



## Oak trees and soil interactions in Mediterranean forests: a positive feedback model

Cristina Aponte, Luis V. García, Ignacio M. Pérez-Ramos, Eduardo Gutiérrez & Teodoro Marañón

### Keywords

Biogeochemical niche; Ecological stoichiometry; Ecosystem functioning; Foliar analysis; Nutrient cycling; *Quercus canariensis*; *Quercus suber*; Soil fertility.

### Nomenclature

Valdés et al. (1987)

Received 2 November 2010

Accepted 28 March 2011

Co-ordinating Editor: Alicia Acosta

---

**Aponte, C.** (corresponding author, [aponte@irnase.csic.es](mailto:aponte@irnase.csic.es)); **García, L.V.** ([ventura@cica.es](mailto:ventura@cica.es)); **Pérez-Ramos, I.M.** ([imperez@irnase.csic.es](mailto:imperez@irnase.csic.es)); **Gutiérrez, E.** ([edugg@irnase.csic.es](mailto:edugg@irnase.csic.es)) & **Marañón, T.** ([teodoro@irnase.csic.es](mailto:teodoro@irnase.csic.es)): Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, PO Box 1052, E-41080 Sevilla, Spain.

### Abstract

**Questions:** What is the spectrum of variability of chemical elements in a Mediterranean forest ecosystem across the different compartments? Do co-existing tree species with different leaf chemical composition and nutrient cycling distinctly modify soil conditions? Could these species-specific, tree-generated soil changes create a potential positive feedback by affecting long-term species distribution?

**Location:** Mixed oak forests of southern Spain, Los Alcornocales Natural Park.

**Methods:** We sampled and chemically analysed five different ecosystem components: leaves, leaf fall, litter and superficial (0–25 cm) and sub-superficial (25–50 cm) soil beneath the canopies of evergreen *Quercus suber* and deciduous *Q. canariensis* trees. We used multiple co-inertia analysis (MCoA) to conjointly analyse the patterns of variability and covariation of eight macro- and micronutrients determined in each of the sampled ecological materials. We implemented a path analysis to investigate alternative causal models of relationships among the chemical properties of the different ecosystem components.

**Results:** Variability in the concentration of chemical elements was related to the nature of their biogeochemical cycles. However, the rank of element concentration was consistent across ecosystem components. Analysis of co-inertia (MCoA) revealed that there was a common underlying multivariate pattern of nutrient enrichment in the ecosystem, which supported the hypothesis of a separation in biogeochemical niches between the two co-existing oak species, with *Q. canariensis* having richer plant tissues and more fertile soil directly under each tree than *Q. suber*. The feasibility of a potential tree–soil positive feedback model was the only statistically validated among several alternative (non-feedback) models tested.

**Conclusions:** In the studied Mediterranean forests, oak species distinctly modify soil fertility conditions through different nutrient return pathways. Further investigation is needed to address whether these tree-generated soil changes could affect seedling establishment and ultimately influence species distribution.

### Introduction

Ecosystem functioning and stability rely on ecosystem interactions. Understanding the complexity and underlying mechanisms of plant–soil interactions is crucial to understand ecosystems and their responses to changing conditions (Ehrendfeld et al. 2005). In forest ecosystems,

trees play a key role in these interactions because of their capacity to modify soil properties and nutrient cycling (Finzi et al. 1998; Lovett et al. 2004). The chemical traits (i.e. mineral content and stoichiometry) of leaves determine the physical and chemical characteristics of leaf fall, which are also affected by the biological processes of

resorption and retranslocation (Pugnaire & Chapin 1993). Furthermore, they influence leaf decomposition and mineralization rates and the incorporation of organic matter and nutrients into the soil (Cornwell et al. 2008). Through distinct nutrient return and the associated changes in soil acidity and nutrient availability, different tree species can alter the habitat and resources of soil organisms and shift the abundance and composition of soil communities (Aponte et al. 2010a, b). Soil biota have a strong influence on the physical (e.g. aeration) and chemical properties of soil (Wardle et al. 2004). In turn, soil characteristics influence tree seedling establishment, survivorship and ultimately tree species distribution (Goldberg 1982; Kobe et al. 1995). Thus, changes in soil communities, soil fertility and nutrient cycling generated by trees could result in changes in the plant community (Mitchell et al. 2007) and lead to a potential positive feedback effect through increasing the fitness of individuals of the same species (Hobbie 1992; Finzi et al. 1998; Gómez-Aparicio & Canham 2008). Feedback effects have ecological and evolutionary consequences for tree populations (Binkley & Giardina 1998; van Breemen & Finzi 1998) and are key to predicting forest dynamics (Weand et al. 2010). Most studies of tree–soil interactions have been conducted in temperate and boreal forests (Knops et al. 2002; Mitchell et al. 2007; Talbot & Finzi 2008), while equivalent studies in Mediterranean forests are less frequent (Gallardo & Merino 1993). Revealing the mechanism of tree–soil interactions will contribute to increase our understanding of the patterns and dynamics of Mediterranean-type ecosystems.

The biogeochemistry of chemical elements depends on their biological control, chemical bonding properties and source of the elements. Nitrogen, S and P form organic molecules with covalent bonds and are irreplaceable and essential elements for plant functioning and growth. However, they are usually limiting in many ecosystems and their uptake is associated with high costs for the plant (Elser et al. 2007; Ågren 2008). In soil, despite their different sources, biological mineralization processes driven by homeostatic organisms control transformations of N, P and S. Therefore, there are significant biochemical reasons to expect restricted variability of these elements (Knecht & Goransson 2004). In contrast, geochemical reactions control the cycle of mineral nutrients such as Ca, K or Mn. These non-limiting elements are usually absorbed in concentrations exceeding the physiological need, and their uptake is poorly regulated. This can lead to highly variable concentrations of these elements in plant tissues, which can reflect soil concentration patterns (Knecht & Goransson 2004). Tree species have a characteristic mineral element composition in leaves, stems and roots. This is a multivariate phenotypic trait that reflects

the genotypic and environmental interactions influencing the chemical content of plants (Garten 1978). The species position in the multivariate space of elemental concentration has been defined as a species “biogeochemical niche” and might reflect differences in the use of nutrients when assessed under similar environmental conditions (Peñuelas et al. 2008).

