Shoot and root biomass production in semi-arid shrublands exposed to long-term experimental N input
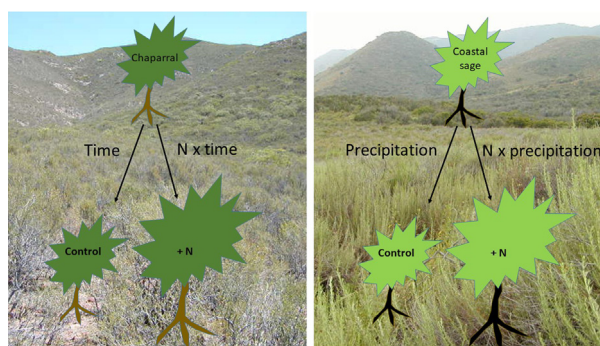
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HIGHLIGHTS

- Nitrogen (N) deposition is high in chaparral and coastal scrub (CSS) ecosystems.
- Root and biomass production were measured in an N addition experiment for 11 years
- N-exposed chaparral initially produced more roots but switched to shoots over time.
- N-exposed CSS had higher rates of productivity during wet years only.
- N responses are often variable because of interactions with water and nutrients.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Anthropogenic nitrogen (N) deposition has affected the primary production of terrestrial ecosystems worldwide; however, ecosystem responses often vary over time because of transient responses, interactions between N, precipitation, and/or other nutrients, and changes in plant species composition. Here we report N-induced changes in above- and below-ground standing crop and production over an 11-year period for two semi-arid shrublands, chaparral and coastal sage scrub (CSS), of Southern California. Shrubs were exposed to 50 kgN ha^{-1} in the fall of each year to simulate the accumulation of dry N deposition, and shoot and root biomass and leaf area index (LAI) were measured every 3 months to assess how biomass production responded to chronic, dry N inputs. N inputs significantly altered above- and below-ground standing crop, production, and LAI; however, N impacts varied over time. For chaparral, N inputs initially increased root production but suppressed shoot production; however, over time biomass partitioning reversed and plants exposed to N had significantly more shoot biomass. In CSS, N inputs caused aboveground production to increase only during wet years, and this interaction between added N and precipitation was due in part to a highly flexible growth response of CSS shrubs to increases in N and water availability and to a shift from slower-growing native shrubs to fast-growing introduced annuals. Together, these results indicate that long-term N inputs will lead to complex, spatially and temporally variable growth responses for these, and similar, Mediterranean-type shrublands.

1. Introduction

Atmospheric nitrogen (N) deposition has fundamentally altered carbon (C) and nutrient cycling in terrestrial ecosystems (Chen et al., 2015; Deng et al., 2017), and these changes are likely to continue with an

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increase in the global population, expansion of agriculture, and burning of fossil fuels (Phoenix et al., 2006; IPCC, 2013). Nitrogen is often the most limiting nutrient for terrestrial net primary production (NPP) (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008), thus, increases in N deposition are likely to increase NPP on a global scale (Zhang et al., 2011; Wieder et al., 2015). However, N inputs may eventually exceed demand, leading to "N-saturation" (Fenn et al., 2010), and increases in N may cause soil acidification (Tian and Niu, 2015) and alterations in plant stoichiometry (Peñuelas et al., 2013), resulting in limitations from other nutrients, especially phosphorus (P) (Vitousek et al., 2010). Increases in N can also alter disturbance regimes (Wood et al., 2006; Talluto and Suding, 2008), change plant-microbial mutualisms (Suding et al., 2005; Allen et al., 2016), and promote invasion of introduced species (Isbell et al., 2013; Simkin et al., 2016; Vourlitis, 2017), which feed-back on NPP.

Here we utilized a long-term field N addition experiment to assess how N enrichment alters above- and below-ground biomass production. We focus on chaparral and coastal sage scrub (CSS) ecosystems because they are globally important ecosystems and relevant models for other semiarid ecosystems, such as Chilean mattoral, Spanish maquis, South African fynbos and thorn-scrub, and Australian kwongan/mallee, which share similar adaptations to drought, fire, and nutrient limitation (DiCastri, 1991; Lavorel et al., 1998; Vogiatzakis et al., 2006). Within San Diego County, chaparral and CSS represent over half of the natural vegetation (Pryde, 1992), while in California, these ecosystems comprise nearly 30% of the natural vegetation (Hornbeck, 1983; Dallman, 1998). Chaparral and CSS are experiencing rapid climate and land use change, alterations to disturbance regimes (Vogiatzakis et al., 2006; Keeley and Brennan, 2012; Syphard et al., 2018), and have been exposed to high levels of atmospheric N deposition for decades (Bytnerowicz and Fenn, 1996; Fenn et al., 2003, 2010).

Anthropogenic N inputs to chaparral and CSS have caused soil acidification (Vourlitis and Fernandez, 2012), and changes in N cycling (Vourlitis and Zorba, 2007; Homyak et al., 2016), plant species diversity (Valliere et al., 2020), the abundance of introduced species (Sigüenza et al., 2006; Valliere et al., 2017; Vourlitis, 2017), and primary production (Vourlitis, 2012; Vourlitis and Hentz, 2016). However, N effects in these semi-arid woodlands are often dependent on precipitation and water availability, leading to variable patterns in ecosystem N responses that vary depending on species, soil type, and/or disturbance (Harpole et al., 2007; Talluto and Suding, 2008; Vourlitis and Pasquini, 2009; Kimball et al., 2014; Homyak et al., 2016; Valliere and Allen, 2016). These variations cause non-linear and/or lagged responses to anthropogenic N inputs that are almost impossible to observe and/or interpret with short-term experiments or observations (Milchunas and Lauenroth, 1995; Isbell et al., 2013; Vourlitis and Hentz, 2016; Vourlitis, 2017). For example, Vourlitis (2012) found that N inputs in a secondary chaparral stand caused a significant decline in aboveground NPP (ANPP) during the first 3 years of fertilization but a significant increase in ANPP after an additional 3 years of fertilization. Similarly, Vourlitis (2017) found that it took at least 4 years of N fertilization in CSS to cause a significant change in plant species composition, and up to 10 years of fertilization to promote the invasion of the introduced species Brassica nigra. These changes occurred after prolonged drought caused shrub dieback, providing space available for nitrophilic (Pivovaroff et al., 2016) and introduced species to exploit (Vourlitis and Pasquini, 2009; Rao and Allen, 2010; Vourlitis, 2017).

Here we extend the aboveground biomass and productivity databases for chaparral (Vourlitis et al., 2009; Vourlitis and Hentz, 2016) and CSS (Vourlitis, 2012), and add unpublished data on root biomass and productivity, to assess how long-term dry season N inputs alter above and below productivity. Root biomass measurements began in 2008, when both ecosystems had already been fertilized for nearly 5 years, and both the root and aboveground biomass data reported here extend over an 11 year period between 2008 and 2018. Root biomass was measured in the surface 0–10 cm layer, which only captures on average about 30% of the root mass density for sclerophyllous shrubs

(Jackson et al., 1996). However, root biomass estimates are sparse for chaparral and CSS woodlands, and other authors suggest that between 40 and 70% of the root density for chaparral shrubs may be found in the upper 10 cm soil layer (Kummerow et al., 1977; Miller and Ng, 1977). Given previous research, we hypothesize that N inputs will (1) significantly increase above- and below-ground biomass stocks and production, and (2) cause a decline in the root:shoot ratio for both chaparral and CSS over time.

2. Materials and methods

2.1. Site location and experimental design

Research was conducted in a chaparral stand located at the Sky Oaks Field Station (SOFS) in NE San Diego, County CA (33°22′51.49″N; 116°37′32.67″W; 1420 m above sea level), and a CSS stand located at the Santa Margarita Ecological Reserve (SMER) in SW Riverside County, CA (33°26′18.14″N; 117°10′52.18″W; 248 m above sea level).

