

# Linking N-driven biodiversity changes with soil N availability in a Mediterranean ecosystem

Teresa Dias · Sónia Malveiro ·  
Maria Amélia Martins-Loução ·  
Lucy J. Sheppard · Cristina Cruz

Received: 24 March 2010 / Accepted: 22 October 2010  
© Springer Science+Business Media B.V. 2010

**Abstract** Nitrogen (N) enrichment has been pinpointed as a main driver for biodiversity change. Most of our knowledge of effects of increased N availability on ecosystems comes from northern Europe and America. Most other ecosystem types have been neglected. In contribution to filling this gap, our study examined the short-term effects of N enrichment in a N-manipulation (doses and forms) field study of a severely nutrient-limited Mediterranean ecosystem located in a Natura 2000 site in Portugal. Our aims were to (a) understand the effects of N enrichment on plant diversity, and to (b) link N-driven plant community changes with changes in soil inorganic N availability. In general, the standing plant community responded to short-term N enrichment with increased richness and evenness. Changes in the plant community occurred through changes in species composition and cover, and were correlated with soil

N, and N and phosphorus availability. Fertilization with 80 kg  $\text{NH}_4\text{NO}_3 \text{ ha}^{-1} \text{ y}^{-1}$  was the treatment which changed plant composition the most, while geophytes, hemicryptophytes and therophytes were the biological types more responsive to N enrichment. *Dittrichia viscosa* was the only species that responded significantly to increased N, i.e., its cover decreased in control plots, but increased in fertilized plots, suggesting that it could be used as an indicator of N enrichment in Mediterranean maquis. Changes in plant richness and evenness were correlated with the mean and/or the variation (standard deviation) of soil inorganic N parameters (e.g. nitrate concentration in the soil solution and the soil's ratio of bioavailable N and phosphorus) measured along the time between the two plant community assessments. However, short- and long-term effects can be quite distinct, thus highlighting the need for further studies.

Responsible Editor: Elizabeth M. Baggs.

T. Dias (✉) · S. Malveiro · M. A. Martins-Loução ·  
C. Cruz  
Universidade de Lisboa, Faculdade de Ciências,  
Centro de Biologia Ambiental (CBA),  
Campo Grande,  
1749-016 Lisboa, Portugal  
e-mail: mtdias@fc.ul.pt

L. J. Sheppard  
Centre of Ecology and Hydrology (CEH),  
Bush Estate,  
Penicuik EH26 OQB, UK

**Keywords** Biodiversity changes · Mediterranean · Soil N availability · Plant groups · N-limited ecosystems

## Introduction

Increased nitrogen (N) deposition has been acknowledged as a main driver of biodiversity change (Sala et al. 2000), constituting a threat to biodiversity (Phoenix et al. 2006; Clarisse et al. 2009). Bobbink et al. (1998) reviewed the effects of increased N availability on biodiversity of various natural and semi-natural ecosys-

tems pointing out that although most global biodiversity is contained within natural and semi-natural ecosystems, most research has focused on forests and trees. They highlighted serious gaps in knowledge of other ecosystems. More than a decade later, Bobbink et al. (2010) acknowledged that ecosystems other than those in northern Europe and America have received insufficient attention. Mediterranean-type ecosystems are included among the ‘neglected ecosystems list’ although they are biodiversity hotspots. In particular, the Mediterranean-type ecosystems located in the Mediterranean Basin constitute one of the most important biodiversity hotspots for vascular plants (Phoenix et al. 2006). Presently, Mediterranean ecosystems are thought to be experiencing the greatest proportional biodiversity change (Sala et al. 2000), with N deposition being estimated to increase three fold by 2050 (Galloway et al. 2004; Phoenix et al. 2006).

Most studies of the effects of increased N availability on Mediterranean-type ecosystems have been made in California (e.g. Fenn et al. 2003; Vourlitis et al. 2007; Allen et al. 2009), while very few have been made on the Mediterranean Basin (Bonanomi et al. 2006; Pinho et al. 2009). This paper describes an integrated field study of short-term effects of increased N availability on a Mediterranean ecosystem located in a Natura 2000 site in southern Portugal (PTCON0010 Arrábida/Espichel). N availability was manipulated in reduced and/or oxidized forms in three equal applications throughout the year corresponding to distinct biological demands and activities. Our aims were:

- (i) Understand the effects of N enrichment on plant biodiversity in a severely N-limited Mediterranean ecosystem. Given that biodiversity is a complex variable that includes taxonomic, functional, spatial and temporal aspects of organism diversity, we focused on species richness (the number of species) and evenness (their relative abundance), which are considered to be the most important indicators (Wilsey and Potvin 2000) of changes in biodiversity;
- (ii) Link N-driven plant community changes with soil inorganic N availability. Biodiversity changes reflect changes in communities that occur in response to gradients of temperature, moisture and soil chemistry (McGill et al. 2006). Besides the spatial heterogeneity, in systems with a marked seasonality such as Mediterranean ecosystems

(Grove and Rackham 2001), the magnitude of the temporal heterogeneity becomes as important as spatial heterogeneity (Cruz et al. 2008). Therefore we attempted to link observed N-driven plant community changes with changes in soil inorganic N availability measured along the time between the two plant community assessments.

## Materials and methods

### Study site

The present study was conducted at Serra da Arrábida in the Arrábida Natural Park, in a Natura 2000 site south of Lisbon, Portugal (PTCON0010 Arrábida/Espichel). The study site (38°29' N–9° 01' W) is located within a region belonging to the sub-humid thermomediterranean bioclimatic domain (Clemente 2002). According to the climatic normal (1971–2000) mean annual precipitation was 730 mm; mean maximum temperature, 27.8°C (August); and mean minimum temperature, 8.1°C (January). Reported data refer to the nearest climatic station (Setúbal, 15 km distance—Instituto Nacional de Meteorologia e Geofísica). The study site is located on a southeast-facing slope (5%) at 130 m a.s.l. that is protected from public access and has not suffered human intervention in the recent decades. Soil is skeletal (topsoil layer of approximately 15 cm) and true profiles cannot be discerned. Silt predominates in the soil (57%), while clay and sand contents are 28% and 15%, respectively (silt-sand-loam—Correia, 1988). In Specht et al. (1988), the soils of Serra da Arrábida are classified as calcic rhodo-chromic luvisols and calcareous chromic cambisols (FAO System). The vegetation consists of a dense maquis (Eunis class F5—Mediterranean maquis), which developed after a fire event 4 years before the beginning of N additions (summer 2003). The vegetation is dominated by *Cistus ladanifer* L., an obligate seeder species (after a fire, there is a massive seedling recruitment from a dormant seed bank—Clemente et al. 1996). Other abundant plant species include *Erica scoparia* L., *Calluna vulgaris* (L.) Hull, *Genista triacanthos* Brot., *Ulex densus* Welw. ex Webb, *Dittrichia viscosa* L., and *Myrtus communis* L. Herbaceous species comprise ≈10% of the total plant cover and many are annual plants which

survive the unfavorable season in the form of seeds and complete their life-cycle during favorable seasons (therophyte species).

