Climatic events inducing die-off in Mediterranean shrublands: Are species responses related to their functional traits?

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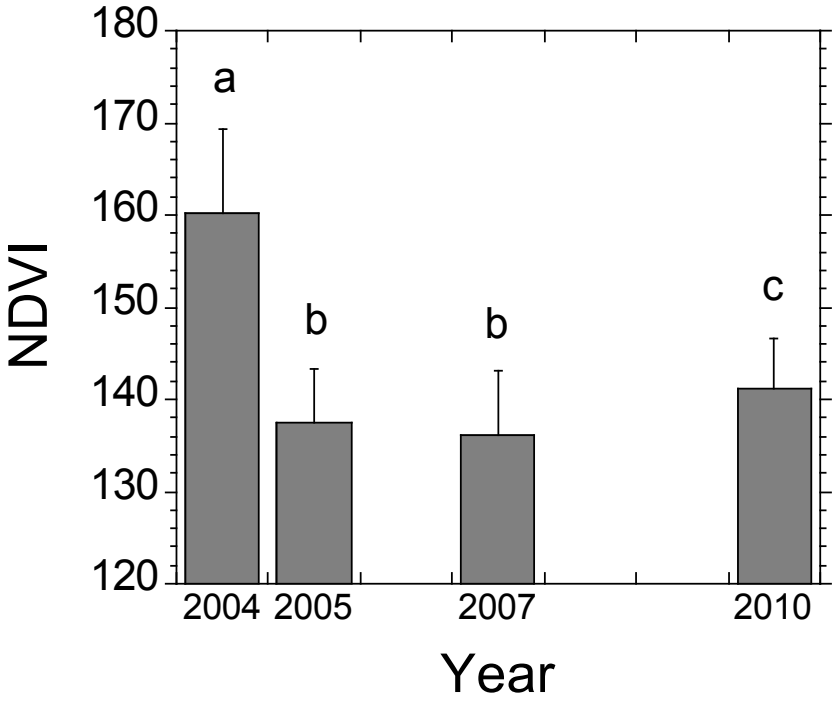
Electronic Supplemental Material

Appendix 1. Photograph of the Doñana shrubland in 2007 following the climatic extreme episode of the 2004-2005 hydrological year. Author: Francisco Lloret.

Appendix 2. Aerial photograph of the study area after the climatic extreme episode of the 2004-2005 hydrological year. Author: Hector Garrido (Equipo de Seguimiento de Procesos Naturales. Estación Biológica de Doñana).

Appendix 3. NDVI and die-off

NDVI values (mean, SD bars, after linearly scaling to values ranging from 0 to 255) from Landsat imagery (30 x 30 m^2 pixels, n = 21) obtained in the site of the Doñana sampled plots. The images were obtained in the same month (November) of the 2007 survey. Images for some years were not available due to clouds. Different letters indicate significant differences (LSM Student's t test $p<0.005$). The figure shows that vegetation canopy dropped in 2005, following the extreme climatic episode, remaining at low values in 2007 (similarly to the observed in 2005). This observation supports that the 2007 sampling is an adequate estimation of the effect on vegetation cover of the 2005 climatic event. Eventually, canopy greenness started to recover after 2007.



Appendix 2. Leaf traits measures

Leaf traits, in particular specific leaf area (SLA; leaf area per unit of dry leaf mass), leaf dry matter content (LDMC; dry mass per unit of water-saturated fresh mass), leaf thickness (Lthick), leaf nitrogen concentration (LN) and leaf chlorophyll (LChl; concentration of chlorophyll per unit of leaf fresh mass) were measured in each selected individual, collecting at least two branches with young, fully expanded leaves from those parts of the plant with the highest light exposure. The branches were placed in plastic bags and transported in a chilled, dark container to the laboratory, where the plants were stored in darkness at 15 °C with the stem bases submerged in water for at least 12 h to fully rehydrate the leaves. A subsample of rehydrated and fully expanded young leaves per individual was removed from the stem and weighed to obtain the lamina fresh mass (the petiole was previously excised). Lthick (average of three measurements per leaf) was measured with an electronic digital micrometer (Palmer-Comecta, Spain). Fresh leaves were scanned, and leaf area was determined by digital analysis of the images using the software Image-Pro Plus 4.5 (Media Cybernetic Inc. USA). Leaves were further oven-dried at 70 °C for 48 h and then weighed to the nearest 0.0001 g. Specific leaf area (SLA) was calculated as the ratio between the area of the lamina and its dry mass. Likewise, LDMC was calculated as the ratio between the dry and the fresh (saturated) weights of the lamina. LN was obtained by elemental analysis (Eurovector EA 3000, Milan, Italy) from additional leaves after being oven-dried (at 70°C for 48 h) and grounded using a stainless steel mill. The chlorophyll

concentration was obtained following the method of Wintermans and de Mons (1965), using methanol for the extraction of chlorophyll in a leaf portion during 24 hours under dark conditions. The absorbance of the supernatant was analyzed by spectrophotometry at 650 (A650) and 655 (A655) nm. The equation used was: leaf chlorophyll content = 25.5×A650+ 4×A665. Leaf chlorophyll content was divided by the leaf fresh mass portion to obtain LChl (μg g^{-1}).

Carbon isotopic ratio (δ13C; ‰, precision of ca. 0.2‰) was obtained from a mixture of leaves collected in each individual, using a continuous flow elemental analyzer-isotopic ratio mass spectrometer (EA Thermo 1112-IRMS Thermo Delta V Advantage).

Reference

Wintermans JFGM, DeMots A (1965) Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. Biochimica Biophysica Acta 109:448–453