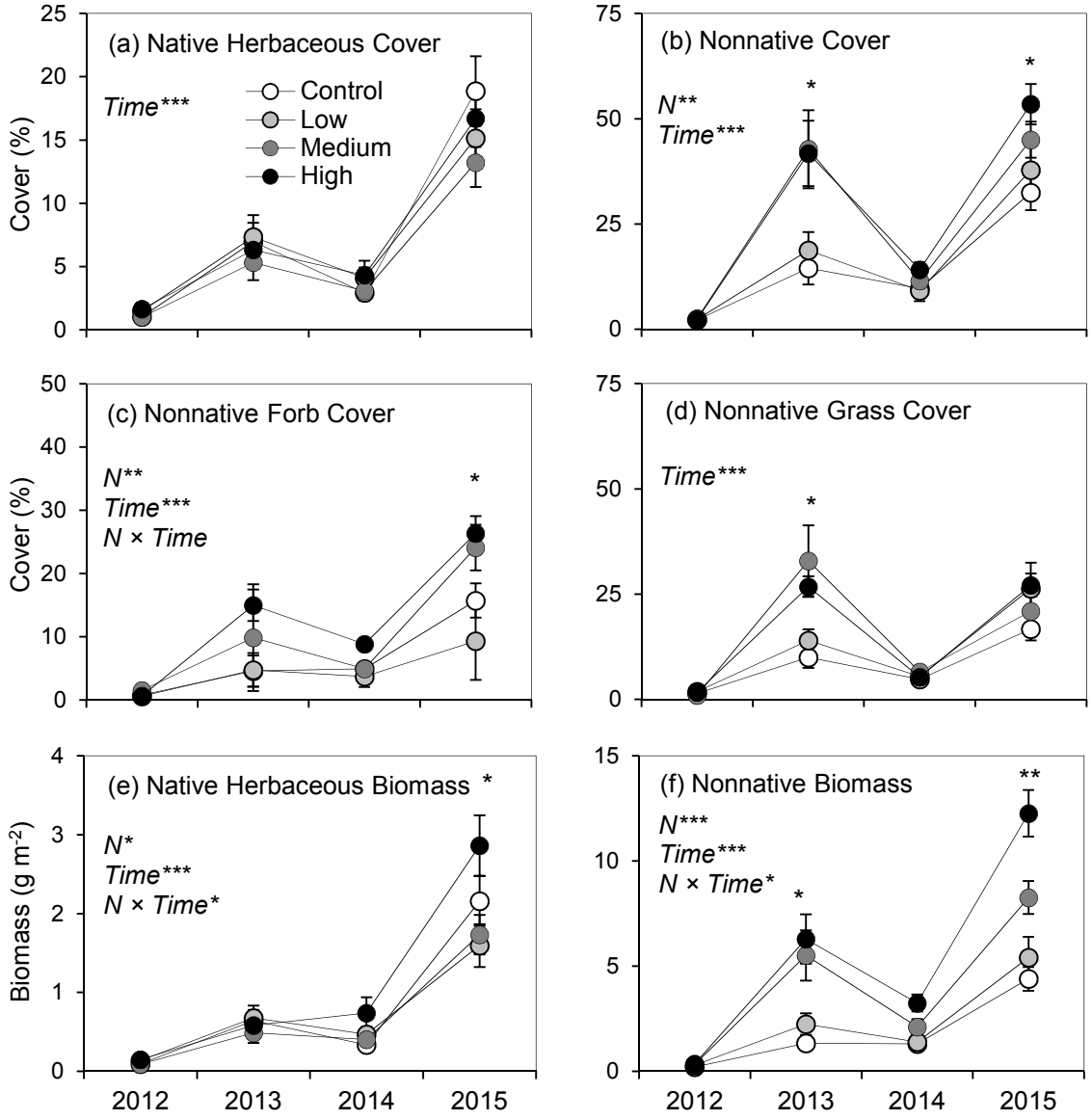

Fig 2.6. Total native herbaceous cover (a), total nonnative cover (b), nonnative forb cover (c), nonnative annual grass cover (d), native herbaceous biomass (e) and nonnative biomass (f) within N addition plots (Control = 0 g N m^{-2}; Low = 0.5 g N m^{-2}; Medium = 1.5 g N m^{-2}; High = 3.0 g N m^{-2}) through time from 2012 to 2015. Values represent means ± SE. Note different *y*-axis labels and scale. Asterisks above data points indicate significant effect of N when analyzed with ANOVA by year. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. Nonsignificant factors are not listed.

Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of *Artemisia californica* seedlings

Abstract

Nitrogen (N) deposition and drought are major drivers of global change that will influence plant-soil feedbacks. I investigated how N availability, N-impacted soil communities and drought affect feedback in seedlings of a drought-deciduous mycorrhizal shrub, *Artemisia californica*. Seedlings were inoculated with soil from either a high or low deposition site or sterilized inoculum and grown with or without supplemental N and under well-watered or drought-stressed conditions. Inoculum, N and water had interactive effects on feedbacks. Seedlings grown in low deposition inoculum exhibited a neutral to positive feedback under drought and had the highest root to shoot ratios and mycorrhizal colonization. Seedlings inoculated with high N-deposition soil experienced a positive feedback when N fertilized and well-watered, but plants allocated large amounts of biomass to shoots and had a negative response to drought. The soil community mediates plant response to varying belowground resource availability. I found N-impacted communities may reduce mycorrhizal colonization and allocation to roots and provide less protection against drought. My results highlight the context dependency of plant-soil feedbacks and the potential for climate change and N deposition to have interactive effects on these relationships.

Introduction

Atmospheric nitrogen (N) deposition and drought are two major components of global change likely to have substantial effects on terrestrial plant communities, both above- and belowground, that will influence plant-soil feedbacks (Tylianakis et al. 2008, Bardgett and Wardle 2010, Van der Putten et al. 2013). Interactions between plants and the soil microbial community are increasingly recognized as important drivers of large-scale vegetation patterns and processes (Van der Putten et al. 1993, Bever et al. 1997, Klironomos 2002) and these aboveground-belowground linkages may play a critical role in mediating ecosystem response to environmental change (Schlesinger et al. 1990, Wolters et al. 2000, Mohan et al. 2014). Many single factor studies have established the response of plants to soil microorganisms is strongly influenced by both soil N (Corkidi et al. 2002, Johnson et al. 2003, Manning et al. 2008) and water availability (Meijer et al. 2011, Meisner et al. 2013), but given the strong potential for these two factors to have complex and interactive effects on ecosystems (Zavaleta et al. 2003, Southon et al. 2012, De Marco et al. 2014, Kimball et al. 2014), multi-factor studies are needed to elucidate potential impacts of co-occurring global change drivers on these relationships.

Nitrogen deposition, the input of reactive N from the atmosphere, is increasing globally due to anthropogenic emissions (Vitousek et al. 1997, Galloway 2005, Galloway et al. 2008), with the potential to negatively impact terrestrial plant diversity and community composition (Bobbink et al. 2010). In southern California, which receives high levels of N deposition due to air pollution (Fenn et al. 2003), N deposition has been implicated in the widespread conversion of coastal sage scrub (CSS) to exotic annual-

dominated grasslands (Allen et al. 1998, Fenn et al. 2003, Cox et al. 2014). Coastal sage scrub is a low growing, drought-deciduous shrub-land plant community, which has been reduced to a fraction of its historic range as a result of land-use change, invasion and increased fire frequency (Westman 1981, Minnich and Dezzani 1998, Talluto and Suding 2008). High soil N may favor exotic annual grasses over native shrub seedlings (Wood et al. 2006, Rao and Allen 2010) reducing native establishment, but there may be other direct and indirect effects of increased N inputs that affect native seedling survival and performance.

Nitrogen deposition can have a number of impacts belowground that might influence plant-soil feedbacks, such as reduced microbial biomass (Johnson et al. 1998, Wallenstein et al. 2006, Treseder 2008), altered microbial enzyme activity (Carreiro et al. 2000, Saiya-Cork et al. 2002) and changes to soil microbial community composition and diversity (Wolters et al. 2000, Eisenhauer et al. 2012). Increased soil N due to anthropogenic N deposition has resulted in a decline of arbuscular mycorrhizal fungal (AMF) diversity in areas of high pollution in southern California, characterized by a loss of large-spored species within the Gigasporaceae and an increase in abundance of small-spored *Glomus* species (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2001, Sigüenza et al. 2006b). These N-impacted mycorrhizal communities may be less mutualistic, especially when soil N availability is high, resulting in reduced growth of natives in these soils (Johnson 1993, Johnson et al. 1997, Sigüenza et al. 2006a). In addition to providing plants with nutrients in exchange for photosynthate, AMF also supply plants with water and improve plant resistance to drought (Augé 2001, Allen

2007). It remains unknown how N deposition influences plant-water relations through changes to the soil microbial community, but less effective mutualists could increase host susceptibility to drought (Smith and Read 2008, Bobbink et al. 2010).

Drought is a second factor that has been cited as a possible cause of conversion of CSS to exotic annual grasslands (Cox and Allen 2008, Kimball et al. 2014). Typical of a Mediterranean climate, precipitation in this region is highly variable year to year (Jones 2000, Pratt and Mooney 2013) and CSS species are subject to seasonal and long-term drought, with extreme drought events expected to increase in the near future due to climate change (Jones 2000, Bell et al. 2004, Griffin and Anchukaitis 2014). Many CSS shrub species are drought-deciduous, avoiding water deficit by shedding their leaves during the summer dry period (Harrison et al. 1971). However, during the winter growing season, these species exhibit high rates of transpiration (Harrison et al. 1971, Jacobsen et al. 2007) and may be quite sensitive to short-term water-stress (Kolb and Davis 1994). Seedling recruitment of CSS shrub species is highly dependent on precipitation (Keeley et al. 2006, Cox and Allen 2008) and plants are particularly vulnerable to drought during seedling stage (Fenner 1987, Moles and Westoby 2004).

Nitrogen deposition can increase plant susceptibility to drought (Wu et al. 2008, Friedrich et al. 2012, Meyer-GrüNefeldt et al. 2013), and one possible explanation for poor CSS seedling establishment under N deposition is that high N availability alters morphological and physiologically plant traits in a way that makes them less able to withstand short- and long-term drought stress. Enhanced soil N availability may reduce biomass allocation to roots, thereby increasing evapotransporative demand and

decreasing root:shoot ratios (Aerts et al. 1991, Reynolds and D'antonio 1996). High soil N can also decrease mycorrhizal colonization of roots (Egerton-Warburton and Allen 2000), and altered AMF diversity and functioning due to N eutrophication could conceivably influence plant-fungal water relations. These effects may negatively impact water uptake and drought tolerance of plants (Bobbink et al. 2010) and be partially responsible for the observed decline of CSS under simultaneous drought and high N conditions (Kimball et al. 2014). Hence, there is a strong need to better understand the combined effects of N deposition and drought on plant growth responses as mediated by the soil microbial community.

In this study I evaluated effects of inoculation with soils from low and high N deposition sites and differential N and water availability on growth and biomass allocation of seedlings of the dominant CSS shrub species, *Artemisia californica* Less. (Asteraceae). This species is highly mycorrhizal (Sigüenza et al. 2006a) and plastic in its growth response to both N (Padgett and Allen 1999, Yoshida and Allen 2001, Sigüenza et al. 2006a) and water (Pratt and Mooney 2013). Sigüenza et al. (2006a) found a strong negative feedback of N-impacted mycorrhizal communities on growth of *A. californica* seedlings, likely due to the selection of inferior mutualists, but this experiment was conducted under well-watered conditions and it is unknown how drought might change these dynamics. The purpose of this study was to investigate the effects of N deposition on growth and biomass allocation of *A. californica* seedlings due to increased N availability and N-impacted soil microbial communities, and to understand how these factors influence the response of seedlings to drought. I hypothesized: (1) plant growth

would differ in low and high deposition soil communities, resulting in different plant-soil feedbacks; (2) seedlings would experience a more negative feedback in N-impacted soil communities when soil N was high (Johnson et al. 1997, Sigüenza et al. 2006a); (3) drought would reduce plant growth, negatively impacting feedbacks, especially in plants grown in N-impacted soils and under high N availability; and (4) belowground resource availability would alter biomass partitioning, with N addition favoring allocation to shoots and drought favoring allocation to roots.

