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Soil-nutrient availability under a global-change scenario in a Mediterranean mountain ecosystem

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Abstract

Changes in rainfall availability will alter soil-nutrient availability under a climate-change scenario. However, studies have usually analyzed the effect of either drier or wetter soil conditions, despite the fact that both possibilities will coexist in many climatic regions of the world. Furthermore, its effect may vary across the different habitats of the ecosystem. We experimentally investigated the effect of three contrasting climatic scenarios on different carbon (C), nitrogen (N), and phosphorus (P) fractions in soil and microbial compartments among three characteristic habitats in a Mediterranean-type ecosystem: forest, shrubland, and open areas. The climatic scenarios were dry summers, according to the 30% summer rainfall reduction projected in the Mediterranean; wet summer, simulating summer storms to reach the maximum historical records in the study area; and current climatic conditions (control). Sampling was replicated during two seasons (spring and summer) and 2 years. The climatic scenario did not affect the nutrient content in the litter layer. However, soil and microbial nutrients varied among seasons, habitats, and climatic scenarios. Soil-nutrient fractions increased with lower soil-moisture conditions (dry scenario and summer), whereas microbial nutrients increased under the wet summer scenario and spring. This pattern was consistent both studied years, although it was modulated by habitat, differences being lower with denser plant cover. Holm oak seedlings, used as live control of the experiment, tended to increase their N and P content (although not significantly) with water availability. Thus, the results support the idea that higher rainfall boosts microbial and plant-nutrient uptake, and hence nutrient cycling. By contrast, a rainfall reduction leads to an accumulation of nutrients in the soil, increasing the risk of nutrient loss by leaching or erosion. These results show that the projected climate change will have significant effects on nutrient cycles, and therefore will have important implications on the ecosystem functioning.

Keywords: carbon, climate change, drought, irrigation, litter, microbial, nitrogen, phosphorus

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Introduction

Soil-nutrient availability is one of the most important limiting factors affecting plant growth (Killham, 1994; Lambers *et al*., 1998). However, this availability is highly heterogeneous, showing a strong spatial and temporal variation (Gallardo & Schlesinger, 1994; Ettema & Wardle, 2002), frequently associated with seasonal and climatic conditions, habitat structure, or root and microbial activity (Díaz-Raviña *et al*., 1993; Gallardo & Schlesinger, 1995; Criquet *et al*., 2004; Monokrousos *et al*., 2004). Under a global-change scenario, where habitat as well as climatic conditions will be altered (Houghton *et al*., 2001), it is not clear how these alterations will affect the dynamics of soil nutrients and its interaction with the plant community (Jensen *et al*., 2003; Andersen *et al*., 2010).

Global-circulation models forecast a generalized reduction in precipitation at a 30–40° of latitude for the coming decades (Houghton *et al*., 2001), which together with the increase in temperature will increase drought, especially during summers. However, climate change is also augmenting variability of precipitation in many areas (Rodrigo, 2002; Beniston *et al*., 2007). Although this is less well documented, it is very likely that the greater aridity in some regions will not cancel the possibility of eventual rainy years (Beniston *et al*., 2007). These sporadic rainy years have a strong impact for regeneration (Castro *et al*., 2005; Holmgren *et al*., 2006; Mendoza *et al*., 2009). However, their possible role under a climate-change scenario has been scarcely explored, despite that they could be a key in the maintenance of the ecosystem structure (Castro *et al*., 2005; Holmgren *et al*., 2006).

Climate change is not the only global-change driver affecting ecosystems. Soil processes can be affected simultaneously by diverse human-driven factors (Sala *et al*., 2000). Interactions among these drivers frequently generate nonadditive responses that cannot be predicted based on single-factor studies (Sala *et al*., 2000). Land-use change is another major driver with impor-

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tant consequences not only for ecosystem functioning itself (Sala *et al*., 2000; Lindenmayer & Fischer, 2006) but also interacts with climate change, exacerbating or ameliorating it effects (Matesanz *et al*., 2009). This becomes especially relevant for Mediterranean ecosystems, in which the profound human alterations undergone over centuries have produced several phases of habitat degradation (Kosmas *et al*., 2002; Matesanz *et al*., 2009).

It is increasingly clear that changes in temperature or precipitation provoked by climate change will alter nutrient cycles (Nadelhoffer *et al*., 1991; Jonasson *et al*., 2006; Rinnan *et al*., 2007; Sardans & Penuelas, 2007), and therefore nutrient availability for plants (Michelsen *et al*., 1999). Differences in carbon (C), nitrogen (N), and phosphorus (P) availability have severe effects for plant communities, as these are fundamental nutrients for plant growth (Killham, 1994; Lambers *et al*., 1998), and because P has strong implications in the water-use efficiency (Graciano *et al*., 2005), modulating plant vulnerability to drought stress. These changes induced by the different climatic conditions, together with the high spatial heterogeneity of soil nutrients and processes associated with changes in habitat quality (Gallardo & Schlesinger, 1995; Criquet *et al*., 2004; Monokrousos *et al*., 2004) would result in a complex situation affecting soil microbial activity (Jensen *et al*., 2003; Cookson *et al*., 2007), and therefore nutrient availability for plants. However, plant-nutrient uptake also depends on soil-water content (Kozlowski & Pallardy, 2002), so it is not clear the way in which differences in soil-nutrient availability will affect plant nutritional status.

The consequences of alterations in rainfall upon soil-nutrient availability due to climate change has been widely addressed for some ecosystem (Illeris *et al*., 2003; Jensen *et al*., 2003; Sardans & Penuelas, 2007; Allison & Treseder 2008; Johnson *et al*., 2008; Andersen *et al*., 2010;), but usually within the same habitat. Only in a few cases has habitat heterogeneity or land use been considered (Cookson *et al*., 2007; Casals *et al*., 2009). However, to our knowledge, there are no field studies that analyze simultaneously the effect of habitat type and climatic change on soil-nutrient availability. This is a key point for understanding nutrient cycling and plant–soil–microbial interactions in a heterogeneous environment at the community level. Studies integrating different climatic scenarios and habitat types are necessary to properly assess the effects of climate change on plant communities for the coming decades.

