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(E)MERGING DIRECTIONS ON AIR POLLUTION AND CLIMATE CHANGE RESEARCH IN MEDITERRANEAN BASIN ECOSYSTEMS

Long-term simulated nitrogen deposition alters the plant cover dynamics of a Mediterranean rosemary shrubland in Central Spain through defoliation

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Abstract Nitrogen (N) deposition due to anthropogenic pollution is a major driver of the global biodiversity loss. We studied the effect of experimental N and phosphorus (P) fertilization (0, 10, 20, and 50 kg N ha^{-1} year^{-1} and 14 kg P ha^{-1} year^{-1} over the background deposition levels) on plant cover dynamics of a rosemary (*Rosmarinus officinalis* L.) shrubland after 8 years of nutrient addition in a semiarid Mediterranean ecosystem from Central Spain. We specifically aimed at testing whether N deposition has the potential to influence the observed expanding trend of woody vegetation into areas dominated by grassland, biological soil crusts, and bare soil. Our results show that N addition loads above 10 kg N ha^{-1} year^{-1} reverted the cover dynamics of shrubs. Under N addition conditions, N was no longer a limiting nutrient and other elements, especially P and calcium, determined the seasonal growth of young twigs. Interestingly, N fertilization did not inhibit the growth of young shoots; our estimates point to a reduced rosemary leaf lifespan that is driving individuals to death. This may be triggered by long-term accumulation of N compounds in leaves, suggesting the need to consider the old organs and tissues in long-lived perennial plants, where N toxicity effects could be more mediated by accumulation processes. Shrublands are a widely distributed ecosystem type in biodiverse Mediterranean landscapes, where shrubs play a key role as nurse plants. Therefore, the disappearance of shrublands may accelerate the biodiversity loss associated with other global change drivers, hamper the recruitment of seedlings of woody species, and, as a consequence, accelerate desertification.

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Introduction

Plants are generally subjected to several co-occurring environmental stresses that may be far from additive (Chapin et al. 1987; Austin et al. 2004; van der Waal et al. 2009). Mediterranean ecosystems are acknowledged as biodiversity hotspots and a prime target for conservation efforts (Myers et al. 2000). High temperatures, high light irradiance, and aridity are three of the main features of Mediterranean ecosystems determining the structure and dynamics of their vegetation (Nardini et al. 2014). To cope with these stressing factors, plants in the Mediterranean biome have developed different mechanisms of resistance (Pugnaire et al. 1996a; Qian et al. 1997; Werner et al. 1999; Balaguer et al. 2002). However, global environmental change is adding stress to natural extreme environmental factors by increasing temperatures, reducing water availability, and increasing air pollution,

including N deposition (Bobbink et al. 2010; Ochoa-Hueso et al. 2011; Dias et al. 2014). Although plant adaptations to natural environmental stresses in Mediterranean ecosystems have been relatively well studied (Bussotti et al. 2014; Garbulsky et al. 2014; Matesanz & Valladares 2014), there is a need of more studies focusing on the effects of anthropogenic stressing factors, including N deposition, to understand, predict, and prevent future undesirable changes in these ecosystems that could lead to a loss of fundamental ecosystem services. Nitrogen deposition, associated with the continuous emission of anthropogenic reactive N into the atmosphere, is a direct driver of biodiversity loss (Sala et al. 2000; Galloway et al. 2004; Dentener et al. 2006; Phoenix et al. 2006; Basto et al. 2015), while it can also alter competitive interactions between plant species and modify plant community composition (Bobbink et al. 2010).

Shrublands are characteristic components of Mediterranean landscapes and are subjected to degradation in the Mediterranean basin where they may indeed cover more land surface of forests (Tomaselli 1977). Nitrogen deposition has the potential to trigger the loss of shrub cover in Mediterranean systems. This has been particularly well demonstrated in ecosystems from California (Padgett and Allen 1999), while in the Mediterranean basin, the evidence of the potential effects of N deposition on shrub cover is much scarcer. The few experimental studies carried out in Mediterranean basin shrublands consistently reveal a variable response to increased N availability that could depend on N form, plant species present, and local edapho-climatic conditions (e.g., soil chemistry and fertility, rainfall regime, water and other limiting nutrient availability, or plant interactions) (Ochoa-Hueso et al. 2011; Dias 2012). A N addition study carried out in a dense Portuguese maquis showed that higher NH_4^+ availability reduced scrub cover promoting soil erosion and N leaching, while higher NO_3^- availability led to biomass accumulation which may increase fire risk (Dias et al. 2014). The role of P availability seems to be particularly relevant in the response of N-saturated Mediterranean-type ecosystems to increased N deposition. For example, in a study carried out in Central Spain, N addition only increased the abundance of nitrophilous annual plants where P availability was naturally high, particularly during wet years (Ochoa-Hueso and Manrique 2014). In dryland ecosystems, shrubs play a crucial role by ameliorating environmental conditions (water, fertility), providing shelter to other plant and animal species, and representing true islands of fertility. Furthermore, shrubs are fundamental to maintain soil structure and fertility after degradation processes (Sardans et al. 2005). Therefore, the loss of this kind of vegetation could compromise a wide array of ecological functions and thus the ability of these systems to provide key ecosystem services, including carbon sequestration.