Materials and Methods

Study Sites

I collected soil for plant inoculations from two sites (Table 1) receiving different levels of N deposition in the Santa Monica Mountains National Recreation Area, California. Rancho Sierra Vista is located in eastern Ventura County on the western end of the mountain range (34.15°N, 118.96°W) and receives low levels of N deposition, modeled at about 8.8 kg N ha^{-1} yr^{-1} (Tonnesen et al. 2007, Fenn et al. 2010). Franklin Canyon is located near the geographic center of the City of Los Angeles (34.12°N, 118.41°W) on the eastern end of the Santa Monica Mountains. The modeled rate of N deposition for Franklin Canyon is about 20.1 kg N ha^{-1} yr^{-1} (Tonnesen et al. 2007), and the area has experienced high levels of N deposition for decades (Egerton-Warburton et al. 2001). Both sites are similar in elevation and, typical of a Mediterranean climate, experience hot dry summers and cooler winters with variable precipitation falling between November and April. Rancho Sierra Vista receives approximately 420 mm

rainfall annually, while Franklin Canyon receives 379 mm. The soils at each site are loamy and similar in texture and soil P (Table 1). While other factors that influence soil microbial communities besides N deposition may differ between sites, my goal was to evaluate the response of seedlings to soil communities representative of sites along the anthropogenic N deposition gradient of the Santa Monica Mountains. Seeds of *A. californica* for my growth experiment were collected from Zuma Canyon (34°04'N, 118°82'W), a site located roughly between the two inoculum collection sites that receives approximately 12.3 kg N ha^{-1} yr^{-1} (Tonnesen et al. 2007, Fenn et al. 2010).

Soil Inoculum

Soil was collected from both sites in December 2012 to be used as whole-soil inoculum. At each site I collected soil cores 0-10 cm in depth beneath the outer canopy of mature *A. californica* shrubs. I collected five cores from 20 individual shrubs over an area of approximately one hectare at each site. Soil was sieved through a 1 cm^2 stainless steel mesh and transported to the laboratory where it was refrigerated until potting. I homogenized the whole-soil inoculum for each site to ensure plants in each treatment were inoculated with a complete soil microbial community representative of each site. Spore density of AMF was similar in the high and low deposition inocula, 136.7 spores g^{-1} (SE ± 8.6) and 156.8 spores g^{-1} (SE ± 21.4) respectively, but the high deposition inoculum was dominated by a few species of small-spored *Glomus*, especially *G. clarum* and *G. deserticola* (Schenck and Perez 1990). I included a sterile control as my third soil treatment for comparison in order to better understand the response of seedlings to the

two live soil communities. The different soil inoculation treatments were prepared as follows:

1. Low deposition: 25 g of whole-soil inoculum from the low N deposition site and 25 g of steam-sterilized whole-soil inoculum from the high N deposition site added to each pot.
2. High deposition: 25 g of whole-soil inoculum from the high N deposition site, and 25 g of steam-sterilized whole-soil inoculum from the low N deposition site added to each pot.
3. Sterilized control: 25 g of steam-sterilized whole-soil inoculum from the low N deposition site, and 25 g of steam sterilized whole-soil inoculum from the high N deposition site added to each pot.

Each pot that received live inoculum also had steam-sterilized soil from the other site added to account for any differences in nutrient availability, while the sterilized control received steam-sterilized soil from both sites. Total KCl extractable N of the low and high deposition inoculum was 13.9 μg N g^{-1} soil (SE ± 3.0) and 37.4 μg N g^{-1} soil (SE ± 6.1) respectively. Steam sterilization did not result in a significant increase of extractable N.

Growth Experiment

I conducted the growth experiment from January to March 2013 in a greenhouse at the University of California, Riverside set at 21/16^{o} day/night temperature to simulate winter growing season conditions for this Mediterranean-type climate. The potting media,

to which inoculum was added, was a 1:1 mix of field soil to silica sand. Sand was added to promote water infiltration and drainage. I collected field soil for potting at my low deposition site, Rancho Sierra Vista, from a mature stand of CSS. Prior to potting, this soil mixture was steam sterilized for 24 hours, followed by a 48-hour incubation period, and then an additional 24-hour steam treatment. I filled 650 ml plastic pots (Deepots; Steuwe and Sons, Corvallis, Oregon, USA) with approximately 500 g of the potting soil mixture. The appropriate inoculum was added to each pot about 10 cm from the surface and mixed into the soil. Extractable N in the potting soil after sterilization and dilution with sand was 12.5 μg N g^{-1} soil (S.E. ± 2.1), which was well below the high N treatment. Prior to planting I leached all pots with 250 ml distilled water. Seeds of *A. californica* were sown into pots and watered daily with distilled water until seedlings germinated and established in each pot, at which point seedlings were thinned to a density of one per pot. I rotated pots within racks and randomly rearranged racks on greenhouse benches weekly.

Nitrogen Fertilization

Seedlings were grown in one of two N treatments. Half of the pots in each inoculum treatment received supplemental N (high N treatment) while the other half did not (low N treatment). In order to simulate the high amounts of soil N that would be available early in the growing season at high N deposition sites, I fertilized each of the high N pots twice early in the experiment, first after seedlings were established in each pot and again one week later. When fertilized, each high N pot received approximately

22.5 μg N g^{-1} soil from ammonium nitrate (NH_4NO_3) in solution for a total of 45 μg N g^{-1} soil total, while the low N plants received an equal volume of distilled water.

Watering Treatments

I initiated watering treatments after two weeks of growth. Half of the pots in each soil treatment were maintained at 60% water holding capacity by weight (well-watered treatment), while the other half were maintained at 20% water holding capacity (drought treatment) for the duration of the experiment. Every 3-5 days depending on greenhouse conditions, individual pots were weighed and watered with distilled water to the desired water holding capacity according to the two treatments. Drought-stressed seedlings were often visibly wilted prior to watering indicating plants in this treatment were consistently water-limited throughout the duration of the experiment.

Harvest

I harvested plants (n = 10) after ten weeks of growth. Seedlings were separated into roots and shoots, dried to constant mass at 60° C and weighed. Subsamples of roots were weighed and measured to determine root length for each plant. Dry leaf tissue was analyzed for percent N content using a Thermo-Finnigan FlashEA 1112 Nitrogen and Carbon Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the University of California, Riverside, Environmental Science Research Laboratory.

Root Colonization

I quantified the colonization of mycorrhizal and non-mycorrhizal fungi in plant roots as percent root length colonized (PRLC). Once roots were dried and weighed, I rehydrated them in distilled water overnight and cleared and stained them with trypan blue (Koske and Gemma 1989). Previous work in our lab has demonstrated drying does not affect microscopic recognition of fungal structures within roots. I employed a modified line-intercept method based on the procedure of McGonigle et al. (1990) to assess percent colonization of mycorrhizal and other non-mycorrhizal fungi within roots and from these measurements calculated PRLC. For each plant, I examined ten randomly selected fine root fragments under the microscope, making observations on presence or absence within ten fields along transects for each fragment. I distinguished between mycorrhizal and other fungi based on visual appearance, including hyphal morphology, size and presence or absence of septa. I found no mycorrhizal colonization and very low levels (< 1 %) of other fungi in the roots of plants grown in sterilized control inoculum, and these were not included in analyses of PRLC.

Calculation of Feedback

I calculated plant-soil feedback as the difference in total biomass of seedlings grown in live soil relative to the mean total biomass of those grown in sterilized controls under the same resource conditions:

$$Relative\ feedback\ = \frac{(Biomass_{Live} - \ Mean\ Biomass_{Sterile})}{Mean\ Biomass_{Sterile}}$$

Using this calculation of feedback, a value less than zero indicates a negative feedback, likely due to belowground pathogens or possibly antagonistic mutualists (e.g. Johnson 1993), while a value greater than zero indicates a positive feedback, likely due to beneficial organisms such as AMF or other soil biota involved in nutrient cycling.

Statistical Analyses

I grew seedlings in three soil inoculum treatments (low deposition, high deposition and sterile control), with or without supplemental N and under well-watered or drought conditions in a full factorial experimental design (3 × 2 × 2). I performed analysis of variance (ANOVA) with inoculum, N, water and the interactions between these as factors on plant response variables measured including root, shoot and total dry mass, relative feedback, root:shoot ratio, PRLC of mycorrhizal and non-mycorrhizal fungi and leaf percent N content. Prior to statistical analysis, I tested all data for normality and homogeneity of variance, transforming data when necessary to meet the assumptions of ANOVA. Following ANOVA, I performed Tukey's honest significant difference test (HSD) to compare means and assign significance at $p<0.05$. I used linear regression to compare rates of mycorrhizal and non-mycorrhizal colonization with plant biomass. Finally, I used polynomial regression to compare root:shoot ratios with the natural logarithm of total biomass to determine the influence of plant size on biomass partitioning (Gedroc et al. 1996), and completed allometric analysis using linear regressions of log-transformed shoot and root biomass (Farrar and Williams 1991,

Staddon and Fitter 1998). Statistical analyses were performed using RStudio Version 0.98.57, RStudio, Inc.

Results

Plant Growth Response

Inoculum ($p < 0.0001$), N ($p < 0.0001$), and water ($p < 0.0001$) and the interactions between these treatments significantly influenced plant biomass (Table 2, Fig. 1). In the low deposition inoculum, mean total biomass did not significantly differ between N and water treatments (Fig.1). Under low N conditions, seedlings grown in low deposition inoculum had significantly higher biomass than those grown in high deposition inoculum (Fig. 1). Plants grown in high deposition inoculum had very low biomass when grown under low N availability or drought conditions (Fig. 1). However, when plants inoculated with high deposition soil were grown under high N and well-watered conditions, they accrued the highest biomass of any treatment (Fig. 1). In the sterile controls, seedlings were significantly larger in the well-watered treatments relative to drought-stressed plants, but N addition did not significantly affect total biomass in this treatment (Fig. 1).

Plant-soil Feedbacks

Relative plant-soil feedbacks differed by inoculum type and with resource availability (Fig. 3). Feedbacks were significantly affected by inoculum type ($p = 0.0005$) and the interaction of inoculum with N ($p = 0.0004$) and water ($p < 0.0001$), indicating

plants responded differently to soil communities from low and high N deposition sites. Water availability significantly influenced feedbacks (p<0.0001), as did the interaction of water and N (p < 0.0001). Nitrogen alone had no significant effect (p = 0.2756). There was also a significant three-way interaction of treatments (p < 0.0001). In the low deposition inoculum, seedlings exhibited a negative feedback when well-watered, regardless of N availability (Fig. 3). However, in the drought treatment, seedlings showed a very positive feedback when soil N was low, and a neutral response when soil N was high. Conversely, seedlings grown in high deposition inoculum exhibited a negative feedback when soil N was low, but a positive to neutral feedback under high N (Fig. 3).

Drought influenced feedbacks differently depending on inoculum type and N availability. Biomass of seedlings grown in low deposition inoculum was unaffected by drought, and drought positively influenced feedbacks in this inoculum (Fig. 3). A similar pattern was observed in seedlings grown in high deposition inoculum under low N, where well-watered plants exhibited a negative feedback, and drought-stressed plants showed a neutral feedback. However, in high deposition inoculum under high N, drought diminished the positive feedback observed under well-watered conditions, resulting in a neutral feedback (Fig. 3).

Biomass Allocation

I also assessed effects of inoculum source, soil N and water on root:shoot ratios of plants, which is instructive in understanding how plants allocate biomass under varying belowground resource availability. Root:shoot ratios of seedlings were significantly

affected by inoculum ($p < 0.0001$), water ($p < 0.0001$) and the interaction between inoculum and water ($p < 0.0001$; Table 2). Nitrogen had no significant effect ($p = 0.15$; Table 2). Average root:shoot ratios were highest in seedlings grown in low deposition inoculum compared to seedlings from other soil treatments, ranging from 0.57 to 0.81 (Fig. 2). In this inoculum, drought resulted in higher allocation to roots, but this difference was only statistically significant under low N (Fig. 2). In high deposition inoculum, mean root:shoot ratios ranged from 0.32 to 0.47 and were not significantly affected by N or water availability (Fig. 2). The lowest root:shoot ratios were observed in seedlings grown in sterile soil, which averaged 0.28 to 0.33, with no significant differences between N and water treatments (Fig. 2). The results of a third-order polynomial regression of root:shoot ratios *vs.* the natural logarithm of total biomass across treatments (Gedroc et al. 1996) indicates that allocation patterns were not correlated with plant size ($p = 0.316$). Furthermore, correlations between log-transformed shoot and root biomass were different in seedlings grown in low deposition ($r = 0.35$, $p = 0.004$) and high deposition ($r = 0.83$, $p < 0.0001$) soils, showing relative growth rates of root vs. shoots differed in low and high deposition soils (Farrar and Williams 1991).

Plant Nitrogen

I analyzed effects of the different soil, N and water treatments on leaf tissue N of seedlings and found a significant influence of inoculum ($p < 0.0001$) and water ($p < 0.0001$), as well as an interactive effect of inoculum, N and water ($p = 0.015$; Table 3). Seedlings grown in the two live inocula tended to have lower percent leaf N than in

sterile controls and exhibited a similar response to N and water availability, except percent leaf N was significantly higher in the high deposition plants in the high N, drought treatment. Across soil and N treatments, drought-stressed plants generally had higher percent leaf N than well-watered counterparts.

