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Universitat Autònoma de Barcelona

Departament de Biologia Animal,
de Biologia Vegetal i d'Ecologia
Unitat d'Ecologia

"Phenology, biomass and community composition changes in European shrublands submitted to experimental warming and drought"

Ph. D. Thesis
Patricia Prieto Calvo

Bellaterra, setembre del 2007

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Memòria presentada per:

Patricia Prieto Calvo
per optar al grau de Doctor

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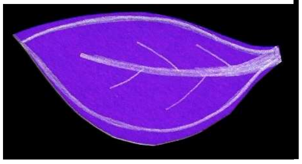
Universitat Autònoma de Barcelona	Universitat Autònoma de Barcelona

Bellaterra, setembre del 2007

Quiero dedicar el esfuerzo de este trabajo a David Calvo

Aspazié, se puya a paret
plenando os foratos
con purnas de bida
dende os alazez.

Asinas deziba
a yaya mia
ye que se feba
as buenas fayenas
ta durar perén.
(P. Ramos)



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Siempre seréis muy bien recibidas/os!

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INTRODUCCIÓN GENERAL

- CAMBIO CLIMÁTICO: OBSERVACIONES Y PROYECCIONES

A lo largo del tiempo el clima ha experimentado cambios como resultado de procesos naturales, como son las variaciones en la producción de energía del sol, variaciones en la órbita de la tierra y variaciones internas del mismo sistema climático (composición de la atmósfera, disposición de los continentes, etc.). Es lo que la Convención Marco de las Naciones Unidas sobre el Cambio Climático denomina **"variabilidad del clima"**.

El cuarto Informe de Evaluación del Grupo Intergubernamental de expertos sobre el Cambio Climático realizado en 2007 ha manifestado, con un nivel muy alto de confianza, que las actividades humanas desde 1750 han resultado en un calentamiento global. **Cambio climático** es el término que el IPCC utiliza para referirse a un cambio en el clima debido tanto a la variabilidad natural como a la actividad humana. Uno de los **factores antropogénicos** contribuyentes a alterar el balance energético del sistema climático es el aumento de la concentración de **gases de efecto invernadero** en la atmósfera. Nos referimos principalmente al dióxido de Carbono (CO_2) cuyo aumento está relacionado con la utilización de combustibles fósiles y con los cambios en los usos de suelo, y también al metano (CH_4) y óxido nitroso (N_2O) debido principalmente a algunas prácticas en la agricultura. Además entre otros factores cabe mencionar los cambios en el **ozono** estratosférico debido a la emisión de precursores químicos de ozono (óxidos de nitrógeno, monóxido de carbono e hidrocarbonos) y los cambios en la cantidad de energía reflejada por la Tierra (**albedo**) debido a cambios en la cubierta vegetal (cambios en el **uso del suelo)** y a la emisión de aerosoles (partículas en suspensión en la atmósfera).

Hay una serie de **observaciones** a nivel global que evidencian que el sistema climático se está calentando. La temperatura es un parámetro que se viene midiendo desde finales del siglo XIX. En el periodo comprendido entre 1906 y 2005 se ha estimado un incremento de la **temperatura media** del aire de 0.74 ºC. A su vez, una mayor temperatura ha producido una disminución de la extensión y espesor de los hielos polares y un retroceso de la superficie de los glaciares. El mayor **derretimiento** de hielo y nieve junto al calentamiento de los océanos ha dado lugar a un aumento medio global del **nivel del mar**.

La **previsión** de la mayoría de los modelos generales climáticos (GCMs) coincide en que en este siglo la **temperatura** puede aumentar entre 1.1 y 6.4 ºC dependiendo de los escenarios socio-económicos y de la emisión de gases de efecto invernadero resultante (IPCC, 2007). Los mayores aumentos de temperatura se esperan en los meses fríos de las zonas situadas en latitudes altas, lo que produciría inviernos más suaves y primaveras más cálidas (Hari & Häkkinen 1991; Ahas *et al.,* 2002; Schwartz *et al.,* 2006).

Los modelos climáticos tienen mucha más dificultad para prever cómo se verán afectadas las **precipitaciones** en las próximas décadas. En algunas zonas como el este de América del Norte y Sudamérica, norte de Europa y norte y centro de Asia se han observado aumentos y en cambio, en otras como la zona del Sáhel, en el Mediterráneo, África del sur y algunas partes del sur de Asia se han observado reducciones (IPCC, 2007). De cualquier manera, aquellos ecosistemas que de por sí ya sufren estrés por periodos de sequía pueden verse gravemente afectados por el aumento de la **evapotranspiración** ligado al aumento de las temperaturas (Le Houérou, 1996; Piñol *et al.*, 1998; De Luís *et al.*, 2001; Peñuelas *et al.*, 2002, 2005).