In this study, we performed a field experiment to test the effect on soil C, N, and P of three contrasting climatic scenarios differing in water availability and its consequences for plant–soil–microbial interaction. The scenarios were (1) current conditions (no manipulation of rainfall availability), (2) more severe summer drought according to a widely accepted IPCC scenario for the area, and (3) heavier summer rainfall simulating eventual rainy years (following maximum average records for the study area). In addition, we performed the study in the main successional habitats in the area: forest, mid-successional shrubland, and open habitat. The experiment was repeated in two consecutive summers, and samplings were performed in spring and summer in order to explore temporal variability and the possibility for lasting consequences of rainfall manipulation. Four specific questions were posed: (1) What is the effect of different climatic scenarios on the soil and microbial C, N, and P fractions? (2) Is the effect of climatic scenario interacting with habitat type? (3) Are the effects consistent through time, both at seasonal and inter-annual level? and (4) What might be the consequences for nutrient cycling?

Materials and methods

Study site

The study was conducted in La Cortijuela, a mountain area at 1650 m asl within the limits of Sierra Nevada National Park (37°05′N, 3°28′W, Granada, SE Spain). The area has a continental Mediterranean climate, with cold winters and hot dry summers. Mean minimum temperature in the coldest month (January) is −1.1 °C, and mean maximum of the hottest month (July) is 29.2 °C. Rainfall is 811 mm yr^{-1}, accumulated mostly during spring and autumn (means 1990–2008). Total rainfall during the experiment was 641.5 mm in 2007 and 874.8 mm in 2008. The experiment was conducted inside a natural 12.4 ha fenced area with ungulate exclosure since 1986, covered by trees (mainly *Pinus sylvestris*, *Pinus nigra*, and *Quercus ilex*), shrubs (mainly *Crataegus monogyna*, *Berberis vulgaris*, *Salvia lavandulifolia*, or *Cytisus scoparius*), and open areas without woody cover (bare soil or with a sparse herbaceous cover). The bedrock is calcareous, with regosols and cambisols as predominant soil types (Delgado *et al*., 1989). Across-habitat soil texture at 0–20 cm depth is 32% sand, 48% silt, and 20% clay, with pH from 6.8 to 8.5 (mean 7.9 ± 0.2; values from Laboratorio Agroalimentario de Atarfe, Junta de Andalucía, Granada; unpublished results).

Experimental design

We performed a fully factorial field experiment crossing habitat type and climatic scenario, each with three levels. For the habitat factor, we selected the three main successional habitats in terms of plant cover in the study area: *open*, open areas with bare soil or sparse grass cover; *shrubland*, covered by the main mid-successional shrubby species in the area (mainly *C. monogyna* and *B. vulgaris*); and *forest*, covered by tree species, mainly *P. sylvestris* and *P. nigra* with scattered individuals of *Q. ilex*. The climatic scenario factor was repre-

sented by three levels differing in water availability during summer, (1) dry summer, (2) wet summer, and (3) current climatic conditions:

(1) The dry-summer scenario was based on the SRES A-2 model by Intergovernmental Panel on Climate Change [Intergovernmental Panel on Climate Change (IPCC), 2001], where a reduction in summer rainfall of 30% was predicted for Mediterranean areas. For this treatment, we built rain-exclusion shelters (Yahdjian & Sala, 2002) formed by a 2×2 m metal frame supporting V-shaped clear methacrylate bands without UV filter (Barlocast®; Faberplast S.L., Madrid, Spain), covering 35% of the surface, and intercepting the same percentage of natural water supply by rain. A 20 cm deep ditch was excavated along the entire shelter to intercept runoff water. Rainout shelters were placed from April to September, simulating drier and longer summers.

(2) The wet-summer scenario was simulated by placing 2×2 m squares on the soil with a water addition system composed of four sprinklers at the corners. Each week, from mid-June to end September (years 2007 and 2008), we added 12 L m^{-2} of water, simulating a summer storm. If a natural storm occurred 1 week, the irrigation pulse was not added. Thus, the total water added during the summer was 180 mm, the equivalent to the mean summer rainfall of the five milder summers of the 1902–2006 series in the study area (Appendix S1).

(3) Current climatic conditions during experiment development. We placed 2×2 m squares without water addition or exclusion, acting as a control for the experiment.

These three climatic levels will be referred to hereafter as dry, wet, and control scenarios, respectively. Eight replicated plots of each climatic scenario were placed in each of the three habitats, for a total amount of 72 study plots (eight replicates × three climatic scenarios × three habitats). Soil-water content was monitored monthly from May to September in all the plots by the time domain reflectometry method (TDR-100, Spectrum Technologies Inc., Plainfield, IL, USA). Each plot was sampled by two perpendicular transects recording the volumetric water content every 0.5 m.

Soil sampling

Soil samples were taken three times during the experiment performance, coinciding with the moments of maximum water stress in soil (end of summer, August 2007 and 2008), and the maximum soil biological activity (mid-spring, May 2008). Soil cores were extracted using a gouge auger (2.5 cm diameter) at two depths, 0–8 and 8–16 cm. Previous studies determined that this was the maximum depth that could be reached in all the habitats (Gomez-Aparicio *et al*., 2008), and we split the soil profile in half. From each study plot, we took at least four cores, which were homogenized within the same depth. Samples were immediately sieved at 2 mm removing stones, roots, and visible plant remains, and stored at 4 °C for extraction. For gravimetric determination of the water content by the difference between fresh and dry weight, a 30 g subsample was oven-dried at 105 °C for 48 h and stored for further analyses. In the same sampling dates, the litter contained in a square 10×10 cm was collected in all plots. Litter samples were oven-dried at 60 °C for 72 h, weighted, and ground for analysis.

Similar soil sampling was also performed in the previous summer (August 2006) and spring (May 2007) until the start of the experiment in order to quantify any possible variation among plots where the climatic scenarios were later simulated. All parameters measured differed among habitats, as expected, but plots where climatic scenarios were later simulated did not differ within each habitat (Appendix S2). We may thus consider that differences detected in the following years were due to the treatment.

Plant-nutrient uptake

Plant–soil–microbial interactions determine nutrient availability and immobilization on a continuous time scale, making it difficult to interpret the interactions of soil and microbes with plant-nutrient acquisition at a particular sampling date (Jonasson *et al*., 2006). To determine the treatment effect on plant nutrient uptake, we sowed five Holm oak (*Q. ilex* L.) acorns in each of the 72 experimental plots. *Q. ilex* is the most abundant tree species in these mountains, and constitutes the natural potential vegetation in the area (Rivas-Godoy & Rivas-Martínez, 1971). Acorns were sowed inside 25×25 cm quadrats within each plot in December 2006, emergence occurred in May–June 2007, and the plants were grown during two complete growing seasons until September 2008, thus coinciding with the time of rainfall manipulation. At the end of the experiment, all surviving seedlings were harvested (extracting roots completely, with the help of a pneumatic hammer), and oven-dried at 60 °C for 72 h, weighed, and ground for analysis of N and P pooling all seedlings (leaves, shoots, and roots together) growing in the same plot.