Root Colonization

I observed mycorrhizal and non-mycorrhizal fungal structures in roots of plants grown in live soil inocula (Table 4). Percent root length colonized by AMF was significantly affected by inoculum ($p < 0.0001$) the interaction of N and water ($p = 0.007$) and the interaction of inoculum, N and water ($p = 0.0316$). The highest levels of PRLC by mycorrhizal fungi were observed in the roots of plants grown in low deposition inoculum, especially when N and water were limiting (Table 4). Seedlings grown in high deposition inoculum showed a different pattern, where PRLC was reduced under low N or drought conditions (Table 4). There were no significant correlations between PRLC by AMF and plant biomass within or among treatments ($p > 0.05$ for all correlations).

Percent root length colonized by non-mycorrhizal fungi was significantly affected by inoculum type ($p < 0.0001$), N ($p = 0.002$) and the interaction of inoculum and N ($p = 0.025$) and inoculum and water ($p = 0.017$). Mean PRLC by non-mycorrhizal fungi ranged from 22.5% to 34.5% in seedlings grown in low deposition inoculum, and did not differ significantly across resource treatments (Table 4). Seedlings grown in the high deposition inoculum had a similar PRLC to those grown in low deposition inoculum under high N and water availability, but significantly lower colonization under low N and

drought treatments (Table 4). There were no significant correlations between PRLC of non-mycorrhizal fungi within or across treatments ($p > 0.05$ for all correlations).

Discussion

Effects of Nitrogen Deposition

I found that plant-soil feedbacks of *A. californica* seedlings differed in low and high deposition soils, and N and water availability influenced feedbacks differently in the two soil communities (Fig. 3). While soil N availability had significant effects on plant growth and feedbacks (Table 2, Fig.3), the results of this study suggest that N deposition may also exert strong indirect effects on native seedling performance through changes to soil biota. Moreover, I observed significant interactions of soil inoculum, N and water on plant performance (Table 2), suggesting seedlings at high N deposition sites may respond differently to drought. These results highlight the critical role of the soil microbial community in mediating plant performance in response to varying environmental conditions, which could have important implications for seedling establishment under global change. Reestablishment or restoration of this species is often limited by seedling recruitment (Eliason and Allen 1997, Allen et al. 2000, Cox and Allen 2008), and the different growth strategies observed might differ in benefits conferred to seedlings in a realistic field setting. For example, native shrub seedlings often face intense competition from invasive grasses and forbs, and increased growth could enhance their competitive ability. Reduced growth of native shrub seedlings in N-impacted soils under drought

could be partially responsible for loss of CSS observed under high N deposition (Talluto and Suding 2008, Fenn et al. 2010, Kimball et al. 2014)

While plant-soil feedbacks were altered in N-impacted soil communities, unlike previous studies (Johnson 1993, Corkidi et al. 2002, Sigüenza et al. 2006a), I found no evidence of N deposition leading to an antagonistic mycorrhizal relationship. In fact seedlings grown in high N deposition inoculum under high N and water availability had the greatest biomass of any treatment (Fig. 1). However, my results suggest N deposition could negatively impact mycorrhizal relationships and plant-soil feedbacks in several ways. The high deposition soil community only led to a positive growth response under high resource availability; under drought conditions, seedlings exhibited a neutral feedback, and under well-watered and low N conditions, seedlings grown in high deposition soils experienced a very negative feedback (Fig. 3). This suggests the benefits of this N-impacted soil community are diminished when N or water are limiting. Furthermore, in high deposition inoculum under low N or drought, AMF colonization was much lower than in seedlings grown in low deposition inoculum (Table 4), indicating that under some conditions, N deposition may severely reduce mycorrhizal activity. This could negatively impact native seedling performance and establishment in the field, given the important role AMF can play in increasing plant nutrition and tolerance to biotic and abiotic stressors (Smith and Read 2008), including drought (Augé 2001, Birhane et al. 2012).

Effects of Drought

My results are particularly informative in the context of global climate change, and effects of drought on seedlings grown in the two soil communities under varying N availability may further illustrate the potential negative impacts of N deposition on dryland species. Contrary to my initial hypothesis, drought only negatively impacted plant growth and feedbacks in high deposition inoculum (Fig. 1, 3). Seedlings grown in N-impacted soils with supplemental N had the most negative response to drought, while biomass of plants inoculated with low deposition soil was unaffected by water-deficit (Fig. 1). Furthermore, when both N and water supply was low, seedlings grown in low deposition inoculum exhibited a positive feedback (Fig. 3). Species from arid and semi-arid environments may respond more positively to AMF under low amounts of pulse-driven precipitation (Birhane et al. 2012), and my results indicate N deposition could minimize the benefit of these mutualistic relationships when water is limiting. This shows native soil communities may help protect against drought-stress and supports the hypothesis proposed by others, that N-impacted AMF communities may increase susceptibility to drought (Smith and Read 2008, Bobbink et al. 2010).

Biomass Allocation

Effects of inoculation, N and water on biomass allocation may help further explain how N deposition will influence plant performance in the field under variable environmental conditions. Plants are often able to adjust biomass partitioning in response to resource availability, increasing allocation to structures that acquire a limiting resource

(Chapin et al. 1987, Poorter and Nagel 2000). After ten weeks of growth, I found no significant effects of N availability on root:shoot ratios, although patterns of allocation differed in low and high deposition soils (Fig. 2), suggesting N deposition may influence plant allocation indirectly via changes to the soil community. Seedlings grown in low deposition inoculum had the highest root:shoot ratios and exhibited a significant increase in root:shoot ratios under drought (Fig. 2), consistent with the theory of optimal partitioning. However, seedlings grown in high deposition soil allocated significantly less biomass to roots, and did not adjust root:shoot ratios in response to N or water availability (Fig. 2). Thus, it appears this species is able increase allocation to roots in response to drought under some conditions, but this effect may be diminished in N-impacted soils. While plant size is often a key determinant of biomass allocation (Gedroc et al. 1996), with plants tending to have higher allocation to roots early in development, I found no significant relationship between plant size and root:shoot ratio within or across treatments, and allometric relationships of shoot and root biomass differed in the two soil communities. Furthermore, seedlings with the highest root:shoot ratios, those grown in low deposition soil, had significantly higher mean biomass than several other treatments. Altogether, this indicates that differences in patterns of allocation are due to treatment effects, and not ontogenetic drift (Farrar and Williams 1991, Gedroc et al. 1996).

Shifts in allocation due to N deposition could have important implications for seedling establishment and large-scale vegetation patterns. Mediterranean plant species typically have higher root:shoot ratios than plants from more mesic biomes (Hilbert and Canadell 1995), possibly as an adaptation to seasonal drought (Lloret et al. 1999). In

addition, seedling survival is positively correlated with root:shoot ratio (Lloret et al. 1999) and rooting depth (Padilla and Pugnaire 2007) in a number of Mediterranean shrub species. Increased allocation to roots, as observed in seedlings inoculated with low deposition soil (Fig 3), could promote survival in this semi-arid ecosystem. Conversely, low root:shoot ratios of seedlings grown in high deposition inoculum (Fig. 3) would be expected to increase susceptibility to drought, and lower allocation to roots could be partially responsible for the significantly lower biomass in drought-stressed plants grown in this inoculum under high N (Fig. 1).

Pathogens and Other Biota

While AMF are known to be important in shaping feedback in this system (Sigüenza et al. 2006a, Bozzolo and Lipson 2013, Hilbig and Allen 2015), pathogens, including both fungi and bacteria, probably also play a significant role. However, my methods only allowed for an assessment of potential fungal pathogens in plant roots. In several treatments, colonization by non-mycorrhizal fungi was higher in roots of seedlings grown in low N deposition inoculum vs. high deposition inoculum (Table 4), even though biomass was significantly lower in the latter (Fig. 1). The majority of these fungi were ascomycetous and probably facultative pathogens. I did not observe any symptoms of disease in plant roots or shoots, but pathogenic activity likely contributed to the negative feedbacks observed under some conditions. It is possible that the high density of *A. californica* shrubs at the low N deposition site promoted a higher pathogen load (Packer and Clay 2000, Van der Putten et al. 2001) relative to the high N deposition

site where shrub density is lower, resulting in higher levels of colonization. Root pathogens are also strongly influenced by soil moisture (Cook and Papendick 1972) and increased pathogenic activity could be responsible for the negative feedbacks observed under well-watered conditions (Fig. 3).

It is likely plant-soil feedbacks were influenced by non-symbiotic organisms in my soils through competition and changes in nutrient cycling. Growth depressions in inoculated plants may be partially attributable to immobilization of nutrients by the microbial community (Barber 1978, Lekberg and Koide 2013). Plants grown in sterile soil generally had higher percent leaf N than plants inoculated with live soil communities (Table 3), which could be evidence plants grown in live soil were competing with soil microbes for N (Kaye and Hart 1997). High levels of N deposition may also increase rates of N mineralization in CSS soils (Vourlitis et al. 2007), and similar changes in microbially-mediated nutrient cycling could have also contributed to the positive feedback observed in high deposition inoculum under high resource availability (Fig. 3).

Methodological Considerations

I used a whole-soil inoculation approach similar to previous studies aimed at understanding feedbacks in this system (Sigüenza et al. 2006a, Bozzolo and Lipson 2013). As such, these inocula likely contained a range of soil biota. While AMF and other fungi likely played an important role in shaping plant responses, other organisms, both free-living and symbiotic, were undoubtedly involved. However, I did not observe any micro-fauna in spore extractions, nor did I find evidence of belowground herbivory when

examining roots under the microscope. It is important to note soils at my collection sites may differ in other factors besides N inputs, but despite these and other limitations inherent to this experimental approach, this study successfully demonstrates how seedlings of this species would respond to actual soil communities at low and high N deposition sites within the study area. This work also calls into question the ecological relevance of similar experiments conducted under well-watered conditions using drought-adapted species. I observed dramatic differences in the direction and magnitude of feedback with watering regime, and water significantly affected a number of parameters. In dryland ecosystems, plants and microbes are frequently water-limited, and attempts to characterize feedbacks in these systems under a single, often artificially high, watering regime may be misleading.

Conclusions

Our understanding of the ecological impacts of N deposition comes largely from temperate biomes (Bobbink et al. 2010). However, there is also evidence from dryland ecosystems other than CSS that N addition can result in plant community shifts, including deserts (Brooks 2003, Báez et al. 2007, Rao and Allen 2010), semi-arid shrublands (Ochoa-Hueso and Manrique 2010, Ochoa-Hueso et al. 2013) and arid and semi-arid grasslands (Schwinning et al. 2005, Bonanomi et al. 2006, Zeng et al. 2010). With N deposition (Galloway et al. 2008) and extreme drought events (Sheffield and Wood 2008) expected to increase globally in the near future, there is a strong need to understand potential interactive effects of these co-occurring global change drivers on both plants

and soils. This is the first study I are aware of to explicitly consider the role of the soil microbial community in shaping plant responses to these two factors simultaneously. I found native soil communities promote higher mycorrhizal activity and allocation to roots and may protect against drought, but N deposition can diminish these potentially beneficial effects through elevated N availability and changes to the soil community. In addition to other stressors, interactive effects of N and drought on plants and soil communities could contribute to vegetation shifts in arid and semi-arid ecosystems under chronic N deposition.

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Table 3.1. Sites used for soil inoculum collection and their geographic locations, elevation, average annual rainfall, modeled N deposition, and soil P and N concentrations.

Site	Latitude (°N)	Longitude (°E)	Eleva-tion (m)	Rain-fall (mm)	N Dep.[a] (kg $ha^{-1}yr^{-1}$)	Soil P[b] (ppm)	Soil N[c] (µg N/g)
Satwiwa	35°15’	118°96’	271	420	8.8	18.8 (±2.9)	13.9 (±3.0)
Franklin Canyon	34°12’	118°41’	275	379	20.1	15.5 (±2.3)	37.4 (±6.1)

[a]Modeled rates of atmospheric nitrogen deposition from Tonneson et al. (2007)
[b]Mean soil phosphorus (Olsen-P) concentrations (0-10 cm depth) and SE.
[c]Mean KCl extractable soil nitrogen concentration (0-10 cm depth) and SE.

Table 3.2. *F* ratios from two-way ANOVA tests of inoculum (I), nitrogen (N) and water (W) on plant responses.

	Source of variance						
	Main effects			Interactions			
Response	Inoculum	Nitrogen	Water	I × N	I × W	N × W	I × N × W
Shoot mass	44.35***	88.64***	310.45***	54.78***	41.49***	49.54***	45.40***
Root mass	15.95***	21.36***	66.45***	17.36***	19.48***	10.21**	15.80***
Total mass	20.99***	69.59***	237.42***	45.65***	38.63***	37.59***	38.89***
Root:shoot	81.36***	0.15	12.62***	0.16	9.06***	0.20	0.89

*,**,***, indicate that *F* ratios were significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Table 3.3. Percent leaf N of seedlings grown in high and low N deposition inoculum and in a sterilized control under differential N and water availability after ten weeks.