Asimismo, también se prevé una mayor frecuencia de **fenómenos meteorológicos extremos** (sequías más largas e intensas en trópicos y subtrópicos, precipitaciones fuertes, olas de calor) que pueden tener mayores efectos sobre la vegetación que pequeños cambios graduales (Peñuelas *et al.,* 2001; Meehl & Tebaldi**,** 2004; Schär *et al.,* 2004; Ciais *et al.,* 2005).

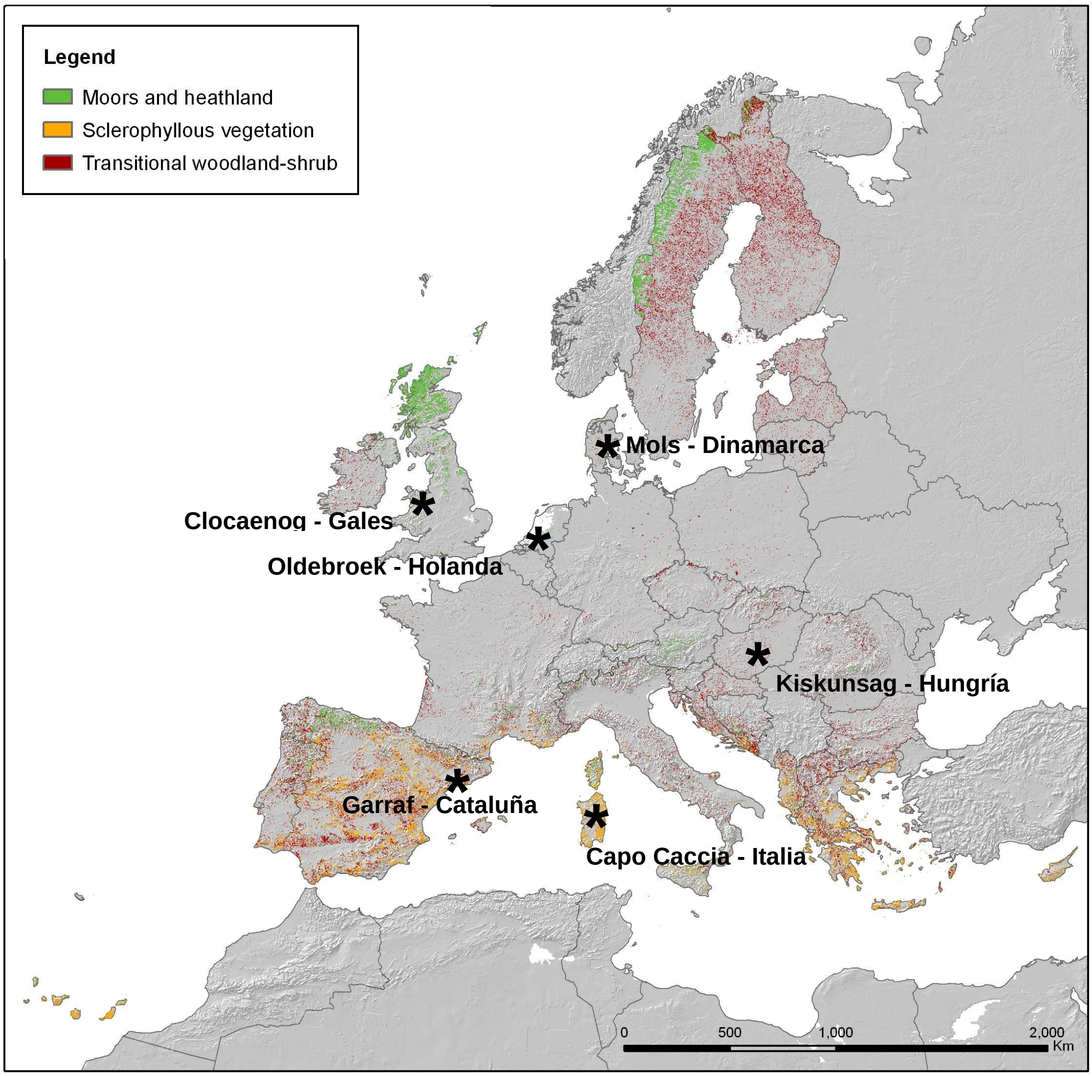
- LA BIOSFERA EN ACCIÓN

El cambio climático puede tener **grandes impactos** a nivel **ambiental, económico** y **social.** Cambios en la agricultura y pérdida de capacidad productiva agrícola en muchas regiones en desarrollo con el consiguiente peligro de hambrunas, migración de población asentada en zonas costeras, disminución de reservas hídricas en determinadas zonas donde el agua potable ya es escasa y aumento de los conflictos violentos asociados a esta problemática, aumento y propagación de enfermedades transmitidas por vectores (malaria y dengue) son sólo alguna de ellas.

Debido a la mayor vulnerabilidad de los países en vías de desarrollo con menor capacidad de adaptación frente a estos cambios, el **Protocolo de Kyoto** propone que sean los países desarrollados los que apliquen políticas para reducir los efectos adversos del cambio climático. La primera y más básica solución a toda esta problemática pasa por realizar un real ejercicio de reflexión sobre el tipo de desarrollo que estamos fomentando. Las decisiones tomadas por las administraciones deberían tener como máxima la búsqueda de la **sostenibilidad**, entendida como un proceso en el que se persigue el bienestar de las personas y también de los ecosistemas tanto presentes como futuros. Por suerte, también contamos con un importante factor moderador del cambio climático, la **biosfera,** y formando parte de ella, los organismos fotosintéticos que ejercen una retroalimentación negativa sobre la cantidad de dióxido de carbono que hay en la atmósfera. Ya se han realizado numerosos estudios a nivel mundial para estudiar los efectos del cambio climático sobre el funcionamiento de las **formaciones boscosas** y estimar su capacidad de absorción de carbono (Phillips *et al.*, 1998; Caspersen *et al.*, 2000; Fang *et al.