In this ecological context, we aimed at studying the impacts of increasing N and P availability on the cover dynamics, the net primary production (NPP), the tissue N:P stoichiometry, and a proxy indicator of leaf lifespan (LL) of a rosemary (*Rosmarinus officinalis* L.) population in a limestone semiarid Mediterranean shrubland in Central Spain after 8 years of N application in the form of NH_4NO_3 (0, 10, 20, and 50 kg N ha^{-1} $year^{-1}$ over the background level of ca. 6–7 kg N ha^{-1} $year^{-1}$) and 1 year of P addition in the form of KH_2PO_4 (14 kg P ha^{-1} $year^{-1}$, alone or in combination with 20 kg N ha^{-1} $year^{-1}$). Given that the dominant mineral N form in soil according to our data was NO_3-N during the 7-year experiment, we predict that rosemary cover may be increasing under current conditions but also that this trend might be reverted under the experimental N treatments due to the accumulation of reduced N (Dias et al. 2014). Secondly, we investigated the proximal causes of the harmful effect that long-term N addition may have caused in rosemary cover. We hypothesize that the rosemary cover decrease reported might be due to either a decay in NPP or a shorter LL, both driving to a reduction in photosynthetic material and triggering plant mortality. However, given that NPP responses to N may also be linked to nutritional imbalances (with P and possibly other nutrients) (Sardans et al. 2005), we hypothesize that responses to long-term N addition will promote growth in combination with P addition but only at low doses—i.e., when both nutrients are limiting. At high doses, N addition will result in a reduction of rosemary cover due to the overriding effects of ammonium toxicity.

Methods

Nitrogen fertilization experiment

We carried out a simulated N deposition experiment in the Nature Reserve El Regajal-Mar de Ontígola, 40° 00′ N, 3° 36′ W, near Madrid urban Area (Spain) (500–600 masl). The climate of the area is continental-Mediterranean with cold and wet winters and warm and dry summers. Plants are active all year round, but there are two characteristic plant activity peaks per year, one in spring and another in autumn (Navarro and Cabezudo 1998). Total rainfall amount is approximately 425 mm $year^{-1}$, predominantly falling between October and May (Rivas-Martínez 1987). In Spain, kermes oak (*Quercus coccifera* L.) chaparral—a tall (around 2 m in our site) shrubland constituted by several individuals growing very close one each other—is frequent in thermic Mediterranean regions with marked semiarid climate (Alcaraz et al. 1987) but it is also present in Mediterranean-continental areas in Madrid province due to local particularities, sharing the space with short shrubs (20–60 cm) like rosemary, thyme, and other chamephytes (Izco 1972). The vegetation in our study site is a mosaic with two clearly different vegetation physiognomies: (1) kermes oak dense tall shrubland (hereafter chaparral

patches) and (2) open interspaces where scattered small sclerophyllous rosemary shrubs are separated by open spaces where bare soil, a diverse and well-developed biological soil crust, and short-lived therophytes during the growing seasons are mixed (Crespo and Barreno 1975; Ochoa-Hueso et al. 2013a) (hereafter rosemary patches). Despite other small shrubs species different than rosemary are present in the rosemary patches at the study site, rosemary is the dominant short shrub and within our plots it is close to monospecific.

In autumn 2007, six randomized blocks were established in flat or slightly sloped areas exclusively covering rosemary patches (Fig. 1). Within each block, four 2.5- × 2.5-m plots were established, resulting in a total of 24 plots. Plot edges were not juxtaposed to avoid cross-contamination between treatments; a 1-m buffer band was disposed between contiguous plots (Fig. 2), a distance considered sufficient given the flatness of the territory. In each block, treatments were randomly assigned to each plot: 0, 10, 20, and 50 kg N ha^{-1} year^{-1}. In one slightly sloped block, the plots were assigned a treatment from low N concentrations in the top of the hill to high N concentrations in lower parts to minimize the effect of unlikely cross-contamination events. A background
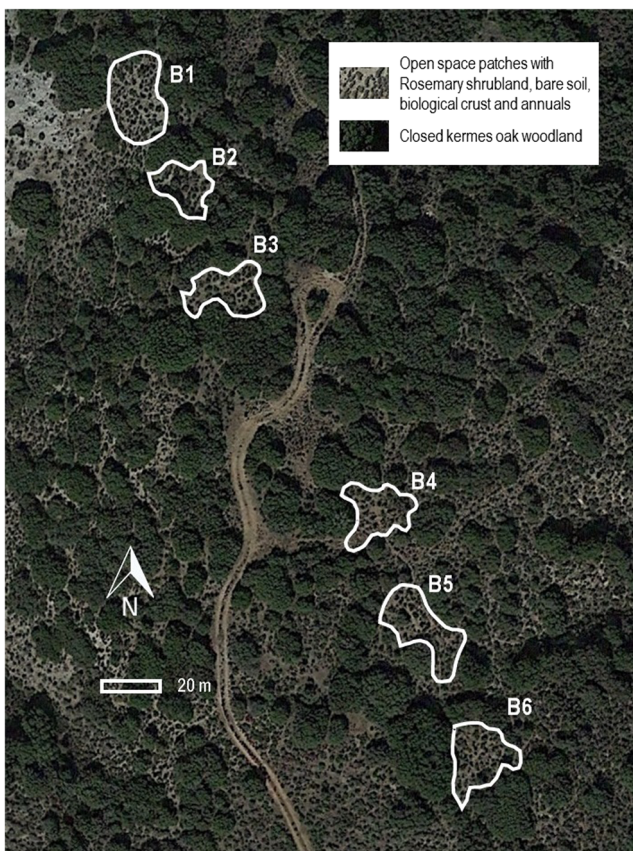