We aimed to investigate tree–soil interactions in Mediterranean forests by comparing two co-existing native oak species, the evergreen cork oak (*Quercus suber*) and the winter-deciduous *Q. canariensis*. Trees with long-lived leaves (i.e. evergreens) tend to occupy nutrient-poor environments and are characterized by their conservative use of nutrients compared to deciduous trees with short-lived leaves (Aerts 1995). Therefore, biogeochemical interactions of trees having contrasting ecological strategies with the soil are expected to be different. We studied five different components of the ecosystem: living leaves, leaf fall, litter, superficial soil and sub-superficial soil, at three different forest sites. Specifically, we pursued four main objectives. First, we aimed to analyse the variability and correlational patterns of chemical elements within and between each ecosystem component. We expected to find less variability for elements that are organically bound and subject to tighter biological control (e.g. N and S) and a more variable stoichiometry for non-limiting mineral elements (e.g. Cu and Zn). Second, we investigated the multivariate patterns of nutrient covariation in the ecosystem. We hypothesized that the transfer of nutrients across leaves, leaf fall, litter and soil would be reflected in a common structure of nutrient covariation in the ecosystem. Third, we aimed to ascertain whether the two co-occurring oak species, *Quercus canariensis* and *Q. suber*, occupy different biogeochemical niches within the ecosystem. We expected strong differences in their positions in the multivariate space defined by their tissue and soil nutrient composition, given their differences in leaf habit. Finally, we evaluated the potential existence of a causal chain that links the ecosystem compartments in a tree–soil feedback cycle, and compared alternative non-feedback hypotheses, using a path analysis. We hypothesized that both oak species distinctly modify soil properties through their contrasting chemical traits and that such tree-generated soil conditions might ultimately affect oak species distribution.

## Methods

### Study area

The study area was located in the Aljibe Mountains of southern Spain. Acidic and nutrient-poor soils (Palexeralfs) exist on an Oligo-Miocene sandstone bedrock that is interspersed with layers of marl sediments, yielding soils

rich in clay (Haploxererts) (nomenclature according to Soil Survey Staff 2010). The area has a sub-humid Mediterranean climate, the annual mean temperature is 16.5 °C and the annual rainfall ranges from 701 to 1331 mm (Anonymous 2005). The dominant vegetation is a mixed forest of evergreen cork oak (*Quercus suber* L.) and winter-deciduous Algerian oak (*Q. canariensis* Willd.). The arborescent shrubs *Phillyrea latifolia* L. and *Pistacia lentiscus* L. are abundant in the understorey (Ojeda et al. 2000). The area has been protected since 1989 as Los Alcornocales (cork oak forests) Natural Park. Three structurally different forest sites (30 km apart) were selected within the study area. These sites were San Carlos del Tiradero, Buenas Noches and La Saucedá. Appendix S1 presents the characteristics of the climate, soil and vegetation of these study sites (see details in Pérez-Ramos et al. 2008; Quilchano et al. 2008). Plant species nomenclature follows Valdés et al. (1987).

### Sampling design

At each site, ten individuals of *Q. suber* and ten individuals of *Q. canariensis* were selected within a 1-ha mixed forest stand, with the exception of the Buenas Noches site, where the forest stand was monospecific, and thereby only ten individuals of *Q. suber* could be selected. All trees (a total of 30 *Q. suber* and 20 *Q. canariensis*) were estimated to be more than 50 years old (Rodríguez-Sánchez, pers. comm.).

The five ecosystem components – leaves, leaf fall, litter, superficial soil (0–25 cm depth) and sub-superficial soil (25–50 cm depth) – were sampled in November 2006. Thirty fully expanded leaves were sampled at each of the four cardinal points for each tree. Leaf fall, litter and soil were sampled beneath the canopy of each selected oak at approximately 2 m from the trunk. Tree species footprint on soil is expected to be more intense within the vertical projection of the canopy, where in general most leaf fall is deposited (Boettcher & Kalisz 1990; Finzi et al. 1998). The selected trees in the mixed forest stands had their nearest hetero-specific neighbour within approximately 4 to 10 m. Accumulated annual leaf fall was collected in four traps (50-cm diameter) located under each tree 1 m above the ground level. The trap content was removed and the leaf fraction separated and dried. Litter was harvested within two 30-cm × 30-cm quadrats placed beneath each of the 50 selected trees. Soil cores were extracted with a cylindrical auger. Four samples of superficial soil (0–25 cm) and four samples of sub-superficial soil (25–50 cm) were taken under each oak tree and pooled into single representative samples.

### Laboratory analyses

All samples of plant material were dried at 70 °C for 48 h and then ground for chemical analyses. Soil samples were air-

dried and sieved (mesh width: 2 mm). pH was determined in a 1:2.5 soil:CaCl 0.01 M solution. Soil carbon was estimated using a total organic carbon analyser (TOC-Vesh). We determined the available soil P using the Bray-Kurtz method; soil NH<sub>4</sub><sup>+</sup> by extraction with KCl (2 M) and steam distillation; CEC with neutral 1 M ammonium acetate; available soil Ca, Mg and K by extraction with neutral 1 M ammonium acetate and atomic absorption spectroscopy. Available micronutrients (Mn, Cu and Zn) were extracted using a neutral 0.05 M EDTA solution and were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES). The total concentration of several nutrients (Ca, K, Mg, P, S, Mn, Cu and Zn) in plant tissues and soils was determined using acid digestion followed by ICP-OES analysis. Plant and soil N were determined by Kjeldahl digestion and subsequent distillation–titration in a Bran-Luebbe autoanalyser. Concentrations of the elements are provided on a dry weight basis. See detailed methods in Jones & Case (1990) and Sparks (1996).

### Statistical analysis

Three sets of analyses were used to address the previously defined objectives. First, we used the coefficient of variation (CV = standard deviation/mean, expressed as a percentage) and bivariate Spearman's correlation to examine the variability and correlational patterns of individual chemical elements in the five studied ecosystem components: leaves, leaf fall, litter, superficial soil and sub-superficial soil. Pooled data from the three sites were used for these variability analyses. We controlled type I error inflation by using the false discovery rate (FDR) procedure recommended by García (2003). Second, we used a multiple co-inertia analysis (MCoA) to investigate the patterns of multivariate covariation of chemical elements both within and between the five components of the ecosystem. This analysis provided a less detailed but more holistic vision of the ecosystem patterns. The results of this analysis were further used to assess differences in the biogeochemical niche of the two co-existing oak species. MCoA is a multi-table technique that enables the simultaneous ordination of several multivariable data sets to explore general patterns in ecosystems; however, it has rarely been used in ecology (Dray et al. 2003; Bady et al. 2004). MCoA maximizes the variance within each individual table and the correlation between the scores of each individual table (individual ordination) and synthetic scores (ordination of the juxtaposed tables) to provide a reference structure. MCoA thus optimizes the covariance between several individual ordinations and a reference ordination that can be viewed as the common structure shared by the individual ordinations. Mathematical details are provided in Chessel & Hanafi (1996).

Multiple co-inertia analysis was performed on the five data sets containing the chemical element concentrations of leaves, leaf fall, litter, superficial, and sub-superficial soil of the two mixed forest stands (the mono-specific forest was excluded from this analysis). Normality of the data was checked and log-transformations were applied when necessary. A preliminary analysis of the five data sets showed that the forest site had a significant effect on most variables, with San Carlos del Tiradero always showing higher levels of fertility, thus concealing differences between the two oak species. To remove differences between sites and obtain comparable values of nutrient concentrations in plant and soil material from the two forest sites, we performed a linear analysis of each dependent variable (i.e. the concentration of each chemical element in the ecosystem components), using the forest site as an independent variable. We used the residuals resulting from the removal of forest site differences, which represented nutrient concentrations relative to a common basal fertility level, to perform the co-inertia analysis. MCoA statistics were computed with the *ade4* library implemented from the R freeware (<http://www.r-project.org/>). The significance of the extracted axes was evaluated using the broken-stick method (King & Jackson 1999). Differences between species on the extracted ordination axes were tested using analysis of variance (one-way ANOVA).