*, 2001; Leinonen & Kramer, 2002). Se han propuesto medidas relacionadas con los bosques y su gestión para intentar reducir la emisión de gases de efecto invernadero a la atmósfera y para aumentar la captación de carbono, y que además pueden crear sinergias con el desarrollo sostenible en términos de mejoras económicas para la población local, abastecimiento de energía renovable o conservación del agua y de la biodiversidad (Albretch & Kandji, 2003; Gullison *et al.*, 2007; IPCC, 2007).

- VULCAN: EL ROL DE LOS MATORRALES.

Menor interés se le ha prestado al rol que pueden jugar otras comunidades como las dominadas por matas o arbustos. Los **matorrales** son una formación cada vez más abundante en todo el mundo debido a diferentes causas. Algunos estudios lo han relacionado directamente con el aumento de temperaturas (Sturm, 2001) y otros han señalado la importancia de los cambios de uso en el territorio y la gestión que se hace en ellos. En las praderas semiáridas de América del Norte el exceso de presión ganadera ha reducido la frecuencia de fuegos lo que ha favorecido a los arbustos por encima de las especies herbáceas (Van Auken, 2000; Brown & Archer, 1999). En el este de la Península Ibérica, los matorrales también han aumentado debido a cambios en nuestras actividades socioeconómicas que han llevado al abandono de una gran superficie de tierras de cultivo, a la reducción del pastoreo y al incremento en la frecuencia de fuegos (Baeza *et al.*, 2002; Lloret *et al.*, 2002). En algunos países como Reino Unido, los matorrales (*heathlands*) son mantenidos por el ser humano ya que son considerados un paisaje singular con gran una gran importancia para el mantenimiento de una mayor diversidad (Alonso, 2004).



Superficie de Europa cubierta por matorral (ver leyenda). Fuente: CORINE LAND COVER 2000.

La tesis que presento a continuación con el título *"Phenology, biomass and community composition changes in European shrublands submitted to experimental warming and drought"* se ha desarrollado como parte del proyecto **VULCAN** (Vulnerabibilty assessment of shrubland ecosystems under climatic changes). VULCAN ha sido un proyecto de investigación llevado a cabo en seis ecosistemas de matorrales europeos (Gales-Reino Unido, Dinamarca, Holanda, Hungría, Cerdaña-Italia y Catalunya-España) durante 2001-2005, continuación de uno anterior (CLIMOOR Project 1998-2001)[1].