Fig. 1 *Left*: Map of the study site and the location of the six experimental blocks (*B1* to *B6*) in the open spaces between kermes oak tall shrublands, where the vegetation is a mixture of scattered rosemary small shrubs and bare soil, a well-developed biological crust, and annual plants during the growing seasons. Photo by courtesy of Google Earth 2016

deposition of ~6 kg N ha^{-1} year^{-1} was estimated based on the CHIMERA model (Vivanco et al. 2009). Nitrogen was applied monthly with a hand sprayer or a watering can in 2-L doses of an aqueous solution of ammonium nitrate (NH$_4$NO$_3$). Due to the seasonality and unpredictability of rains in Mediterranean climate, it is common that the reactive dry N accumulate in soils and become suddenly available in high concentration peaks with the onset of rains (Fenn et al. 2003; Ochoa-Hueso et al. 2013b). The treatment simulates dry deposition because the water from the aqueous solution evaporates a few minutes after application, and only when natural precipitations occur the treatment has an effect. However, to avoid interference due to our experimental treatment, control plots are also watered with the same volume of water. The use of a 1:1 proportion of oxidized and reduced N is in agreement with rain chemistry measurements at the study site (Ochoa-Hueso et al. 2013c). Despite initially the solution was applied monthly from October to June, during summers (July–September), and since 2012 all year, fertilization was applied in a 3-month basis, adjusting the total N load.

Rosemary shrubs had been previously found to be unresponsive to N fertilization even though P addition promoted their growth (Sardans et al. 2005), suggesting that P could be the real limiting nutrient in sites similar to our study area where P is usually immobilized under insoluble forms (calcium phosphate). Therefore, to assess the effects of increased P availability and its interactions with N deposition, two new plots with the same vegetation characteristics were set up in early March 2015 in each previously defined block (Fig. 2), summing a total of 36 plots, six per block. The new plots were assigned to one of the following treatments: (1) 0 kg N ha^{-1} year^{-1} + 14 kg P ha^{-1} year^{-1} and (2) 20 kg N ha^{-1} year^{-1} + 14 kg P ha^{-1} year^{-1}. This amount of added P is sufficient to increase P availability to enhance plant growth in P-depleted soils (Kanzler et al. 2015). The application of N in these new plots was done following the methodology described above. Phosphorus was added once per year in a 2-L aqueous solution in the form of KH$_2$PO$_4$. This form has been chosen because our results over 7 years showed no response of rosemary plants to soil K measured levels.

Soil analysis

We collected and pooled three soil cores (2.0 cm diameter × 0–4 cm depth) in each of the 24 plots as described in Ochoa-Hueso et al. (2013b) by the end of each of the following seasons: autumn 2008, winter 2008, spring 2009, summer 2009, spring 2010, spring 2011, autumn 2011, autumn 2013, autumn 2014, spring 2015, and autumn 2015 (Fig. 3). Soil extractable NO$_3$-N and NH$_4$-N were included in a N dataset (N24) containing six variables with 24 data each (N treatment plots only) representing soil extractable NH$_4$ and NO$_3$ data collected in autumn 2014, spring 2015, and autumn 2015.

The variables are as follows: $[NO_3]_{autumn\ 2014}$, $[NH_4]_{autumn\ 2014}$, $[NO_3]_{spring\ 2015}$, $[NH_4]_{spring\ 2015}$, $[NO_3]_{autumn\ 2015}$, and $[NH_4]_{autumn\ 2015}$.

Extractable P for soil samples from autumn 2008 until autumn 2013 was extracted following the Burriel and Hernando (1950) method (see Ochoa-Hueso et al. 2013b); also, extractable P of samples from spring 2010 until spring 2016 was measured in the same extract made with 1 M KCl. A P dataset