	Nitrogen Treatment			
	Low N		High N	
Soil inoculum	Well-watered	Drought-stressed	Well-watered	Drought-stressed
Low deposition	2.53de	3.52abc	2.49e	3.03cde
High deposition	2.94cde	3.34ab	2.43e	3.97ab
Control	3.33bcd	4.13ab	3.41bcd	4.13a

Values followed by different letters are significantly different at $P<0.05$.

Table 3.4. Mean percent root length colonization (PRLC) of roots by mycorrhizal (AMF) and other non-mycorrhizal (NMF) fungi of seedlings grown in high and low N deposition inoculum and under differential N (Low N or High N) and water (Well-watered or Drought-stressed) availability.

	Inoculum Treatment							
	Low deposition				High deposition			
	Well-watered		Drought-stressed		Well-watered		Drought-stressed	
	Low N	High N	Low N	High N	Low N	High N	Low N	High N
AMF	6.5a	5.6a	14.4a	5.3a	0.7c	4.9ab	1.3bc	0.2c
NMF	22.2a	32.0a	34.5a	31.4a	6.9b	32.6a	5.3b	7.5b

Values in the same row followed by different letters are significantly different at $p < 0.05$.

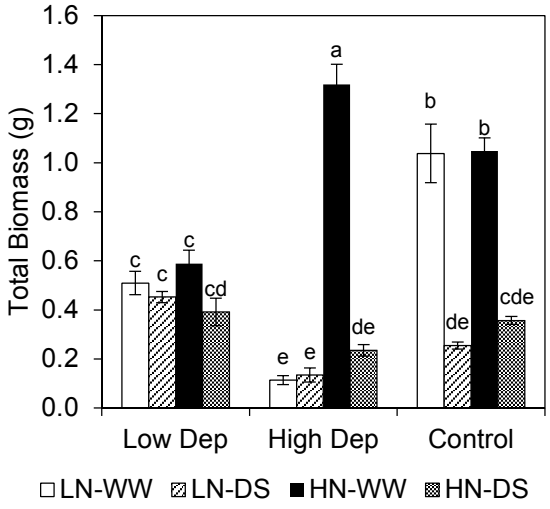

Figure 3.1. Total plant biomass of seedlings after ten weeks ± SE. Data represent average values of ten plants. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site, Control = sterilized control. Different letters above bars indicate significant differences (Tukey's HSD test, $P < 0.05$).

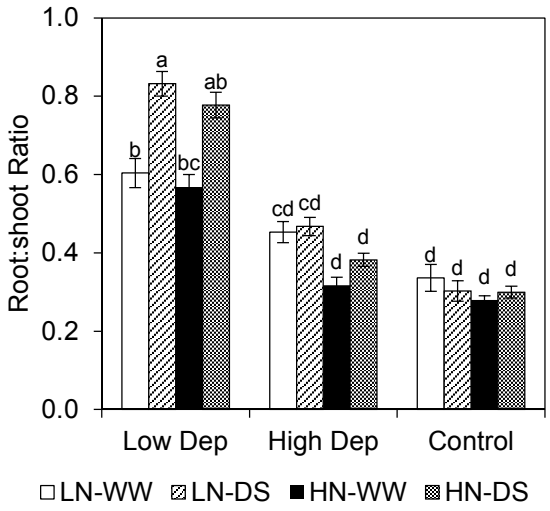

Figure 3.2. Root:shoot ratios of seedlings after ten weeks ± SE. Data represent average values of ten plants. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site, Control = sterilized control. Different letters above bars indicate significant differences (Tukey's HSD test, $P < 0.05$).

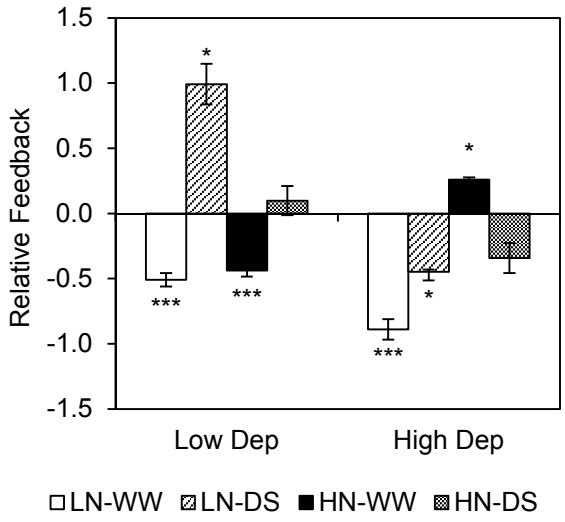

Figure 3.3. Relative feedback calculated as the difference in biomass relative to sterilized controls at ten weeks ± SE. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site. *,**,***, indicate significance relative to sterilized controls at $P \leq 0.05$, 0.01 and 0.001, respectively (ANOVA, Tukey's HSD test).

Nitrogen enrichment contributes to positive responses to soil microbial communities in three invasive plant species

Abstract

Increased resource availability and feedbacks with soil biota have both been invoked as potential mechanisms of plant invasion. Nitrogen (N) deposition can enhance invasion in some ecosystems, and this could be due to elevated soil N availability or shifts in soil biota. In a two-phase, full-factorial greenhouse experiment, I tested effects of N addition and N-impacted soil communities on the growth of three plant species invasive in California: *Bromus diandrus, Centaurea melitensis* and *Hirschfeldia incana*. In phase one, plants were grown individually in pots and inoculated with sterile soil, soil from control field plots or soil from high N addition plots, and with or without supplemental N. In phase two, I grew conspecifics in soils conditioned in phase one. I hypothesized growth responses would differ across species due to species-specific relationships with soil biota, but overall increased N availability and N-impacted soil communities would enhance growth. In phase one, *Centaurea* had the greatest growth response when inoculated with N-impacted soil, while *Bromus* and *Hirschfeldia* performed best in low N soil communities. However, in phase two all species exhibited positive growth responses in N-impacted soil communities under high N availability. These results suggest N deposition could facilitate invasion due to direct impacts of soil N enrichment on plant growth, as well as through feedbacks with soil biota.

Introduction

Resource availability plays a key role in mediating nonnative plant invasion (Vitousek and Walker 1989, Huenneke et al. 1990, Davis et al. 2000), and elevated soil nitrogen (N) due to anthropogenic N deposition may increase community invasibility (Dukes and Mooney 1999, Weiss 1999, Fenn et al. 2003). Feedbacks between plants and soil biota can also influence invasion success (Klironomos 2002, Callaway et al. 2004, Van der Putten et al. 2007, Pringle et al. 2009), and N deposition might alter soil microbial communities in ways that could further promote invasion (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2001, Sigüenza et al. 2006a, Sigüenza et al. 2006b). However, the response of invasive plants to increased soil N and N-impacted soil communities is likely species-specific, and once established plants can exert strong effects on soil biota (Kourtev et al. 2002, Belnap et al. 2005, Hawkes et al. 2005) that might influence subsequent growth and performance of conspecifics. In this study, I asked how N deposition influences the response of three invasive plant species in southern California to native soil communities and how grow responses change once invasives have conditioned soils.

The invasion of ecosystems by nonnative species poses a serious ecological and economic threat worldwide (Vitousek et al. 1997b, Pimentel et al. 2000), and multiple hypotheses have been invoked in an attempt to identify generalizable mechanisms. The theory of fluctuating resource availability (Davis et al. 2000) posits that a community becomes more invasible as unused resource availability increases. Under this hypothesis, the release or enrichment of a resource is expected to enhance the performance of

arriving invaders (Davis et al. 2000). Most terrestrial ecosystems are N limited, and multiple studies across biomes have found increased invasion with N addition (Burke and Grime 1996, Wedin and Tilman 1996, Brooks 2003, Rao and Allen 2010). As global rates of N deposition continue to rise (Galloway et al. 2004), this could increase the success of future invasions (Dukes and Mooney 1999, Bradley et al. 2010).

In addition to resource availability, invasion success can be strongly influenced by biotic factors, including soil fungi (Klironomos 2002, Callaway et al. 2004, Reinhart and Callaway 2006, Pringle et al. 2009). Species differ in their associations with soil biota, such as mutualists and pathogens, and therefore their effects on soil microbial communities, as well as their responses to these changes, resulting in plant-soil feedbacks (Bever et al. 1997). For example, a plant species may accumulate host-specific soil pathogens that inhibit the subsequent growth of conspecifics, resulting in a negative feedback (Mills and Bever 1998). Conversely, plants may promote the accumulation of beneficial soil mutualists, such as arbuscular mycorrhizal (AM) fungi, resulting in positive feedback (Zhang et al. 2010). Further, the influence plants exert on soils will also impact co-occurring plant species, and these feedbacks may be an important driver of community-level processes, such as invasion (Bever et al. 1997, Klironomos 2002).

Feedbacks between plants and soil biota have been hypothesized to be an important driver of plant invasion, with many invasive species exhibiting positive responses to soil communities (Klironomos 2002, Levine et al. 2006, Reinhart and Callaway 2006). For example, de la Pena et al. (2010) found that despite initial biotic resistance of native soils to invasion by *Carpobrotus edulis* in Mediterranean sand dunes,

once this species establishes, biotic resistance is diminished through the promotion of a soil community that enhances growth of this species, to the detriment of native plant species. Introduced plants may benefit from a lack of host-specific soil pathogens, promoting their invasiveness (Keane and Crawley 2002, Mitchell and Power 2003). However, while release from natural enemies belowground may contribute to positive feedback in invasive plants (Klironomos 2002, Kulmatiski et al. 2008), mutualists, such as AM fungi, can also be involved. These fungi form symbiotic relationships with plant roots, enhancing nutrient and water uptake in exchange for photosynthetic carbon (Smith and Read 2010). Arbuscular mycorrhizae play an important role in shaping plant community structure and diversity (van der Heijden et al. 1998), and may be critical determinants of invasion success (Callaway et al. 2004, Pringle et al. 2009).

Invasion can be influenced by AM fungi in a number of ways. Some nonnatives can reduce mycorrhizal density, and as invasive species are often less dependent on these associations, this results in increased performance relative to mycorrhizal natives (Stinson et al. 2006, Callaway et al. 2008, Vogelsang and Bever 2009, Owen et al. 2013). This interaction forms the basis of the "degraded mutualism" hypothesis (Vogelsang and Bever 2009). Alternatively, invading plant species may alter the mycorrhizal community by selecting for the most beneficial fungal species present, thereby reinforcing their competitive dominance, a phenomenon referred to as the "enhanced mutualism" hypothesis (Reinhart and Callaway 2006, Zhang et al. 2010). Finally, AM fungi can hinder invasion when a species promotes fungi that benefits heterospecifics more than conspecifics, contributing to a negative plant-soil feedback (Bever 2002). These

responses will depend on a number of factors, including the mycorrhizal status of the plant host, the identity of the host species and fungal symbiont(s) and the growth response of the plant (Pringle et al. 2009), as well as environmental conditions such as soil fertility (Johnson et al. 1997, Hoeksema et al. 2010).

Resource availability strongly influences interactions between plants and AM fungi (Johnson et al. 1997, Hoeksema et al. 2010), and this could impact the response of invading plants to native soil communities and the trajectory of plant invasions. Increased soil N due to anthropogenic N deposition may promote invasion via changes to the soil community. For example, high soil N may reduce root colonization and sporulation of some large-spored fungal species, thereby lowering mycorrhizal diversity and concomitantly increasing the proportion of small-spored fungal species, which has been shown to favor invasive annual grasses over native plant species (Egerton-Warburton and Allen 2000, Egerton-Warburton et al. 2001, Sigüenza et al. 2006b). Nutrient limitation may be a driver of local adaptation in mycorrhizae (Johnson et al. 2010), and increased soil N may also select for less beneficial fungi, resulting in a parasitic, rather than mutualistic, relationship (Johnson et al. 1997).

In southern California, anthropogenic N deposition has been implicated in the widespread invasion of coastal sage scrub (CSS) by annual grasses and forbs native to the Mediterranean Basin (Fenn et al. 2003, Talluto and Suding 2008, Cox et al. 2014, Goldstein and Suding 2014, Kimball et al. 2014). While elevated soil N appears to favor invasives in this system, there is also evidence that the soil microbial community plays an important role mediating these effects (Egerton-Warburton and Allen 2000, Sigüenza et

al. 2006a, Bozzolo and Lipson 2013). Research in this ecosystem has demonstrated differential responses of both native and nonnative plant species to microbial inoculation and N addition (Padgett & Allen 1999; Yoshida & Allen 2001; Sigüenza et al. 2006; Bozzolo & Lipson 2013), strongly suggesting a potential influence of N deposition on invasion though plant-soil feedbacks (Bever et al. 1997). For example, Sigüenza et al. (2006) found that growth of a native CSS species was inhibited by N-impacted soil communities, while an invasive annual grass, *Bromus madritensis*, responded positively.