Ambos proyectos han tenido como **objetivo global** el aportar conocimientos y reducir incertidumbres acerca del funcionamiento de los matorrales europeos y del rol que pueden desempeñar éstos como fuente o sumidero de carbono bajo la perspectiva del cambio climático. Además, los resultados que se desprenden de los estudios realizados durante 7 años en estos matorrales pueden ser aplicados a la gestión que se lleve a cabo en ellos en el marco de un clima cambiante.

En las parcelas de los diferentes países participantes se instaló una novedosa **manipulación experimental** para aumentar la temperatura y para reducir el agua disponible en **campo,** simulando los efectos del cambio climático previstos para las décadas futuras (IPCC, 2007; Peñuelas *et al.*, 2005).

El tratamiento de calentamiento consiste en un toldo que cubre la vegetación automáticamente por la noche durante todo el año. Este toldo evita que la radiación infrarroja emitida por suelo y plantas se escape al exterior lo que aumenta la temperatura del aire en torno a 1 ºC dependiendo del lugar y del periodo (Beier *et al.*, 2004).

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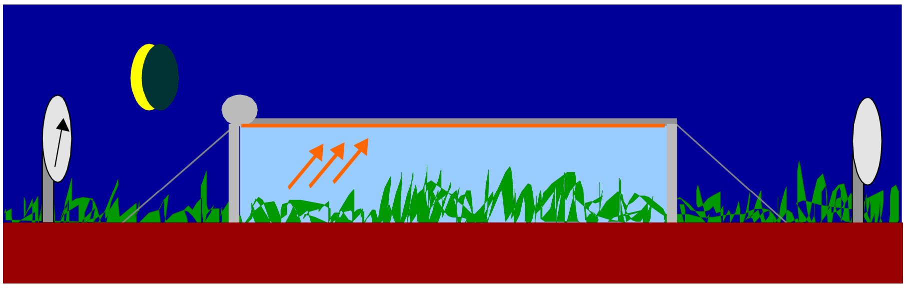
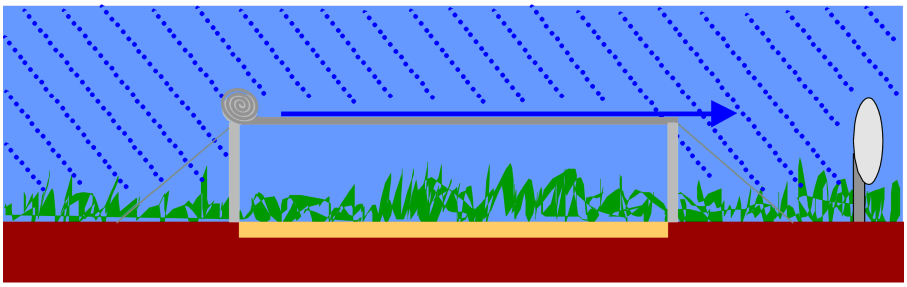
[1] En la página http://www.vulcanproject.com/ pueden encontrarse la descripción y las características básicas de cada uno de los ecosistemas estudiados.



El tratamiento de sequía consiste en un toldo impermeable que cubre la vegetación automáticamente cuando llueve durante las estaciones de crecimiento (en primavera para todos los países y también en otoño para la parcelas de Garraf). Tras el episodio de lluvia, el toldo se vuelve a recoger. La reducción de la humedad del suelo también depende del lugar y del periodo considerado (Beier *et al.*, 2004).



Estos tratamientos de **calentamiento** y **sequía** experimentales no afectan otras condiciones bióticas y abióticas como la intensidad de luz recibida y la libre circulación de viento, fauna y semillas y su montaje no produjo perturbaciones en el suelo ni en las plantas (ver detalles en Beier *et al.*, 2004; Peñuelas *et al.*, 2004).

La ventaja de este tipo de diseño que combina manipulaciones experimentales en un gradiente geográfico, es que permite inferir las respuestas de la vegetación a las nuevas condiciones climáticas a una mayor **escala espacial** y a un **largo plazo** (Chapin *et al.*, 1995; Shaver *et al.*, 2000; Rustad *et al.*, 2001; Dunne *et al.*, 2004).

- LA TESIS EN CINCO CAPÍTULOS

Los **resultados** que presento en este trabajo de investigación han sido organizados en **cinco capítulos** en formato de artículos, aunque la información es complementaria y lo que aporta cada uno de ellos nos ayuda a entender los resultados globalmente.

1- Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought. Reductions in primary productivity in the heat and drought year of 2003.

2- Effects of experimental warming and drought on photosynthesis shoot elongation and biomass accumulation in the dominant species of a Mediterranean shrubland.