(P24) was put together with four variables with 24 data each (N treatment plots only) representing soil extractable P measures, grouped to obtain mean values for 2 to 3-year periods. The variables are as follows: $[P_2O_5]_{2008–2009}$ (mean of Burriel-Herrando extractions for autumn 2008, winter 2008, spring 2009, and summer 2009), $[P_2O_5]_{2010–2011}$ (mean of Burriel-Herrando extractions for spring 2010, spring 2011, and autumn 2011), $[PO_4]_{2010–2011}$ (mean of KCl extractions for
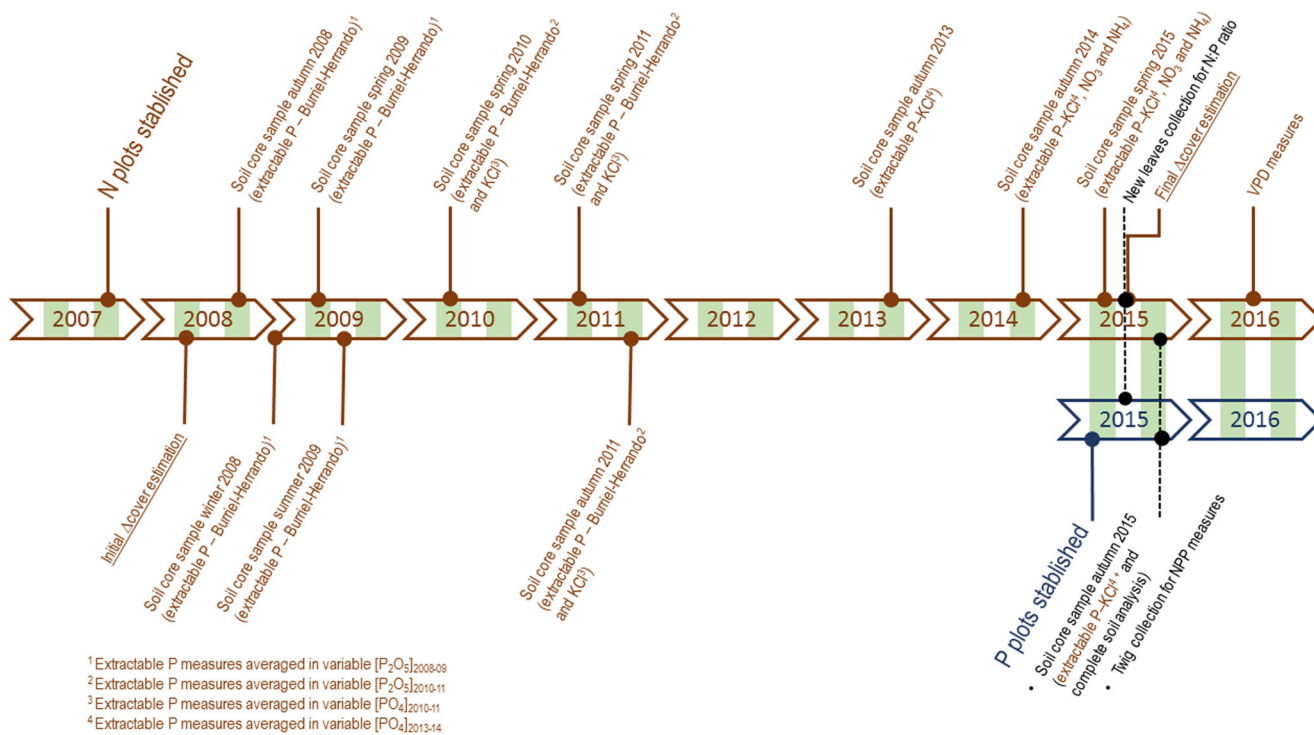

Fig. 3 Timeline showing the establishment of the N-treated plots in 2007 and the P-treated plots in 2015, the timing of the soil data collection, Dcover data collection, the leaf tissue collection for tissue chemistry analysis, and the twig collection for NPP estimation

spring 2010, spring 2011, and autumn 2011), and $[PO_4]_{2013-2014}$ (mean of KCl extractions for autumn 2013, autumn 2014, spring 2015, and autumn 2015).

In autumn 2015, a complete soil nutrient analysis was performed in samples collected for both the 24 eight-year-old plots and the 12 new P plots. We collected data for extractable NO_3, NH_4, PO_4, total C, total N (organic N + inorganic N) and total P, pH, and extractable K, Mg, Ca, Fe, Mn, Zn, and Cu. A first soil nutrient (Sn36) dataset groups 15 variables with 36 data each (24 N treatment and 12 new P treatment plots). The variables are all the measures from autumn 2015 (see above). A second soil micronutrient (Sm24) dataset includes three variables with 24 data each (N treatment plots only) representing soil micronutrient data collected on autumn 2015. The micronutrients selected for Sm24 are those affecting rosemary NPP according to our data analysis presented below. Variables are Na, Ca, and Mn (extractable).

Rosemary measures

To analyze the dynamics of rosemary population, we accounted for four response variables. The main response variable studied was Δcover (difference in % rosemary at the beginning and the end of the 7-year period). In order to understand the mechanisms involved in the cover dynamics response, we measured several physiological response variables, including net primary production (NPP as dry weight biomass production per twig), N:P stoichiometry in new leaves, and the plant volume defoliated (VD) that serves as a proxy of LL.

Δcover was calculated in the 24 N-treated plots based on the assumption that the horizontal geometric projection of each individual shrub has a shape similar to an ellipse. We measured firstly the maximum horizontal diameter of each individual (D_1) and secondly its perpendicular horizontal diameter intersecting with the former one in the geometric center of the projection of the shrub (D_2). The ellipse area of each individual was estimated by means of the formula $\pi \cdot D_1 \cdot D_2$, and the plant cover in each plot was then expressed as the summed area of the ellipses in each plot on a percentage basis of the total plot area. This percentage cover was measured twice, first in spring 2008 and second in summer 2015. The evolution of rosemary cover (Δcover) is the increment in shrub cover, calculated as follows:

$$\Delta\text{cover} = \%\text{cover}_{2015} - \%\text{cover}_{2008}$$

Both in 2008 and 2015 all plots located in rosemary patches were rather covered by rosemary short shrubs or deprived of woody vegetation cover, and kermes oak recruitment was not reported within our plots by the end of the period. Therefore, a positive Δcover value indicates an expansion of rosemary over bare soil, biological crust, and annual forbs space, and a negative value indicates the opposite dynamics, i.e., decay in short shrub encroachment and expansion of bare soil, biological crust, and annual forbs.