Chemical analyses

Within 24 h from soil sampling, three subsamples of 15, 15, and 7.5 g of soil were extracted for 1 h in agitation with 75 mL of 2 M KCl, 0.5 M K_2SO_4, and 0.5 M $NaHCO_3$ respectively, and filtered through Whatman GF-D filter. Another subsample was fumigated with $CHCl_3$ for 24 h in vacuum to release the nutrients in the microbial biomass (fumigation-extraction method, Jenkinson & Powlson, 1976), after which the soil was extracted with 0.5 M K_2SO_4 and 0.5 M $NaHCO_3$ and filtered as above. Fumigated and nonfumigated extracts were frozen at −20 °C until analyzed (Schinner *et al*., 1995).

From the dried subsample, soil organic-matter content (SOM) was determined by the incineration at 550 °C with a thermobalance (Leco TGA 701, St. Joseph, MI, USA) to constant weight (Sparks, 1996), whereas total C (C_{tot}) and N (N_{tot}) were determined by combustion at 850 °C (Leco TruSpec autoanalyzer), and total inorganic C (TIC) was measured by acidification with $HClO_4$ in a TIC analyzer (UIC CM-5014). The difference between C_{tot} and TIC gave the total organic C (C_{org}). Ammonium (NH_4^+) and nitrate (NO_3^-) were determined from KCl extracts by the Kjeldhal method (Bremner & Keeney, 1965) with a Buchi distillation unit B-324 and a Metrohm SM Titrino 702 titrator. These two elements were

combined into inorganic N (N_{inorg}). From K_2SO_4 extracts (fumigated and nonfumigated), we determined the dissolved organic C (DOC) and dissolved organic N (DON) with a Shimadzu TOC-V CSH analyzer (Kyoto, Japan). Microbial C and N (C_{micro} and N_{micro}, respectively) were determined by the difference in DOC and DON between fumigated and nonfumigated subsamples. Inorganic P (P_{inorg}) was determined in nonfumigated $NaHCO_3$ extracts by the Olsen method (Watanabe & Olsen, 1965) with a Perkin Elmer 2400 spectrophotometer (Waltham, MA, USA). Microbial P (P_{micro}) was measured as the difference in P between the fumigated and nonfumigated extracts. Concentration values in the microbial fraction were not corrected for extraction efficiency, as the main objective of the study is to analyze the effect of the different treatments on nutrient availability rather than determine the total nutrient immobilization. For simplicity, we refer to SOM, extractable soil N and P, dissolved organic fractions (DON and DOC), and microbial nutrient content (C, N and P) as the soil-nutrient fractions and microbial fractions, respectively, hereafter. The C and N content in litter were determined by combustion at 850 °C (Leco TruSpec autoanalyzer), and P by the molybdovanadate method [Association of Official Analytical Chemists (AOAC), 1975]. The proportion of C, N, and P was referred to the dry weight of the 10×10 cm sample and expressed as $g\,m^{-2}$. *Q. ilex* seedlings were also analyzed for N, and P following the same procedure.

Data analysis

Differences in soil volumetric water content were analyzed between habitats and climatic scenarios by a repeated-measures ANOVA. The effect of habitat and climatic scenarios on SOM and C, N, and P forms in soil and microbes was analyzed using a factorial ANOVA for each nutrient form followed by Bonferroni's correction. As simulations of climatic scenarios were applied only in summer, and we used a different number of factors for each season (spring and summer), we analyzed the two seasons separately instead of analyzing all the data together with RM-ANOVA. For spring, we used habitat and climatic scenario as independent factors. For the summer analysis, we also included year (2007 and 2008) as a factor, which allowed us to evaluate both interannual variability and the cumulative effect of one or two climatic simulations (one in 2007 and a second in 2008). As depth had a consistent effect on all soil and microbial nutrient fractions (higher concentrations in the upper soil profile), we eliminated this factor from the analysis, pooling data from two depths (differences among depths are shown in Appendix S3). For litter, we performed similar analyses for the concentration and total pool of C, N, and P. For N and P concentrations and pools in *Q. ilex* seedlings, we performed one-way ANOVAs to test the effect of climatic scenario within the habitat. We used this approach instead a two-way factorial model because we were using seedlings as a live control of the effect of climatic conditions, rather than focusing on differences among habitats. To fulfill normality and homoscedasticity assumptions, variables were log-transformed when necessary. Fisher's *post hoc* PSLD test was used for differences within groups. Values are given throughout the articleas mean ± SE. Analyses were made using JMP 7.0 (SAS Institute Inc. 2007, Cary, NC, USA).

Results

Soil-water availability

The volumetric soil-water content was significantly different among habitats ($F_{2,715} = 499.68$; $P < 0.0001$) and climatic scenarios ($F_{2,715} = 1214.71$; $P < 0.0001$; Fig. 1). Forest was the habitat with the highest soil moisture (15.1 ± 0.2%), followed by shrubland (14.6 ± 0.2%) and open (12.4 ± 0.2%). Among climatic scenarios, the highest values in soil moisture appeared under the wet-summer scenario (16.4 ± 0.2%), followed by control (13.8 ± 0.2%), and dry-summer scenario (11.9 ± 0.2%; Fig. 1). The climatic-scenario simulations translated therefore in concordant differences in soil moisture.

Litter nutrient pool and concentration

The litter-nutrient concentration was determined mainly by habitat effect (Table 1), with higher values in open (C: 34.67 ± 0.37%; N: 1.07 ± 0.04%; P: 1.11 ± 0.04 $mg\,g^{-1}$) than in shrubland (C: 34.68 ± 0.51%; N: 1.13 ± 0.03%; P: 0.81 ± 0.02 $mg\,g^{-1}$) or forest (C: 35.94 ± 0.77%; N: 0.73 ± 0.02%; P: 0.60 ± 0.01 $mg\,g^{-1}$) pooling scenarios and seasons. However, as different habitats differed in the total litter mass, (forest 5762 ± 231 $g\,m^{-2}$; shrubland 1684 ± 111 $g\,m^{-2}$; open 801 ± 59 $g\,m^{-2}$), the total nutrient pool contained in litter followed the opposite pattern (Table 1), with higher values in forest (C: 2690 ± 183.94 $g\,m^{-2}$; N: 56.07 ± 3.98 $g\,m^{-2}$; P: 4.41 ± 0.28 $g\,m^{-2}$) than in shrubland (C: 506.52 ± 22.51 $g\,m^{-2}$; N: 16.97 ± 1.02 $g\,m^{-2}$; P: 1.21 ± 0.08 $g\,m^{-2}$) or open (C: 214.76 ± 16.56 $g\,m^{-2}$; N: 6.64 ± 0.81 $g\,m^{-2}$; P: 0.70 ± 0.07 $g\,m^{-2}$). The climatic scenario had no effect, either on litter concentration or total nutrient pool during spring or summer. No differences among years appeared in total litter-nutrient pool or concentration, except a slight decrease in summer-N concentration in 2008 (0.99 ± 0.03% in 2007 and 0.97 ± 0.03% in 2008; Table 1).