3- Acceleration of the onset of shrubland species spring growth in response to an experimental warming along a north-south gradient in Europe.

4- Flowering of *Globularia alypum* and *Erica multiflora* in Mediterranean garrigue, and its response to precipitation and to experimental drought and warming.

5- Experimental Drought and Warming decrease diversity and slow down post-fire succession in a Mediterranean shrubland.

- LA ABSORCIÓN DE CARBONO.

En los dos primeros artículos de esta tesis, abordo el tema de la absorción de carbono por los matorrales bajo los tratamientos de calentamiento y sequía.

En el **primer capítulo** he estudiado los efectos de los tratamientos en la productividad primaria aérea de los seis matorrales europeos distribuidos en un gradiente de temperatura (8.2 - 15.6 ºC) y precipitación (511 - 1427 mm), así como en el crecimiento de las especies dominantes.

Además, incluyo los efectos de los tratamientos durante la **ola de calor** que sufrió Europa en 2003. Como se comentó anteriormente, se prevé que este tipo de fenómenos extremos sean más frecuentes en un clima cambiante (Meehl & Tebaldi, 2004; Schär *et al.*, 2004) y que afecten gravemente a la capacidad de secuestro de carbono por parte de la vegetación (Ciais *et al.*, 2005).

La actividad de la vegetación a escala global tiene diferentes controles climáticos. Ramakrishna *et al.*, (2003) estimaron que la disponibilidad de agua, la temperatura y la radiación limitan el crecimiento de la vegetación en un 40, 33 y 27 % respectivamente de la superficie de la tierra con cubierta vegetal y que la mayoría de los cambios observados en el clima iban en la dirección de reducir estas limitaciones. A escala global hay evidencias sobre el **incremento de la actividad** de la vegetación en respuesta al calentamiento en latitudes medias-altas del hemisferio Norte. La concentración de CO_2 crece en invierno y se reduce en verano principalmente debido al crecimiento estacional de la vegetación. En las últimas décadas, se ha observado un aumento de esta oscilación estacional que puede ser explicada por una prolongación de la estación de crecimiento y por un aumento de las tasas fotosintéticas (Keeling *et al.*, 1996; Myneni *et al.*, 1997).

Sin embargo, como ya han puesto de manifiesto algunos estudios realizados en el marco de los proyectos VULCAN y CLIMOOR, los efectos del cambio climático a nivel de ecosistema son complejos debido a la gran **variabilidad de respuestas** de las plantas a los tratamientos según las variables medidas, las especies, estaciones u años, sitios y periodos de experimentación (Beier *et al.*, 2004; Emmet *et al.*, 2004; Llorens *et al.*, 2003a, b, 2004a, b, Lloret *et al.*, 2004; Peñuelas *et al.*, 2004; Llorens & Peñuelas 2005).

El efecto del **calentamiento** sobre el crecimiento de las plantas y sobre la productividad primaria, dependerá entre otras cosas del efecto que tenga sobre las tasas fotosintéticas, las pérdidas de CO_2 por respiración, la producción de VOCs (Körner & Larcher, 1988; Long, 1991; Huntingford *et al.*, 2000; Peñuelas & Llusià, 2002), la duración de la estación de crecimiento (Peñuelas & Filella, 2001; Beier *et al*., 2004) y la mineralización de nutrientes (Shaver & Chapin, 1995; Robinson *et al.*, 1997; Jonasson *et al*., 1999; Emmett *et al*., 2004; Schmidt *et al*., 2002).

En los ecosistemas situados más al **norte,** donde la actividad de las plantas se ve limitada en algunos periodos por las bajas temperaturas, se prevé que las plantas serán especialmente sensibles al **calentamiento** (Aerts *et al.*, 2006) y que un aumento de las temperaturas conllevará un mayor crecimiento y un aumento de la productividad primaria (Chapin *et al.*, 1995). Sin embargo, en los ecosistemas situados más al **sur,** donde el agua es más limitante para las plantas, se prevé que el crecimiento y la productividad estarán más afectados por la **sequía** (Peñuelas *et al.*, 2001, Terradas, 2001, Kovács-Lang *et al.*, 2000).

Parcelas experimentales en Clocaenog (Gales-Reino Unido).