#### Experimental design and fertilization schedule

The experimental design consisted of 12 plots, of 400 m<sup>2</sup> each, but to prevent the boundary effect and dilution processes, all measurements and analyses were performed within an internal 100 m<sup>2</sup> square. Each treatment had three replicates (3 plots). To prevent N 'contamination' through runoff from fertilized plots, the experimental plots were distributed in three rows along the slope, with the controls being located in the top row.

Estimated background N deposition is 5.2 kg ha<sup>-1</sup> yr<sup>-1</sup> (2.9 kg NO<sub>x</sub>+2.3 kg NH<sub>y</sub>—[http://webdab.emep.int/Unified\\_Model\\_Results/AN/](http://webdab.emep.int/Unified_Model_Results/AN/)). In Portugal, scattered protected areas represent 21% of the continental territory, making the Portuguese landscape very patchy, with distinct land uses in close proximity. Therefore the chosen N doses were lower than the N deposition reported for other areas in Mediterranean-type ecosystems (145 kgN ha<sup>-1</sup> y<sup>-1</sup>—Fenn et al. 2003; Meixner and Fenn 2004) but high enough to assure that this study can be useful to establish 'worst case' scenarios for N enrichment in this type of habitat. Given that the study site is close (<30 km) to urban and industrial areas and that important agricultural activities overlap partially with the Natura 2000 site, we decided to test the effects of reduced and oxidized N (mimicking combined inputs from urban/industrial sites and agriculture) and of only reduced N (mimicking close proximity to an agricultural source). N availability was modified by the addition of 40 and 80 kgN ha<sup>-1</sup> y<sup>-1</sup> in the form of NH<sub>4</sub>NO<sub>3</sub> (40AN and 80AN, respectively) and 40 kgN ha<sup>-1</sup> y<sup>-1</sup> as a 1:1 mixture of N-NH<sub>4</sub>Cl and N-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (40A). Beginning in January 2007, the fertilizer was homogenously added, by hand, in three equal applications throughout the year: spring, summer and middle autumn/winter. Fertilizer granules dissolved rapidly (1–7 days depending on the fertilization period) after application. N additions took place in January, April and August 2007 and January 2008.

#### Soil sampling

Five soil-sampling locations were identified per plot, corresponding to the four corners and the centre. From each sample location, soil samples (2 cm diameter and

15 cm depth) were removed. Soil samples were sieved and stored at 4°C until analyses. Sampling took place in May, August and October 2007 and February and April 2008, corresponding to the distinct seasons. Individual soil samples (five per plot) were used to determine soil moisture and concentrations of nitrate, ammonium, inorganic N and phosphate. Bulk soil samples (equal mixtures of the five soil samples from each experimental plot) collected in April 2008 were analyzed for soil characterization (Table 1).

#### Vascular plant community composition assessments

The vegetation at the study site was sampled within one 5×5 m square per experimental plot (within the internal 100 m<sup>2</sup>). Percentage of vascular plant species cover (herbaceous included), and of bare soil were recorded. Each species' cover was calculated from the total projected crown area (calculated from two perpendicular diameters, assuming elliptical shape). The vascular plant community composition was assessed in the Spring (June 2007, May 2008).

#### Soil chemical analysis

From each individual soil sample (five per plot), 10 g fwt of soil were used to gravimetrically determine soil

**Table 1** Response of soil chemical composition (N, nitrogen; [N-NO<sub>3</sub>-], extractable nitrate; [N-NH<sub>4</sub>+], extractable ammonium; [inorgN], sum of extractable nitrate and ammonium; pH; OM, organic matter; P, phosphorus; K, potassium; and Mg, magnesium) to distinct N availabilities (Control, 40A, 40AN and 80AN). Soil sampling took place in April 2008 (1 year after the beginning of the N additions)

Soil properties	Control	40A	40AN	80AN
N (%)	0.1±0	0.1±0	0.1±0	0.1±0
[N-NO <sub>3</sub> -] (μg g <sup>-1</sup> )	7.7±1.7	8.2±1.5	5.3±1.7	9.2±5.0
[N-NH <sub>4</sub> +] (μg g <sup>-1</sup> )	1.2±0.4	1.2±0.7	2.0±0.6	2.3±0.8
[inorgN] (μg g <sup>-1</sup> )	8.9±1.6	9.4±1.9	7.3±1.8	11.5±4.4
pH (H <sub>2</sub> O)	5.0±0	5.6±0.5	5.7±0.3	6.3±0.6
OM (%)	2.8±0.4	2.6±0.3	3.1±0.1	3.1±0.9
P (μg g <sup>-1</sup> )	5.0±1.2	3.3±0.3	4.0±0.6	4.7±1.2
K (μg g <sup>-1</sup> )	98±21	118±30	132±22	141±25
Mg (μg g <sup>-1</sup> )	77±13	95±7	89±4	88±1

There were no significant differences between treatments (ANOVA  $p < 0.05$  followed by a Bonferroni test). Values represent the mean ( $n=3$  experimental plots per treatment) ± SE

water content (Kern EG300 3 M). Five g (fwt) of soil were used to prepare soil water extracts (1:10–w/v). Soil extracts were shaken (Cassel Agitator) for 1 h at room temperature, centrifuged (Eppendorf Centrifuge 5403) at 5,000 rpm for 20 min at 4°C and the supernatant collected and analyzed colorimetrically (spectrophotometer Tecan Spectra Rainbow A-5082) for nitrate, ammonium and phosphate. Nitrate ( $\text{N-NO}_3^-$ ) was determined using a modification of the Cataldo method (Matsumura and Witjaksono 1999), ammonium ( $\text{N-NH}_4^+$ ) using a modified Berthelot reaction (Cruz and Martins-Loução 2000), inorganic N as the sum of the water extractable  $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$  and phosphate ( $\text{P-PO}_4^{3-}$ ) using the Fiske and Subarow method (1925). Nitrate, ammonium, inorganic N and phosphate were expressed as  $\mu\text{gN}$  or  $\text{P}$  per gram of dry soil and  $\text{mM}$  of N or P in the soil solution (based on soil water content).