Soil and microbial nutrient content

Soil and microbial nutrient fractions differed among seasons, habitats, and climatic scenarios (Table 2). In spring 2008, habitat affected SOM, C_{org}, DOC, N_{tot}, N_{inorg}, and C_{micro}, but had no effect on DON, P_{inorg}, N_{micro}, or P_{micro}. For those fractions with a significant habitat effect, overall there were higher values in open for most of them, except for DOC (highest in forest) and N_{inorg} (highest in shrubland). Despite the heterogeneous effect of habitat, none of the nutrient fractions

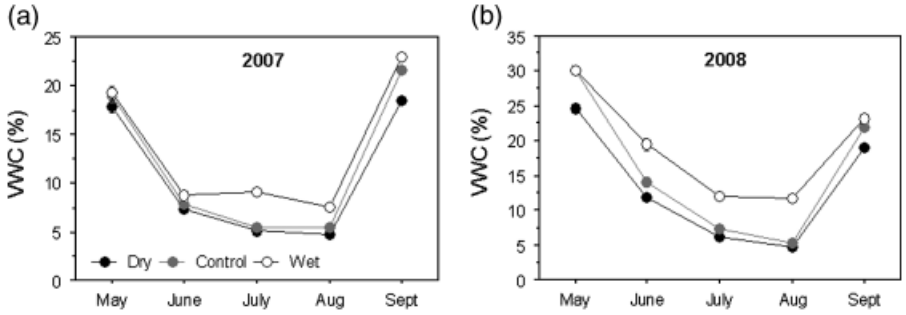

Fig. 1 Volumetric water content (VWC, in %) during experiment development (2007 and 2008) under the different climatic scenarios simulations: dry summer (black circles), control (grey circles), and wet summer (open circles). Habitats are pooled. Means are given ± SE.

Table 1 Results of the factorial ANOVA testing differences in nutrient total carbon (C), nitrogen (N) and phosphorus (P) pool and concentrations in (a) litter and (b) *Quercus ilex* seedlings

	C	N	P
(a) Litter			
Spring concentration			
Habitat	**4.59***	**34.43*****	**22.69*****
Scenario	1.85	0.19	0.01
H × S	1.20	0.96	0.99
Spring pool			
Habitat (H)	**69.17*****	**34.16*****	**49.74*****
Scenario (S)	0.83	0.89	2.03
H × S	1.10	1.74	2.53
Summer concentration			
Habitat	1.89	**32.55*****	**80.05*****
Scenario	0.88	0.79	0.17
Year	2.14	**14.26*****	1.85
H × S	0.63	0.87	1.24
H × Y	1.44	0.22	**6.53****
S × Y	0.96	2.35	1.52
H × S × Y	0.35	2.40	1.29
Summer pool			
Habitat	**87.09*****	**76.94*****	**84.94*****
Scenario	0.23	0.04	0.20
Year (Y)	0.04	0.97	0.11
H × S	0.17	0.30	1.01
H × Y	0.26	0.78	0.04
S × Y	0.25	0.08	0.17
H × S × Y	0.16	0.16	0.21

Significative values ($P<0.05$) after Bonferroni correction are bold-signaled:
*$0.05 \leq P<0.01$.
**$0.01 \leq P<0.001$.
***$P \leq 0.001$.

was affected by climatic scenario (Table 2), indicating that the effect exerted by this factor in the previous summer is lost during spring.

By contrast, soil and microbial nutrient fractions during summer were globally affected by habitat and climatic scenarios. Habitat affected most fractions (Table 2), generally with lower values in forest (Fig. 2). Climatic scenario effect was significant for all soil and microbial nutrient fractions except N_{inorg} (Table 2). A significant interaction between habitat and climatic scenario appeared in some cases as a consequence of changes in the pattern of climatic simulation across habitats (Table 2; Fig. 2). However, two general trends could be distinguished. First, either the dry or the control scenario showed the highest values for most of the soil fractions, whereas the wet scenario showed the lowest values. This was particularly consistent for DON, DOC, and P_{inorg} (Fig. 2c–e) and also appeared in some habitats for SOM, C_{org}, and N_{tot}. Second, microbial N and P showed the opposite pattern, with a clear trend to increase in the wet scenario (Fig. 2i and j). Microbial C peaked for the dry scenario in shrubland, but did not show a clear pattern across climatic scenarios. Overall, forest was the habitat where differences among climatic scenarios were lower, especially patent for P fractions (Fig. 2e and j). All soil and microbial fractions with the exception of SOM and N_{tot} varied among seasons (Fig. 3). In general terms, soil nutrients increased their concentrations during summer, whereas microbial nutrient concentrations increased in spring (Fig. 3). The pattern across seasons and across climatic scenarios was therefore similar, with an increase in inorganic nutrient availability under dry conditions and a reduction with higher moisture availability.

Yearly variation presented a significant effect for DON, P_{inorg}, N_{micro}, and P_{micro} (Table 2), and had a consistent effect across habitats. DON and N_{micro} followed the same pattern, being consistently lower in 2008, after two climatic simulations in the three habitats (Fig. 4a and b). P_{inorg} also was lower after two climatic simulations, but the trend reversed for P_{micro} except in the dry scenario (Fig. 4d). Although yearly variations

Table 2 Results of the factorial ANOVA for differences in habitat, climatic scenario, and their interactions on the different elements: soil organic matter (SOM), organic carbon (C_{org}), dissolved organic carbon (DOC), total nitrogen (N_{tot}), inorganic nitrogen (N_{inorg}), dissolved organic nitrogen (DON), phosphorus (P_{inorg}), microbial carbon (C_{micro}), microbial nitrogen (N_{micro}), and microbial phosphorus (P_{micro}) during spring (2008) and summer (2007 and 2008)