En el **segundo capítulo**, me he centrado en la absorción de carbono en el matorral estudiado en el Parque Natural de Garraf (Barcelona-España). En él estudio la sensibilidad de los procesos relacionados con la captación de carbono por la vegetación a nivel de hoja (fluorescencia, fotosíntesis, conductancia estomática), planta (elongación de ramas) y cubierta (acumulación de biomasa). Este capítulo tiene un gran interés ya que no son muchos los estudios que comparan los efectos del cambio climático en las variables ecofisiológicas y los efectos integrados en el tiempo y en el espacio sobre la acumulación de biomasa (Chapin & Shaver, 1996; Parson *et al.*, 1994; Kudo & Suzuki, 2003).

En el área Mediterránea, el verano ha sido considerado como el periodo más estresante para la actividad de las plantas (Larcher, 2000). El **agua** juega un papel imprescindible en el crecimiento ya que de ella depende la disponibilidad de nutrientes en el suelo y es básica para mantener la turgencia en las células (Munns *et al.*, 2000). Ante la falta de agua las plantas responden cerrando **estomas**. Al cerrar estomas, se reduce la entrada CO_2 y en consecuencia también la **fotosíntesis** todo lo cual junto con los cambios que la humedad puede generar en la **disponibilidad de nutrientes** puede reducir el crecimiento y la acumulación de biomasa en la comunidad (Chapin, 1980; Sardans & Peñuelas, 2004, 2005; Sardans *et al.*, 2006).

En esta zona, los efectos de un aumento en las **temperaturas** dependerán en gran medida de los efectos que tengan sobre la disponibilidad de agua. De esta manera, por ejemplo, se prevé que en las estaciones más cálidas el calentamiento de lugar a un aumento de la **evapotranspiración** (Piñol *et al.,* 1998) y que el calentamiento y la sequía puedan reducir las tasas fotosintéticas debido al **sobrecalentamiento** de las hojas (Peñuelas *et al.,* 1998; Peñuelas & Llusià, 2002, Llorens *et al.*, 2004). Sin embargo, en **invierno** el calentamiento puede aliviar las bajas temperaturas que se pueden llegar a alcanzar en esa zona y a la que muchas especies esclerófilas mediterráneas son sensibles (Mitrakos, 1980; Oliveira & Peñuelas, 2000, 2001, 2004; Ogaya & Peñuelas, 2003a, b, 2007).

Temperatura y agua determinan también la **superficie de hojas** donde directamente se lleva a cabo la captación de luz para la asimilación de carbono vía fotosíntesis. Particularmente, en especies mediterráneas se ha visto que existe una relación entre la disponibilidad de agua y la longitud de los brotes, el tamaño de la hoja o el índice de área foliar (Kyparissis *et al.,* 1997; Kramer *et al.,* 2000; Mutke *et al.,* 2003). También se ha observado que algunas especies bajo condiciones de sequía pueden modificar su patrón de asignación de carbono para por ejemplo, reducir la pérdida de agua por las hojas o aumentar su adquisición por las raíces (Sharp & Davies, 1989; Weltzin *et al.*, 2000). En este segundo capítulo he estudiado también los efectos de los tratamientos experimentales en caracteres de la estructura de la comunidad como la proporción de contactos vivos y muertos y la proporción de los contactos de hojas y tallos (ver método “pin-point” en capítulo 2).

El estudio se ha realizado durante un periodo de tiempo suficiente **(1998-2005)** como para detectar la dirección de los cambios y posibles diferencias entre respuestas primarias (por ejemplo cambios en el crecimiento de los individuos establecidos en las parcelas) y secundarias (cambios en el reclutamiento de nuevos individuos) a los tratamientos (Arft *et al*, 1999; Hollister *et al.*, 2005).

Siete años de experimentación también dan la posibilidad de estudiar las respuestas a los tratamientos teniendo en cuenta la **variabilidad interanual** de las condiciones ambientales. En este estudio se incluyen dos años con periodos especialmente duros, el año 2003 (ola de calor) y el año 2005 en el que se dio la primavera más fría y seca antecedida además por un otoño de 2004 también muy seco.