SOFS is on a 4–10° SE-SW facing slope, and is dominated by the evergreen shrubs Adenostoma fasciculatum H. & A. and Ceanothus greggii A. Gray, which make up 98% of the total plant biomass (Vourlitis and Pasquini, 2009). The stand is recovering from a wildfire that occurred in July 2003, or 2 months prior to initiation of the fertilization treatments (Vourlitis and Hentz, 2016). The soil is an Ultic Haploxeroll of the Sheephead Series (Moreno and Oechel, 1992) with a sandy loam texture and a bulk density of 1.34 g/cm^3 (Vourlitis et al., 2009).

The SMER research site has not experienced fire for over 40 years. The site is on a 9–11° S-SW facing slope, and is dominated by summer-deciduous shrubs, Artemisia californica Less. (California sage) and Salvia mellifera Greene (Black sage), which make up 95% of the total plant biomass (Vourlitis and Pasquini, 2009; Vourlitis, 2012). Soil is weathered Gabbro material of the Las Posas Series (Knecht, 1971), with a sandy clay loam texture and an average bulk density of 1.22 g/cm^3 (Vourlitis et al., 2009).

Annual precipitation data for both sites were obtained from the Western Regional Climate Center (www.wrcc.dri.edu; Accessed June 14, 2020). Data for SOFS are for Oak Grove, CA (NWS ID: 045710) and data for SMER are from the Santa Rosa Plateau (NWS ID: 045623). The average (±sd) annual precipitation since fertilization began (2003) is 382 ± 193 mm for SOFS and 414 ± 226 mm for SMER. Most of the precipitation (85–90%) for both sites is from rain that occurs from November–April; however, SOFS receives some snow in the winter and monsoonal rainfall during the summer months.

The experimental layout at both sites consists of a randomized design where four-10 × 10 m plots received 50 kgN ha^{-1} y^{-1} (added N) and an additional four-10 × 10 m plots served as un-manipulated controls. This rate is about 2-times higher than the N deposition reported for the most polluted low-elevation shrublands, but is similar to values observed for high-elevation forests in southern California (Fenn et al., 2010). Plots are arranged in pairs along the slope gradient, with one control and one added-N plot paired together (Vourlitis and Fernandez, 2012). Granular N fertilizer was added each year in the fall (September–October) since 2003, and evenly broadcast using a handheld spreader as either NH_4NO_3 (2003–07), $(NH_4)_2SO_4$ (2007–09), or urea (2009-present). Plots are fertilized in the fall because the majority of the N deposition to southern California shrublands (ca. 90%) accumulates as dry deposition during the summer and fall (Bytnerowicz and Fenn, 1996; Fenn et al., 2003). Estimated N deposition is 2–4 kgN ha^{-1} y^{-1} for each study area (Vourlitis, 2017), thus, control plots received 2–4 kgN ha^{-1} annually while N plots received 52–54 kgN ha^{-1} y^{-1} since September 2003.

2.2. Field sampling

Field sampling occurred quarterly (ca. January, April, July, and October) each year during the 2008–18 study period except 2014, when field sampling occurred in the fall only.

All plants >20 cm in height were measured within a 2-meter radius (12.57 m^2) quadrat in the center of each plot, while plants <20 cm were measured in a 0.25 m radius (0.20 m^2) quadrat centered within each 12.57 m^2 quadrat. Vegetation volume was calculated as the product of canopy area (A) and maximum height, where $A = \pi D^2/4$ and D is the average shrub diameter calculated from measurements of the maximum and perpendicular diameters (Vourlitis, 2012; Bonham, 1989). Vegetation biomass was estimated from shrub volume using regression equations that were developed for individual species sampled from both study sites (Table 1).

Root biomass was measured in the upper 10 cm soil layer from soil core samples obtained every 3 months (except in 2014) as described above (Laurenroth, 2000). During each sampling, 2–4 soil sub-samples were randomly collected in each plot using a 4.7 cm diameter x 10 cm depth bucket auger (Vourlitis and Hentz, 2016). Each soil sub-sample was transferred to a sterile plastic bag, and was stored in a freezer (−20 °C) until analysis, which was typically within a week of sampling. Soil was passed through a 2 mm mesh sieve to remove the roots, and retained roots were washed, oven-dried at 70 °C, and weighed using a digital balance. The dry mass of roots for each sub-sample was first converted to a volumetric standing crop (gdw/m^3) by dividing the dry root mass by the volume of the soil core sampler and then converted to a standing crop per unit area (gdw/m^2) by multiplying the volumetric standing crop by the sampling depth (0.1 m). Plot-level estimates of root biomass were calculated as an average of the 2–4 subsamples taken per plot, and because of the difficulties associated with identifying roots of different species, no attempt was made to quantify species differences in root production or standing crop.

Leaf area index (LAI) was measured in the center of each plot every 3–4 months using a PAR-ceptometer (AccuPAR LP-80, Decagon Devices, Inc., Pullman, WA, USA). Measurements were made above the canopy and at 8 points below the canopy in the center of each plot. The 8 measurement points were made in all cardinal directions from the center of the plot, and given that the length of the PAR-ceptometer is approximately 1 m, the diameter of the LAI measurement "footprint" was about 2 m, which overlapped with the measurements of shrub volume and biomass. LAI was calculated from the above and below canopy measurements using the PAR-ceptometer software assuming a spherical leaf angle distribution.

Table 1
Regression equations for estimating total aboveground biomass from measurements of shrub volume. Biomass was measured from direct harvests and volume was measured as the average canopy area times maximum plant height (see methods) ($n = 10$–26 depending on shrub). Biomass and volume were LN-transformed, and the coefficients below are linear regression coefficients of LN-biomass (y-axis) vs. LN-volume (x-axis) where were m is the slope, b is the intercept, and r^2 is the coefficient of determination. Also shown are the F-ratios from the linear regression, the degrees of freedom (df: regression, error) and the P-values. General equations were developed by combining all data for all shrubs or herbaceous plants.
Modified from Pasquini (2008).

Species name	m	b	R^2	F	df	P
Adenostoma fasciculatum	0.883	−4.784	0.99	1223	1,19	<0.0001
Artemisia californica	0.861	−5.753	0.99	2428	1,24	<0.0001
Hirschfeldia incana	1.149	−11.525	0.78	29	1,8	0.0007
Camissonia spp.	0.409	−2.748	0.80	32	1,8	0.0005
Ceanothus greggii.	0.716	−4.199	0.95	351	1,19	<0.0001
Cryptantha spp.	0.736	−5.440	0.87	52	1,8	0.0001
Dendromecon rigida	0.590	−3.111	0.96	210	1,8	<0.0001
Eriodictyon trichocalyx	0.763	−4.541	0.94	120	1,8	<0.0001
Eriogonum fasciculatum	0.787	−4.959	0.99	139	1,3	0.0071
Gnaphalium spp.	0.716	−4.199	0.93	154	1,12	<0.0001
Heliathemum scoparium	0.662	−3.897	0.72	20	1,8	0.0020
Phacelia spp.	0.716	−5.192	0.95	163	1,8	<0.0001
Salvia mellifera	0.935	−6.455	0.99	1939	1,16	<0.0001
Stephanomeria virgata	0.421	−3.090	0.89	62	1,8	<0.0001
Shrubs (general)	0.870	−5.488	0.97	3195	1116	<0.0001
Herbaceous (general)	0.720	−5.087	0.79	234	1,62	<0.0001

2.3. Theory, calculation, and data analysis

Missing data for 2014 were interpolated as a seasonal average from measurements made in 2013 and 2015 (e.g., winter data in 2014 was calculated as the average of winter data collected in 2013 and 2015). Above- and belowground rates of annual biomass production were calculated using two different methods (1) as the sum of the positive increments, where only increases in biomass over time are summed, and (2) the min-max method, where biomass production is calculated as the difference between the maximum and minimum standing crop (Lauenroth, 2000; Sala and Austin, 2000). For the sum of positive increments, above- and below ground biomass increments were calculated for each 3 month measurement interval, positive increments were summed, negative increments were set to zero, and annual rates of production were calculated as the sum of the positive increments. For the min-max method, minimum values measured for each 3 month measurement interval were subtracted from maximum values measured during each interval.