Factor	SOM	C_{org}	DOC	N_{tot}	N_{inorg}	DON	P_{inorg}	C_{micro}	N_{micro}	P_{micro}	df
Spring											
Habitat (H)	**11.20*****	**5.85****	**10.83*****	**17.58*****	**4.19***	1.65	1.97	**4.12***	1.87	3.00	2
Scenario (S)	1.23	0.45	0.78	1.49	1.55	0.68	0.41	1.45	1.47	0.49	2
H × S	0.61	0.66	0.63	0.69	1.26	0.87	0.35	0.59	1.51	0.38	4
Summer											
Habitat	**45.79*****	**33.02*****	1.80	**71.21*****	**9.04*****	**15.94*****	**5.15****	**6.44****	**7.51*****	**4.27***	2
Scenario	**13.69*****	**7.45****	**28.11*****	**10.41*****	3.03	**21.81*****	**15.34*****	**8.62*****	**7.54*****	**9.36*****	2
Year (Y)	3.05	2.17	0.28	0.71	2.02	**18.91*****	**66.47*****	4.60	**8.36****	**3.80***	1
H × S	5.06	**3.80****	**3.71***	**4.11****	**6.41*****	3.12	**5.74****	**4.32****	0.91	0.60	4
H × Y	1.21	0.13	1.49	1.82	**10.25*****	0.82	0.26	0.75	1.10	0.80	2
S × Y	0.17	0.67	0.56	0.21	1.85	0.22	0.02	0.21	0.11	2.02	2
H × S × Y	0.07	0.14	0.88	0.09	1.21	0.56	0.33	0.53	0.33	0.93	4

All variables except C_{org} were log-transformed. Year factor join both the effect of interannual variation and the cumulative effect of one or two climatic simulations. Significative values ($P < 0.05$) after Bonferroni correction are bold-signaled.
*$0.05 \leq P < 0.01$.
**$0.01 \leq P < 0.001$.
***$P \leq 0.001$.

were found, the same pattern across climatic scenarios persisted in both study years.

Microbial C : N ratio

Microbial C to N relations significantly varied among seasons ($F = 53.21$, $P < 0.0001$), with higher values in summer (27.7 ± 1.9) than in spring (8.4 ± 0.5; pooling habitats and scenarios), indicating higher N immobilization by microbes during the spring period. No effect of habitat was detected in C : N relation during spring or summer but, in contrast, although climatic scenario showed no effect in spring, it had a strong effect during summer ($F = 12.27$, $P < 0.0001$): overall, the C : N ratio was higher under the dry-summer scenario (mean 39.6 ± 4.4; pooling habitats and depths), whereas this relation was lower under the wet-summer scenario (18.6 ± 2.0; Fig. 5). Differences among seasons and among climatic scenarios indicated higher N immobilization by microbes per mass unit with higher soil moisture.

Seedlings N and P

There were no differences in N and P concentrations in *Q. ilex* seedlings among climatic scenarios for any of the habitats studied ($P > 0.05$ in all cases). The overall mean N and P concentrations were $0.62 \pm 0.02\%$ and $0.68 \pm 0.04\%$ mg g^{-1}, respectively. However, as seedling mass was slightly greater for those seedlings growing under the wet-summer scenario, N and P total pool contained tended to increase (Table 3), although these differences were not significant.

Discussion

Changes in soil-moisture availability as a consequence of climate change have the potential to alter soil-nutrient availability and soil–plant–microbial interactions (Emmett *et al*., 2004). The possible impact has been analyzed considering either drier (Sardans & Penuelas, 2007; Andersen *et al*., 2010) or milder conditions (Illeris *et al*., 2003; Johnson *et al*., 2008), trying to simulate the most plausible scenario for the coming decades in those ecosystems. However, this is the first study considering simultaneously these two possible scenarios across different habitats that make up the ecosystem, despite that climate and land-use changes are the two main drivers of global change at the planetary scale. Only by integrating the different climatic scenarios under a gradient of habitat quality could we precisely assess the impact of climate change and predict its consequences on the different compartments involved in nutrient dynamics (i.e. nutrient input to soil and plant assimilation).

Our experimental results reveal strong differences in soil and microbial nutrient concentrations among seasons, years, habitats, and climatic scenarios. Microbial nutrient content increased in spring, whereas soil nutrients did during summer. Seasonal variations in soil and microbial nutrients have been reported from different ecosystems worldwide (Díaz-Raviña *et al*.,