Third, we used a path analysis with d-sep tests to evaluate the feasibility of the proposed causal relationships among the chemical properties of the ecosystem components (Shipley 2000a). We hypothesized a tree–soil feedback model based on previous studies and on the empirical patterns derived from the multiple co-inertia analysis. We also tested several alternative non-feedback models (see below). Causal relationships between variables were combined to form directed graphs (the path models). These directed graphs imply a series of independence relations between pairs of variables, either directly or after conditioning on other variables. The relationships among variables are then translated into a structure of variances and covariances that can be tested against the observed data. A d-separation test of independence among variables can be used with small sample sizes because it does not rely on asymptotic methods and, since it does not iteratively parameterize any path coefficients, it also avoids problems of convergence. The overall fit of the model was evaluated using the  $\chi^2$  statistic. A model fits the data significantly when the causal assumptions cannot be falsified ( $P \geq 0.05$ ). Otherwise, the model is rejected (when  $P < 0.05$ ) as a feasible explanation of the overall observed relationships between the explored variables. The validation of a causal model does not imply that the hypothesis is true, but that it is plausible, given the

empirical data (Shipley 2000b, 2009 for a concise overview on d-separation and path analysis).

## Results

### Variability of chemical elements in leaves, leaf fall and litter

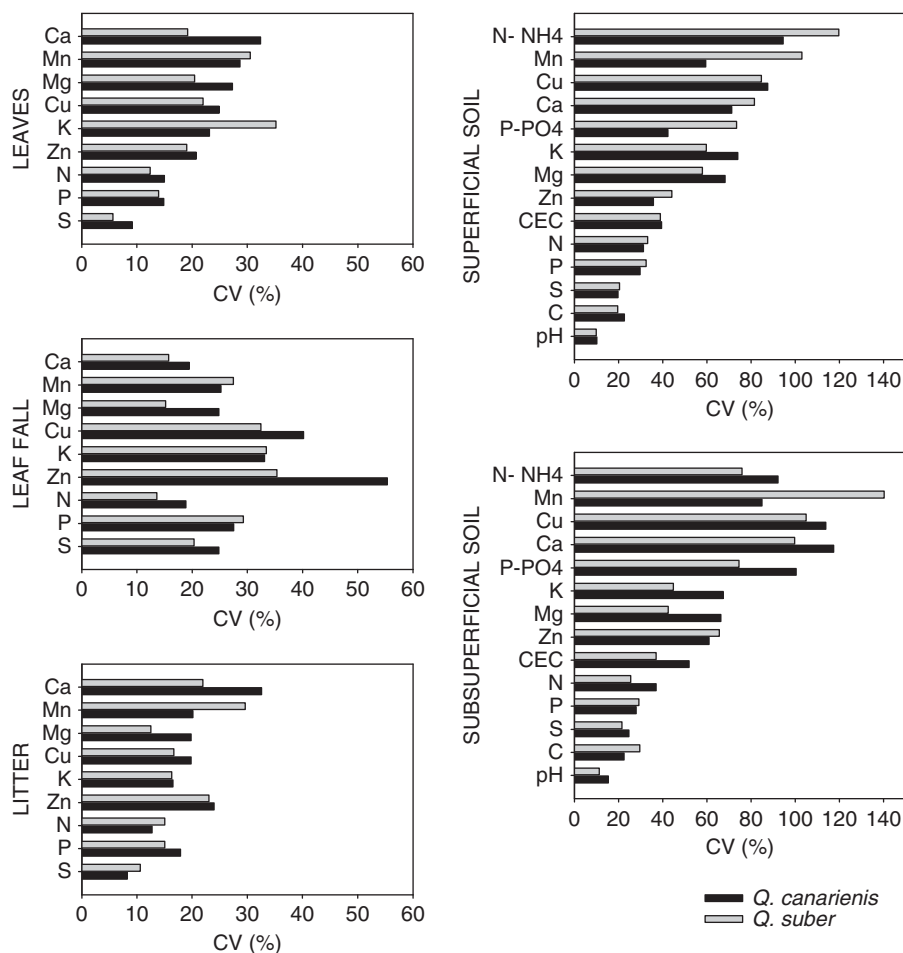
A similar ranking of element concentrations was found for all plant materials, independent of the site and oak species (Appendix S2). Elements were ranked as:  $N > K > Ca > Mg > S > P > Mn > Zn > Cu$ . The main change was observed for K, since its relative abundance decreased from leaves to leaf fall and litter. In general, the nutrient concentration in leaves, leaf fall and litter tended to be higher for *Q. canariensis* than for *Q. suber*. In leaves, S, P and N were the least variable elements (Fig. 1). The variability of S, P and N in leaves and litter was similar for the two oak species, while *Q. canariensis* tended to have higher S and N variability in leaf fall. Other elements (such as Ca, Mn and K in leaves, and Cu and Zn in leaf fall) showed higher variability (30–40%) and larger differences between the two oak species (Appendix S2).

Most elements were significantly and positively related within each of the three components: 22, 26 and 22 of the 36 possible correlations ( $\approx 65\%$ ) were significant in leaves, leaf fall and litter, respectively (Appendix S3). Organically bound elements (N, P and S) and macronutrients, such as Ca and Mg, had positive correlations in all of the ecosystem components.

### Variability of soil chemical properties

Soil beneath *Q. canariensis* tended to be less acidic than soil beneath *Q. suber* (pH 4.5 versus 4.1) and tended to have a higher content of N (0.20 versus 0.16%). Cation exchange capacity (CEC), nutrient availability and total element content were generally higher in soils under the deciduous *Q. canariensis*. The main exception was found for the available and total K in soil, with higher values beneath *Q. suber* (Appendix S4).

Among the soil variables, pH had the lowest CV for both superficial ( $\approx 10\%$ ) and sub-superficial soil ( $\approx 14\%$ ), due to its logarithmic scale, followed by the organically bound elements C, S, P and N ( $< 35\%$ ) (Fig. 1). Higher variability was recorded for the available and extractable N ( $N-NH_4$ ) and P ( $P-PO_4$ ) than for the total element concentration. Some total elements were more variable in the sub-superficial soil (Zn, Ca, Cu and Mn), while others had a similar CV in the superficial and sub-superficial soil (Mg and K). More significant correlations among chemical elements were found in the soil than in the plant tissues: 100% of all possible correlations among total nutrient content in superficial soil and



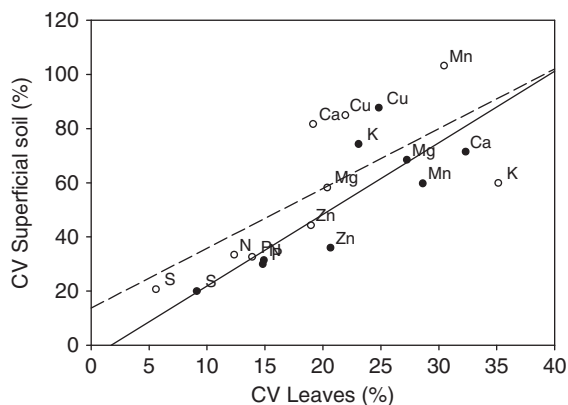
**Fig. 1.** Coefficient of variation (CV) for the total chemical elements in the leaves, leaf fall, litter, superficial soil (0–25 cm) and sub-superficial soil (25–50 cm) of the two studied oak species. In the soil graphs, CEC = cation exchange capacity, N-NH<sub>4</sub> = available N, P-PO<sub>4</sub> = available P.