Orthogonal regression was used to assess the correspondence between the above- and belowground biomass production derived from the sum of positive increments method vs. the min-max method. Orthogonal regression was used because both methods have the potential for error, thus, ordinary least-squares regression would not be appropriate (Leng et al., 2007). Regressions were performed on LN-transformed data ($n = 80$) by first assessing if the intercept was significantly different from zero, and if not, by forcing the intercept through zero.

Annual data were analyzed using a repeated-measures ANOVA to assess whether N addition and time caused significant ($p < 0.05$) variations in response variables. Box's M and Mauchly's tests were used to test the assumptions of equality and compound-symmetry (sphericity), respectively ($p \leq 0.10$) of the between-group covariance matrices. Interactions between N addition and rainfall on standing crop, biomass production, and the R:S ratio were assessed using linear regression, because previous research indicated the possibility of interactions between precipitation and N addition (Vourlitis, 2012). First, biomass, productivity, and R:S were LN-transformed, then differences between the LN-transformed values from N and control (C) treatments were calculated as LN(N)-LN(C). Log-differences were then regressed against annual precipitation to assess if the relative effects of N on standing crop or production varied due to annual variations in precipitation.

Analyses were conducted using NCSS statistical software (Version 7, NCSS, LLC, Kaysville, UT, USA). Data were LN-transformed to fulfill assumptions of normality and heteroscedasticity.

3. Results

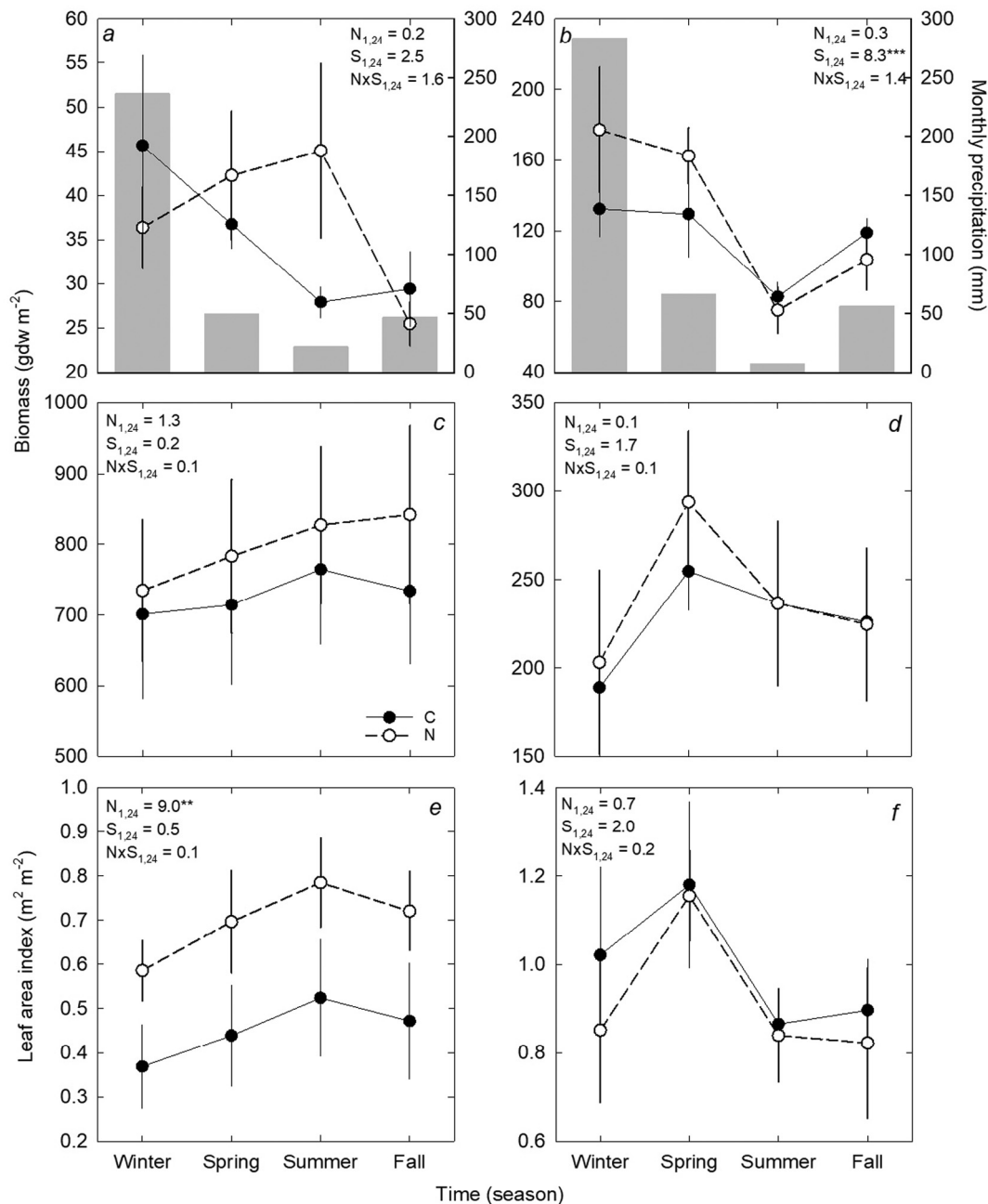### 3.1. Estimating aboveground biomass and production

Equations for the dominant chaparral (*A. fasciculatum* and *C. greggii*) and coastal sage scrub (CSS) (*A. californica* and *S. mellifera*) shrubs had r^2

Table 2
Orthogonal regression results between root and aboveground biomass production estimated from the sum of positive increments and min-max methods of estimation. Regression was first conducted with an intercept, but if the intercept was not significantly different from zero the regression was re-run with the intercept forced through zero. Shown are mean (±95% confidence intervals) of the intercept (b), slope (m), and coefficient of determination (R^2) for both the control and N treatments combined for the annual estimates of biomass production calculated for the chaparral at Sky Oaks Field Station (SOFS) and the coastal sage scrub at Santa Margarita Ecological Reserve (SMER) for the study period. Data were LN + 1 transformed prior to orthogonal regression ($n = 80$). NA = not applicable because the regression was forced through zero.