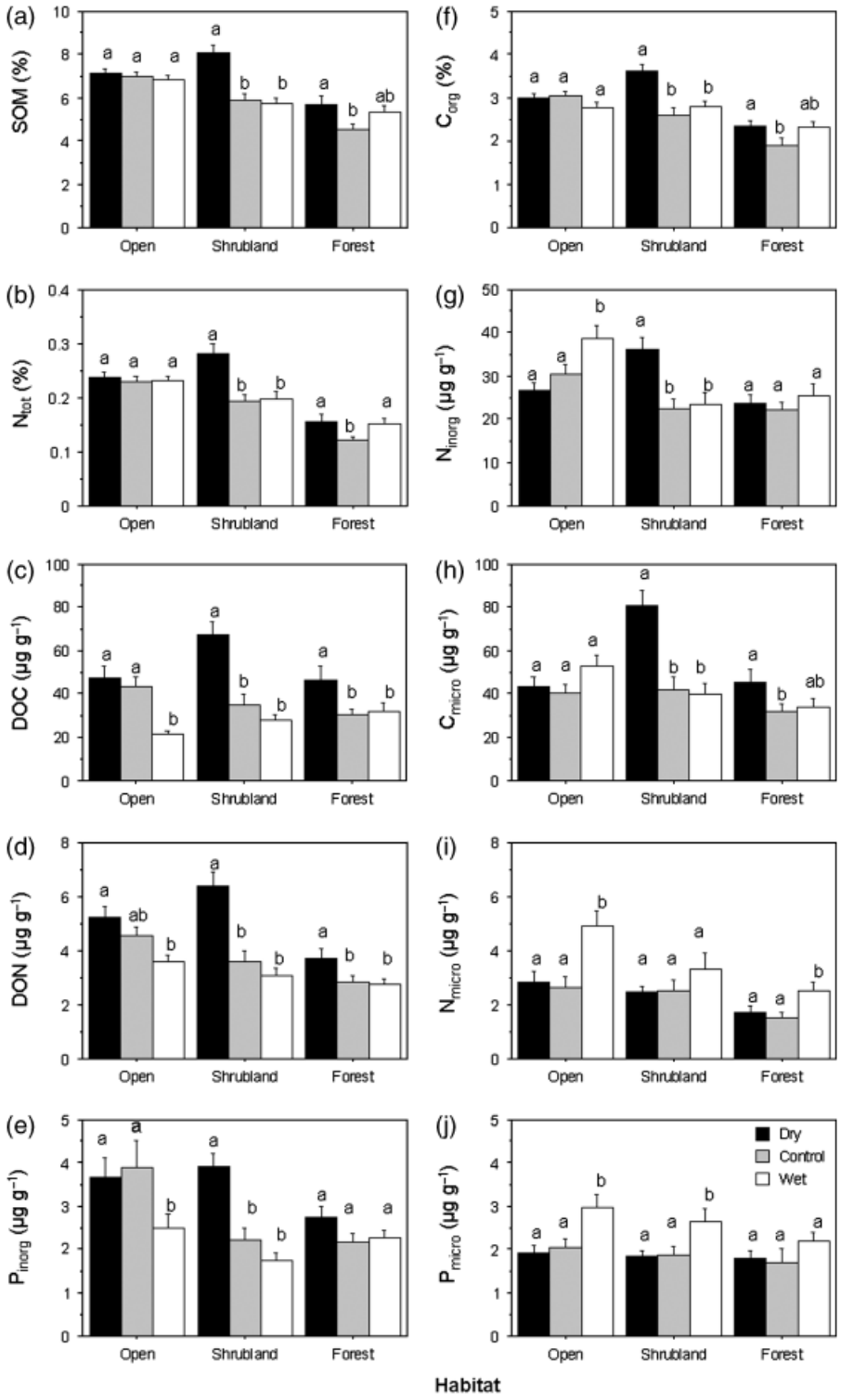

Fig. 2 Mean soil organic matter (SOM), and soil (C_{org}, DOC, N_{tot}, N_{inorg}, DON, and P_{inorg}) and microbial (C_{micro}, N_{micro}, and P_{micro}) fractions during summer among the three studied habitats: open, shrubland, and forest, and the three different climatic scenarios: dry summer scenario (black bars), control (grey bars), and wet summer scenario (open bars). Differences among climatic scenarios within habitat are indicated by different letter. Depths and years are pooled. Error bars represents SE. Concentration values in the microbial fractions were not corrected for extraction efficiency. C_{org}, organic carbon; DOC, dissolved organic carbon; N_{tot}, total nitrogen; N_{inorg}, inorganic nitrogen; DON, dissolved organic nitrogen; P_{inorg}, phosphorus; C_{micro}, microbial carbon, N_{micro}, microbial nitrogen; P_{micro}, microbial phosphorus.

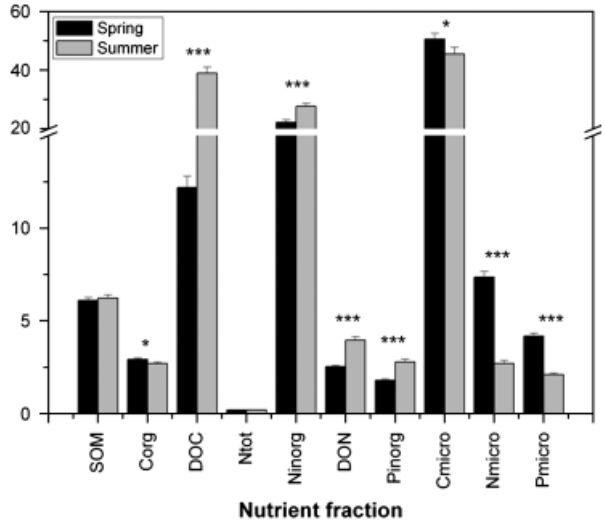

Fig. 3 Soil and microbial nutrients variations among seasons: spring (2007, black bars), and summer (2007 and 2008 data pooled, grey bars). Different habitats, climatic scenarios, and depths are pooled. Significative differences among depths after Bonferroni correction are indicated: $*0.05 \leq P < 0.01$; $**0.01 \leq P < 0.001$; $***P \leq 0.001$. Error bars representsSE. SOM, soil organic matter, in %; C_{org}, organic carbon, in %; DOC, dissolved organic carbon, in μg g^{-1}; C_{micro}, microbial carbon, in μg g^{-1}; N_{tot}, total nitrogen, in %; N_{inorg}, inorganic nitrogen, in μg g^{-1}; DON, dissolved organic nitrogen, in μg g^{-1}; N_{micro}, microbial nitrogen, in μg g^{-1}; P_{inorg}, inorganic phosphorus, in μg g^{-1}; P_{micro}, microbial phosphorus, in μg g^{-1}. Concentration values in the microbial fractions were not corrected for extraction efficiency.

1993; Miller *et al*., 2009), and are a response of temperature and soil-moisture differences (Mlambo *et al*., 2007). During spring, when the temperature is not too high and water is not a limiting factor, nutrients available in soil can be lower due to the greater nutrient demand by plants and microbes (Wardle, 1992), as well as to higher leaching or run-off (Singh *et al*., 1989; Srivastava, 1992). By contrast, during the summer drought, plant and microbes demand and leaching decline, at the same time that evaporation could augment upward nutrient movement (Austin *et al*., 2004 and references therein), thereby increasing the soil extractable nutrient concentration. Finally, habitats and climatic scenarios showed a strong effect for almost all the nutrient forms analyzed in summer. Moreover, there were differences related to the number of simulations of the climatic events (1 or 2 years). We could not ascertain whether this was due solely to the number of events, to interannual variability, or both, but in any case provided consistent effects for key components of the nutrient cycle such as DON, P_{inorg}, N_{micro}, and P_{micro}. All this support the idea that climate change may profoundly alter nutrient availability and soil–plant–microbial interactions in a short period, and that these changes may happen across the main habitats at the ecosystem level.

Climatic scenarios effect

Climatic scenarios consistently affected all soil and microbial fractions except N_{inorg} during summer. In