86% in the sub-superficial soil were positive and significant (Appendix S3).

**Links among ecosystem components**

On average, variability in the superficial soil (CV = 55%) and the sub-superficial soil (75%) was higher than in the plant material (20%). When comparing the variability of elements across the five ecosystem components, we detected a high degree of correlation among them ( $r_s > 0.6$ ), resulting in a consistent pattern of nutrient variability among ecosystem components (Fig. 2). The organically bound nutrients (N, P, S) had the lowest CVs, whereas other elements, such as Ca and Cu, had the highest variability.

Correlations between the total concentration of the same element in the different components of the ecosystem were significant in most cases (Table 1), with Ca and Mn having the strongest relationship. Correlations between the superficial and sub-superficial soil components tended to be higher than correlations among the tree-



**Fig. 2.** Correlation between the CV values of the nutrient concentrations in leaves and superficial soil (total values) for *Q. canariensis* (filled dots, solid regression line,  $r^2 = 0.683$ ) and *Q. suber* (hollow dots, dashed regression line,  $r^2 = 0.511$ ).

derived components (leaves, leaf fall and litter). The elements K, Cu and Zn showed negative relationships between the plant and soil components.

**Table 1.** Correlations of the total concentration of chemical elements between the five ecosystem components. LV (Leaves), LF (Leaf fall), LI (Litter), TOP (Superficial soil), SUB (Sub-superficial soil). Significant correlations ( $< 0.05$ ) are in bold (\*\* $P < 0.001$ ; \* $P < 0.01$ ; \* $P < 0.05$ )

	LF	LI	TOP	SUB		LF	LI	TOP	SUB
N					Ca				
LV	<b>0.52***</b>	0.09	0.09	0.03		<b>0.76***</b>	<b>0.72***</b>	<b>0.43**</b>	0.18
LF		0.27	<b>0.33*</b>	<b>0.33*</b>			<b>0.81***</b>	<b>0.57***</b>	<b>0.39**</b>
LI			<b>0.32*</b>	<b>0.38**</b>				<b>0.74***</b>	<b>0.70***</b>
TOP				<b>0.77***</b>					<b>0.73***</b>
K					Mg				
LV	<b>0.35**</b>	0.17	<b>-0.34**</b>	<b>-0.32*</b>		0.17	<b>0.58***</b>	0.27	0.25
LF		<b>-0.32*</b>	<b>-0.49***</b>	<b>-0.31*</b>			0.26	<b>-0.40**</b>	<b>-0.43**</b>
LI			<b>0.40**</b>	-0.01				<b>0.42**</b>	<b>0.31*</b>
TOP				<b>0.61***</b>					<b>0.84***</b>
S					P				
LV	0.18	<b>0.45**</b>	<b>0.38**</b>	<b>0.29*</b>		<b>0.51***</b>	<b>0.31*</b>	0.22	0.25
LF		0.25	<b>0.26*</b>	-0.02			0.14	0.15	0.23
LI			<b>0.61***</b>	<b>0.29*</b>				<b>0.58***</b>	<b>0.38**</b>
TOP				<b>0.48***</b>					<b>0.71***</b>
Mn					Zn				
LV	<b>0.69***</b>	<b>0.62***</b>	<b>0.37**</b>	0.27		0.27	0.23	0.19	-0.08
LF		<b>0.85***</b>	<b>0.49**</b>	<b>0.44**</b>			<b>0.41**</b>	<b>-0.44**</b>	<b>-0.62***</b>
LI			<b>0.63***</b>	<b>0.60***</b>				0.16	0.04
TOP				<b>0.92***</b>					<b>0.82***</b>
Cu									
LV	<b>0.63***</b>	0.07	<b>-0.59***</b>	<b>-0.57***</b>					
LF		<b>0.30**</b>	<b>-0.61***</b>	<b>-0.62***</b>					
LI			-0.24	-0.16					
TOP				<b>0.78***</b>					

### Patterns of covariation in the ecosystem

All individual ordinations showed a similar trend of nutrient enrichment. In every component of the tree–soil system (leaves, leaf fall, litter, superficial soil and sub-superficial soil), all chemical variables were positively related to the first axis for which most of the elements showed high loadings (Table 2). Chemical elements covaried in the forest ecosystem components along a multivariate gradient of nutrient enrichment that was retained in the first extracted axis of the individual ordinations.

Multiple co-inertia analysis arranged the axes extracted from each ecosystem component (individual ordination) along common synthetic axes, revealing the relationships and structures of the whole data set. The first individual axis of every component was strongly related to other first axes and with the common synthetic axis extracted from the whole data set ordination (high factor loadings  $> 0.95$ ; Fig. 3, Appendix S5). This highlighted the strong match between the multivariate structures of the individual data sets and showed that the pattern of nutrient enrichment was consistent across all ecosystem components. Therefore, those trees with leaves having a higher nutrient concentration also presented a higher nutrient concentration in their leaf fall and litter and occupied richer soils.

The first axis of each component (individual ordination) and the first common synthetic axis separated the samples of the two studied oak species (Fig. 3; Appendix S6). The average scores of *Q. canariensis* were significantly higher than those of *Q. suber* in all main axes. This result demonstrates that all leaves, leaf fall, litter and soil of *Q. canariensis* tended to have higher nutrient concentrations.

### Tree–soil positive feedback model

Multiple co-inertia analysis showed that all material shared a common multivariate pattern of nutrient enrichment based on which chemical element concentrations in the plant and in the soil were strongly related. In addition, the distribution of the two species along the multivariate axes indicated that the two oaks differed in the nutrient richness of their associated soils and plant tissues. This relationship suggests the existence of a positive feedback mechanism between trees and soil, in which trees of different oak species, via differences in their nutrient return, distinctly modify soil conditions. This, in turn, could also influence species distribution.

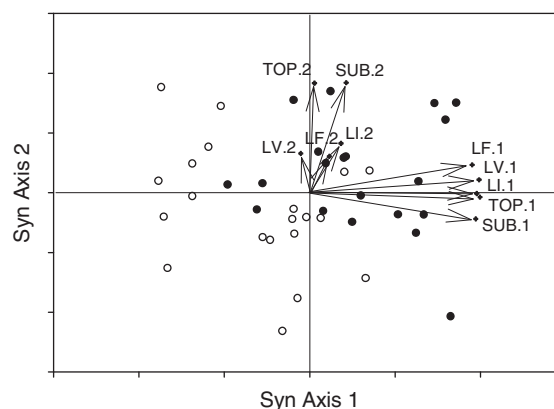
To reveal the causal relationship that linked the tree and soil components and determine whether our hypothesized feedback model would fit the empirical

**Table 2.** Loading of the variables onto the first (1.) and second (2.) individual ordination axes of each ecosystem component (names as in Table 1). Available (Avail.) concentrations of elements in soil were measured by extraction with ammonium acetate (Ca, K and Mg) or with ethylenediaminetetraacetic acid (EDTA, for S, Mn, Cu and Zn).