NPP was measured in October 2015, by the end of the autumn activity peak and after the summer drought which could interfere with the plant response to N in a dose-dependent manner. We randomly collected five twigs from five rosemary shrubs growing in each plot—including those treated with P. The twigs collected for the measurement fully grew during a plant activity peak posterior to the establishment of the P-treated plots, and therefore we assume that this biomass production was affected by the treatment in equal condition that the one exposed to the N treatment. Each twig was processed in the laboratory to separate the last growth from the older parts of the plant. While old parts of the stems present a gray chapped epithelium and scarcity of leaves, the new growth of the stems can be easily discerned by the russet color of the epithelium, the abundance of new leaves, and especially by the presence of ramifications in the base of the new annual growth. In apices where annual growth was scant and neither new twigs nor ramifications were discernible, we assumed that the last leaves, with a lighter green color, developed in the immediate previous growing season. New growths were cut from the twigs, counted, oven dried at 65 °C to constant weight, and weighted. The mean value per twig in each plot of the new growths' dry weight in grams was used as an NPP estimate.

N:P stoichiometry in plant tissues is a reliable indicator of the limitation and co-limitation levels of both N and P in the soil (Tessier and Raynal, 2003) and is often studied as an indicator of the ecosystem response to N deposition (Sardans et al. 2012). Data on N:P stoichiometry of leaves from the last activity peak were collected by the end of June 2015, using the same collection process described above for NPP, in all the 36 plots including new P-treated plots. The tissue collected for the chemical analysis grew in an activity peak posterior to the establishment of the P-treated plots, and therefore we assume this tissue was affected by the treatment. Twigs were oven dried at 65 °C to constant weight and then milled. Due to its hardness and high resin content, leaf dried tissues were first frozen in liquid N, crushed in a mortar, and then powdered in a ball mill for chemical analysis. The plant powder was digested following the Kjeldahl protocol and analyzed for total P (ammonium molybdate method, Murphy and Riley 1962) and organic N.

The VD measures where estimated in the 24 N-treated plots by the end of June 2016. For each individual shrub, VD was visually estimated by two independent researchers previously trained as 3D volume included in the shrub geometric space that was lacking leaves, in a scale from 0 to 100% in intervals of 10%. The final value for each shrub was the mean value estimated by both researchers. The VD value for each plot was calculated as the mean value of the VD estimated for all the

individual shrub within the plot. The volume of the plant that is lacking leaves is inversely proportional to the volume populated with leaves, and therefore VD is the result of an equilibrium between the leaf production (proportional to NPP) and the leaf fall (explained by LL). Controlling for NPP, VD is therefore a reliable proxy of LL: for a similar NPP, a higher VD will indicate shorter LL. A plot showing a higher VD but same NPP than other growing under optimal conditions would be indicating premature defoliation.

Statistical analysis

Δcover dataset including 24 observations for N-treated plots was firstly analyzed by means of a one-way ANOVA model considering only the N treatment and secondly by means of ANCOVA models considering the N treatment as factor and the soil phosphorus dataset P24 and soil micronutrient dataset Sm24 as covariates. The NPP dataset including 36 observations for both N- and P-treated plots was firstly analyzed by means of two-way ANOVA models considering the N and P treatments and their interaction. Secondly, we developed linear models where the soil nutrient dataset Sn36 was used as independent variables. Finally, we performed ANCOVA models combining the N and P treatments as factors and Sn36 as covariates excluding in this case extractable NO_3, NO_2, NH_4, and PO_4 measurements that would not be independent of the factors. The N:P ratio dataset including 36 observations for both N- and P-treated plots was also analyzed by means of a two-way ANOVA including both N and P treatments as factors. The VD dataset including 24 observations for N-treated plots was analyzed by means of ANCOVA models using the soil nitrogen dataset N24 as covariates. Despite N24 and the N treatment are not completely independent, we decided to use this dataset as covariate in the VD models because it helps to understand the intrinsic variability of the soils in each block and the differential effect of reduced and oxidized forms of N. All the above models were validated checking the homoscedasticity and normality and visualizing the Leverage and Cook's distance values for all the observations. In the case of NPP, models including factors were fitted using the sum contrast matrix. All linear models where performed using a [3, −1, −1, −1] contrast matrix, as previous prospection of the data for all the variables considered in this study clearly revealed no differences between the three N addition levels but pointed to differences with the control.