The soil bulk samples used for determining organic matter, pH and concentrations of N, phosphorus, potassium and magnesium were dried at 35°C. Soil pH (Crison micro pH 2002) was measured in a 2.5:1 (v/v) water extract. Organic matter was determined according to ISO norm 10694 by loss on ignition overnight at 600°C (Nabertherm L3/11/C6). Analysis of total N was done according to ISO standard 13878 by dry combustion using an elemental analyzer (Leco CNS). Extractable phosphorus and potassium were quantified by a modification of the Egner-Riehm method using plasma emission spectrophotometry with an optical detector (ICP-OES), following extraction using ammonium lactate 0.1 M and acetic acid 0.4 M, pH 3.65–3.75. Magnesium was extracted with ammonium acetate 1 M, pH 7 and quantified by atomic absorption spectrophotometry with flame atomization.

#### Community diversity indices and differences over time

Richness and evenness of the vascular plant communities were calculated (Krebs 1989). The standing plant community was functionally diverse, comprising summer semi deciduous, evergreen sclerophyllous (Correia 1988), ericaceous, leguminous shrubs (Barradas et al. 1999), herbaceous and young trees (Table 2). The changes in the number of plant species due to the N additions were approached by grouping them into therophyte or perennial (plants that live for more than 2 years, including those hemicryptophytes that in some

cases can be therophytes—Table 2), since these two plant groups show distinct temporal responses and dependence on soil nutrient availability (Marschner et al. 1997). Changes over time ( $t_0$ ,  $t_1$ ) were calculated as follows:

#### Parameter Changes (%)

$$= \frac{(\text{Parameter}_1 - \text{Parameter}_0)}{(\text{Parameter}_1 + \text{Parameter}_0)/2} \times 100$$

Soil parameters were calculated as mean with variation along the experimental period, i.e., average and standard deviation of the five sampling occasions (spring, summer and autumn 2007 and winter and spring 2008) per plot.

#### Statistics

Summary statistics of community responses (richness and evenness) were compared for the different treatments. The two-way ANOVA was applied to assess the existence of significant interactions between time and treatment for plant richness and evenness and differences in community parameters per treatment were analyzed by a one-way ANOVA, followed by a Bonferroni test ( $p < 0.05$ ), or by a Games-Howell test whenever homogeneity of variances was not confirmed by the Levene's test. Linear correlations between the changes in plant community and soil inorganic N availability were also studied (Pearson's correlations). In all cases, preliminary analyses were performed to ensure there was no violation of the assumptions regarding the tests' application. SPSS software, version 17.0, was used for all tests.

## Results

### Soil chemical composition

The soil was characteristically low in N and P. Addition of 40 or 80  $\text{kgN ha}^{-1}\text{yr}^{-1}$  did not significantly increase soil N concentration, which remained at 0.1% for total N and  $\approx 10 \mu\text{g g}^{-1}$  for inorganic N irrespective of N treatment (Table 1). Accordingly, organic matter was also low for all treatments. Potassium and magnesium concentrations were in the range normally found in Portuguese agricultural

**Table 2** List of the vascular plant species observed in the two consecutive assessments (June 2007 and May 2008, the first and second springs of N fertilization) in response to distinct N availabilities (Control, 40A, 40AN and 80AN)

PG		Family	Plant species	Control	40A	40AN	80AN
Perennials	Phanerophyte	Cistaceae	<i>Halimium halimifolium</i>	+			
			<i>Cistus crispus</i>			(-)	+
			<i>Cistus ladanifer</i>	+	+	+	-
			<i>Cistus monspeliensis</i>		+		(+)
			<i>Cistus salvifolius</i>	+	+	+	-
		Ericaceae	<i>Arbutus unedo</i>				-
			<i>Calluna vulgaris</i>	+	+	+	+
			<i>Erica arborea</i>		+	+	
			<i>Erica scoparia</i>	+	+	+	+
			<i>Erica umbellata</i>				+
		Fabaceae	<i>Genista triacanthos</i>	+	+	+	0
			<i>Ulex densus</i>	+	-	+	+
		Fagaceae	<i>Quercus coccifera</i>		+	+	0
			<i>Quercus suber</i>			0	
		Lamiaceae	<i>Lavandula stoechas</i>	+	-	+	0
			<i>Rosmarinus officinalis</i>		(+)		+
			<i>Salvia sclareoides</i>	(-)			(+)
		Myrtaceae	<i>Myrtus communis</i>	+	0	+	-
		Oleaceae	<i>Olea europaea var sylvestris</i>			+	+
			<i>Phillyrea angustifolia</i>				+
		Pinaceae	<i>Pinus pinaster</i>		-		+
		Rhamnaceae	<i>Rhamnus alaternus</i>			+	
		Rosaceae	<i>Rubus ulmifolius</i>	(-)	(+)	-	
		Thymelaeaceae	<i>Daphne gnidium</i>	0	+	-	(+)
	Chamaephyte	Anacardiaceae	<i>Pistacea lentiscus</i>	-	-	+	-
		Asteraceae	<i>Dittrichia viscosa*</i>	-	+	+	+
			<i>Helichrysum stoechas</i>	(-)			-
			<i>Phagnalon saxatile</i>				(-)
		Boraginaceae	<i>Lithodora prostrata</i>	0		+	(+)
		Rubiaceae	<i>Rubia peregrina</i>				(+)
	Geophyte	Asphodelaceae	<i>Asphodelus ramosus</i>	(-)			(+)
		Asteraceae	<i>Carlina corymbosa</i>			(+)	
		Iridaceae	<i>Gladiolus illyricus ssp. reuteri</i>		(+)	(+)	(+)
			<i>Iris xiphium</i>				(+)
		Liliatae	<i>Urginea maritima</i>		(+)		(-)
	Hemicryptophyte	Orobanchaceae	<i>Orobanche latisquama</i>	(+)			
		Ranunculaceae	<i>Anemone palmata</i>	(+)			
		Apiaceae	<i>Daucus carota</i>			(-)	
			<i>Andryala ragusina</i>	-			
		Asteraceae	<i>Cynara sp</i>	(-)			
			<i>Leontodon taraxacoides</i>	-	-	+	0
			<i>Pulicaria odora</i>			(-)	(+)
		Campanulaceae	<i>Campanula rapunculus</i>		0	(-)	
		Cyperaceae	<i>Carex flacca</i>	+	+	+	-