	LV.1	LV.2	LF.1	LF.2	LI.1	LI.2
N	0.48	-0.59	0.82	-0.10	0.62	0.54
Ca	0.89	0.29	0.79	0.40	0.81	-0.45
K	0.71	-0.13	0.30	-0.91	0.60	-0.49
Mg	0.69	0.31	0.84	0.11	0.86	-0.27
S	0.85	-0.12	0.94	-0.07	0.95	0.19
P	0.76	-0.17	0.76	-0.18	0.82	0.33
Mn	0.51	0.56	0.44	0.18	0.25	-0.36
Zn	0.49	-0.04	0.72	0.12	0.67	-0.05
Cu	0.28	-0.50	0.79	0.00	0.38	0.61
		TOP.1	TOP.2	SUB.1	SUB.2	
pH		0.67	-0.02	0.56	0.31	
C		0.70	0.43	0.60	0.60	
N		0.81	0.41	0.75	0.45	
N-NH <sub>4</sub> <sup>+</sup>		0.27	0.63	0.31	0.26	
P-PO <sub>4</sub> <sup>3-</sup>		0.17	0.83	0.21	0.63	
CEC		0.77	0.08	0.82	-0.39	
Avail. Ca		0.78	0.19	0.74	0.00	
Avail. K		0.75	0.13	0.37	-0.07	
Avail. Mg		0.53	-0.41	0.64	-0.48	
Avail. S		0.21	0.15	0.60	0.12	
Avail. Mn		0.75	0.23	0.57	0.50	
Avail. Zn		0.45	-0.05	0.47	-0.03	
Avail. Cu		0.67	-0.42	0.59	-0.05	
Ca		0.90	0.20	0.75	0.09	
K		0.70	-0.48	0.61	-0.70	
Mg		0.82	-0.37	0.70	-0.64	
S		0.72	-0.05	0.86	-0.07	
P		0.82	0.01	0.76	-0.19	
Mn		0.75	-0.01	0.63	0.55	
Zn		0.87	-0.18	0.70	-0.13	
Cu		0.89	-0.28	0.44	-0.01	

observations, we used path analysis to test several alternative models (see Fig. 4, although not all alternative models are shown). The main underlying hypotheses were: (1) oak species affect soil conditions via nutrient return, and in turn this affect species distribution and generates a positive feedback effect; (2) species modify superficial soil conditions via nutrient return but species distribution is only affected by sub-superficial soil properties, thus there are no feedback effects; and (3) soil affects species distribution, but trees have no effect on soil conditions.

For the d-sep analysis, we generated a data set ( $n = 40$ ) comprised of the MCoA first extracted axis of the individual ordinations, which was used as the variables accounting for the multivariate nutrient composition of each ecosystem component, and a dummy variable that was used to separate oak species. Leaf fall was used as a proxy for nutrient return because both leaf fall and leaf



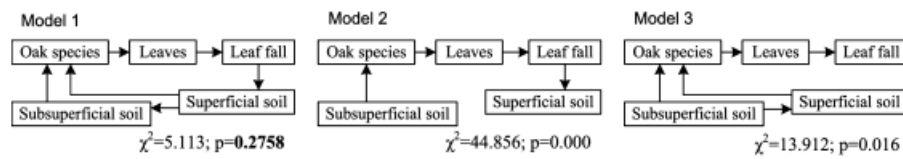
**Fig. 3.** Representation of the projected individual ordination axes (see abbreviations in Table 2) and the tree scores on the synthetic common axes of the multiple co-inertia analysis (MCoA). Filled symbols represent *Q. canariensis* trees, and hollow symbols represent *Q. suber* trees.

litter were equally related to leaves ( $r_s = 0.66$ ) and this distorted the order of the causal chain (Shipley 2000a). The analysis showed that the structure of variance and covariance of the interrelated variables from the alternative models based on the positive feedback hypothesis fit the empirical observations (i.e. Model 1;  $\chi^2 = 10.99$ ,  $P = 0.2021$ ). Models based on the other two alternative non-feedback hypotheses were rejected ( $P < 0.05$ ). These results indicate that the existence of a tree-soil positive feedback was feasible given our empirical data set.

## Discussion

### Variability of tree and soil chemical composition

We examined the variability and relationships of the chemical compositions of five components in the forest ecosystem. We determined that there is reciprocal control of their elemental composition resulting from the exchange of chemical elements. The two studied oak species, *Q. suber* and *Q. canariensis*, showed similar patterns of variability in the concentration of chemical elements in their tissues and surrounding soil. In contrast, we found an important difference between the variability of the chemical properties measured in the tree system (leaves, leaf fall and litter; CVs of approximately 20–30%) and those quantified in the soil components (CVs of 55–75%). Similarly, Ladanai et al. (2010) found no differences between variability in the chemical composition of the needles and in the soil humus of two conifer species in Sweden, but observed a large difference in the variability between the two ecosystem components (20% for needles, 60% for humus). Plants must control their internal chemical balance within certain biological ranges that normally differ from the chemical composition of the external environment. On the contrary, the spatial heterogeneity of chemical element sources and



**Fig. 4.** Examples of the alternative causal models tested using the d-sep method. Model 1 = tree–soil positive feedback effect; Model 2 = species affect the superficial soil via nutrient return, but there is no feedback; Model 3 = only soil affects species distribution, there is no feedback and no species effect on soil.

processes in the soil (e.g. mineral weathering and organic matter mineralization) as well as the “footprint” effect of the different plant species, fungal hyphae and root exudates may increase the variability of element concentrations in the soil (Ladanai et al. 2010).

Despite the differences in ranges of CVs, we found a strong coupling between the variability patterns in the different ecosystem components. Those elements with great variance of concentration in the leaves tended also to have a great variance in the other components. A similar but weaker coupled relationship between leaves and soil has been found in boreal forests (Merila & Derome 2008; Ladanai et al. 2010). These results confirmed the initial hypothesis that there is a general gradient between chemical elements with low variability (S, P and N) and elements with high variability (Ca, Mn, and K) in the forest ecosystem that depends on the nature of their biogeochemical cycles. The variability of elements bonded to organic molecules with significant physiological function or uptake cost will show a rather constant stoichiometry across ecosystem compartments reflecting the biological control of their cycles, as opposed to other non-limiting elements under geochemical control (Chapin 1980; Ågren 2008; Ladanai et al. 2010).