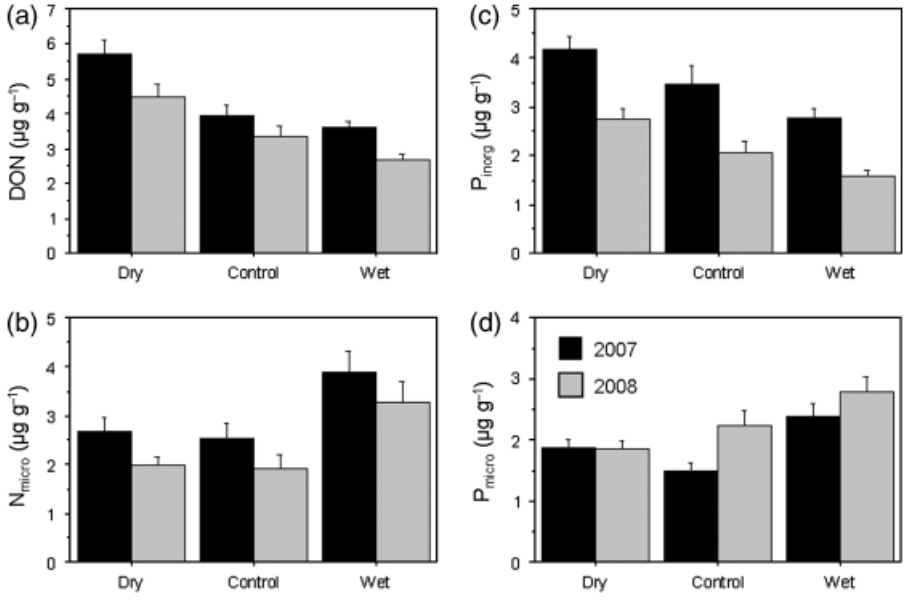

Fig. 4 Mean concentrations of dissolved organic nitrogen (a), microbial nitrogen (b), inorganic phosphorus (c), and microbial phosphorus (d) during 2007 (black bars) and 2008 (grey bars). Error bars represents SE. Concentration values in the microbial fractions were not corrected for extraction efficiency.

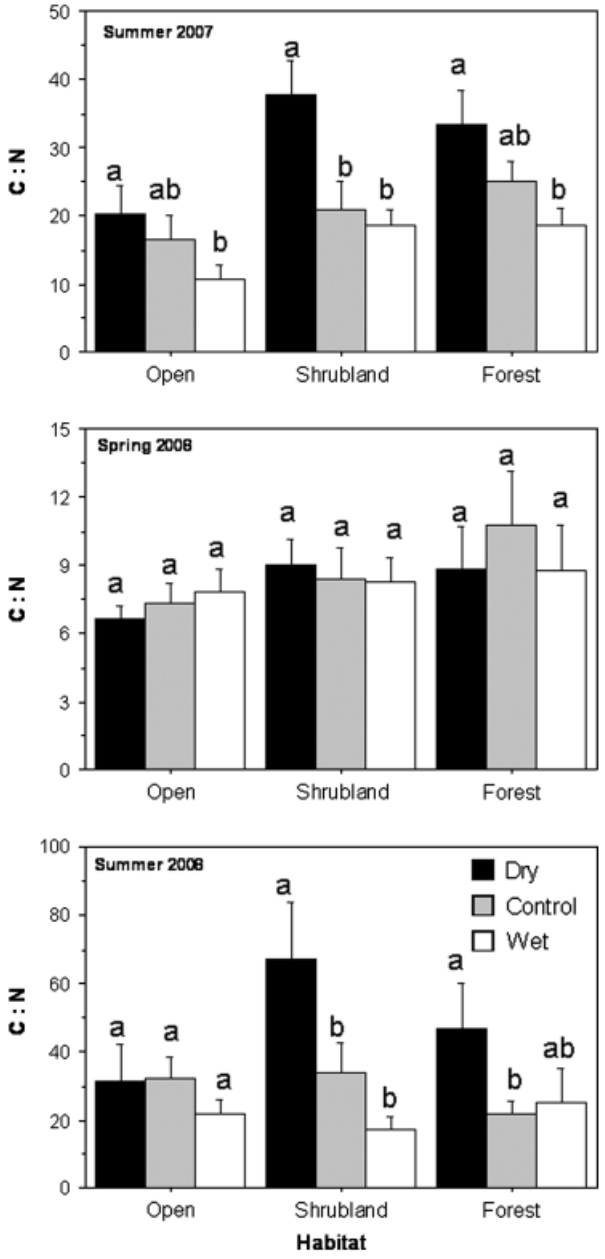

Fig. 5 Mean microbial C : N ratios among the three studied habitats: open, shrubland, and forest, and the three different climatic scenarios: dry summer scenario (black bars), control (grey bars), and wet summer scenario (open bars). Differences among climatic scenarios within habitat are indicated by different letter. Depths are pooled. Error bars represents SE.

general terms, soil-nutrient concentrations decreased with soil-moisture increase, from dry to wet climatic scenarios, whereas the pattern was the opposite for microbial fractions. Usually, soil-nutrient availability is inversely related to microbial activity (Ross & Sparling, 1993; Killham, 1994; Criquet *et al*., 2004; Monokrousos *et al*., 2004), as our results confirm. The decrease in soil

Table 3 Mean ± SE values of nitrogen (N) and phosphorous (P) total pool contained in *Quercus ilex* seedlings growing under the different climatic scenarios

	Dry	Control	Wet
N			
Open	13.64 ± 2.32	17.61 ± 7.24	20.92 ± 3.28
Shrubland	9.36 ± 1.54	7.38 ± 0.49	9.13 ± 1.52
Forest	11.56 ± 0.94	10.01 ± 1.18	12.82 ± 1.20
P			
Open	1.20 ± 0.29	1.21 ± 0.41	2.07 ± 0.46
Shrubland	0.97 ± 0.19	0.76 ± 0.14	1.20 ± 0.23
Forest	1.32 ± 0.18	1.33 ± 0.14	1.55 ± 0.17

nutrients under the wet scenario is explained by the positive relationship between microbial biomass and soil moisture (Santruckova, 1992; Kandeler & Bohm, 1996), as well as the higher plant uptake (Kozlowski & Pallardy, 2002), resulting in the opposite case with drier conditions. Mild years have therefore strong implications for ecosystem functioning, since they activate N and P cycling, two of the most limiting resources in Mediterranean areas (Sardans & Penuelas, 2007). Furthermore, this pattern was reinforced by seasonal variations increasing microbial uptake during spring as well as the soil fractions during summer. However, differences in soil moisture also altered microbial C : N relations. In general terms, lower soil moisture increased C : N relation, both among seasons and scenarios, this pattern being consistent through habitats. These differences have been commonly addressed to an alteration of microbial community composition (Austin *et al*., 2004; Schimel *et al*., 2007). Lower C : N relations should be interpreted as a bacterial-dominated community, whereas higher C : N values indicates a fungi dominance (Ross & Sparling, 1993; Schimel *et al*., 2007). In our case, bacteria may be responsible for most microbial activity during spring, whereas fungi predominate in summer, especially under the dry summer scenario. Thus, fungi dominance would increase under the drier conditions expected for the coming decades.

Although differences were found between the two sampled summers, the same pattern among climatic scenarios was repeated in 2007 and 2008 for some soil and microbial nutrients. The overall between-year pattern reinforced the results found by climatic scenarios simulations, increasing soil nutrients (DON and P_{inorg}) during the drier 2007, whereas the milder 2008 increased microbial P immobilization. However, this was not the case of N_{micro}, which was higher in 2007. Because 2007 was the first year with scenarios simulations and in 2008 accumulated 2 consecutive years of

simulations, it is difficult to determine the source of variation.