The purpose of this study was to understand effects of soil N availability and N-impacted soil communities on the performance of three invasive plant species of southern California. This was completed in a two-phase factorial greenhouse experiment. In the first phase, I grew individuals of three invasive plant species in pots inoculated with soil from field plots subject to simulated N deposition, control field plots or sterile soil, and plants were grown with or without supplemental N addition. In the second phase, I grew the same species in soils conditioned in phase one, under the same conditions. I hypothesized that higher N availability would lead to increases in plant growth overall, but that responses would differ across plant species, due to species-specific responses to soil biota and N availability. I also hypothesized growth responses to live soil treatments would remain positive once plants had conditioned the soil, especially under high N availability and in soils previously impacted by N enrichment. An alternative hypothesis is that growth responses will be negative once plants have conditioned the soil, due to accumulation of soil pathogens.

Materials and Methods

Study Site

Soil to be used as whole-soil inoculum was collected from plots that are part of an experimental N addition gradient, located in the foothills of the Santa Monica Mountains, California (34.15°N, 118.96°W). The site receives approximately 8.8 kg N ha^{-1} yr^{-1} of background N deposition, most of which falls as dry deposition (Tonnesen et al. 2007). Typical of a Mediterranean climate, precipitation is seasonal, falling mostly during the cooler winter months, and summers are hot and dry. Average yearly rainfall for the site is 420 mm, but has been below average since 2012, with rainfall totals during the winter growing season, November to May, ranging from 33% 44% of 30-year normals. Vegetation at the site is mature CSS with *Artemisia californica* the dominant shrub species. Native and nonnative annuals and herbaceous perennials are present in shrub interspaces.

Study Species

I selected three nonnative plant species for this study: *Bromus diandrus* Roth (Poaceae), *Centaurea melitensis* L. (Asteraceae) and *Hirschfeldia incana* L. (Brassicaceae). These species are native to the Mediterranean Basin and invasive in California. They are also three of the dominant nonnatives at my field site and represent a variety of plant functional groups. *Bromus* is an annual C_3 grass, *Centaurea* is an annual forb and *Hirschfeldia* is an annual to perennial forb. Seeds of each species were collected

to be used in the growth experiment from invaded CSS adjacent to experimental N addition plots during spring and summer 2013.

Soil Inoculum

I collected soil from plots that receive supplemental N and unfertilized control as part of a multi-year N addition experiment. Fertilized plots have received 3 g N m^{-2} annually in the fall since 2011 to simulate the accumulation of dry N deposition during the summer dry period. This is equivalent to 30 kg N ha^{-1} yr^{-1}, which is the high range of modeled N deposition rates in the region (Tonnesen et al. 2007, Fenn et al. 2010). In December 2013, I collected five 10 cm deep soil cores from around the drip-line of mature *Artemisia* shrubs within 10 replicate plots for each treatment. I collected soil inoculum from the drip-line of shrubs, as this area is likely to have actively growing roots, and therefore presumably higher levels of microbial activity compared to shrub interspaces or soils with older, suberized roots. Soil was transported back to the lab where it was sieved through a 1 cm^2 stainless steel mesh. Root fragments were cut into 1 to 2 cm fragments and mixed back into the inoculum. I homogenized soil from each of the two plot types (N fertilized and unfertilized controls) to produce two whole-soil inocula with soil microbial communities characteristic of native CSS soils and N-impacted CSS soils. I also included a sterilized control treatment consisting of inocula from both sites which was steam-sterilized in a process including a 24 hour steam-sterilization, followed by a 48 hour incubation period and a second 24 hour sterilization period. Total extractable N of the fertilized and unfertilized soils was 58.6 μg N g^{-1} (SE ± 8.9) and 19.8

μg N g^{-1} (SE ± 1.4). Sterilization did not result in a significant increase in total extractable N in either fertilized (t-test; t_6 = -0.003, $P > 0.05$) or unfertilized inoculum (t-test; t_6 = 0.001, $P > 0.05$). To account for differences in available N in the two live inocula, when adding these I also included the same amount of sterilized inoculum from the other plot type, prepared as follows:

1. CSS Inoculum: 25g live soil from unfertilized control plots and 25 g sterilized soil from N addition plots added to each pot
2. N+CSS Inoculum: 25g live soil from N addition plots and 25 g sterilized soil from unfertilized control plots added to each pot
3. Sterilized Control: 25 g steam sterilized soil from unfertilized control plots and 25 g sterilized soil from N addition plots added to each pot

Growth Experiments

I used a 1:1 mixture of field-collected soil from undisturbed CSS adjacent to experimental plots and silica sand for potting, to which live soil inocula was added. I mixed sand with field soil using an electric cement mixer and steam-sterilized it as described above. Sand was added to improve water drainage and infiltration and to facilitate the recovery of root biomass when plants were harvested. Total KCl extractable N of our soil was 15.0 μg N g^{-1} (SE ± 0.7). Each pot (650 ml Deepots; Steuwe and Sons, Corvallis, Oregon, USA) was filled with 500 g of soil mix, with inoculum mixed in about 10 cm from the soil surface. Growth experiments were conducted from November 2013

to February 2014 in a greenhouse at the University of California, Riverside. High and low daily greenhouse temperatures were approximately 20° C and 16° C.

Phase 1

In the first phase, I grew 20 replicates of each species (3) by inoculum (3) by N (2) treatment (total number of pots = 360). Seeds of each species were sown directly in pots and watered with distilled water until germination, about five days for all species. Once seedlings emerged, I thinned them to a density of one per pot. I then switched to watering as needed every 3-5 days with distilled water and initiated N treatments. Half of the pots in each soil inoculum treatment received two applications of supplemental N to simulate high soil N availability as a result of elevated N deposition early in the growing season. The first N treatment was applied after plants had established in each pot and the second ten days later. Each time I applied approximately 20 μg N g^{-1} of soil from ammonium nitrate (NH_4NO_3) in solution to the high N pots, for a total of 40 μg N g^{-1}. Low N pots received an equal amount of distilled water each time. Twice per week, I rotated pots within racks and randomly re-distributed pot racks on greenhouse benches to account for differences in microclimate. After 60 days of growth, I clipped plant shoots off at the soil surface from all pots. Ten replicates of each treatment were allocated for root and soil analyses and the other ten for Phase 2 of the experiment.

Phase 2

In the second part of the experiment, I examined the response of my study species to the soils conditioned in Phase 1. After plant shoots were harvested from Phase 1, the ten replicates of each treatment reserved for Phase 2 were allowed to air-dry on greenhouse benches for two weeks. Soils within pots were left intact, and thus contained the root systems of plants grown in Phase 1. Prior to planting, I added a CSS nutrient solution (Padgett & Allen 1999; Table 1), so nutrients other than N would not be limiting to plant growth. Mean total extractable N in pots at the end of Phase 1 ranged from 2.4 to 5.2 μg N g^{-1} and did not differ significantly among species or treatments (ANOVA; $F_{17,72}$ = 1.33, P = 0.2753). All pots also received approximately 10 μg N g^{-1} in solution from NH_4NO_3, which restored available N to levels comparable to the beginning of Phase 1. I then re-seeded conspecifics into pots and maintained the same watering and N fertilization regime as in Phase 1under identical greenhouse conditions. I again grew plants for 60 days before harvesting.

Harvests

At each harvest, I separated plants into roots and shoots. Roots were carefully removed from the soil and washed with distilled water. Roots and shoots were dried at 60° C and weighed. I estimated root biomass in the pots at the end of Phase 1 that were reserved for Phase 2 using regressions of root and shoot biomass of fully harvested plants. Dried leaf tissue from was ground and analyzed for percent C and N using a Thermo-Finnigan FlashEA 1112 Nitrogen and Carbon Analyzer (Thermo Fisher

Scientific, Waltham, Massachusetts, USA) at the University of California, Riverside, Environmental Science Research Laboratory.

Root colonization

After drying and weighing roots, I rehydrated them in distilled water and stained them with trypan blue (Koske and Gemma 1989). For each plant, I mounted ten randomly selected fine root fragments on slides and assessed percent colonization of mycorrhizal and other non-mycorrhizal fungi in a procedure based on McGonigle et al. (1990). For each fragment, I made ten observations and noted presence or absence of fungi within roots. I distinguished between coarse AM fungi, fine AM endophyte and other fungi based on staining, hyphal diameter and morphology and the presence or absence of septa. Percent colonization of all fungi was low (< 2.5%) in roots of plants grown in sterile soil at each harvest and these were not included in my statistical analyses of percent colonization.

Calculation of inoculum response

I calculated relative inoculum responses in order to better illustrate the response of these study species to the different live soil inoculum treatments and understand how these relationships vary with N availability. Relative inoculum response was calculated as the difference in total biomass of plants grown in live soil inoculum relative to the mean total biomass of those grown in sterilized soil under the same N availability:

$$Relative\ inoculum\ response\ = \frac{(Biomass_{Live} - \ Mean\ Biomass_{Sterile})}{Mean\ Biomass_{Sterile}}$$

Using this calculation, a value greater than zero indicates a positive response to soil inoculation relative to sterile controls, while a value less than zero indicates a negative response.

Statistical analysis

My experimental design included three species, *Bromus*, *Centaurea*, and *Hirschfeldia*, grown in each of the three soil inoculum treatments (CSS Inoculum, N+CSS Inoculum and Sterile) and with or without supplemental N (High N and Low N) in a full factorial design. For each harvest, I analyzed data for the three species separately, performing two-way ANOVA with inoculum, N and the interaction between inoculum and N as factors. Relative inoculum responses for each harvest were also analyzed using two-way ANOVA, with species included as a factor along with inoculum type and N treatment. Prior to analysis, data were tested for the assumptions of ANOVA and transformed as necessary (log, arcsine, square root). Following ANOVA, I performed Tukey's honest significant difference test (HSD) to compare means and assign significance at $P < 0.05$. I also ran linear regressions to compare biomass from Phase 1 with biomass in Phase 2. Analyses were performed using RStudio Version 0.98.57, RStudio, Inc.

Results

Phase 1

During Phase 1 of the experiment, growth of *Bromus* was significantly affected by inoculum treatment ($P < 0.0001$) but not by N availability ($P = 0.11$) or the interaction of inoculum and N ($P = 0.83$; Table 2, Fig. 1a). Plants grown in the CSS inoculum had the greatest aboveground biomass and were on average 21% larger than those inoculated with N+CSS soil inoculum and 49% larger than plant grown in sterile soil (Fig. 1a). Belowground biomass was significantly influenced by inoculum type ($P < 0.0001$), with plants grown in CSS inoculum having higher mean root biomass ($F_{5,149} = 18.24$, $P < 0.0001$; Table 3). Percent N content of leaves was not significantly affected by inoculum or N individually ($P > 0.05$), but was influenced by their interaction ($P = 0.0067$), with N fertilization leading to a significant increase in tissue N only in sterile soil ($F_{5,23} = 3.71$, $P = 0.0175$; Fig. 2a). I observed very low levels of root colonization by AM fungi (< 2.2 %) and 5.2% to 10.6 % colonization by fine AM endophytic fungi, with no significant differences among treatments (Table 4). Colonization by nonmycorrhizal fungi was significantly affected by inoculum type, with plants grown in N+CSS inoculum on average 45% more colonized (Table 4).

Shoot biomass of *Centaurea* was significantly affected by both inoculum type ($P < 0.0001$) and N treatment ($P = 0.0088$; Table 2). Relative to plants grown in sterile soil, shoot biomass was on average 84% higher in N+CSS inoculated plants and 79% higher in CSS inoculum, indicating a positive growth response in both live soil inoculation treatments (Fig. 1b, 3a), with more positive responses observed under low N availability.

Belowground biomass was significantly affected by inoculum type ($P < 0.0001$) and N treatment ($P = 0.0012$), but not the interaction ($P = 0.1662$), and followed a similar trend as aboveground biomass ($F_{5,149} = 34.56$, $P < 0.0001$; Table 3). Percent N of leaf tissue was generally higher in N fertilized plants, but this difference was only significant in the CSS inoculum treatment ($F_{5,23} = 5.70$, $P = 0.0025$; Fig. 2b). *Centaurea* had the highest levels of colonization by AM fungi of the three species, but there were no significant differences among treatments within *Centaurea* (Table 4). Colonization by fine AM endophyte was significantly higher in plants inoculated with N+CSS soil, with the highest percent colonization occurring in plants also receiving supplemental N (Table 4).

Both inoculum ($P = 0.0003$) and N ($P = 0.0156$) significantly influenced growth of *Hirschfeldia* (Table 2, Fig 1c). The highest shoot biomass was observed in plants inoculated with CSS soil and grown under high N (Fig. 1c), which were on average 46% larger than those grown in sterile soil. There were no other significant differences in shoot growth among treatments (Fig. 1c). There were also no significant differences in belowground biomass across treatments ($F_{5,133} = 2.14$, $P = 0.0646$; Table 3). Percent N of leaves was significantly affected by the interaction of inoculum type and N ($P = 0.0174$), with the highest N content found in plants grown in sterile soil with supplemental N ($F_{5,23} = 3.08$, $P = 0.0349$; Fig. 2c). *Hirschfeldia* had very low levels (< 0.6%) of AM and fine endophytic fungi. There was a significant inoculum by N effect on the percent colonization of nonmycorrhizal fungi, with low N plants grown in CSS soil having the highest colonization (Table 4).