#### Multivariate patterns of nutrient covariation

Both the bivariate correlations and the multiple co-inertia analysis showed that most chemical elements covaried along a main enrichment gradient common to all ecosystem components. Results indicated that in all the tree components (leaves, leaf fall and litter), the abundance of organically bound elements (N, P and S) was associated with high levels of other macronutrients (Ca, Mg and K) and micronutrients (Zn, Cu and Mn). Patterns of covariation of leaf nutrients across a variety of plant species and individuals have been documented previously (Garten 1976; Alonso & Herrera 2001, 2003). Nutrients in plants are related to the intrinsic chemical properties and biochemical functionality in cell metabolism. Thus, relationships between concentrations of elements may not vary randomly, and may be a consequence of similarity in physiological function or nutrient uptake and accumulation (Garten 1976, 1978; Sterner & Elser 2009).

A common pattern of fertility was found for the superficial and sub-superficial soil. Higher concentrations and availability of mineral nutrients was related to higher soil C content and pH. Most nutrients in forest soils are bound to soil organic matter. The abundance of these nutrients can be directly related to soil C content in acidic soils (Fisher & Binkley 2000). At the same time, soil acidity influences mineral solubility, mineral weathering and the distribution of cations in the exchange complex. Therefore, soil acidity controls inorganic nutrient availability.

The multiple co-inertia analysis showed that the multivariate pattern of nutrient covariation remained consistent across the five ecosystem components considered in this study. Nutrient cycling in ecosystems involves the storage and transfer of elements. The significant correlation found among most chemical element between different components of the ecosystem, particularly for structural and organically bound elements (Ca, Mn, N, P), suggests that nutrient concentration and stoichiometry is transferred across ecosystem components through the organic matter cycle (Perakis et al. 2006; Cornwell et al. 2008; Merila & Derome 2008). Other elements (i.e. K, Cu and Zn), in contrast, showed a lack of coupling between vegetation and soil chemical compositions (see also Wood et al. 2006; Ladanai et al. 2010). Several mechanisms can result in the lack of a relationship between element concentrations in leaves (plant system) and soil: protective mechanisms in the root cell environment might impede excess uptake of potentially toxic elements (Hall 2002; Domínguez et al. 2009), and plants can experience antagonistic interactions of other elements, inhibiting uptake (Madejón et al. 2006).

A remarkable result obtained from the MCoA was the significant separation of the two co-existing oaks species in the multivariate space defined by the chemical composition of each (individual ordination) and all (common synthetic ordination) ecosystem components. This result suggested a separation in their biogeochemical niches, which would be the combined result of genotypic interactions (absorption and accumulation capacity, and functional stoichiometry) and environmental interactions (Garten 1978; Peñuelas et al. 2008). The deciduous *Q. canariensis* had higher multivariate scores than the evergreen *Q. suber*, reflecting its higher nutrient concentrations



in leaves, litter and soil. These results support our hypothesis and are in accordance with the previously reported differences between deciduous and evergreen species of boreal and temperate forests in terms of resource acquisition and cycling strategies (Hobbie 1992; Aerts 1995).

### Tree–soil positive feedback effects in Mediterranean forests

In this study, we have demonstrated the potential feasibility of a tree–soil positive feedback effect by implementing a causal modelling approach and testing several alternative competing models. In this context, feedback effects can be modelled as cyclic causal relationships that must be understood as a fixed image of a dynamic system occurring in time (Shipley 2000a). Nevertheless, causal modelling only tests whether the hypothesized model fits the empirical data, thus it is feasible and could eventually occur, but it does not prove that the model fitting the data is the correct one and that those relationship actually occur.

Feedback effects must be interpreted cautiously because it is often difficult to discern the origin of the cyclic causal relationship. Our analyses showed that differences between the two species were highly significant in the plant components as well as in the surface soil, whereas differences were attenuated in the sub-superficial soil. This suggests that tree species modify soil conditions through a top-down cascade. In accordance, our results showed that *Q. canariensis* trees produced leaves with a higher nutrient content than the co-occurring *Q. suber* trees. These differences in the chemical composition of leaves were transferred to the leaf fall and litter of each species, leading to a differential nutrient return that distinctly modified soil conditions underneath each oak species. Thus, *Q. canariensis* trees increased soil fertility levels through a higher nutrient input. In addition, as a consequence of the elevated base input (particularly Ca cations), they could have mitigated soil acidity and increased nutrient availability, both in the surface and the subsurface soil.

To confirm the existence of a feedback effect, the modifications created by a tree species must have an impact (positive or negative) on the performance and fitness of individuals of the same species (Ehrensfield et al. 2005; Kardol et al. 2006; Kulmatiski et al. 2008). According to our initial hypothesis, the more fertile soil conditions generated by the deciduous oak and the nutrient-poorer environments created by the evergreen cork oak could differentially benefit their own recruits and ultimately influence long-term species distribution. Previous studies in Mediterranean oak forests have shown that winter-deciduous trees and evergreen cork oaks have different regeneration niches (Maltez-Mouro et al. 2005). Winter-deciduous trees usually have a higher probability of successful recruitment in microsites closer to

conspecific adults and with a high percentage of canopy cover and litter mass, whereas evergreen oak seedling performance is enhanced in microsites with lower litter mass and canopy cover (Maltez-Mouro et al. 2009). On the other hand, the strong positive relationship found between litter mass and soil fertility (García et al. 2006) in these mixed oak forests is in accordance with our hypothesis that a leaf fall-induced increase of soil fertility might differentially influence recruitment and species distribution. Furthermore, a recent study conducted in the same study area, where seeds of both species were planted beneath individuals of the two species, concluded that *Q. canariensis* seedlings grew faster than those of *Q. suber* beneath the canopy of a con-specific adult (Pérez-Ramos et al. 2010).

Evidence that plants can create positive feedback effects through their influence on nutrient cycling has previously been reported for temperate and boreal forests (Hobbie 1992; Aerts 1995). Low nutrient content and structural traits associated with the long-lived leaves of evergreen trees produce poor-quality litter that decomposes slowly, minimizing nutrient loss and keeping a low rate of nutrient mineralization that might help such trees to maintain their position in infertile sites (Cornelissen et al. 1999). In contrast, deciduous trees have a higher nutrient concentration and specific leaf area that produces high decomposition rates, further increasing soil fertility and enhancing the competitive ability of their own seedlings (Hobbie 1992; Antúnez et al. 2001).

The ability of a species to control nutrient cycling and enhance its own fitness has important ecological implications (van Breemen & Finzi 1998; Post & Palkovacs 2009). In our study, the hypothesized model suggests that evergreen and deciduous oaks could generate a positive feedback effect that would confer stability in species interactions and maintain the structure and diversity associated with the two co-existing species (Ojeda et al. 2000; Aponte et al. 2010a). Recent studies have demonstrated that climate change affects nutrient availability and accumulation in plants (Sardans & Peñuelas 2004, 2007), which implies changes in the biogeochemical niche of the species, and affects their relationships with nutrient cycling (Peñuelas et al. 2008). These alterations in tree–soil interactions may change interspecific competitive relationships and subsequently modify the species composition of vegetation and soil biota.

### Conclusions and future directions for tree–soil feedback effect research

Chemical elements varied in concentration depending on their biogeochemical cycles, but this variability was consistent across the five ecosystem components studied.