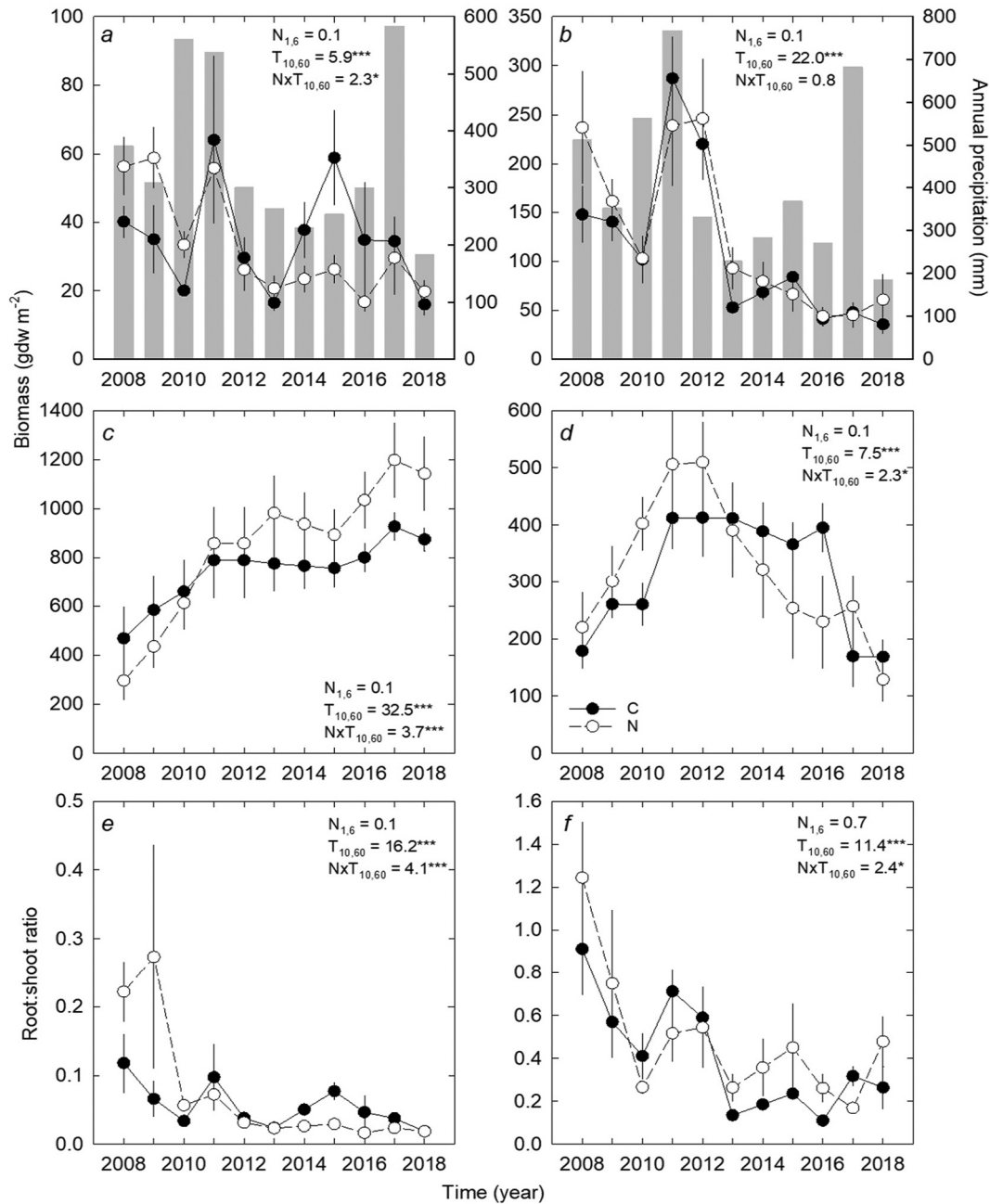| Site	Variable	b (±95% CI)	m (±95% CI)	R^2
SOFS	Root biomass	NA	0.987 (0.967, 1.004)	0.98
	Shoot biomass	NA	0.995 (0.974, 1.015)	0.98
SMER	Root biomass	0.645 (0.080, 1.118)	0.875 (0.789, 0.976)	0.80
	Shoot biomass	NA	0.996 (0.978, 1.013)	0.99

Fig. 1. Root (a and b) biomass (lines and symbols, left-hand axis) and monthly precipitation (bars, right-hand axis), and shoot (c and d) biomass and leaf area index (e and f) for chaparral plots at Sky Oaks Field Station (a, c, and e) and coastal sage scrub plots at the Santa Margarita Ecological Reserve (b, d, and f) averaged between 2008 and 2018. Also shown are the results of a 2-way ANOVA (F-statistic, factor and error degrees of freedom), and p-value ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$) for nitrogen addition (N), season (S), and the N × S interaction. Data for biomass and leaf are index are means ($\pm$se; $n = 4$), and data for monthly precipitation are means.

values ≥0.95, indicating a high level of confidence in estimating above-ground biomass from measurements of shrub volume (Table 1). Other species exhibited higher variation, for example *Heliathemum scoparium* and *Hirschfeldia incana* had the lowest r² values (0.72 and 0.78, respectively), but these species were considerably less abundant than the principal species listed above. Even so, we realize that errors in biomass estimates derived from shrub volume measurements alone may arise because of N-induced changes in stem diameter, density, and/or leaf thickness (Sala and Austin, 2000); however, periodic measurements indicate that specific leaf area (SLA) and stem density and diameter have not changed due to N addition (Davis, 2008). Shrub volume measurements may also fail to account for variations in leaf production, which may be especially important for semi-deciduous CSS shrubs, but some of this information is available from the measurements of LAI.

Estimates of above- and below-ground biomass production calculated from the sum of positive increments method were similar to those calculated from the min-max method (Table 2). Orthogonal regression intercepts were not significantly different between the biomass production methods for chaparral shoot and root biomass and CSS shoot biomass, and the slopes were not significantly different from 1 (Table 2), indicating that both methods provided similar estimates of biomass production along the full range of production values. In contrast, the intercept of the orthogonal regression for CSS root biomass was significantly different from 0 and the slope was significantly different from 1, indicating a slight bias between the estimates of root production calculated from the different methods (Table 2). Coefficients of determination ranged from 0.80 (CSS roots) to 0.99 (CSS shoots), indicating good-excellent goodness of fit between the two methods.

Fig. 2. Root (a and b) biomass (symbols and lines, left-hand axis) and annual precipitation (bars, right-hand axis) and shoot (c and d) biomass and the root:shoot ratio (e and f) for chaparral plots at Sky Oaks Field Station (a, c, and e) and coastal sage scrub plots at the Santa Margarita Ecological Reserve (b, d, and f) for the 2008–18 study period. Also shown are the results of a repeated-measures ANOVA (F-statistic, factor and error degrees of freedom), and p-value (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) for nitrogen addition (N), time (T), and the N × T interaction. Data for biomass and root:shoot ratio are means ($\pm$se; $n = 4$), and data for precipitation are annual totals.
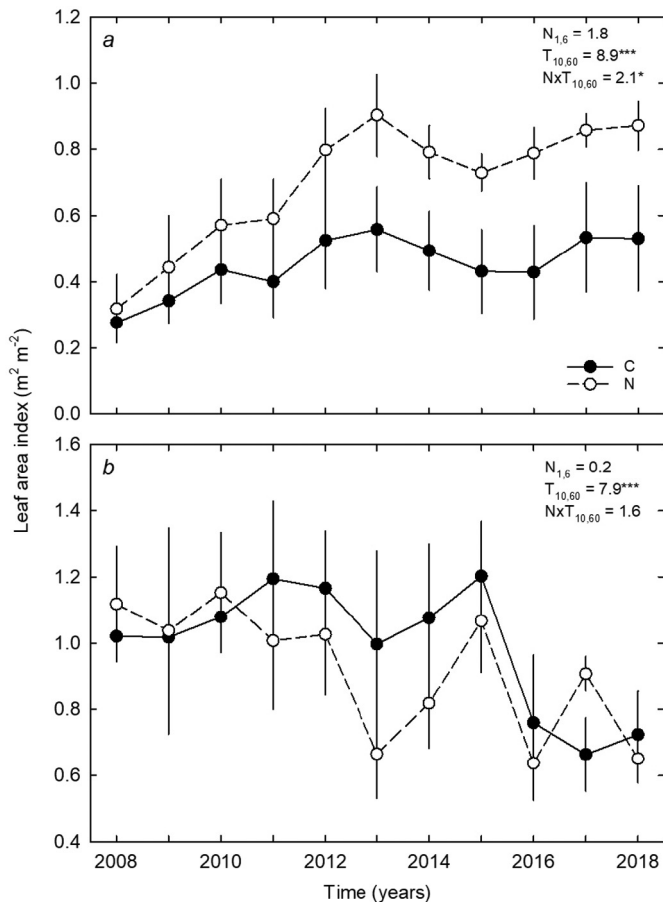
Given the close correspondence between the two methods, and the fact that both methods are prone to error, we decided to use the root and shoot production estimates calculated from the sum of positive increments for the analyses here because this method utilized all of the seasonal data available.