**Table 2** (continued)

PG		Family	Plant species	Control	40A	40AN	80AN
Annuals	Ther. or Hem.	<i>Fabaceae</i>	<i>Anthyllis vulneraria</i>				(+)
		<i>Gentianaceae</i>	<i>Blackstonia perfoliata</i>	–	–	(–)	0
			<i>Centaureum erythraea</i>	–	–	(–)	–
		<i>Hypericaceae</i>	<i>Hypericum sp</i>	–	–	–	
		<i>Poaceae</i>	<i>Agrostis sp</i>	(+)			
			<i>Avenula sp</i>	(+)			
			<i>Brachypodium phoenicoides</i>	–	0	–	+
			<i>Arrhenatherum album</i>		+		(–)
			<i>Dactylis glomerata</i>				(+)
			<i>Sanguisorba hybrida</i>			(–)	(–)
		<i>Asteraceae</i>	<i>Centaurea melitensis</i>	(+)			
			<i>Crepis capillaris</i>		(+)		
			<i>Picris echioides</i>		(–)		+
		<i>Fabaceae</i>	<i>Lotus sp</i>	(+)	(+)	+	
			<i>Trifolium sp</i>	(–)		(–)	(–)
	Therophyte		<i>Vicia sp</i>		(+)		
		<i>Asteraceae</i>	<i>Evax pygmaea</i>				(+)
			<i>Filago minima</i>	–	(–)	(+)	(–)
			<i>Chrysanthemum coronarium</i>		(+)		
			<i>Galactites tomentosa</i>	–	(–)	(–)	(–)
			<i>Matricaria recutita</i>	(–)	(–)	(–)	
			<i>Senecio jacobaea</i>	(+)		(–)	
			<i>Sonchus sp</i>	(–)		(–)	(+)
		<i>Lamiaceae</i>	<i>Stachys arvensis</i>	(+)	(+)	(+)	
		<i>Poaceae</i>	<i>Briza maxima</i>			(+)	(+)
			<i>Briza minima</i>		(–)		(–)
			<i>Briza minor</i>	(–)		0	(+)
		<i>Primulaceae</i>	<i>Anagallis arvensis</i>	(+)	–	+	+
		<i>Apiaceae</i>	<i>Apiaceae</i>	(+)			(+)
		<i>Asteraceae</i>	<i>Asteraceae</i>	(–)	(–)	(+)	
Unknown	nd	<i>Fabaceae</i>	<i>Fabaceae</i>				(–)
		<i>Liliaceae</i>	<i>Liliaceae</i>	(+)		(+)	
		<i>Poaceae</i>	<i>Poaceae</i>	0		–	0
			<i>Vulpia sp</i>	(–)	(–)		(–)
		<i>Rubiaceae</i>	<i>Galium sp</i>			(+)	(+)
			<i>Rubiaceae</i>				(+)
		Increased	+	10	12	19	13
		Decreased	–	10	9	5	8
		Maintained	0	3	3	2	6
		Appeared	(+)	11	9	8	18
		Disappeared	(–)	11	7	12	10

Plant species were grouped according to their biological type (PG— $n=3$  experimental plots per treatment)

\*for *Dittrichia viscosa* refer to statistically significant differences between treatments (ANOVA  $p<0.05$  followed by a Bonferroni test)

soils, therefore not limiting, and were similar in all treatments. Soil pH (water) was slightly acidic, but the pH tended to increase with the applied N dose (Table 1).

### Changes in vascular plant community

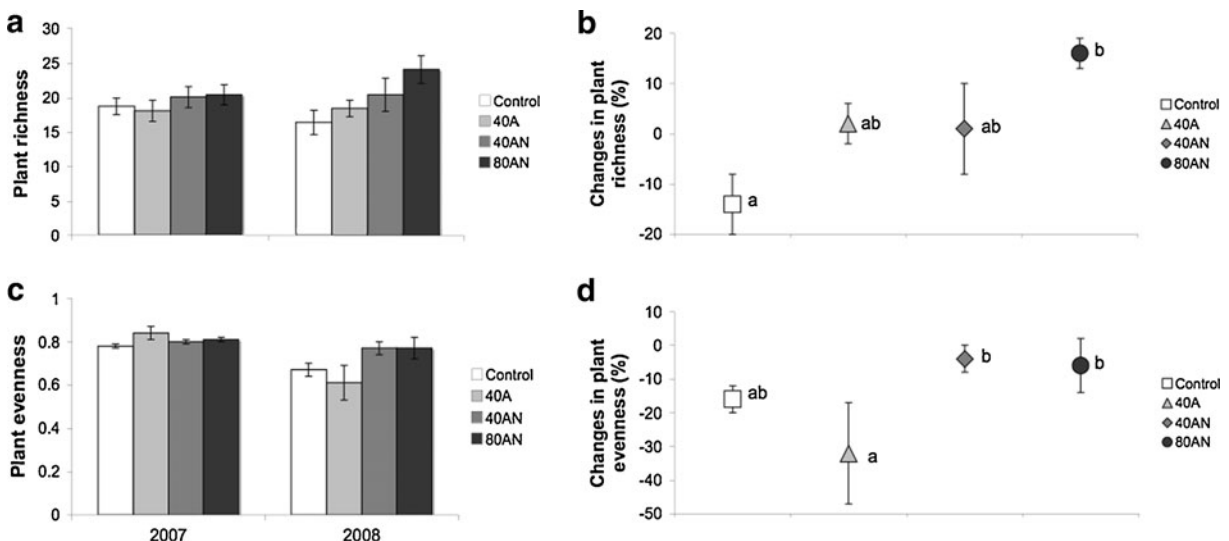
In contrast to the soil N concentration (Table 1), the standing vascular plant community responded to increased N availability (Table 2, Fig. 1). At the beginning of the experiment (Spring 2007), plant communities were similar in all treatments (based on richness and evenness—Fig. 1-a, c). However, differences between treatments were observed 1 year later, in Spring 2008 (Fig. 1-b, d).

The number of plant species (richness—Fig. 1-a, b) decreased in non-fertilized plots (control), did not change in both treatments receiving 40 kgN ha<sup>-1</sup> yr<sup>-1</sup> (40A and 40AN), but increased in the 80AN plots. Data show that 1 year of 80AN was enough to cause a significant increase in the number of plant species in relation to the control  $\approx 30\%$ , as well as in relation to the starting point (Spring 2007),  $\approx 16\%$ .