For each response variable, best-fitting models were selected using the Akaike information criterion for small samples (AIC_C), a Bayesian likelihood-based measure of model fit. The method, based on the information theory, allows to select the models that minimize the Kullback–Leibler distance between the model and the truth (Burnham and Anderson 2002). Model selection was carried out using a stepwise regression procedure. To avoid relying on a single best model when alternative models are similarly well fitted (Whittingham et al. 2006), we calculated AIC_C weights (w_i), which provides a relative weight of evidence for each model. Models were compared to the best-fitting model by means of the Kullback–Leibler evidence ratio (K-L) ($w_i/w_{\text{best-fitting}}$). Models that resulted to be less than four times worse than the best-fitting model (K-L ratio ≤ 4) are reported as "similar" to the best-fitting model (Wagenmakers and Farrell 2004). The parameter significance in the best-fitting model and the similar models was checked to find significant factors or covariates affecting the response variable. All analyses are performed using R version 3.2.2 (R Core Team 2016) in Rstudio (version 0.99.879).

Results

We found that 7 years of N addition reduced rosemary shrub cover. Changes in cover did not differ from zero or were negative in the N-treated plots, showing a trend to recession or stabilization of shrub encroachment, while it was positive under control conditions, indicating encroachment over the same period (Fig. 4a and Table 1). The Δcover best-fitting model also points to a significant role of soil extractable P measured in 2010–2011, Na, and Ca (Suppl. Tables 1A, B, C).

Regarding the proximal causes of this decrease in cover over time in response to nutrient addition, neither N fertilization (Fig. 4b) nor P fertilization did affect NPP, which was better explained by linear models including only soil measures. All NPP best-fitting models showed significant effects of pH, Na, Ca, Mn, and P_2O_5 (Suppl. Tables 2A, B, C, D). In the NPP best-fitting model, P_2O_5 and Ca had markedly high p values and positive slopes (Suppl. Table 1A and Fig. 5), suggesting an effect mediated by P that was not detected with our P treatment. In contrast, N addition significantly increased VD in rosemary plants (Fig. 4c), and NH_4^+ measures from spring 2015 and autumn 2015, but not spring 2014, were significant covariates in the two best-fitting models (Table 2 and Suppl. Table 3). Finally, N:P stoichiometry was not significantly affected by N and P additions (F statistic = 1.947 on 4 and 31 df, p value = 0.13). The N:P ratio was on average $13.14 \pm 1.4SE$, and it was not significantly altered by N nor by P addition treatments (Fig. 4d).

Discussion

In this study, we have shown that increased N deposition has the potential to reduce rosemary cover due to deleterious effects of excessive N on rosemary annual growth. Rosemary is a widespread shrub in the Mediterranean basin that is capable of colonizing nutrient-poor calcareous soils (Sardans et al.
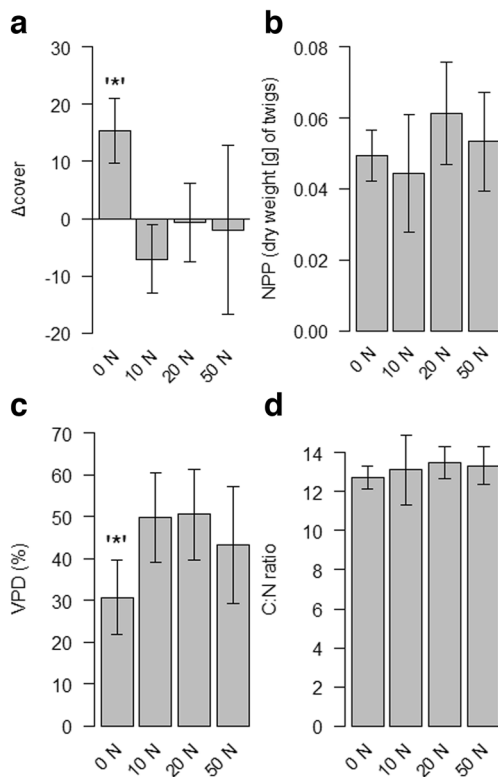
Fig. 4 Plot of mean values and standard errors. **a** Cover change of rosemary shrub between 2008 and 2015 for the different N fertilization treatments. **b** NPP measured as twig annual growth. **c** VD. **d** N:P stoichiometry in leaves of new bourgeons; all variables presented for the different N fertilization treatments. 0 N, 10 N, 20 N, and 50 N refer to the treatments with 0, 10, 20, and 50 kg N ha^{-1} year^{-1}, respectively. Significant different groups ($p < 0.05$) in all best-fitting models are marked with the *asterisk* symbol

2005), which makes observations from our site transferable to other similar locations throughout the Mediterranean Basin. Therefore, based on our local results, we predict that a small further increase in chronic N deposition levels in the Mediterranean basin could lead to a widespread decay of this type of shrublands. Shrub cover in semiarid ecosystems influences soil moisture via shading (Valladares et al. 2016) and nutrient availability due to litter accumulation (Maestre and Cortina 2005; Gimeno-García et al. 2001). By means of these mechanisms, short shrubs act as nurse plants in Mediterranean systems making easier the growth and survival of herbaceous

Table 1 Best-fitting ANCOVA results for Δcover (%)

	Estimate	Std. error	t value	Pr(>|t|)
(Intercept)	24.270	16.549	1.467	0.158053
N treatment	4.994	1.139	4.385	0.000286
[PO$_4$]$_{2010–2011}$	13.987	5.314	2.632	0.015971
Na	−2.661	1.018	−2.614	0.016614

Multi R-squared = 0.5629; F statistic = 8.587; p value = 0.00073; df = 5; AICc = 185.53