3.2. Seasonal patterns in standing crop and leaf area index

Average seasonal trends in root production differed more for chaparral control and N plots (Fig. 1a) than CSS (Fig. 1b), but temporal differences were not significantly different for either vegetation type. For chaparral, control plots had higher root biomass in the winter and the lower biomass in the summer and fall, while N plots had peak root biomass in the summer (Fig. 1a). Root biomass in CSS was similar for control and N plots, and was highest in the winter and lowest in the summer. Trends in root biomass in CSS, and control plots in chaparral, were coincident with seasonal variations in precipitation (Fig. 1a and b).

Both shoot biomass and leaf area index (LAI) exhibited similar seasonal trends. For the chaparral stand, both shoot biomass (Fig. 1c) and LAI (Fig. 1e) were lower in winter and increased into the summer, and the N plots had slightly higher shoot biomass and significantly higher LAI than the control plots. For the SMER CSS, both shoot biomass (Fig. 1d) and LAI (Fig. 1f) were low in the winter, increased to a peak in the spring, and declined into the summer and fall, and there were no differences between N and control plots.

Fig. 3. Mean ($\pm$se; n = 4) leaf area index for chaparral plots at Sky Oaks Field Station (a) and coastal sage scrub plots at the Santa Margarita Ecological Reserve (b) for the 2008–18 study period. Also shown are the results of a repeated-measures ANOVA (F-statistic, factor and error degrees of freedom), and p-value (*p < 0.05; **p < 0.01; ***p < 0.001) for nitrogen addition (N), time (T), and the N × T interaction.

3.3. Annual patterns in standing crop and leaf area index

Annual variations in root biomass varied significantly over time for both sites and there was a statistically significant N × time (N × T) interaction for chaparral (Fig. 2a and b). Root biomass exhibited peaks in 2011, which was a wetter than average year (Fig. 2a), and again in 2015 for the chaparral control plots at SOFS (Fig. 2a), which was also a wetter than average year, but in general, there was a decline in root biomass over the study period, especially for CSS (Fig. 2b). Root biomass for chaparral was on average higher for N plots during the first 3 years but lower for the last 5 years of the study period, accounting for the significant N × T interaction (Fig. 2a).

Shoot biomass varied significantly over time and there was a significant N × T interaction for both study sites (Fig. 2c and d). For chaparral, shoot biomass increased consistently over the study period, and was lower for N plots during the first 2 years but higher for N plots during the final 6 years (Fig. 2c). For CSS, aboveground biomass increased for both treatments until 2011, but thereafter, biomass declined consistently for N plots (Fig. 2d). In contrast, shoot biomass for control plots was similar for 2011–16, and then declined by >2-fold by the end of the study period (Fig. 2d). Thus, the significant N × T interaction appeared to be due to the relatively higher shoot biomass for N plots until 2011 followed by the relatively higher shoot biomass for control plots thereafter.

The root:shoot (R:S) ratio varied significantly over time and there was a significant N × T interaction for both study sites (Fig. 2e and f). The chaparral R:S was higher for both plots during the first 3 years of the study period and declined thereafter (Fig. 2e). Nitrogen plots had

a higher R:S ratio than control plots during the first 2 years of the study, however, control plots had a slightly higher R:S during the final 5 years. For CSS, the R:S ratio declined consistently over time, and N plots had a higher R:S than control plots in about half of the years studied.

LAI varied significantly over the study period for both sites, and there was a significant N × T interaction for chaparral (Fig. 3a and b). LAI increased over the study period for chaparral, but the increase was higher for N plots accounting for the significant N × T interaction (Fig. 3a). For CSS, LAI was consistently between 1 and 1.2 m^2/m^2 for both N and control plots during the first 5 years of the study period, but LAI declined starting in 2012 for the N plots and 2015 for the control plots (Fig. 3b). The decline in LAI coincided with a decline in aboveground standing crop (Fig. 2d), and excluding 2015, a decline in annual precipitation that occurred during the latter half of the study period (Fig. 2a and b).

3.4. Patterns in biomass production

Annual variations in root production were statistically significant for both sites and there was a significant N × T interaction for chaparral (Fig. 4a and b). Root production in chaparral N plots was consistently higher than control plots during the first 3 years but consistently lower during the last 6 years, and excluding the peak in root production in 2011, which was a wet year (Fig. 2a), both treatments exhibited a general decline in root production over time (Fig. 4a). In contrast, differences in CSS root production between N and control plots were negligible, and root production was between 2- and 4-fold higher in 2011–12 compared to other years.
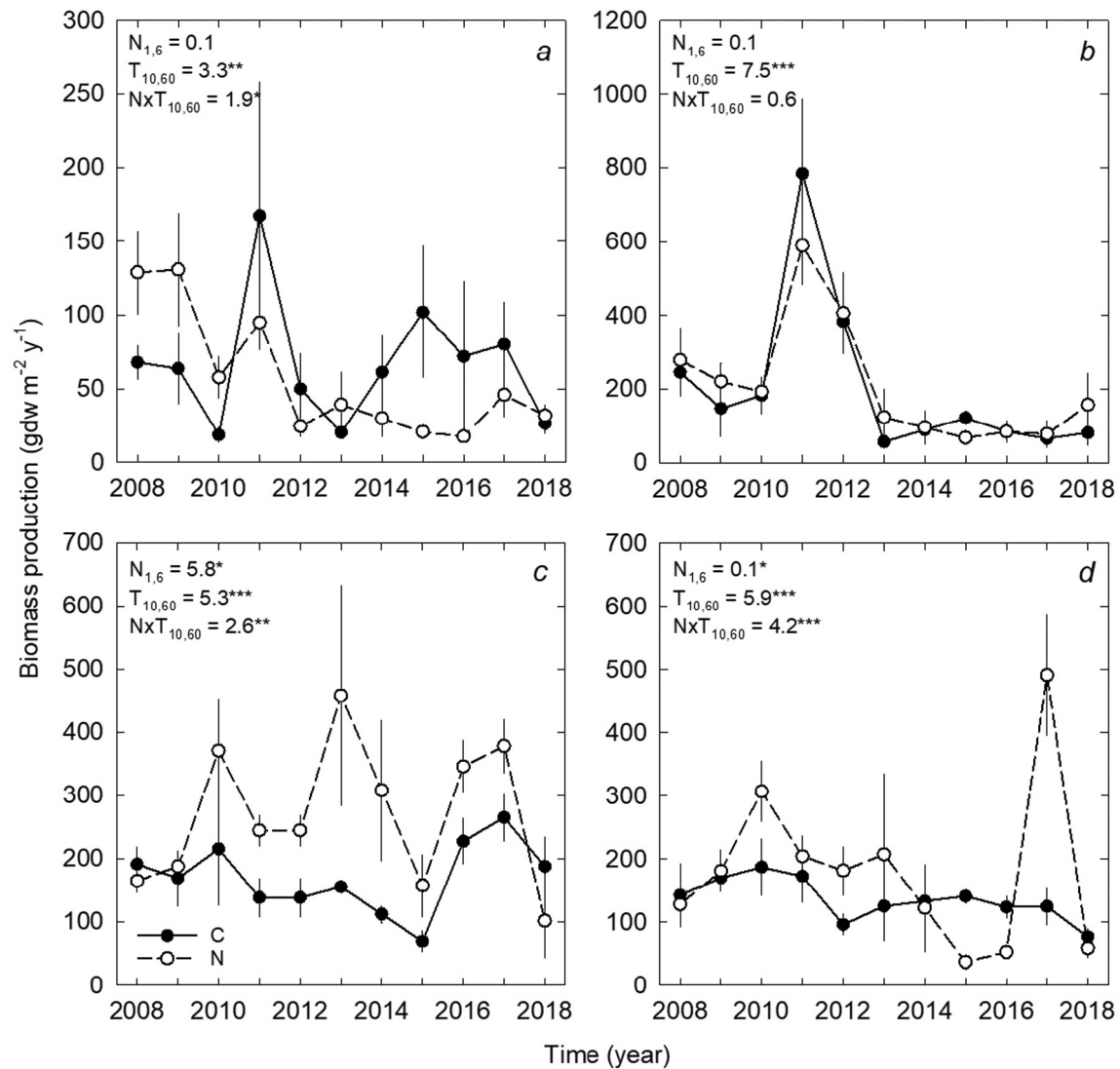
Rates of shoot production varied significantly over time and there was a significant N × T interaction for both sites (Fig. 4c and d). In chaparral, N plots had a higher rate of shoot production in 8 of the 11 years studied (Fig. 4c). In CSS, differences in shoot production between control and N plots were more subtle, and N plots tended to have higher shoot production in 2009–11 but lower shoot production in 2012–15 than control plots (Fig. 4d). Both treatments in CSS exhibited peak shoot production in 2015, which was a wet year (Fig. 2a and b), but excluding that year, both treatments had a decline in shoot production over the study period (Fig. 4d).