Plant species evenness decreased in control plots (Fig. 1-c, d) but decreased even more in the 40A plots.

However, it did not change in the plots fertilized with ammonium nitrate, either with 40AN or 80AN.

Changes in the plant community (Fig. 1) involved species appearance and disappearance and/or changes in plant species cover (Table 2). In control plots, the number of plant species that increased their cover was the same as the ones that decreased it (10) and only three species stayed the same. Eleven ‘new’ species were detected and another eleven were ‘lost’. Fertilization with 40A resulted in twelve species that increased their cover, nine that decreased and three that maintained it. In the second spring after the beginning of the N addition, nine ‘new’ species and seven ‘losses’ were observed. Fertilization with 40AN resulted in nineteen species that increased their cover, five that decreased and two that maintained it. In spring 2008, eight ‘new’ species and twelve ‘losses’ were observed. The treatment which changed plant species the most was the 80AN with thirteen species that increased their cover, eight that decreased and six that maintained it. After 1 year of treatment, eighteen ‘new’ species and ten ‘losses’ were recorded. The biological types more responsive to the N enrichment were the geophytes, the hemicryptophytes and the therophytes (Table 2).



**Fig. 1** Response of the vascular plant community to distinct N availabilities (Control, 40A, 40AN and 80AN) in terms of plant richness (a–b) and evenness (c–d). Community assessments were performed in the first and second Springs of N fertilization: 2007 and 2008. Changes in each parameter between 2008 and 2007 were positive or negative and were

calculated as described in [materials and methods](#). Different letters refer to statistically significant differences between treatments (ANOVA  $p < 0.05$  followed by a Bonferroni test). There were no significant interactions between treatment and time ( $p < 0.001$ ). Values represent the mean ( $n = 3$  experimental plots per treatment)  $\pm$  SE



In terms of plant cover, seven groups of plant species could be identified: (a) *Dittrichia viscosa* was the only species which responded significantly to increased N, i.e., its cover decreased in control plots, but increased in fertilized plots (Table 2). Similarly, in the second spring of N addition, *Gladiolus illyricus* ssp *reuteri* only appeared in fertilized plots; (b) cover of *Cistus ladanifer* (the dominant plant species), *C. salvifolius*, *Genista triacanthos* and *Carex flacca* tended to decrease only in the 80AN; (c) *Ulex densus*, *Lavandula stoechas* and *Anagalis arvensis* tended to respond negatively to fertilization with ammonium as the only N source (40A); (d) in spring 2008, *Orobancha latisquama* (a parasitic plant) and *Anemone palmate* only appeared in the control plots. (e) *Briza maxima* and *Galium* sp were only observed in the second spring of the N addition in plots receiving ammonium nitrate (40AN and 80AN); (f) *Calluna vulgaris* and *Erica scoparia* increased their cover in all treatments and (g) *Centaureum erythraea* and *Hypericum* sp decreased their cover irrespective of the treatment.

#### Linking plant community changes with soil N

No relation was found between changes in the whole-plant community and the soil total N pool (Table 1, Fig. 1). However, when the distinct N fractions (e.g. inorganic N and ratio of reduced to oxidized N), or the ratio between bioavailable N/P were considered, some correlations became apparent (Table 3).

Changes in plant richness were positively correlated with plant richness in 2008 ( $r=0.79$ ), the mean of nitrate concentration in the soil solution ( $r=0.73$ ), the changes in perennial richness ( $r=0.63$ ), the variation of nitrate concentration in the soil solution ( $r=0.63$ ) and the mean and variation of soil inorganic N ( $r=0.66$  and  $0.60$ , respectively); and negatively correlated with the variation (standard deviation) of the ratio between bioavailable N/P ( $r=-0.75$ ).

Analyzing the changes in richness for the two main plant types (perennials and therophytes—Table 2) independently showed that these behaved differently in terms of the number of parameters with significant correlations, and the identity of the variable with which a significant correlation was found (Table 3). Changes in perennial richness were positively correlated with the mean of nitrate concentration in the soil solution ( $r=0.73$ ), with changes in the plant richness

( $r=0.63$ ), and with the mean of inorganic N in the soil, and in the soil solution ( $r=0.61$  and  $0.60$ , respectively). Changes in therophyte richness were negatively correlated with the variation of soil's ratio of reduced to oxidized inorganic N ( $r=-0.58$ ).

Changes in species evenness were positively and strongly correlated with plant evenness in 2008 ( $r=0.97$ ). Positive correlations were also obtained with the variation of soil nitrate concentration ( $r=0.46$ ) and the mean of inorganic N in the soil, and in the soil solution ( $r=0.49$  and  $0.46$ , respectively). Changes in species evenness were negatively correlated with plant evenness in 2007 ( $r=-0.64$ —Table 3).

## Discussion

### Short-term response of a N-limited ecosystem to increased N availability

In contrast to most studies (see Bobbink et al. 2010 for worldwide review) our data suggest that increased N availability did not lead to a loss of diversity of vascular plants (Fig. 1). On the contrary, increased N availability, created by the addition of 40 or 80 kgN ha<sup>-1</sup>yr<sup>-1</sup>, seemed to promote biodiversity. The few other studies reporting increased biodiversity in response to N enrichment (Calvo et al. 2005; Allen et al. 2009; Pinho et al. 2009) were also carried out at sites with low soil N contents. In fact, and according to Bobbink et al. (1998), increasing the N availability in extremely nutrient-poor soils (Table 1) can increase biodiversity (Fig. 1, Table 2). Thus it seems that the initial ecosystem N status is an important factor in predicting the ecosystem's response to N enrichment (Emmett 2007).