Parcelas en el Parque Natural del Garraf (Cataluña-Barcelona)

- LA FENOLOGÍA DEL CRECIMIENTO VEGETATIVO Y DE LA FLORACIÓN

La temperatura y el agua son también dos de las variables ambientales que más claramente influencian los **ciclos de vida** de la mayoría de los seres vivos. En las últimas década, el estudio de la fenología de las plantas ha cobrado un gran interés en la investigación sobre el cambio climático debido a su gran **sensibilidad** al aumento de temperatura, su bajo coste y su baja dificultad ya puede realizarse simplemente mediante una inspección visual (Schwartz, 1999; Menzel, 2000; Osborne *et al.*, 2000; Roetzer *et al.*, 2000; Peñuelas & Filella, 2001; Spark & Menzel, 2002, Walther *et al.*, 2002; Piao *et al.*, 2006; Linderholm, 2006; Menzel *et al.*, 2006). De hecho, a nivel nacional existen redes de voluntarios que detectan cada año el momento en que se produce una determinada fenofase de una determinada especie[2]. El gran volumen de información que puede ser recogido puede ser utilizado para la detección y seguimiento del cambio climático mundial. Por eso se están haciendo esfuerzos en Europa y Norte América para coordinar las redes fenológicas ya existentes y estandarizar las observaciones[3].

Muchos estudios sobre fenología de plantas han concluido que existe una clara relación entre las fenofases que ocurren en primavera (aparición de hojas, brotes y floración) y la **temperatura** de los meses anteriores (Fitter *et al.*, 1995; Osborne *et al.*, 2000; Fitter & Fitter, 2002; Peñuelas *et al.*, 2002; Walther *et al.*, 2002; Chen *et al.*, 2005; Crepinsek *et al.*, 2006; Piao *et al.*, 2006; Menzel *et al.*, 2006). De hecho, se ha

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[2] Páginas sobre proyectos de seguimiento de fenología en España: http://www.fundacionglobalnature.org, http://www.fundacionmigres.org, http://www.jatunsacha.org/espanol/voluntariado/bilsa_info.htm, http://www.mapa.es/rmarinas/lasreservas/tabarca/proyectos_iniciativas/proyect_2.htm, http://www.seo.org

[3] Red Europea para el seguimiento de la fenología: http://www.dow.wau.nl/msa/epn/index.asp
Red Norteamericana: http://www.uwm.edu/Dept/Geography/npn/index.html

relacionado el aumento de temperatura de las últimas décadas con **avances** de la fenología de diferentes especies en amplias zonas geográficas (Menzel & Fabian, 1999; Peñuelas & Filella, 2001; Rotzer & Chmielwski, 2001; Parmesan & Yohe, 2003). Estimas recientes sugieren que las fenofases de plantas que ocurren entre primavera y verano en Europa se están avanzando como media 2.5 días por cada 1 ºC de incremento de temperatura (Menzel *et al.*, 2006).

El avance del momento en que las plantas comienzan a crecer podría significar un alargamiento de la **estación de crecimiento**, es decir, del tiempo en el que la vegetación está activa y por lo tanto del carbono que pueden fijar (Menzel & Fabian, 1999; Schwartz, 1999; Menzel, 2000; Pop *et al.*, 2000; Peñuelas & Filella, 2001; Linderholm, 2006).

Algunos autores también han puesto de manifiesto que la aparición anticipada de hojas y brotes en algunas especies, especialmente de aquellas distribuidas en zonas más frías, podría conllevar un mayor riesgo de daños por **heladas** tardías (Kellomaki *et al.*, 1995; Walther *et al.*, 2002).

En el **tercer capítulo** de esta tesis, me he planteado si las especies dominantes de los seis matorrales europeos estudiados podrían aprovechar unas condiciones más cálidas para comenzar a crecer antes. Las especies que he estudiado son *Calluna vulgaris*, *Empetrum nigrum* y *Vaccinium myrtillus* en Reino Unido, *C. vulgaris* en Holanda, *C. vulgaris* y *Deschampsia flexuosa* en Dinamarca, *Populus alba* en Hungría, *Cistus monspeliensis* en Italia y *Erica multiflora* y *Globularia alypum* en España. Aunque hemos estudiado tanto los efectos de los tratamientos de calentamiento como los de la sequía, en este capítulo sólo he incluido los resultados que hacen referencia al calentamiento al que la fenología ha sido mucho más sensible.

La combinación de manipulaciones experimentales, con el estudio de diferentes matorrales europeos y además con el estudio de diferentes especies coexistentes en un mismo matorral en varios años, nos permitirá inferir si las posibles respuestas al calentamiento dibujan un **gradiente** de mayor a menor intensidad de norte a sur o si en cambio las respuestas son específicas o dependientes de las especificidades ambientales que se den cada año.

Planta de *Erica multiflora* con nuevos brotes.