Across all species and treatments in Phase 1, relative inoculum responses were significantly ($F_{11,239}$ = 85.98, P < 0.0001) affected by species identity (P < 0.0001), nitrogen (P = 0.0005) and the interaction of species and inoculum (P < 0.0001), species and nitrogen (P < 0.0001) and species, inoculum and nitrogen (P = 0.0459). *Bromus* exhibited a positive growth response to N+CSS inoculum only when N fertilized, but had a positive response to the native CSS inoculum regardless of N availability (Fig. 3a). *Centaurea* plants had a positive growth response to both soil inoculation types, but this was reduced in plants receiving supplemental N. *Hirshcfeldia* plants grown in CSS inoculum under high N exhibited a positive inoculum response, but in all other live inoculum treatments plants showed a neutral response to inoculation.

Phase 2

In the second phase of the experiment, inoculum (P = 0.0081), N (P < 0.0001) and the interaction of these two factors (P < 0.0001) had significant effects on growth of *Bromus* (Table 2, Fig. 1d). The highest shoot biomass was observed in the two live inoculum treatments under high N, where biomass was 65% and 69% higher in plants receiving supplemental N in the CSS and N+CSS inoculated plants respectively (Fig. 1d). Plants receiving supplemental N had higher percent N content in leaf tissue in the N+CSS inoculum, but not in any other soil treatment ($F_{5,23}$ = 3.77, P = 0.0164; Fig. 2d). In this phase, plants grown in the N+CSS soil under high N availability had significantly higher colonization of fine endophyte compared to low N plants (Table 4). Plants grown in CSS soil without added N had the highest colonization of nonmycorrhizal fungi (Table 4).

Centaurea shoot biomass was again significantly affected by both inoculum ($P < 0.0001$) and N ($P < 0.0001$; Table 2, Fig. 1e). Effects of the two live inocula on shoot biomass were similar in both treatments, with N fertilized plants 50% to 55% higher than low N plants in the CSS and N+CSS treatments respectively. Plants grown in sterile soil were again significantly smaller than those inoculated with live soil (Fig.1e). Percent leaf N was highest in plants inoculated with N+CSS soil under high N availability ($F_{5,23} = 4.57$, $P = 0.0073$; Fig. 2e). Plants receiving supplemental N generally had lower colonization of AM fungi, but higher colonization by fine endophyte. In plants grown in CSS inoculum, colonization by nonmycorrhizal fungi was significantly reduced by N addition (Table 4).

In this phase, shoot biomass of *Hirschfeldia* was not significantly affected by inoculum type ($P = 0.0599$), but was influenced by N ($P < 0.0001$) and the interaction of inoculum and N ($P < 0.0001$; Table 2, Fig. 1f). In the two live inocula, N fertilized plants were on average 68% larger than low N plants (Fig 1f), but N had no significant effect on plants grown in sterile soil (Fig. 1f). There were no significant differences in percent leaf N across treatments ($F_{5,23} = 0.83$, $P = 0.5479$; Fig. 2f), and there were no effects of inoculum or N on percent colonization of roots by soil fungi (Table 4).

Across all species and treatments in Phase 2, relative inoculum responses were significantly ($F_{11,107} = 22.53$, $P < 0.0001$) affected by species identity ($P < 0.0001$), nitrogen ($P < 0.0001$) and the interaction of species and nitrogen ($P = 0.0003$). In this phase, *Bromus* exhibited a positive growth response in both live inoculum treatments when N fertilized, but a negative response when N availability was low (Fig. 3b).

Centaurea plants again showed the most positive inoculum response, regardless of inoculum type, but this effect was minimized in plants receiving supplemental N (Fig 3b). In the CSS soil inoculum, *Hirschfeldia* plants exhibited a negative growth response when N availability was low, but a neutral response under high N, while in the N+CSS soil inoculum, plants showed a positive inoculum response under high N availability and no response when N was low (Fig. 3b).

Finally, I explored potential relationships between plant performance in each phase using regression. For each species, biomass in Phase 2 was not significantly correlated with biomass, percent colonization of roots or extractable N of soils from Phase 1 ($P > 0.05$ for all correlations). I also did not find any significant correlations between plant biomass and percent colonization of mycorrhizal or nonmycorrhizal fungi in either phase of the experiment ($P > 0.05$).

Discussion

The success of invasive plants is often enhanced by soil N addition (Huenneke et al. 1990, Weiss 1999, Davis et al. 2000, Brooks 2003, Cox et al. 2014) and positive responses to soil biota (Klironomos 2002, Reinhart and Callaway 2006, Van der Putten et al. 2007, Pringle et al. 2009). Here I show that these two factors may increase growth of three nonnative invaders in California, which could potentially influence plant-soil feedbacks between native and invasive species under chronic N deposition. Initially in Phase 1, all species exhibited neutral to positive responses to native soil communities. Contrary to My hypotheses, in the first phase of the experiment N addition was less

important than soil community in determining plant growth, and only one species, *Centaurea,* performed better in native soil communities previously impacted by simulated N deposition. However, once species had conditioned soils, all exhibited positive growth responses in N-impacted soil communities under high N conditions. While positive responses to soil biota in invasive plants are often predicted (Klironomos 2002, Reinhart and Callaway 2006, Pringle et al. 2009), I found that when soil N was limiting, two species, *Bromus* and *Hirschfeldia* experienced neutral to negative inoculum responses. This work highlights the importance of soil N in determining plant responses to soil microbial communities and has important implications for plant invasion under anthropogenic N deposition.

Previous work has shown that the response of invasive species to N addition can be strongly influenced by the soil microbial community (Yoshida and Allen 2001, Sigüenza et al. 2006a, Bozzolo and Lipson 2013), and invasives may benefit from changes in the soil fungal community resulting from N deposition (Sigüenza et al. 2006a). However, plant responses may change once plants have conditioned soil communities, and this is the first study of which I are aware to utilize a two-phase approach to explore the effects of N enrichment on the response of invasive plant species to soil microbial communities. There is limited evidence that N-induced changes to plant-soil feedbacks may alter plant community structure (Manning et al. 2008), and the positive inoculum responses I observed under N enrichment may contribute to the success of these species in the field. In southern California, N deposition appears to facilitate the invasion of native CSS shrublands by nonnative grasses and forbs (Allen et

al. 1996, Cox et al. 2014, Kimball et al. 2014), including the species investigated here, but the underlying mechanisms are not fully understood. This study provides evidence that N enrichment may enhance the growth of invasive plant species due to both elevated soil N availability and through plant-mediated changes to soil microbial communities.

My results illustrate how the interacting dynamics of N deposition and plant responses to soil microbial communities might operate through multiple stages of the invasion process (Theoharides and Dukes 2007). The first phase of the experiment assessed the response of plants to soil communities of native CSS soils and native soils impacted by simulated N deposition. All species exhibited neutral to positive inoculum responses, showing soil communities of native CSS vegetation may not provide any "biotic resistance" against arriving invaders (Levine et al. 2004). However, the direction and magnitude of responses changed once plants had conditioned soils. Results from the second phase of the experiment show that once plants have established they may influence the subsequent performance of conspecifics through changes to the soil community, and N enrichment has the potential to alter these relationships. The negative inoculum responses observed in Phase 2 in the absence of N enrichment are particularly revealing. This shows that while invading species may initially respond positively to soil communities of a novel environment, as is often predicted (Klironomos 2002, Reinhart and Callaway 2006), they may limit the success of subsequent generations through feedbacks with soil biota. When N availability is high, however, these negative effects appear to dissipate, indicating that in addition to enhancing soil N availability, N deposition may promote the success of invasives through changes to soil communities.

There are several strong lines of evidence that suggest observed inoculum responses are due to effects of soil biota on plant performance. First, while many studies take a "black box" approach to plant-soil interaction experiments (Ehrenfeld et al. 2005, van der Putten 2010), I found clear differences in mycorrhizal and nonmycorrhizal fungal colonization of plant roots that may partially explain growth responses to live soil inocula. Second, while plant biomass differed among treatments in the initial conditioning phase, the possibility that responses in Phase 2 are due to nutrient depletion can be excluded, as I found no negative correlations between biomass in Phase 2 with biomass in Phase 1 (Kardol et al. 2006, Pernilla Brinkman et al. 2010). Furthermore, nutrients (minus N) were added to simulate extractable levels of CSS soils (Padgett and Allen 1999). It also appears unlikely that N accumulation in pots contributed to the greater N responses in Phase 2, as mean soil extractable N at the end of Phase 1 (beginning of Phase 2) was low and did not differ significantly among treatments. In addition to the effects of mycorrhizal and nonmycorrhizal fungi on inoculum responses, competition with soil microbes for soil N may have also played an important role (Kaye and Hart 1997). For example, in Phase 2, after two phases of N treatments, soil microbes may have been less N-limited, allowing greater for uptake by plants, resulting in great growth responses with N addition. It is interesting to note, however, that while growth responses were overall greater in high N inoculum in Phase 2, foliar N content was generally lower in all treatments. Finally, all species responded similarly to sterilized controls in both phases of the experiment, strongly suggesting that changes to inoculum responses were due to biotic factors, or interactions between soil biota and N availability.

Ecologists have long sought to identify generalizable mechanisms of plant invasion (Elton 2000, Levine et al. 2003), and the difficulty in doing so is likely due in part to species-specific variability in responses to biotic and abiotic factors. Species differ in effects on soil microbial communities and responses to soil biota, forming the basis of microbially mediated plant-soil feedbacks (Bever et al. 1997, Ehrenfeld et al. 2005). While feedbacks with soil biota may contribute to plant invasion (Klironomos 2002, van der Putten 2010), as is often predicted, the response of invasive species to soil microbial communities will depend on the identity of both plants and soil microbes (Klironomos 2003), as well as the biotic and abiotic context (Hoeksema et al. 2010), including nutrient availability (Johnson et al. 1997). Species exhibit various nutritional strategies and responses to soil nutrient availability (Chapin 1980), and these factors have the potential to interact (Gustafson and Casper 2004, Innes et al. 2004, Sigüenza et al. 2006a, Bozzolo and Lipson 2013). For example, Gustafson and Casper (2004) found that N addition negated negative feedback in one grass species, but had variable effects on another. In another experiment using *Centaurea* and a grass and mustard species closely related to the ones used here, *Brassica nigra* and *Bromus rubens*, Bozzolo and Lipson (2013) found *Brassica* and *Bromus* grew equally well in live and sterile soil, while *Centaurea* grew best in sterile soil. Further, only *Bromus* and *Centaurea* were responsive to N addition (Bozzolo and Lipson 2013). This stands in contrast to my results, where I observed positive inoculum responses in all species, especially under N addition. However this previous study only used soil collected from a low N deposition site, and did not include a soil conditioning phase (Bozzolo and Lipson 2013). While N enrichment appears to

contribute to positive responses to soil biota in all three species studied here, species differed in their relationships with soil fungi. For example *Bromus* associated mostly with fine AM fungi*, Centaurea* formed mycorrhizae with both coarse and fine AM fungi and *Hirschfeldia* was found to be nonmycorrhizal. Such differences in microbial associations may be responsible for the variable growth responses observed among plant species.

The response of *Bromus* to live inoculum treatments appears to have been mediated by both mycorrhizal and nonmycorrhizal fungi. Initially, plants responded less positively to the N-impacted soil community relative to the unfertilized CSS soil community, and these plants also had significantly higher colonization of potentially pathogenic fungi. Then in Phase 2, plants exhibited a positive inoculum response in N-impacted soils under high N and had higher colonization of fine AM fungi and lower colonization of nonmycorrhizal fungi. Plants grown in CSS soils under low N availability showed a negative inoculum response and higher colonization of fungal pathogens. Hilbig and Allen (2015) also found that fine AM fungi contribute to positive inoculum responses in this species in soils from a high N deposition site, and this may counteract potential negative impacts of fungal pathogens. Others have reported higher mycorrhizal colonization of *Bromus* in N rich soils (Parker and D'antonio 2008), and this may be partially responsible for increased invasion of this species under N deposition (Going et al. 2009). Typically a reduction in mycorrhizal colonization is expected with N fertilization, as plants are able to directly take up sufficient N without the added carbon cost of mycorrhizal association (Smith and Read 2010). However, chronic N addition, may select for more aggressive AMF fungi that maintain high rates of colonization even

when soil N availability is high (Johnson et al. 1997, Corkidi et al. 2002). While natives in this system may be negatively impacted by these AM fungal strains (Sigüenza et al. 2006a), invasives, such as *Bromus* may benefit, as demonstrated here. Previous accounts regarding the response of this species to soil microbial inoculation is mixed, with some reporting little or no response to microbial inoculation (Vogelsang 2004) and others reporting a positive growth response relative to growth in sterile soil (Bennett and Strauss 2013, Hilbig and Allen 2015). My results suggest that one possible cause of this variability in responses is differences in soil N availability or historical N inputs, with N-impacted soil communities promoting more positive plant growth responses. Plant soil feedbacks iinvolving native species and invasive annual grasses could promote invasion of this ecosystem if invasive respond positively to soil communities. For example, Siguenza et al. (2006a) found native species respond negatively to N-impacted soil communities, while an invasive grass, *Bromus madritensis* (L.), responded positively.