Potential interactions between precipitation and N input on root and shoot standing crop, root and shoot production, and the R:S ratio were assessed using linear regression of the LN-difference between N and control plots (dependent axis) vs. precipitation (Fig. 5). Only the LN-difference in aboveground biomass (Fig. 5a) and the R:S ratio (Fig. 5b) for CSS at SMER were found to be significantly related to annual precipitation, with N plots having higher aboveground biomass and lower R:S ratio as annual precipitation increased.

Cumulative root production increased rapidly for chaparral N plots for the first 4 years of the study period but leveled off thereafter, while control plots exhibited a linear increase in cumulative root production over time; however, N and control plots produced nearly identical (730–740 g/m^2) root biomass over the study period (Fig. 6a). In CSS, cumulative root production exhibited a sigmoidal increase over time, and both control and N plots produced 2246–2298 g/m^2 of root biomass over the study period (Fig. 6b).

Chaparral N plots had a significantly ($p < 0.05$) higher rate of cumulative shoot production than control plots, and by the end of the study period, mean ($\pm$se; $n = 4$) cumulative shoot production was 1871 $\pm$ 133 g/m^2 for control plots and 2964 $\pm$ 344 g/m^2 for N plots (Fig. 6c). In CSS, mean cumulative shoot production was 1495 $\pm$ 55 g/m^2 for control plots and 1974 $\pm$ 346 g/m^2 for N plots, but differences were not statistically significant at the end of the study (Fig. 6d).

Cumulative total (above + belowground) biomass production for chaparral at SOFS was qualitatively similar to that observed for shoot biomass, and by the end of the study, N plots produced nearly 1.5

Fig. 4. Mean (±se; n = 4) root (a and b) shoot (c and d) biomass production for chaparral plots at Sky Oaks Field Station (a and c) and coastal sage scrub plots at the Santa Margarita Ecological Reserve (b and d) for the 2008–18 study period. Also shown are the results of a repeated-measures ANOVA (F-statistic, factor and error degrees of freedom), and p-value (*p < 0.05; **p < 0.01; ***p < 0.001) for nitrogen addition (N), time (T), and the N × T interaction.

times more biomass than control plots (Fig. 6e). Trends in total cumulative biomass production for N and control plots in CSS were sigmoidal over time, and by the end of the study period, control plots produced 3742 ± 233 g/m² while N plots produced 4274 ± 627 g/m² of biomass (Fig. 6f).
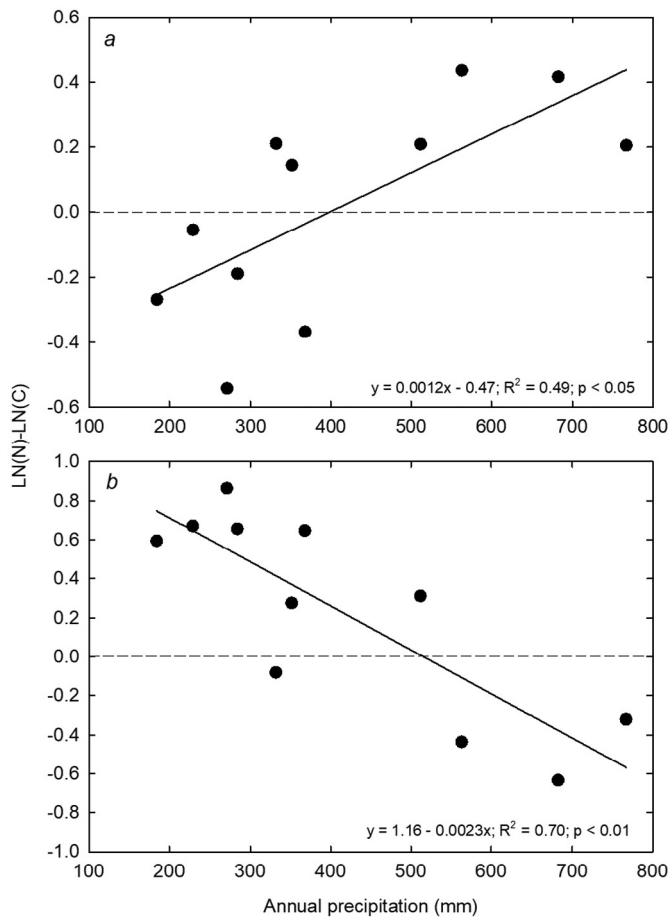
4. Discussion

4.1. Seasonal variations in biomass and LAI

Peak root biomass was found to occur in winter for all sites and treatments except the N treatment in the chaparral stand at SOFS. These results are similar to those reported by Phillips et al. (2019), but differ than those reported by Kummerow et al. (1978), who found that peak root density occurred in the spring and summer for many chaparral species. However, Kummerow et al. (1978) reported that late spring and summer rainfall may have shifted the peak later in the year during their study. Root growth is known to be sensitive to variation in rainfall and soil moisture (Kummerow et al., 1978; Phillips et al., 2019), and except for chaparral N plots, our measurements of peak biomass were during the winter months when rainfall was highest. Furthermore, our measurements were of surface roots, which

likely have an earlier peak than deeper roots because of earlier surface soil warming and drying (Kummerow et al., 1978). In contrast, root biomass for the N treatment in chaparral peaked in summer, and was more closely associated with changes in aboveground biomass and LAI than rainfall. Similar results have been reported for other N-fertilized chaparral and Chilean matorral shrubs, where plants exposed to N and/or P shifted root biomass production to support the increase in shoot production (Kummerow et al., 1982).