Plant species richness and composition (Fig. 1 and Table 2) were consistent with an early phase of a post-fire succession for a Mediterranean ecosystem (Clemente et al. 1996). Immediately after a fire, plant diversity is low (Thompson 2005) since the only plant species are those whose germination is stimulated by high temperatures (e.g. *C. ladanifer*). However, fire is also a disturbance factor which favours the establishment of ruderal plant species resulting in a diversity peak usually observed 3 years after fire (Thompson 2005), which is then followed by a sharp decline in the next 2 years corresponding to the decrease of ruderals (Casal 1987; Trabaud



**Table 3** Pearson's correlation coefficients between changes in plant community and soil inorganic N and bioavailable N/P along the time period between the two plant community assessments

Variables:	Changes in:	Plant richness	Perennial richness	Therophyte richness	Plant evenness
Plant richness 2007		0.17	-0.11	0.02	0.30
Plant richness 2008		0.79**	0.40	0.15	0.23
Changes in plant richness		1.00	0.63*	0.19	0.03
Changes in perennial richness		0.63*	1.00	-0.23	0.15
Changes in therophyte richness		0.19	-0.23	1.00	0.29
Plant evenness 2007		0.16	-0.01	-0.24	-0.64*
Plant evenness 2008		0.10	0.20	0.20	0.97**
Changes in plant evenness		0.03	0.15	0.29	1.00
[N-NO <sub>3</sub> <sup>-</sup> ] mean		0.53	0.40	0.22	0.26
[N-NO <sub>3</sub> <sup>-</sup> ] sd		0.53	0.29	0.35	0.46*
[N-NO <sub>3</sub> <sup>-</sup> ] in soil solution mean		0.73**	0.73**	0.13	0.43
[N-NO <sub>3</sub> <sup>-</sup> ] in soil solution sd		0.63*	0.39	0.49	0.34
[N-NH <sub>4</sub> <sup>+</sup> ] mean		0.49	0.51	-0.32	0.31
[N-NH <sub>4</sub> <sup>+</sup> ] sd		0.35	0.42	-0.40	0.42
[N-NH <sub>4</sub> <sup>+</sup> ] in soil solution mean		0.32	0.44	-0.39	0.37
[N-NH <sub>4</sub> <sup>+</sup> ] in soil solution sd		0.11	0.22	0.13	0.13
[inorgN] mean		0.66*	0.61*	-0.08	0.49*
[inorgN] sd		0.60*	0.33	0.34	0.36
[inorgN] in soil solution mean		0.55	0.60*	-0.12	0.46*
[inorgN] in soil solution sd		0.55	0.54	0.03	0.57
[N-NH <sub>4</sub> <sup>+</sup> ] / [N-NO <sub>3</sub> <sup>-</sup> ] mean		0.50	0.37	-0.26	-0.11
[N-NH <sub>4</sub> <sup>+</sup> ] / [N-NO <sub>3</sub> <sup>-</sup> ] sd		0.31	0.30	-0.058*	-0.21
[N-NO <sub>3</sub> <sup>-</sup> ] / [inorgN] mean		-0.53	-0.31	-0.02	-0.02
[N-NO <sub>3</sub> <sup>-</sup> ] / [inorgN] sd		0.22	-0.13	-0.36	-0.30
[inorgN] / [P-PO <sub>4</sub> <sup>3-</sup> ] mean		-0.45	-0.30	-0.18	0.49
[inorgN] / [P-PO <sub>4</sub> <sup>3-</sup> ] sd		-0.75**	-0.46	0.06	0.52

\*\*Correlation is significant at the 0.01 level (2-tailed)

\*Correlation is significant at the 0.05 level (2-tailed)

1987). Plant community was assessed on the forth and fifth years after the fire, thus explaining the decrease in plant richness observed in control plots (Fig. 1). N enrichment prevented the natural decline in species richness mostly through the appearance and maintenance of geophyte, and therophyte species (Table 2).

The referred decrease in the number and/or cover of ruderal species (e.g. *Dittrichia viscosa*—Table 2) is likely to be responsible for the decrease of plant evenness in the control plots (Fig. 1-a, b), which is in agreement with the fact that plant communities are naturally uneven (Wilsey and Potvin 2000; Naeem 2009). The addition of ammonium as a single N form (40A) may have been a disturbance factor, resulting in

the greatest decline in plant evenness. On the contrary, addition of ammonium and nitrate (40AN and 80AN) may have enabled nitrate-preferring plant species to coexist with those that tolerate ammonium, thus resulting in smaller decreases in plant evenness when compared to the other treatments. Maintenance of high plant evenness in response to fertilization with ammonium nitrate has also been observed by Manning et al. (2006).

Assuming N limitation as an important factor of environmental severity, increased N availability, which often promotes the decrease of slow growing conservative species and the settlement of fast growing exploitative species (Emmett 2007), would increase species richness (Grime 1973). The revised

Grime's humped-back model (Michalet et al. 2006) predicts that the development of the plant community increases the N limitation, so that exploitative species (species with intermediate strategies and competitive ones) would benefit from living under the influence of conservative species (stress-tolerant species). However, as the N limitation is alleviated by the N additions, facilitation would decrease given that exploitative species would be able to exist away from the canopy of the conservative species and therefore the number of species would increase (Fig. 1 and Table 2). Accordingly, and assuming as conservative species *C. ladanifer*, *C. salvifolius*, *G. triacanthos* and *U. densus* (Correia 1988; Clemente 2002), and as exploitative species *D. viscosa* (Karageorgou et al. 2002), the observed biodiversity increase in Arrábida (Fig. 1) may be explained by the coexistence of the remaining conservative with the incoming exploitative. Similar biodiversity changes in response to N availability have been observed for epiphytic lichens (Mitchell et al. 2005; Pinho et al. 2009). The N-induced biodiversity increases, corresponding to the coexistence of conservative and exploitative species, have probably not been observed in many north temperate ecosystem studies, which typically have higher N availability than our study site. Nevertheless it is important to keep in mind that data refer to very short-term effects so that the biodiversity increase may only be an initial response while the long-term effects may be quite different. In the longer term and according to the humped-back model there might be a loss of more N conservative species as the system becomes more nutrient rich (Michalet et al. 2006) and therefore its response may become similar to those in which the majority of studies have been performed.

#### Linking plant community changes with soil N availability

The variation in the soil bioavailable N/P was the soil parameter that was more correlated with the changes in plant richness (Table 3), pointing to the dependence of species on adequate supplies of both N and P. Since most semi-natural ecosystems, and Mediterranean ones in particular (Sardans and Peñuelas 2004), are N and often also P limited (Table 1), Fanelli et al. (2008) also found that the soil's N/P was the best explanatory variable for Mediterranean herbaceous plant communities arranged along a gradient of ruderality.