Previous studies have found *Centaurea melitensis* to be highly mycorrhizal (Callaway et al. 2001, Callaway et al. 2003). However, these two studies reported negative responses to colonization by AM fungi when plants were grown individually; responses were only positive when plants were grown in competition (Callaway et al. 2001, Callaway et al. 2003). Bozzolo and Lipson (2013) found a different result, where plants performed best in sterile soil relative to those grown in live soil. My results stand in contrast to this work and clearly demonstrate that this species can exhibit positive inoculum responses when grown individually, and these positive responses are likely due to colonization by mycorrhizal fungi. Initially, *Centaurea* was highly colonized by coarse

AM fungi, however after soil conditioning, there was a shift to higher colonization of fine AM fungi, particularly when N availability was high. This shows that while this species may associate with species of AM fungi that predominate in native CSS soils (Egerton-Warburton and Allen 2000, Sigüenza et al. 2006a, Sigüenza et al. 2006b), it may select for species of fine AM fungi, especially under high N conditions. It is also possible that this species is a better competitor for N than soil microbes, and this could have been partially responsible for the observed increase in growth and foliar N in *Centaurea* plants receiving supplemental N. Regardless of differences among treatment, I observed a highly positive response to live soil inoculation across treatments and in both phases of the experiment. This clearly demonstrates that performance of this species may be enhanced by soil biota, which could generate positive feedbacks with fine AM fungi under some conditions.

One of the more surprising results of this study was the positive inoculum response observed in *Hirschfeldia* in high N treatments. Like many annual species in the Brassicaceae, *Hirschfeldia* does not form mycorrhizae (Gerdemann 1968, Oliveira et al. 2005), and my methods are unable to explain these positive inoculum responses. There are other accounts of this species responding positively to soil biota; Bonanomi et al. (2008) found that removal of the soil microbial community through soil solarization and chemical fumigation inhibited the growth of *Hirschfeldia* seedlings relative to untreated control plots in the field, but it is unknown what soil microorganisms were involved. The positive growth responses I observed only occurred in plants receiving supplemental N, and it is conceivable that growth promoting bacteria or other soil biota involved in

nutrient cycling may have facilitated these growth responses. For example, rhizobacterium associating with a related species, *Brassica napus*, have been found to enhance N uptake and promote higher biomass (Bertrand et al. 2000). *Hirschfeldia* has been shown to be highly responsive to simulated N deposition in the field (Allen et al. 1996), and is a frequently observed nonnative in high N-polluted sites in southern California (Cione et al. 2002, Wood et al. 2006). While I are unable to explain the positive growth response of *Hirschfeldia* plants grown in N-impacted soil communities with the methods employed for this study, this could contribute to the success of this species under elevated N deposition.

While the positive inoculum responses I observed in these species could influence plant-soil feedbacks in the field, favoring invasion, a true test of plant-soil feedback requires pair-wise comparisons with native species, or at the very least, responses of species to soils conditioned by heterospecifics (Bever et al. 1997, Kulmatiski et al. 2008). My experiment did not close this feedback loop, as I did not include native species in my design. There are also further limitations inherent to my experimental approach. I homogenized my field-collected inocula within treatments prior to addition to pots in Phase 1, as my goal was to have plants condition soils that contain the full range of organisms present in CSS. While this approach has been utilized in similar experiments (Kardol et al. 2006, Mangan et al. 2010), this could confound results due to a potential lack of independence among treatments. While I focused on fungi colonizing plant roots, it is likely other soil organisms played an important role. For example, competition between soil microbes and plants for N played a role in generating observed inoculum

responses (Schimel et al. 1989, Kaye and Hart 1997), and this could explain the greater growth responses in high N treatments in Phase 2, if competition for N from microbes was less intense. However, I did not measure microbial N of soils and could not test this directly. My approach also did not incorporate other factors that could influence plant-soil interactions, such as competition between plants or litter inputs, and further research is needed to determine the importance of these processes in the field.

Conclusions

Global change factors such as N deposition may increase the success of biological invasions (Dukes and Mooney 1999), but effects of N enrichment on plant performance is often mediated by soil biota. The results of this study clearly demonstrate N enrichment can alter interactions between three invasive plant species and soil microbial communities. These relationships have important implications for community invasibility and invasion success under N deposition. Invasive plants benefitting from increased soil N availability and positive feedbacks with soil biota may be more difficult to control or spread more quickly. With rates of anthropogenic N deposition expected to continue to rise globally in the near future (Vitousek et al. 1997a, Galloway et al. 2004), a better understanding of how N enrichment influences both aboveground and belowground aspects of plant invasions is needed.

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Table 4.1. Modified coastal sage scrub nutrient solution from Padgett and Allen (1999) applied to all pots before seeding Phase 2.

Element	Nutrient solution (mM)	Specific Compound
Cl	3	HCl
Ca	1.2	$CaCO_3$
S	1.2	$MgSO_4$
Na	1	NaOH
Mg	0.6	MgO
P	0.16	KH_2PO_4
K	0.14	KCl
B	0.003	H_3BO_3
Zn	0.001	$ZnCl_2$
Mn	0.0001	$MnSO_4$
Cu	0.0001	$CuSO_4$
Fe	5 mg l^{-1}	Fe EDTA

Table 4.2. Results from two-way ANOVA tests of inoculum (I), nitrogen (N) and the interaction of inoculum and nitrogen (I × N) on aboveground plant biomass for each species in Phase 1 and Phase 2 of the experiment.

		Phase 1			Phase 2		
Plant species	Source	d.f.	*F*	*P*	d.f.	*F*	*P*
Bromus diandrus	I	2	13.475	**< 0.0001**	2	5.024	**0.0081**
	N	1	2.642	0.1069	1	238.084	**< 0.0001**
	I × N	2	0.182	0.8342	2	38.108	**< 0.0001**
Centaurea melitensis	I	2	117.166	**< 0.0001**	2	28.619	**< 0.0001**
	N	1	7.113	**0.0088**	1	44.102	**< 0.0001**
	I × N	2	0.098	0.9072	2	2.623	0.0772
Hirschfeldia incana	I	2	8.907	**0.0003**	2	2.891	0.0599
	N	1	6.021	**0.0156**	1	152.242	**< 0.0001**
	I × N	2	1.546	0.2176	2	14.552	**< 0.0001**

Table 4.3. Percent N of leaf tissue for each species grown in three inoculum types, native CSS soils (CSS), native soils subject to experimental N deposition (N+CSS) and a sterile control (Sterile) and under high and low N availability (Low N, High N) for both phases of the experiment. Letters within rows represent significant differences at $\alpha = 0.05$ from ANOVA.

	Inoculum treatment					
	CSS		N+CSS		Sterile	
Plant Species	Low N	High N	Low N	High N	Low N	High N
Phase 1						
Bromus diandrus	2.50ab	3.22ab	2.36ab	3.01ab	2.26b	3.68a
Centaurea melitensis	2.34bc	3.47a	2.76abc	2.87ab	1.64c	2.43abc
Hirschfeldia incana	1.98b	3.32ab	2.58ab	3.01ab	2.85ab	3.47a
Phase 2						
Bromus diandrus	2.07ab	2.51ab	1.88b	3.14a	1.87b	2.03ab
Centaurea melitensis	1.93b	2.18ab	2.17ab	2.53a	2.26ab	2.35ab
Hirschfeldia incana	1.72	1.85	1.41	2.05	1.85	1.79

Table 4.4. Mean (n = 10) percent colonization of roots by arbuscular mycorrhizal (AM) fungi, fine endophytic (FE) fungi and nonmycorrhizal (NM) fungi for each species inoculated with native coastal sage scrub soil (CSS) or native soils impacted by simulated nitrogen deposition (N+CSS) and grown with (High N) or without (Low N) supplemental nitrogen for Phase 1 and Phase 2 of the experiment. Significant sources of variation are shown from ANOVA, including inoculum (I), nitrogen (N) and the interaction (I × N). Different letters within species for each phase represent values were significantly different at α = 0.05.

	Phase 1							Phase 2						
	CSS		N+CSS					CSS		N+CSS				
Plant Species	Low N	High N	Low N	High N	I	N	I × N	Low N	High N	Low N	High N	I	N	I × N
Bromus diandrus														
AM fungi	2.0	2.2	1.6	2.0	-	-	-	0.6	0.8	0.6	0.4	-	-	-
	± 0.5	± 0.7	± 0.4	± 1.1				± 0.2	± 0.6	± 0.2	± 0.2			
FE fungi	7.0	5.2	6.2	10.6	-	-	-	6.2ab	5.6ab	5.0b	10.2a	-	-	*
	± 0.9	± 2.4	± 1.2	± 2.0				± 0.8	± 0.8	± 1.4	± 1.8			
NM fungi	10.0bc	8.0c	15.0ab	18.0a	***	-	-	50.4a	36.6ab	31.2b	34.8ab	*	-	-
	± 1.3	± 1.1	± 1.3	± 2.3				± 3.7	± 2.2	± 4.0	± 7.3			
Centaurea melitensis														
AM fungi	23.6	33.2	18.4	20.4	-	-	-	7.6ab	4.2ab	7.8a	1.8b	-	*	-
	± 2.7	± 7.2	± 3.9	± 3.5				± 0.1	± 1.3	± 2.1	± 0.1			
FE fungi	1.6c	2.2c	5.6b	10.2a	***	**	*	6.0b	18.6a	4.6b	12.4ab	-	*	-
	± 0.2	± 0.8	± 0.7	± 1.0				± 2.1	± 3.3	± 1.4	± 1.4			
NM fungi	12.8	6.2	10.2	6.4	-	-	-	12.6a	2.2b	7.0ab	9.6ab	-	*	**
	± 2.4	± 1.7	± 2.1	± 1.1				± 2.9	± 0.1	± 1.1	± 1.6			
Hirschfeldia incana														
AM fungi	0.6	0.0	0.0	0.4	-	-	-	0.2	0.2	0.4	0.0	-	-	-
	± 0.6			± 0.2				± 0.1	± 0.1	± 0.2				
FE fungi	0.6	0.2	0.0	0.2	-	-	-	0.2	0.0	0.2	0.2	-	-	-
	± 0.4	± 0.1		± 0.1				± 0.1		± 0.1	± 0.1			
NM fungi	17.6a	4.0b	8.2ab	13.8a	-	-	***	5.6	6.6	10.4	6.4	-	-	-
	± 4.0	± 1.0	± 1.6	± 1.6				± 1.0	± 2.3	± 1.1	± 1.0			

*,**,***, indicate that *F* ratios were significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

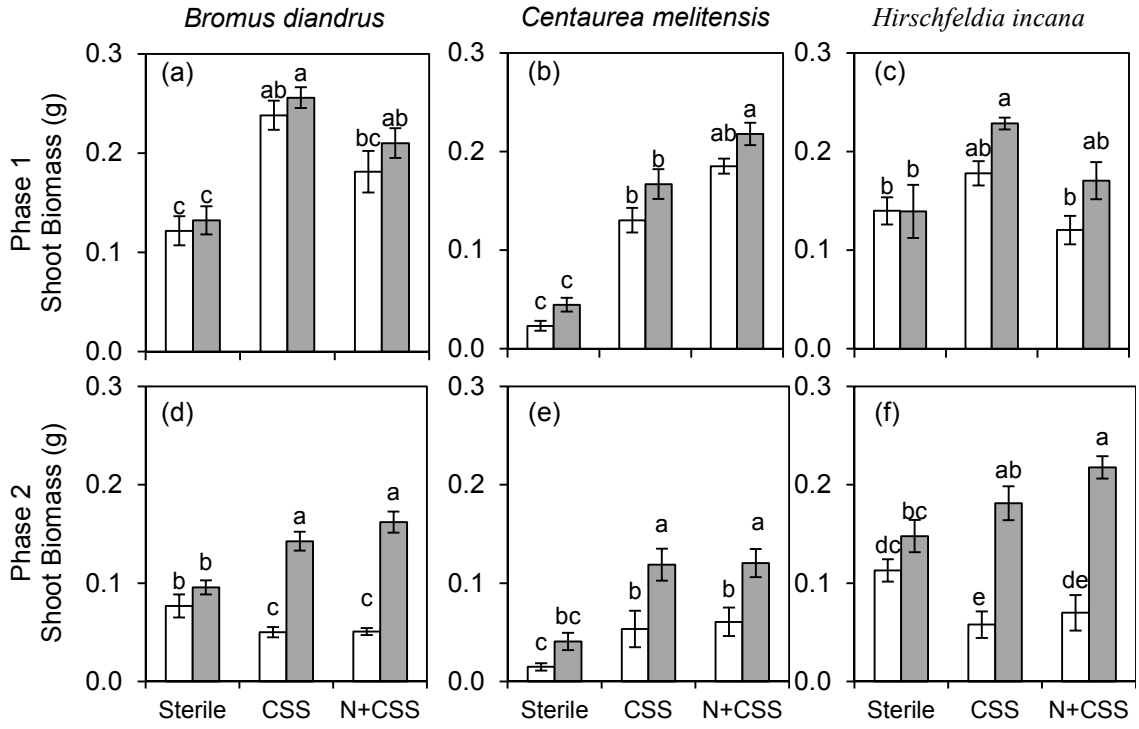

Figure 4.1. Mean dry aboveground biomass of plants for each species grown in each inoculum type, native CSS soils (CSS), native soils subject to experimental N deposition (N+CSS) and sterile controls (Sterile) and with (shaded bars = High N) or without (white bars = Low N) supplemental N for Phase 1 (a-c) and Phase 2 (d-f). Error bars represent standard errors of the mean. Letters represent significant differences within species for each phase at $\alpha = 0.05$ from ANOVA.