Aboveground standing crop and LAI peaked in the summer-fall for chaparral and in the spring for CSS shrubs, which is comparable to other studies of CSS and chaparral shrubs (Gray and Schlesinger, 1981; Gray, 1982). Many CSS shrubs, such as *A. californica* and *Salvia leucophylla*, are summer deciduous and respond rapidly to winter rains, grow vigorously until early summer, then shed leaves and dieback during the summer (Gray and Schlesinger, 1981). In contrast, many chaparral shrubs are evergreen, which initiate stem and leaf growth more slowly after winter rains and continue to growth well into the summer and fall (Gray, 1982). These phenological differences are caused by differences in rooting volume and depth, as chaparral shrubs generally have deeper root systems than CSS and have access to water and nutrients later into the summer when surface water and nutrients are depleted (Hellmers et al., 1955; Kummerow et al., 1977). However,

Fig. 5. Log difference in (a) average annual aboveground biomass and (b) root:shoot ratio as a function of the total annual precipitation for coastal sage scrub at the Santa Margarita Ecological Reserve. Data are calculated as LN (N) – LN (control). Positive values indicate a relatively higher value in N plots while negative values indicate relatively higher values in control plots.

there are also physiological differences between CSS and chaparral shrubs that can explain seasonal responses to water and nutrients, as CSS has higher rates of water use (Pivovaroff et al., 2016) and N uptake that are more closely coupled with growth than chaparral (Gray and Schlesinger, 1983). These differences in water and N utilization may also help explain the interaction between precipitation and N observed for CSS but not chaparral.

4.2. Effects of N on biomass and productivity

We hypothesized that N inputs would significantly increase above- and below-ground biomass stocks and production but also cause a decline in the root:shoot ratio for both chaparral and CSS. Our data support this hypothesis, but significant N × time interactions indicate that the N effects were not consistent over time. For chaparral, N inputs caused shifts in above- and below-ground standing crop, production, and R:S ratio that were consistent over time, but for CSS, N × time interactions were more variable.

The chaparral studied here was a secondary stand that was recovering from a fire that occurred in July 2003, approximately 2 months prior to the first N application (Vourlitis and Hentz, 2016). Trends in aboveground standing crop and LAI are broadly indicative of a post-fire chaparral stand, with rapid initial increases in biomass followed by a gradual leveling-off during the second decade of succession (Black, 1987; Riggan et al., 1988). However, plots exposed to N inputs had higher root biomass, lower shoot biomass, and a higher R:S ratio than control
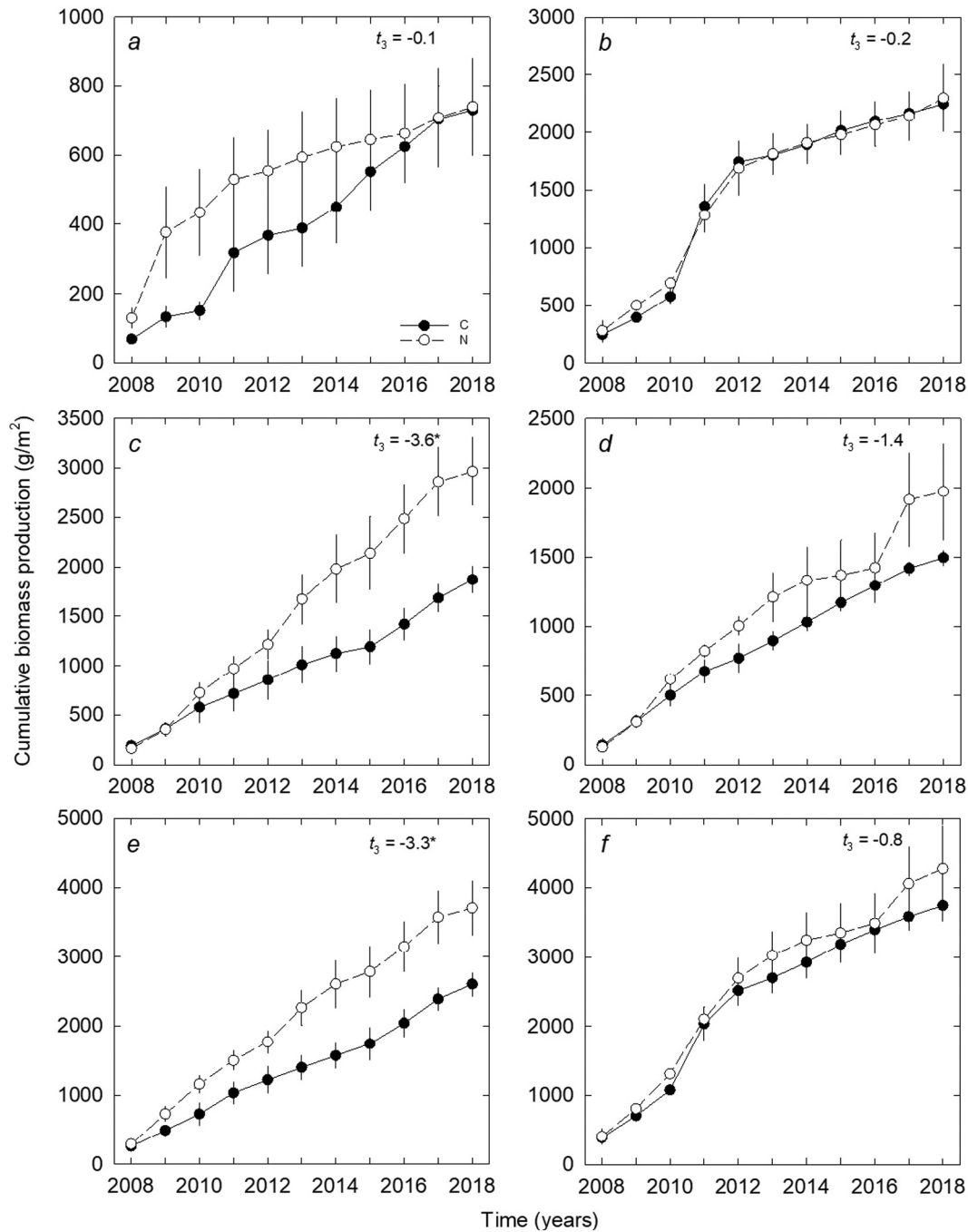
plots until 2010, or about 7 years after fire and N addition (Fig. 2). These data indicate an N-induced shift in above- and belowground biomass production, which implies a shift in above- and below-ground resource limitations (Poorter and Nagel, 2000). Nitrogen addition can cause limitations in other nutrients because N enrichment can alter tissue stoichiometry (Vitousek et al., 2010; Peñuelas et al., 2013), and chaparral soil exposed to N acidified rapidly, in the first 1–2 years after the initiation of N fertilization (Vourlitis and Fernandez, 2012), which implies losses of base cations (Roem et al., 2002). Thus, increases in tissue N concentration, coupled with leaching losses of nutrients, should have resulted in an increase in tissue N:P and/or N:cation ratios. However, Vourlitis et al. (2009) and Lawrence (2015) found that N:P, N:K, N:Ca, and N:Mg ratios did not change over the first 10 years of fertilization. The initially higher R:S ratio of N exposed shrubs, coupled with the stable N:nutrient stoichiometry, implies that shrubs utilized the additional N to increase root size and volume to acquire other nutrients (Smithwick et al., 2013). With stable N:nutrient stoichiometry, additional N inputs could eventually stimulate aboveground production as shrubs became larger, the canopy began to close, and presumably, light became more limiting to photosynthesis (Poorter and Nagel, 2000).