Nitrate concentrations at the site were comparable to those determined for other sites within the same study area (Cruz et al. 2008) and for other Mediterranean ecosystems (Gallardo et al. 2006). It is possible that nitrate being the predominant form of inorganic N (Table 1) in such a post-fire scenario (Knicker 2007; Meixner et al. 2006) accounts for the correlation between soil nitrate and the changes in plant and perennial richness and in plant evenness (Table 3). Since plants differ in their tolerance to ammonium (Cruz et al. 2003; Kronzucker et al. 2003), the response of the particular system to the variation of the inorganic N present as nitrate is expected to depend on its stage in the ecological succession (Cruz et al. 2003), and on its species composition. Early successional species prefer nitrate, and are relatively ammonium intolerant while late successional are more ammonium tolerant (Kronzucker et al. 2003). Thus, soil nitrate concentration may shape plant communities such as the standing plant community which is in an early stage of succession and likely to be dominated by species that prefer nitrate. Similarly to soil nitrate, soil inorganic N was also correlated with the changes in plant and perennial richness and in plant evenness (Table 3), which may reflect the strong correlation between the two N fractions (data not shown).

The variation in the ratio of reduced to oxidized inorganic N was the only parameter showing correlation with the changes in therophyte richness (Table 3). For a therophyte plant, the transition from seed in the seed bank to seedling or juvenile may depend on the initial seed density, while the transition from juvenile to reproductive adult and from adult plant to seed might depend on the corresponding density in the population (Kluth and Bruelheide 2005). Therefore, changes in the ratio of reduced to oxidized inorganic N may have prevented therophyte plants from using the increased N availability to complete their life cycle (van den Berg et al. 2008) in the first and/or second years of N addition.

#### Understanding biodiversity changes due to increased N availability in a Mediterranean ecosystem

Although *D. viscosa* is quite common within the Mediterranean, it is considered a ruderal plant species due to its abundance in areas that have been modified and altered by anthropic activities (Murciego et al. 2007). Therefore the significantly contrasting response

of *D. viscosa* in control and fertilized plots (Table 2) suggests that this species could be regarded as an indicator of N enrichment in Mediterranean maquis, similarly to *Deschampsia flexuosa* in boreal ecosystems (Bobbink et al. 2010). In the following years of this study, other plant species may prove to be consistent indicators of: low N, presence of *Orobanchelatisquama* and *Anemone palmata*; N enrichment, presence of *Gladiolus illyricus* ssp. *reuteri*; ammonium enrichment, decrease of *Ulex densus*, *Lavandula stoechas* and *Anagallis arvensis*; nitrate and ammonium enrichment, presence of *Briza maxima* and *Galium* sp (Table 2).

Mediterranean ecosystems are subjected to dry/wet cycles of varying duration and intensity (Jarvis et al. 2007). As a result, nutrients (N in particular) may be present in the soil but their concentration in the soil solution can be high (when the soil is drying and/or when there is a nutrient flush) or low (when the soil is water saturated and/or nutrient content is low). Thus, N is only truly available when in solution, making N in the soil solution a more reliable measure of availability in these ecosystems. This may explain the correlation between the changes in plant and in perennial richness with nitrate concentration in the soil solution and not with soil nitrate.

In the present study, both the mean and the variation of N availability along the year were correlated with the changes in plant community (Table 3), perhaps due to the characteristics of Mediterranean ecosystems. Plants prefer specific patterns of N availability (Gallardo et al. 2006; Cruz et al. 2008) which, as in many other biological phenomena, are characterized by a mean and amplitude of variation, with its effects becoming functionally important.

The observed response of *D. viscosa* to N enrichment should be further assessed as a management tool for forecasting, and determining real-time actions to prevent biodiversity changes in response to real N enrichment. Finally, this is a first integrated field study on how European Mediterranean vegetation responds to N enrichment from different forms and doses and highlights the fact that longer-term studies are needed to explore the generality of what has been observed in the present study.

**Acknowledgements** This study was supported by the Fundação para a Ciência e Tecnologia (FCT) through the project PTDC/BIA-BEC/099323/2008; Teresa Dias for her PhD grant BD/25382/2005 (FCT). We are grateful to Parque Natural da Arrábida for

making available the experimental site and allowing the N manipulation experiment to which this paper refers, and also to COST 729 for funding the authors participation on the conference (organized by COST 729, ESF and NinE) “Nitrogen Deposition and Natura 2000: Science and Practice in Determining Environmental Impacts” (18–20 May, Brussels—Belgium) which set the cornerstone for the present paper. Finally we are grateful to Steve Houghton for helping with the manuscript’s preparation and to the three anonymous reviewers for the comments and suggestions which greatly improved the present paper.

## References

- Allen EB, Rao LE, Steers RJ (2009) Impacts of atmospheric nitrogen deposition on vegetation and soils at Joshua Tree National Park. In: Webb RH, Fenstermaker LF, Heaton JS, Hughson DL, McDonald EV, Miller DM (eds) The Mojave Desert: Ecosystem processes and sustainability. University of Nevada Press, Las Vegas, pp 78–100
- Barradas MCD, Zunzunegui M, Tirado R, Ain-Lhout F, Novo FG (1999) Plant functional types and ecosystem function in Mediterranean shrubland. *J Veg Sci* 10:709–716
- Bobbink R, Hornung M, Roelofs JGM (1998) The effects of airborne Nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *J Ecol* 86:717–738
- Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman J-W, Fenn M, Gilliam F, Nordin A, Pardo L, de Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol Appl* In press
- Bonanomi G, Caporaso S, Allegrezza M (2006) Short-term effects of nitrogen enrichment, litter removal and cutting on a Mediterranean grassland. *Acta Oecol* 30:419–425
- Calvo L, Alonso I, Fernández AJ, De Luis E (2005) Short-term study of effects of fertilisation and cutting treatments on the vegetation dynamics of mountain heathlands in Spain. *Plant Ecol* 179:181–191
- Casal M (1987) Post-fire dynamics of shrubland dominated by papilionaceae plants. *Ecol Mediter* 13:87–98
- Clarisse L, Clerbaux C, Dentener F, Hurtmans D, Coheur P-F (2009) Global ammonia distribution derived from infrared satellite observations. *Nat Geosci* 2:479–483
- Clemente AS (2002) Dinâmica da vegetação após o fogo na Serra da Arrábida. PhD Dissertation, Universidade de Lisboa
- Clemente AS, Rego FC, Correia OA (1996) Demographic patterns and productivity of post-fire regeneration in Portuguese Mediterranean maquis. *Int J Wildland Fire* 6:5–12
- Correia OCA (1988) Contribuição da fenologia e ecofisiologia em estudos da sucessão e dinâmica da vegetação mediterrânica. PhD Dissertation, Universidade de Lisboa
- Cruz C, Martins-Loução MA (2000) Nitrogen in a sustainable environment: a matter of integration. In: Martins-Loução MA, Lips SH (eds) Nitrogen in a sustainable ecosystem. Backhuys, The Netherlands, pp 415–419
- Cruz C, Lips H, Martins-Loução MA (2003) Nitrogen use efficiency by a slow-growing species as affected by CO<sub>2</sub> levels, root temperature, N source and availability. *J Plant Physiol* 160:1421–1428