Differences in soil and microbial nutrient availabilities were not reflected in the *Q. ilex* N and P concentrations. The lack of difference in nutrient seedling concentration among those scenarios in which soil and microbial concentrations varied may indicate two important facts: on one hand, seedlings did not take advantage of the greater soil-nutrient availability under the dry scenario, probably for the lack of enough water to take them up in solution (Kozlowski & Pallardy, 2002). On the other hand, seedlings did not reduce nutrients under the wet scenario, where microbial retention was higher and soil availability was lower. In fact, the higher seedling growth under this scenario increased the total N and P pool. Thus, higher soil moisture increased nutrient uptake by microbes and plants, boosting nutrient circulation among the different compartments of the cycle. This result indicates that seedlings and microorganisms are not competing for resources, and that a higher microbial nutrient immobilization has positive effects for seedling performance at a seasonal scale (Jonasson *et al*., 2006).

Patterns across habitat

Habitat was an important factor determining nutrient availability, microbial immobilization, and overall plant–microbial interactions as reported in many other studies (Gallardo & Schlesinger, 1995; Criquet *et al*., 2004; Monokrousos *et al*., 2004; Cookson *et al*., 2007). Differences among habitats are expected, as differences in plant cover determine the soil-nutrient input by different litter quantity and quality (Santa-Regina *et al*., 1997; Holmgren *et al*., 2000). Besides these differences among habitats, litter nutrient content was not affected by climatic scenarios, probably due to the small scale of the study plots, which did not affect to leaf-nutrient content of trees or shrubs or decomposition in the soil.

Although the main effects of the different climatic scenarios were consistent, increasing soil nutrients under the drier conditions and increasing microbial immobilization under the wetter scenario, habitat was able to modulate them. The lack of differences between dry scenario and control in open for all soil and microbial fractions suggest the already limiting conditions of current summers in this habitat, where drought reduces microbial biomass to the minimum levels. Owing to this drought limitation, it is in this habitat where a wet summer is especially important to activate microbial activity and nutrient cycling. However, shrubland, and especially forest, had the capacity to partially compensate the effect of the different climatic scenarios. Although differences in reduction appeared in covered habitats for various fractions (N_{inorg}, DON, N_{micro}), this was particularly clear in the case of P, which did not vary its concentration in soil or microbes among scenarios in forest. According to these results, soil sensitivity to changes in precipitation (either higher or lower) varies with the plant cover. That is, denser canopies increase habitat resilience (*sensu* Holling, 1973), making them less prone to alter nutrient dynamics. This interaction of climatic scenarios effects by the different habitats should be taken into account for predictions and forecasting models for the effect of climate change on nutrient cycling.

Conclusions

The interaction between soil, microbes, and plants in relation to nutrient cycle is a complex network deeply affected by habitat structure and climatic conditions. Under a global-change scenario, where climatic and land-use change are expected (Houghton *et al*., 2001), this interaction will be altered. Wetter scenarios induce higher microbial activity, increasing therefore the mineralization rate (Killham, 1994) and mid-term nutrient availability for plants (Jonasson *et al*., 2006). On the contrary, a dryer climate reduces microbial nutrient uptake, increasing soil availability. However, these effects would be modulated by the different habitats: dense-covered habitats as forests are able to ameliorate the effects of the different climatic scenarios. By contrast, habitats with sparse plant cover are more dependent on milder conditions to enhance microbial activity and nutrient cycling. The higher nutrient availability in soil under drier conditions could not be exploited by plants, presumably due to the lack of enough water to take them up in solution. This higher nutrient pool in soil, together with the higher torrential rainfall predicted for the coming decades (Houghton *et al*., 2001) may increase the risk of nutrient loss by leaching or erosion (De Luis *et al*., 2003; Ramos & Martinez-Casasnovas, 2004), leading to a short to middle-term nutrient loss and soil impoverishment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Summer rainfall (June-August) for the study area during the 1902–2006 series. Data from 1902–1990 are inferred from La Cartuja meteorological station (Granada; $R^2 = 0.75$; $P < 0.0001$). Water amount for the simulated rainy summer was added according to the mean summer precipitation of the five mildest years (1915, 1930, 1940, 1952 and 1967), giving a value of 180 mm. We took the five highest values by two principal reasons: in one hand we wanted to simulate extreme (although natural) events; in the other hand, during these mild years evapotranspiration diminishes, making differences even stronger.

Appendix S2. Mean values and results of one way-ANOVA (df = 2; $N = 144$) exploring differences among plots where climatic scenarios were later simulated during the previous summer (2006) and spring (2007) to experiment development of the different elements: soil organic matter (SOM), organic carbon (C_{org}), dissolved organic carbon (DOC), microbial carbon (C_{micro}), total nitrogen (N_{tot}), inorganic nitrogen (N_{inorg}), dissolved organic nitrogen (DON), microbial nitrogen (N_{micro}), inorganic phosphorus (P_{inorg}), and microbial phosphorus (P_{micro}). The two depths (0–8 and 8–16 cm) are pooled. Concentration values in the microbial fractions were not corrected for extraction efficiency. As expected, there were no differences among plots prior to climatic scenarios simulation for any of the nutrient forms analyzed. We may thus consider that differences detected in the following years were due to the treatment.

Appendix S3. Soil and microbial nutrients variations (SOM: soil organic matter, in %; C_{org}: organic carbon, in %; DOC: dissolved organic carbon, in μg/g; C_{micro}: microbial carbon, in μg/g; N_{tot}: total nitrogen, in %; N_{inorg}: inorganic nitrogen, in μg/g; DON: dissolved organic nitrogen, in μg/g; N_{micro}: microbial nitrogen, in μg/g; P_{inorg}: inorganic phosphors, in μg/g; P_{micro}: microbial phosphors, in μg/g) among soil depths: upper (0–8 cm, black bars), and lower (8–16 cm, grey bars). Different habitats and climatic scenarios are pooled. Concentration values in the microbial fractions were not corrected for extraction efficiency. Significant differences among depths after Bonferroni correction are indicated: * $0.05 \leq P < 0.01$; ** $0.01 \leq P < 0.001$; *** $P \leq 0.001$. Error bars represents standard error. Overall, SOM was 1.3 times higher in the upper profile than in the lower, C_{org} 1.3 times, DOC 2.0 times, C_{micro} 1.9 times, N_{tot} 1.4 times, N_{inorg} 1.3 times, DON 1.7 times, N_{micro} 2.3 times, P_{inorg} 1.9 times, and P_{micro} 2.3 times.

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