For CSS, the N × time interaction was likely due to an interaction between N input and rainfall. Previous research found that the N effects on biomass production in CSS were often a function of annual rainfall (Vourlitis, 2012; Kimball et al., 2014), and our data suggest that both aboveground biomass and the R:S ratio exhibited a statistically significant interaction between N input and precipitation (Fig. 5). N-induced increases in aboveground biomass were only observed during wetter than average years. This increase in aboveground biomass caused a significant N-induced decline in the R:S ratio as annual precipitation increased, indicating that CSS has a highly flexible response to variations in both rainfall and N. This flexible response is due to the close coupling between growth, water, and nutrient uptake of CSS shrubs, which are summer deciduous and reliant on rapid C gain when both water and nutrients are available (Gray and Schlesinger, 1983). However, changes in plant community composition may also help explain the variable N response over time. Dominant species such as *A. californica* and *S. mellifera* experienced higher rates of dieback in N plots during the 2012–16 drought (Vourlitis, 2017), which is reflected in the aboveground biomass, LAI, and R:S data. Openings in the canopy were filled by the invasion of *Brassica nigra* (Vourlitis, 2017), which responded to the increase in rainfall in 2017 with an increase in LAI and shoot production in the N plots. These patterns are illustrated by looking at aboveground biomass for the woody shrubs (*A. californica* and *S. mellifera*) and *B. nigra* in 2011, which was a wet year (precipitation = 767 mm) before the 2012–16 drought, and 2017, which was a wet year (682 mm) after the 2012–16 drought (Fig. 7). Immediately before the drought, nearly all of the aboveground standing crop consisted of *A. californica* and *S. mellifera*, while *B. nigra* was rare and only encountered in the N plots. After the drought, the standing crop of *A. californica* and *S. mellifera* declined to <50% of the 2011 values, while *B. nigra* biomass increased and was nearly equal to the woody shrub biomass in the N plots (Fig. 7). These data indicate significant drought-induced dieback of the dominant woody shrubs, but in the N plots, the open space was exploited by *B. nigra*.

4.3. Comparisons to other studies

Our estimates of root biomass from the upper 0–10 cm soil layer ranged from 16 to 64 gdw/m^2 for control plots and 17–59 gdw/m^2 for N plots in chaparral and 36–287 g/m^2 for control plots and 44–245 gdw/m^2 for N plots in CSS. These estimates undoubtedly miss a great deal of root biomass in these shrublands given their reportedly deep root depth (Hellmers et al., 1955; Kummerow et al., 1977; Miller and Ng, 1977; Jackson et al., 1996). Hellmers et al. (1955) in their pioneering work found that chaparral roots can penetrate several meters into the
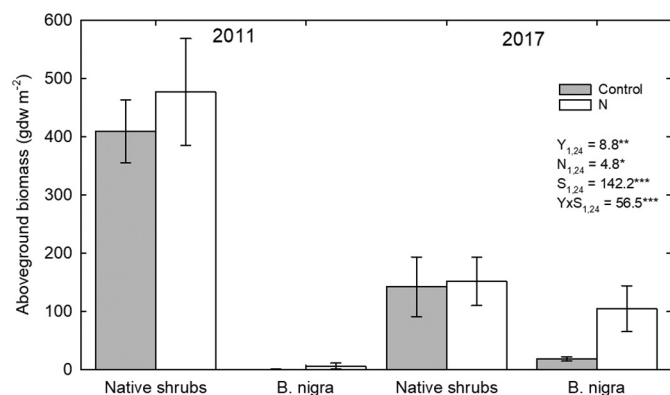
Fig. 6. Mean ($\pm$se; n = 4) cumulative root (a and b), shoot (c and d), and total (root + shoot) biomass (e and f) production for chaparral plots at Sky Oaks Field Station (a, c, and e) and coastal sage scrub plots at the Santa Margarita Ecological Reserve (b, d, and f) for the 2008–18 study period. Also shown are the results of a paired t-test (degrees of freedom and t-statistic) for the final total cumulative biomass production between control and N treatments. *(p < 0.05).

soil and underlying parent material; however, Kummerow et al. (1977) suggested that most of the fine roots were found in the upper 60 cm soil layer. Miller and Ng (1977) found that most of the fine roots were located in the upper 30 cm soil layer, but some species, such as *A. fasciculatum*, could grow roots up to 1 m deep. Data from Kummerow et al. (1977) and Jackson et al. (1996) indicate that on average of 35% of roots are in the upper 10 cm soil layer. Using this estimate, chaparral root biomass estimates adjusted for the upper 10 cm soil layer would be between 47 and 65 gdw/m^2 (Kummerow et al., 1977) and 20–312 gdw/m^2 (Miller and Ng, 1977), which agree well with our values. Unfortunately, studies on root production are scare, but Mooney and Rundel (1979) estimated root production to be 128 gdw

m^{-2} y^{-1} for mature chaparral, which is comparable to the 19–168 and 17–130 gdw m^{-2} y^{-1} calculated for control and N plots, respectively, reported here.

Studies of aboveground standing crop are far more abundant for chaparral and CSS. Peak aboveground standing crop and annual biomass production were reported to be 1172–1418 gdw m^{-2} and 255–355 gdw m^{-2} y^{-1}, respectively for CSS (Gray and Schlesinger, 1981; Gray, 1982). These estimates of standing crop are 2–3 times higher than those reported here; however, our estimates of aboveground biomass production are similar. Estimates of chaparral standing crop are reportedly between 1400 and 7624 gdw m^{-2} (Specht, 1969; Mooney et al., 1977; DeBano and Conrad, 1978; Mooney and Rundel, 1979; Rundel and

Fig. 7. Mean (±se; n = 4 plots/treatment) aboveground biomass for native shrubs (*Artemisia californica* + *Salvia mellifera*) and *Brassica nigra* in control (shaded-bars) and N plots (white-bars) in 2011 and 2017 for the coastal sage scrub stand at the Santa Margarita Ecological Reserve. Both years were wetter than average years (annual rainfall = 767 and 682 mm for 2011 and 2017, respectively) that were separated by a prolonged drought that occurred from 2012 to 16. Also shown are the results of a 3-way ANOVA (F-statistic, factor and error degrees of freedom), and p-value for nitrogen addition (N), year (Y), and species (S), and the Y × S interaction. Only significant main effects and interactions are shown. *p < 0.05; **p < 0.01; ***p < 0.001.

Parsons, 1980; Gray, 1982), which are substantially higher than the 470–928 gdw m^{-2} for control plots and 296–1198 gdw m^{-2} for N plots observed here. However, these published estimates are for mature stands, and those for successional stands 1–2 decades post fire are between 120 and 1400 gdw m^{-2} (Black, 1987; Guo, 2001), which is similar to the successional stand studied here. Aboveground production was 69–265 gdw m^{-2} y^{-1} for control plots and 102–458 gdw m^{-2} y^{-1} for N plots, which is similar to the 120 gdw m^{-2} y^{-1} reported for similar secondary stands (Black, 1987) and the 60–670 gdw m^{-2} y^{-1} reported for mature stands (Specht, 1969; Mooney et al., 1977; Mooney and Rundel, 1979; Rundel and Parsons, 1980).

5. Conclusions

Chronic N addition to Southern Californian coastal sage scrub (CSS) and chaparral shrublands caused significant alterations to above- and below-ground standing crop, production, and leaf area index (LAI); however, N induced impacts varied over time. For chaparral, N inputs initially increased root production but suppressed shoot production, presumably because plants used additional N to increase uptake of non-N nutrients. However, over time biomass partitioning reversed and plants exposed to added N grew significantly more shoots, presumably because aboveground resources became more limiting as shrub size increased and the canopy started to close. These relatively slow and consistent variations in standing crop and growth were not observed in CSS, rather N inputs caused aboveground production to increase only during wetter years. This interaction between added N and precipitation was due in part to a highly flexible growth response of CSS shrubs to increases in N and water availability and to a shift from slower-growing native shrubs to fast-growing introduced annuals. Together, these results indicate that long-term N inputs from atmospheric N deposition will lead to complex, spatially and temporally variable growth responses for these, and similar, Mediterranean-type shrublands.

CRediT authorship contribution statement

George Vourlitis: conceptualization and methodology, formal analysis, resources, writing, visualization of the data, and supervision. Jeff Jaureguy: investigation, data curation, original writing, visualization, formal analysis; Leticia Marin: investigation, data curation, formal

analysis; Charlton Rodriguez: investigation, data curation, original writing, visualization, formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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