- Cruz C, Bio AMF, Jullioti A, Tavares A, Dias T, Martins-Loução MA (2008) Heterogeneity of soil surface ammonium concentration and other characteristics, related to plant specific variability in a Mediterranean-type ecosystem. *Environ Pollut* 154:414–423
- Emmett BA (2007) Nitrogen saturation of terrestrial ecosystems: some recent findings and their implications for our conceptual framework. *Water Air Soil Pollut* 7:99–109
- Fanelli G, Lestini M, Sauli AS (2008) Floristic gradients of herbaceous vegetation and P/N ratio in soil in a Mediterranean area. *Plant Ecol* 194:231–242
- Fenn ME, Baron JS, Allen EB, Rueth HM, Nydick KR, Geiser L, Bowman WD, Sickman JO, Meixner T, Johnson DW, Neitlich O (2003) Ecological effects of nitrogen deposition in the western United States. *Bioscience* 53:404–420
- Fiske CF, Subbarow Y (1925) The colorimetric determination of phosphorous. *J Biol Chem* 66:375–400
- Gallardo A, Paramá R, Covelo F, Fernández-Alés R (2006) Differences between soil ammonium and nitrate spatial pattern in six plant communities. Simulated effect on plant populations. *Plant Soil* 279:333–346
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226
- Grime JP (1973) Competitive exclusion in herbaceous vegetation. *Nature* 242:344–347
- Grove AT, Rackham O (2001) The nature of Mediterranean Europe. Yale University Press, London
- Jarvis P, Rey A, Petsikos C, Wingate L, Rayment M, Pereira J, Banza J, David J, Miglietta F, Borghetti M, Manca G, Valentini R (2007) Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the ‘Birch effect’. *Tree Physiol* 27:929–940
- Karageorgou P, Levizou E, Manetas Y (2002) The influence of drought, shade and availability of mineral nutrients on exudate phenolics of *Diurichia viscosa*. *Flora* 197:285–289
- Kluth C, Bruehlheide H (2005) Effects of range position, inter-annual variation and density on demographic transition rates of *Hornungia petraea* populations. *Oecologia* 145:382–393
- Knicker H (2007) How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. *Biogeochemistry* 85:91–118
- Krebs CJ (1989) Ecological methodology. Harper and Row, New York
- Kronzucker HJ, Siddiqi MY, Glass ADM, Britto DT (2003) Root ammonium transport efficiency as a determinant in forest colonization patterns: a hypothesis. *Physiol Plant* 117:164–170
- Manning P, Newington JE, Robson HR, Saunders M, Eggers T, Bradford MA, Bardgett RD, Bonkowski M, Ellis RJ, Gange AC, Grayston SJ, Kandeler E, Marhan S, Reid E, Tschirko D, Godfray CJ, Rees M (2006) Decoupling the direct and indirect effects of nitrogen deposition on ecosystem function. *Ecol Lett* 9:1015–1024
- Marschner H, Kirkby EA, Engels C (1997) Importance of cycling and recycling of mineral nutrients within plants for growth and development. *Bot Acta* 110:265–273
- Matsumura S, Witjaksono G (1999) Modification of the Cataldo method for the determination of nitrate in soil extracts by potassium chloride. *Soil Sci Plant Nutr* 45:231–235
- McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. *Trends Ecol Evol* 21:178–185
- Meixner T, Fenn ME (2004) Biogeochemical budgets in a Mediterranean catchment with high rates of atmospheric N deposition—importance of scale and temporal asynchrony. *Biogeochemistry* 70:331–356
- Meixner T, Fenn ME, Wohlgemuth P, Oxford M, Riggan P (2006) N saturation symptoms in chaparral catchments are not reversed by prescribed fire. *Environ Sci Technol* 40:2887–2894
- Michalet R, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Pugnaire FI, Valiente-Banuet A, Callaway RM (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecol Lett* 9:767–773
- Mitchell RJ, Truscot AM, Leith ID, Cape JN, Van Dijk N, Tang YS, Fowler D, Sutton MA (2005) A study of the epiphytic communities of Atlantic oak woods along an atmospheric nitrogen deposition gradient. *J Ecol* 93:482–492
- Murciego AM, Sánchez AG, González MAR, Gil EP, Gordillo CT, Fernández JC, Triguero TB (2007) Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Diurichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain). *Environ Pollut* 145:15–21
- Naem S (2009) Gini in the bottle. *Nature* 458:579–580
- Phoenix GK, Hicks WK, Cinderby S, Kuylensstierna JCI, Stock WD, Dentener FJ, Giller KE, Austin AT, Lefroy RDB, Gimeno BS, Ashmore MR, Ineson P (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Glob Chang Biol* 12:470–476
- Pinho P, Branquinho C, Cruz C, Tang S, Dias T, Rosa AP, Máguas C, Martins-Loução MA, Sutton M (2009) Assessment of critical levels of atmospheric ammonia for lichen diversity in a cork-oak woodland, Portugal. In: Sutton M, Reis S, Baker S (eds) *Atmospheric ammonia*. Springer, pp 109–120
- Sala OE, Chapin FS III, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NLR, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774
- Sardans J, Peñuelas J (2004) Increasing drought decreases phosphorous availability in an evergreen Mediterranean forest. *Plant Soil* 267:367–377
- Specht RL, Rundel PW, Westman WE, Catling PC, Majer JD, Greenslade (1988) Mediterranean type ecosystems. A data source book. Kluwer Academic Publishers, Dordrecht
- Thompson JD (2005) Plant evolution in the Mediterranean. Oxford University Press, New York
- Trabaud L (1987) Dynamics after fire of sclerophyllous plant communities in the Mediterranean Basin. *Ecol Mediter* 13:25–37
- van den Berg LJJ, Peters CJH, Ashmore MR, Roelofs JGM (2008) Reduced nitrogen has a greater effect than oxidised nitrogen on dry heathland vegetation. *Environ Pollut* 154:359–369
- Vourlitis GL, Pasquini S, Zorba G (2007) Plant and soil N response of southern Californian semi-arid shrublands after 1 year of experimental N deposition. *Ecosystems* 10:263–279
- Wilsey BJ, Potvin C (2000) Biodiversity and ecosystem functioning: importance of species evenness in an old field. *Ecology* 81:887–892