Multiple co-inertia analysis revealed that there was a common multivariate pattern of nutrient enrichment in the ecosystem that separated the biogeochemical niches of the two oak species. Thus the oak species, through their differential nutrient return, could distinctly modify soil properties and, in turn, affect species distribution. This hypothesized feedback dynamic might be crucial for the stability of the forest ecosystem and the co-existence of the two oak species.

Theoretical models based on observational data (as conducted in this study) constitute an important step in the study of tree–soil interactions. We have found evidence supporting the initial feedback hypothesis, however, further investigations are needed to conclusively assert the existence of feedback effects in these forest dynamics. Future research in the area should include demographic studies that investigate feedback effects on seedling performance. Spatially explicit designs would allow modelling of complex situations in mixed forests where species footprints on soil properties overlap. In addition, experimental studies could contribute to determine the rate and persistence of these feedback effects. Upcoming research incorporating these recommendations may lead to a greater understanding of the role plant–soil interactions play in ecosystem functioning.

### Acknowledgements

We are grateful to Ana Pozuelos, María Navarro and Manuel del Pozo for field and laboratory assistance. We thank the Consejería de Medio Ambiente (Andalusian Government) and Marco Antonio Tena, then Director of Los Alcornocales Natural Park, for the facilities and support to carry out fieldwork. We thank Prof. Alicia Acosta and two anonymous reviewers for their critical comments for the improvement of this paper. This study was supported by a FPI-MEC grant to CA, the Spanish MEC projects Dinamed (CGL2005-5830-C03-01) and Interbos (CGL2008-4503-C03-01), and European FEDER funds. This research is part of the Globimed (<http://www.globimed.net>) network in forest ecology.

### References

- Aerts, R. 1995. The advantages of being evergreen. *Trends in Ecology and Evolution* 10: 402–407.
- Ågren, G.I. 2008. Stoichiometry and nutrition of plant growth in natural communities. *Annual Review of Ecology, Evolution and Systematics* 39: 153–170.
- Alonso, C. & Herrera, C.M. 2001. Patterns made of patterns: variation and covariation of leaf nutrient concentrations within and between populations of *Prunus mahaleb*. *New Phytologist* 150: 629–640.
- Alonso, C. & Herrera, C.M. 2003. Developmental and spatial covariation of nutrients in growing leaves of *Daphne laureola* and their relationships with herbivory. *New Phytologist* 159: 645–656.
- Anonymous. 2005. *PORN/PRUG/PDS Parque Natural Los Alcornocales*. Junta de Andalucía, Consejería de Medio Ambiente, Sevilla, ES.
- Antúñez, I., Retamosa, E.C. & Villar, R. 2001. Relative growth rate in phylogenetically related deciduous and evergreen woody species. *Oecologia* 128: 172–180.
- Aponte, C., García, L.V., Marañón, T. & Gardes, M. 2010a. Indirect host effect on ectomycorrhizal fungi: leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks. *Soil Biology and Biochemistry* 42: 788–796.
- Aponte, C., Marañón, T. & García, L.V. 2010b. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. *Biogeochemistry* 101: 77–92.
- Bady, P., Dolédec, S., Dumont, B. & Fruget, J.-F. 2004. Multiple co-inertia analysis: a tool for assessing synchrony in the temporal variability of aquatic communities. *Comptes Rendus Biologies* 327: 29–36.
- Binkley, D. & Giardina, C. 1998. Why do tree species affect soils? The warp and woof of tree–soil interactions. *Biogeochemistry* 42: 89–106.
- Boettcher, S.E. & Kalisz, P.J. 1990. Single-tree influence on soil properties in the mountains of eastern Kentucky. *Ecology* 71: 1365–1372.
- Chapin, F.S. III. 1980. The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics* 11: 233–260.
- Chessel, D. & Hanafi, M. 1996. Analyse de la co-inertie de K nuages de points. *Revue de Statistique Appliquée* 44: 35–60.
- Cornelissen, J.H.C., Pérez-Harguindeguy, N., Díaz, S., Grime, J.P., Marzano, B., Cabido, M., Vendramini, F. & Cerabolini, B. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytologist* 143: 191–200.
- Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Queded, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Bodegom, P.v., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V. & Westoby, M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11: 1065–1071.
- Domínguez, M.T., Madrid, F., Marañón, T. & Murillo, J.M. 2009. Cadmium availability in soil and retention in oak roots: potential for phytostabilization. *Chemosphere* 76: 480–486.
- Dray, S., Chessel, D. & Thioulouse, J. 2003. Co-inertia analysis and the linking of ecological data tables. *Ecology* 84: 3078–3089.
- Ehrenfeld, J.G., Ravit, B. & Elgersma, K. 2005. Feedback in the plant–soil system. *Annual Review of Environment and Resources* 30: 75–115.

- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. & Smith, J.E. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10: 1135–1142.
- Finzi, A.C., Canham, C.D. & Van Breemen, N. 1998. Canopy tree–soil interactions within temperate forests: species effects on pH and cations. *Ecological Applications* 8: 447–454.
- Fisher, R. & Binkley, D. 2000. *Ecology and management of forest soils*. 3rd ed. John Wiley & Sons, New York, NY, US.
- Gallardo, A. & Merino, J. 1993. Leaf decomposition in two mediterranean ecosystems of Southwest Spain: influence of substrate quality. *Ecology* 74: 152–161.
- García, L.V. 2003. Controlling the false discovery rate in ecological research. *Trends in Ecology and Evolution* 18: 553–554.
- García, L.V., Maltez-Mouro, S., Pérez-Ramos, I.M., Freitas, H. & Marañón, T. 2006. Counteracting gradients of light and soil nutrients in the understorey of Mediterranean oak forest. *Web Ecology* 6: 67–74.
- Garten, C.T. 1976. Correlations between concentrations of elements in plants. *Nature* 261: 686–688.
- Garten, C.T. Jr. 1978. Multivariate perspectives on the ecology of plant mineral element composition. *The American Naturalist* 112: 533–544.
- Goldberg, D.E. 1982. The distribution of evergreen and deciduous trees relative to soil type: an example from the Sierra Madre, Mexico, and a general model. *Ecology* 63: 942–951.
- Gómez-Aparicio, L. & Canham, C.D. 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. *Ecological Monographs* 78: 69–86.
- Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* 53: 1–11.
- Hobbie, S.E. 1992. Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution* 7: 336–339.
- Jones, J.B. & Case, V.W. 1990. Sampling, handling and analyzing plant tissues samples. In: Westerman, R. (ed.) *Soil testing and plant analysis*. pp. 389–427. Soil Science Society of America, Madison, WI, USA.
- Kardol, P., Bezemer, T.M. & Putten, W.H. 2006. Temporal variation in plant–soil feedback controls succession. *Ecology Letters* 9: 1080–1088.
- King, J.R. & Jackson, D.A. 1999. Variable selection in large environmental data sets using principal components analysis. *Environmetrics* 10: 67–77.
- Knecht, M.F. & Goransson, A. 2004. Terrestrial plants require nutrients in similar proportions. *Tree Physiology* 24: 447–460.
- Knops, J.M.H., Bradley, K.L. & Wedin, D.A. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters* 5: 454–466.
- Kobe, R.K., Pacala, S.W., Silander, J.A. & Canham, C.D. 1995. Juvenile tree survivorship as a component of shade tolerance. *Ecological Applications* 5: 517–532.
- Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M. 2008. Plant–soil feedbacks: a meta-analytical review. *Ecology Letters* 11: 980–992.
- Ladanai, S., Ågren, G. & Olsson, B. 2010. Relationships between tree and soil properties in *Picea abies* and *Pinus sylvestris* forests in Sweden. *Ecosystems* 13: 302–316.
- Lovett, G.M., Weathers, K.C., Arthur, M.A. & Schultz, J.C. 2004. Nitrogen cycling in a northern hardwood forest: do species matter? *Biogeochemistry* 67: 289–308.
- Madejón, P., Marañón, T. & Murillo, J.M. 2006. Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. *Science of the Total Environment* 355: 187–203.
- Maltez-Mouro, S., García, L., Marañón, T. & Freitas, H. 2005. The combined role of topography and overstorey tree composition in promoting edaphic and floristic variation in a Mediterranean forest. *Ecological Research* 20: 668–677.
- Maltez-Mouro, S., García, L. & Freitas, H. 2009. Influence of forest structure and environmental variables on recruit survival and performance of two Mediterranean tree species (*Quercus faginea* L. and *Q. suber* Lam.). *European Journal of Forest Research* 128: 27–36.
- Merila, P. & Derome, J. 2008. Relationships between needle nutrient composition in Scots pine and Norway spruce stands and the respective concentrations in the organic layer and in percolation water. *Boreal Environment Research* 13: 35–47.
- Mitchell, R.J., Campbell, C.D., Chapman, S.J., Osler, G.H.R., Vanbergen, A.J., Ross, L.C., Cameron, C.M. & Cole, L. 2007. The cascading effects of birch on heather moorland: a test for the top-down control of an ecosystem engineer. *Journal of Ecology* 93: 540–554.
- Ojeda, F., Marañón, T. & Arroyo, J. 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): a comprehensive account. *Biodiversity and Conservation* 9: 1323–1343.
- Peñuelas, J., Sardans, J., Ogaya, R. & Estiarte, M. 2008. Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. *Polish Journal of Ecology* 56: 613–622.
- Perakis, S., Maguire, D., Bullen, T., Cromack, K., Waring, R. & Boyle, J. 2006. Coupled nitrogen and calcium cycles in forests of the Oregon Coast Range. *Ecosystems* 9: 63–74.
- Pérez-Ramos, I.M., Zavala, M.A., Marañón, T., Díaz-Villa, M.D. & Valladares, F. 2008. Dynamics of understorey herbaceous plant diversity following shrub clearing of cork oak forests: a five-year study. *Forest Ecology and Management* 255: 3242–3253.
- Pérez-Ramos, I.M., Gómez-Aparicio, L., Villar, R., García, L.V. & Marañón, T. 2010. Seedling growth and morphology of three oak species along field resource gradients and seed mass variation: a seedling age-dependent response. *Journal of Vegetation Science* 21: 419–437.
- Post, D.M. & Palkovacs, E.P. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play.

- Philosophical Transactions of the Royal Society – Biological Sciences* 364: 1629–1640.
- Pugnaire, F.I. & Chapin, F.S. 1993. Controls over nutrient resorption from leaves of evergreen Mediterranean species. *Ecology* 74: 124–129.
- Quilchano, C., Marañón, T., Pérez-Ramos, I., Noejovich, L., Valladares, F. & Zavala, M. 2008. Patterns and ecological consequences of abiotic heterogeneity in managed cork oak forests of Southern Spain. *Ecological Research* 23: 127–139.
- Sardans, J. & Peñuelas, J. 2004. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. *Plant and Soil* 267: 367–377.
- Sardans, J. & Peñuelas, J. 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. *Functional Ecology* 21: 191–201.
- Shipley, B. 2000a. *Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference*. Cambridge University Press, Cambridge, UK.
- Shipley, B. 2000b. A New Inferential Test for Path Models Based on Directed Acyclic Graphs. *Structural Equation Modeling* 7: 206–218.
- Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. *Ecology* 90: 363–368.
- Soil Survey Staff. 2010. *Keys to soil taxonomy*. 11th ed. USDA-Natural Resources Conservation Service, Washington, DC, US.
- Sparks, D.L. 1996. *Methods of soil analysis. Part 3. Chemical methods*. Soil Science Society of America and American Society of Agronomy, Madison, WI, US.
- Sterner, R.W. & Elser, J.J. 2009. Ecological stoichiometry. In: Levin, S.A., Carpenter, S.R., Godfray, C.J., Kinzig, A.P., Loreau, M., Losos, J.B., Walker, B. & Wilcove, D.S. (eds.) *The Princeton guide to ecology*. pp. 376–385. Princeton University Press, Princeton, NJ, US.
- Talbot, J.M. & Finzi, A.C. 2008. Differential effects of sugar maple, red oak, and hemlock tannins on carbon and nitrogen cycling in temperate forest soils. *Oecologia* 155: 583–592.
- Valdés, B., Talavera, S. & Fernández-Galiano, E. 1987. *Flora vascular de Andalucía Occidental*. 2 vols. Ketres, Barcelona, ES.
- van Breemen, N. & Finzi, A.C. 1998. Plant–soil interactions: ecological aspects and evolutionary implications. *Biogeochemistry* 42: 1–19.
- Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. 2004. Ecological linkages between aboveground and belowground biota. *Science* 304: 1629–1633.
- Weand, M.P., Arthur, M.A., Lovett, G.M., Sikora, F. & Weathers, K.C. 2010. The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. *Biogeochemistry* 97: 159–181.
- Wood, T., Lawrence, D. & Clark, D. 2006. Determinants of leaf litter nutrient cycling in a tropical rain forest: soil fertility versus topography. *Ecosystems* 9: 700–710.

## Supporting information

**Appendix S1.** Characteristics of the three studied forest sites.

**Appendix S2.** Description of the chemical composition of the leaves, leaf fall and litter. Median and coefficient of variation (%).

**Appendix S3.** Correlation of chemical elements within the five ecosystem components considered in this study. Significant correlations ( $P < 0.05$ ) are in bold (\*\*\*)  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ).

**Appendix S4.** Description of the chemical properties of the superficial and sub-superficial soil. Median and coefficient of variation (%).

**Appendix S5.** Loading of the first (.1) and second (.2) individual ordination axes (LV = leaves, LF = leaf fall, LI = litter, TOP = superficial soil and SUB = sub-superficial soil) onto the common synthetic axes (SYN.1 and SYN.2).

**Appendix S6.** ANOVA for the oak species effect comparing *Q. suber* and *Q. canariensis* scores on the multivariate axes of the five ecosystem components (LV = leaves, LF = leaf fall, LI = litter, TOP = superficial soil and SUB = sub-superficial soil) and the synthetic common axis (SYN). Only data from mixed forest sites (La Saucedá and Tiradero) were included ( $n = 40$ ).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.