**Table 2** Best-fitting ANCOVA model for VD (%)

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	236.192	70.045	3.372	0.00320
N treatment	−4.216	1.365	−3.088	0.00606
[NO$_3$]$_{autumn\ 2015}$	−5.490	2.280	−2.407	0.02640
[NH$_4$]$_{spring\ 2015}$	−25.652	8.136	−3.153	0.00524
[NH$_4$]$_{autumn\ 2015}$	9.024	3.016	2.992	0.00749

Multi R-squared = 0.5926; F statistic = 6.909; p value = 0.0013; df = 6; AICc = 192.94

species (Pugnaire and Lázaro 2000; Brooker et al. 2008, McIntire and Fajardo 2014) and increasing the productivity and the phylogenetic diversity of the plant community (Pugnaire et al. 1996b; Moro et al. 1997; Valiente-Banuet and Verdú 2007). Consequently, a reduction in shrub cover may indirectly enhance the loss of ecosystem services that is directly attributable to N deposition (Bobbink et al. 2010; Ochoa-Hueso et al. 2011). Furthermore, shrub facilitation makes also possible the recruitment of seedlings and saplings of trees and bigger woody species whose long-term success often relies on facilitation interactions (Rousset and Lepart 1999; Gómez-Aparicio et al. 2005a, b; Kunstler et al. 2006; Cuesta et al. 2010; Puértolas et al. 2010); hence, a reversion in the cover dynamic of shrub cover might impede the recruitment of kermes oak and threat this chaparral system. Finally, both the decay in rosemary expansion and the likely consequential limitation of kermes oak encroachment may result in an acceleration of desertification (Maestre et al. 2009).

We have shown that total levels of N deposition over 6–16 kg N ha^{-1} year^{-1} (i.e., 0–10 kg N ha^{-1} year^{-1} + background deposition) have the potential to revert the trend of expansion of the dominating woody vegetation. The observed shift in rosemary cover corresponds to a population dynamic change from encroachment to stabilization or likely recession (Fig. 6). Our results demonstrate that there is a natural expansion trend of the rosemary shrubland over the bare soil where biological crust and some annual forbs develop in control plots, whereas this trend would be at least neutralized, if not reverted if N deposition increases in our study sit—our 10 N treatment shows a clear negative Δcover, whereas the 20 N and 50 N treatments show negative mean values but the deviations are too high as to allow us to state they are nonnegative (Fig. 4a). This range of N deposition values coincides with the range of the average critical load (10 to 15 kg N ha^{-1} year^{-1}) recently published in a study conducted with several plant species (Payne et al. 2013). The expansion trend found under the control conditions was reinforced by observations of recruitment of new rosemary individuals in our control plots and in the wider study area (personal observations). Furthermore, rosemary is an obligate seeder (Sardans et al. 2005), and we

Fig. 5 Plot of NPP (mean dry weight of new twigs) as function of phosphorus and calcium levels in soils. The *dotted lines* represent the plane of predicted values for the best-fitting model (Mod10) assuming constant mean values for the rest of independent variables

Seasonal growth of sprouts fitted to two main soil elements
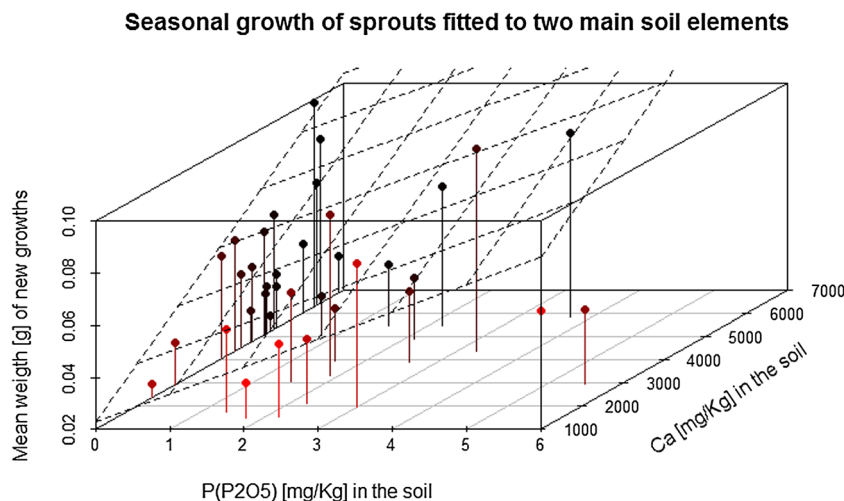
have observed rosemary seed activity and a healthy soil seedbank at the study site (unpublished data).

To better understand the processes causing this N deposition-dependent cover change, we studied how N fertilization affected some leaf physiological parameters of rosemary shrub individuals. The N:P ratio value estimated for all our plots without significant differences between treatments is around 13, a level indicating that P and N are co-limiting nutrients (Tessier and Raynal 2003; Güsewell 2004), meaning either that both are equally limiting plant growth or that nutrient is actually a limiting factor. Our N and P fertilization experiments show no detectable effects on rosemary NPP; therefore, the N:P ratio indicates no N-P-limitation in El Regajal at the current background deposition level. We can conclude

that, above the level at which N is a limiting nutrient, N deposition has no detectable deleterious effect on NPP and on the growth of new bourgeons. However, despite not affecting new leaf tissues, chronic N addition increased the VD of plants reducing the plant's photosynthetic material. Along with the NPP results, we deduce that the higher VD of N-fertilized plants indicate premature leaf fall and therefore a shorter LL. Thus, the recession of shrub cover not related to a decrease in plant growth is caused by adult individuals' death due to defoliation (Fig. 6).