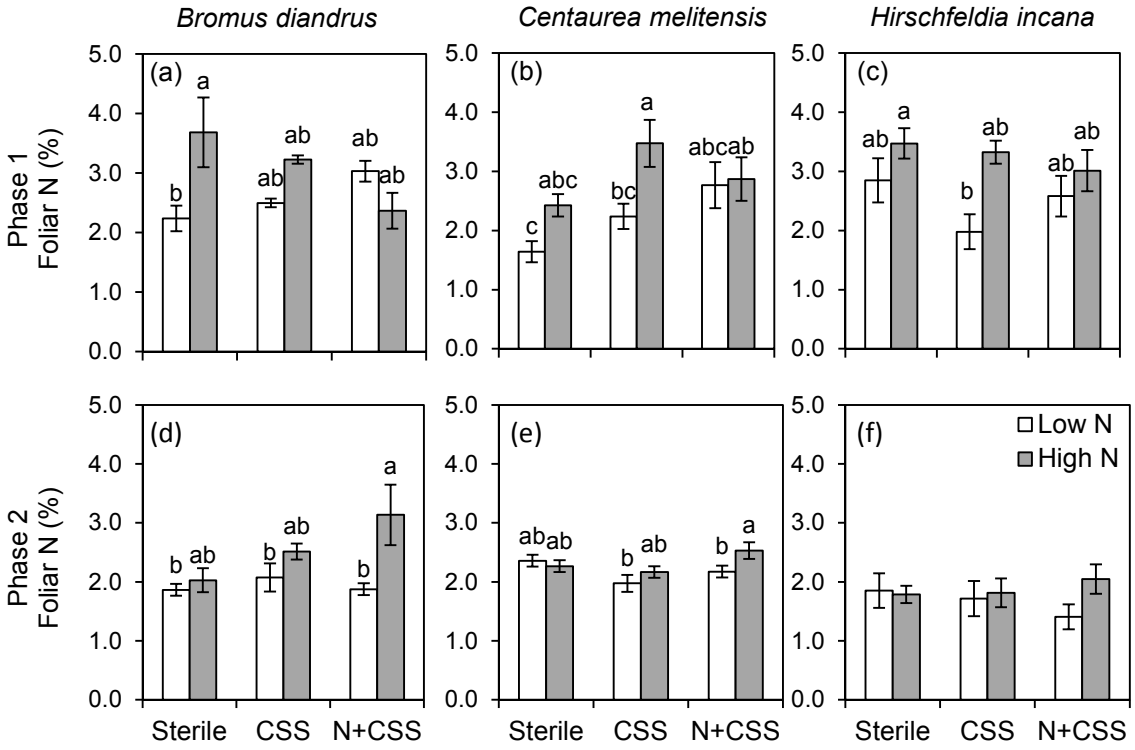

Figure 4.2. Percent foliar N of leaf tissue for each species (n = 5) grown in three inoculum types, native CSS soils (CSS), native soils subject to experimental N deposition (N+CSS) and a sterile control (Sterile) and under high and low N availability (shaded bars = High N; white bars = Low N) for Phase 1 (a-c) and Phase 2 (d-f). Error bars represent standard errors of the mean. Letters represent significant differences within species for each phase at $\alpha = 0.05$ from ANOVA.

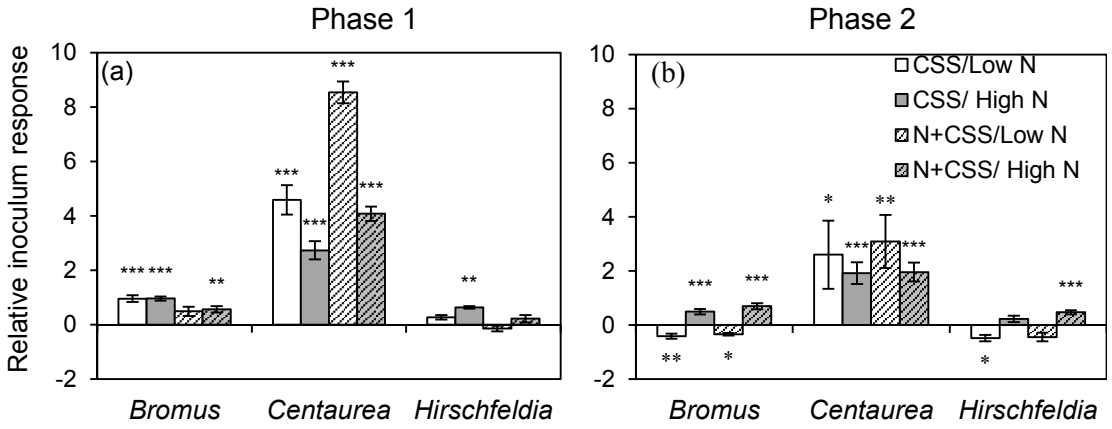

Figure 4.3. Mean relative inoculum response for each species grown in each live inoculum type, native CSS soils (CSS = non-patterned bars) and native soils subject to experimental N deposition (N+CSS = patterned bars) and with (shaded bars = High N) or without (white bars = Low N) supplemental N for Phase 1 (a) and Phase 2 (b). Error bars represent standard errors of the mean. Asterisks indicate mean plant biomass was significantly different than that of plants grown in sterile controls under the same conditions (ANOVA, Tukey's HSD; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$).

Conclusions

Anthropogenic activities, such as the burning of fossil fuels and resulting impacts on climate and nutrient cycling, or the spread of nonnative plant species, have the potential to profoundly impact native ecosystems (Vitousek et al. 1996, Pimentel et al. 2000, Breshears et al. 2005, Galloway et al. 2008). These drivers of global environmental change are expected to increase dramatically in the future, and may also have complex and interactive effects on native plant communities (Zavaleta et al. 2003, Bradley et al. 2010, Valliere and Allen 2016a). I explored the ecological impacts of anthropogenic N deposition, drought and invasive plant species at multiple scales in the Santa Monica Mountains of southern California, finding these factors pose a significant threat to the native coastal sage scrub (CSS) of the region.

At the landscape level, native plant diversity of CSS was negatively correlated with atmospheric N deposition resulting from high levels of atmospheric N pollution. Particularly affected were native forb species, which may be less competitive under high N conditions (Suding et al. 2005). Elevated N availability was also associated with increased cover of nonnative plant species, which may also negatively impact co-occurring natives. While large expanses of CSS are protected within the Santa Monica Mountains National Recreation Area, the eastern portion of the park is subject to high levels of atmospheric N pollution originating from the greater Los Angeles area. The resulting inputs of reactive N will undoubtedly continue to influence vegetation dynamics

in the future, with important implications for the long-term management and conservation of native plant communities, such as CSS.

I also explored the impact of N availability on CSS through an N addition field experiment at a low deposition site, using four realistic levels of N deposition. I measured effects on plant community composition, productivity, decomposition and plant traits over four years (2011-2015), a period of time coinciding with the historic California drought. All levels of N addition resulted in increased soil N availability over time, resulting in significant changes in vegetation and increased rates of decomposition. The dominant native CSS shrub *Artemisia californica* responded to N addition with increased leaf area, and higher leaf little production early in the study. However, after two years of drought, native shrub cover within high N plots declined dramatically due to dieback of branches, resulting in higher production of woody litter. Shrubs within high N plots also exhibited reduced water-use efficiency, which likely contributed to loss of shrub cover during the extended drought. Together, this supports the hypothesis that N deposition may increase plant susceptibility to drought (Bobbink and Lamers 2002, Jones et al. 2004, Meyer-GrüNefeldt et al. 2013).

While native shrub cover declined over time, herbaceous species, both native and nonnative, increased in cover and biomass over time, likely due to the opening of the shrub canopy. This was especially true in high N addition plots, where nonnative annual forbs and grasses increased significantly in cover and biomass. This is consistent with ecological theory predicting enhanced invasion success as unused resources, here soil N availability, increase (Davis et al. 2000, Davis and Pelsor 2001). This is also consistent

with previous observational and experimental work in CSS, where N deposition resulted in increased growth of nonnative annuals (Allen et al. 1996, Fenn et al. 2003, Cox et al. 2014), especially under reduced precipitation (Kimball et al. 2014).

I also found significant effect of N addition on feedbacks between plants and soil microbial communities, and these dynamics may influence the response of plant communities to other components of global change, such as drought and nonnative plant invasion (Wolters et al. 2000, Wardle et al. 2004, Bardgett and Wardle 2010). Using a full-factorial greenhouse experiment, where I manipulated N and water availability and inoculated plants with high and low N deposition soil communities, I found both N availability and N-impacted soil communities influenced the response of *Artemisia californica* seedlings to drought. Seedlings inoculated with native soil microbial communities had reduced biomass relative to those grown in sterile soil, and aboveground biomass was unaffected by N addition or drought. However, these seedlings exhibited the highest root:shoot ratios, especially under drought conditions, which may increase drought tolerance. Conversely, seedlings grown in soil from a high N deposition site attained the greatest shoot biomass under high resource availability, but when grown under low N or drought conditions, plants were significantly smaller than those from other soil inoculation treatments. These seedlings also exhibited the lowest root:shoot ratios and reduced colonization of arbuscular mycorrhizal fungi. Together, these results suggest the soil microbial community plays an important role mediating the response of native shrub seedlings to N and water availability. While native soil communities may

provide protection against drought through beneficial mycorrhizal fungi and increased allocation to roots, N deposition appears to alter this effect (Valliere and Allen 2016a).

Nitrogen addition also altered feedbacks between soil biota and nonnative annual plant species, potentially contributing to increased invasion under high N deposition (Valliere and Allen 2016b). Using a two-phase plant-soil feedback approach, I tested the response of three nonnative plant species, *Bromus diandrus*, *Centaurea melitensis*, and *Hirschfeldia incana*, to N availability and N-impacted soil communities. In the first phase, where plants were inoculated with native soil communities from high and low N addition plots, species showed little response to N addition across inoculum treatments, and two species attained the greatest biomass in low deposition soil. However, in phase two, once soil had been conditioned by conspecifics, plants exhibited significantly greater biomass under N addition, and N availability and N-impacted soil microbial communities contributed to positive plant-soil feedbacks in all species. These responses were also linked to root colonization of soil fungi. *Bromus diandrus*, for example, associated mostly with fine endophytic arbuscular mycorrhizal fungi, especially under high N conditions. These results highlight the potential impacts of N deposition on plant-soil feedbacks of nonnative plant species, possibly contributing to increased invasion success under future global change.

Mediterranean-type ecosystems, such as CSS of California, represent a biodiversity hotspot, and these systems may be particularly vulnerable to global environmental change (Sala et al. 2000, Phoenix et al. 2006, Ochoa-Hueso et al. 2011). With increased rates of N deposition (Galloway et al. 2008), climate change and global

change-type drought (Bell et al. 2004, Breshears et al. 2005) and the spread of nonnative plant species (Vitousek et al. 1996, Levine and D'Antonio 2003) all expected to increase dramatically in the future, these results have important implications for the long-term persistence and conservation of CSS in the region. Elevated N deposition was found to have ecological impacts at a number of scales, from plant traits to community and landscape level changes. This work contributes to the growing body of evidence that anthropogenic N deposition may result in reduced plant diversity (Bobbink and Lamers 2002, Simkin et al. 2016) and more invaded ecosystems (Dukes and Mooney 1999, Cox et al. 2014). Several potential mechanisms of vegetation change under N deposition were identified, including feedbacks with soil biota. This highlights the importance of above-belowground linkages, and the potential for global change drivers to alter these relationships (Bardgett and Wardle 2010).

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