Our VD best-fitting models show a significant effect of ammonium nitrate measured on spring 2015 and autumn 2015 on the defoliation measured by the end of the spring 2016, but previous soil measures of NH_4^+ and the NO_3^-
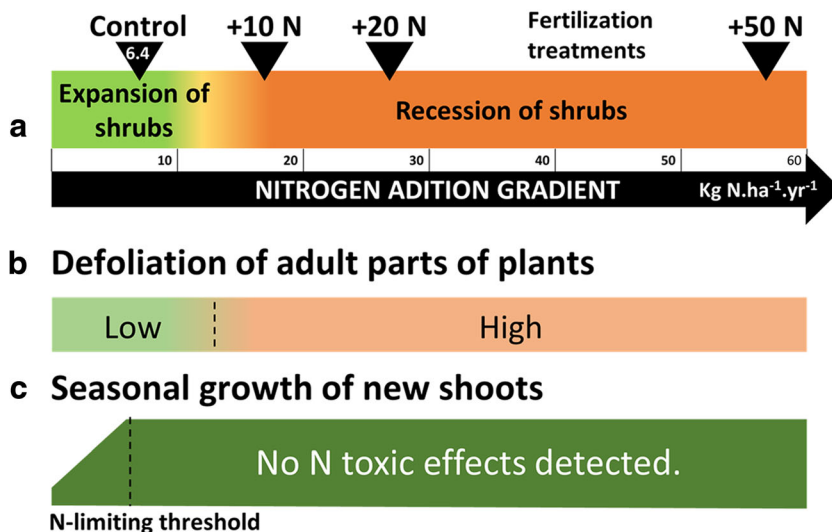

Fig. 6 Schematic display of our main conclusions. **a** Background N deposition and experimental levels of N fertilization and the response of shrub cover over the 7-year experiment. **b** Defoliation levels scaled to the N addition gradient scale above. Note that at the control treatment, there is defoliation in lower than in the experimental fertilization treatments. **c**

Growth of annual shoots scaled to the same N addition gradient scale. Note that there are no differences between the four experimental treatments because the background deposition is over the N-limiting load, and there is no growth decrease observed due to toxicity

measures do not have any effect. The presence of high levels of NH_4^+ in soil, despite apparently not affecting the new twigs NPP, causes long-term harms that are detectable in leaves from the former activity peaks. NH_4^+ measures prior to spring 2015 may have had an effect on leaves from older cohorts, but we might have been unable to detect this effect due to the fact that these cohorts of leaves did already reach the end of their natural life cycle even in the control plots. That being so, the results suggest that rosemary leaves have a maximum LL of 3 activity peaks (1.5 years) in our study site, a value not unreasonable given that rosemary is a semideciduous species with a mean LL of ~11 months (Villar and Merino 2001; Granati and Varone 2004). While growing bourgeons may have a control over the use of N, chronic exposition to sap with high concentration of NH_4^+ may accumulate this element in tissues over time, driving them to reach toxic levels. Other studies have reported similar N accumulation processes in mosses (Pesch et al. 2008) and coniferous trees (Blanes et al. 2013). For example, Blanes et al. (2013) observed that, in N-limited sites, N content in leaves typically decreases in older needles of *Abies pinsapo*, whereas it increases with leaf age in N-saturated sites; the overload of N in trees at N-saturated sites exceeded the homeostatic regulation capacity in terms of foliar stoichiometry.

Our results show that long-term N fertilization experiments are essential to assess the actual effects of N deposition on shrub dynamics. In short-term experiments, in which accumulation processes may not occur, the response of woody vegetation to chronic N deposition might be hidden. We also suggest the importance of accounting for the response of older plant organs—as opposed to only new ones—to better characterize the ecophysiological response of woody plants as a diagnostic tool in chronic N deposition/fertilization studies. Neglecting them may hinder the finding of evidences of effects mediated by chemical accumulation processes, even in long-term experiments.

Conclusions

We have shown that long-term reactive-N enhanced availability in soils damages old leaves of rosemary shrubs and reduces LL, not affecting NPP during the plant activity peaks. These findings point to the fact that N deposition may harm rosemary plants due to its accumulation in old leaf tissues by reaching toxic concentrations. Nitrogen deposition is a global change driver that may drive shrub cover, particularly in rosemary populations, to decay in Mediterranean ecosystems. The effects of N deposition on shrub cover may also enhance the loss of herbaceous plant biodiversity and productivity, reversing the chaparral-thicket encroachment and, therefore, accelerating the desertification due to climate change. Further ecosystem-level studies that confirm these hypotheses are strongly desirable and would allow shaping a general model of how N deposition in semiarid shrublands alters the spatial and temporal dynamic response of the plant community. Finally, future empiric studies approaching N deposition effects on woody vegetation may consider accumulation processes by means of studying long-term effects of N addition on woody plants' old tissues.

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