Por otra parte, así como existe un amplio acuerdo en el avance que produce el calentamiento en las fenofases que tienen lugar en primavera, no lo hay tanto en relación a la señal ambiental que conduce las fenofases que ocurren en **otoño** (caída de hojas, crecimiento y floración) y que en algunos casos son más difíciles de "monitorizar" (Worrall, 1999; Walther *et al*., 2002; Sparks & Menzel, 2002).

La mayoría de especies de plantas mediterráneas aprovechan la primavera para crecer y florecer y así evitan el calor y sequía del verano y el frío del invierno. Pero hay excepciones como *Erica multiflora* y *Globularia alypum*, las especies de arbustos dominantes en las parcelas situadas en Garraf (Barcelona), que separan estas fenofases y pueden crecer tanto en primavera como en otoño (aunque el primero es mucho más importante) y florecer en otoño-invierno (Llorens *et al*., 2004b; Llorens & Peñuelas, 2005). Aprovechado esta característica, en el **cuarto capítulo** de esta tesis he estudiado qué efectos podrían tener un aumento de temperatura y una sequía más acentuada sobre la fenología de la floración de estas especies.

Algunos estudios han mostrado el importante rol que tiene la temperatura en la **floración no primaveral** de especies mediterráneas (Nilsen & Muller, 1981; Picó & Retana, 2001, Peñuelas *et al*., 2002; Jato *et al*., 2004; Ogaya & Peñuelas, 2004). Sin embargo, las especies que se estudian florecen justamente después del verano, lo que hace suponer que las lluvias de final de verano y de otoño pueden tener una gran influencia en esta fenofase.

Estudios con plantas mediterráneas han observado que una reducción en la disponibilidad de agua puede ocasionar **retrasos** en el momento de floración y **reducciones** en la producción de flores y frutos, lo que puede afectar a medio plazo al mantenimiento de la propia población, la relación con los polinizadores y el aporte alimenticio a herbívoros (Gordon *et al*., 1999; Ogaya & Peñuelas, 2004; Peñuelas *et al*., 2004; Llorens & Peñuelas, 2005; Giménez-Benavides *et al*., 2007).

Además, **diferentes respuestas** al cambio climático de las especies de plantas coexistentes en una comunidad pueden dar lugar a cambios en la estructura y funcionamiento de los ecosistemas debido a cambios en las relaciones inter-específicas de competencia o a la pérdida de sincronización con otras especies como los polinizadores o los herbívoros (Corlett & Lafrankie, 1998; Hughes, 2000; Peñuelas & Filella, 2001; Fitter & Fitter, 2002; Gordo & Sanz, 2005).

Detalle de una flor de *Globularia alypum*.

- DIVERSIDAD DE ESPECIES VEGETALES

Cambios en la presencia y abundancia de las distintas especies coexistentes en una comunidad en respuesta a variaciones en el clima pueden dar lugar a cambios en el funcionamiento del matorral, expresado por ejemplo como **productividad** del ecosistema (Tilman *et al.,* 1997; Hector *et al.,* 1999; Pfisterer & Schmid, 2002; Symstad *et al.,* 2003). En dos de los artículos de esta tesis he estudiado los cambios en la **diversidad** de plantas que podrían sufrir los matorrales bajo unas condiciones más cálidas y secas.

En el **primer capítulo** de la tesis me centro en los efectos sobre el número de especies vegetales presentes (riqueza) en los seis matorrales que forman parte del proyecto europeo. La hipótesis acerca de los efectos que tendrían los tratamientos es que éstos serían más intensos en los ecosistemas menos maduros donde se esté dando un mayor reclutamiento de nuevos individuos y especies.

La zona de Garraf sufrió dos incendios en 1982 y 1994 que transformaron una comunidad dominada por *Pinus halepensis* en un matorral dominado por *Erica multiflora* y *Globularia alypum*. En 2005, el último año de toma de datos para esta tesis, esta comunidad todavía estaba aumentando su cobertura vegetal, todavía estaba reclutando más especies y todavía los arbustos rebrotadores ganaban en abundancia a *Pinus halepensis.* Es decir, el matorral de estudio era una comunidad que estaba aún en un proceso de **recuperación**. La vegetación mediterránea tiene una gran capacidad para recuperar su estructura y composición específica después de los incendios y se le considera una **autosucesión** puesto que la mayoría de las especies que existían antes del fuego pueden rebrotar o germinar después del fuego (Tárrega *et al.,* 1997; Ojeda *et al.,* 1996; Lloret *et al.,* 2002; Lloret, 1998; Pausas & Verdú, 2005).

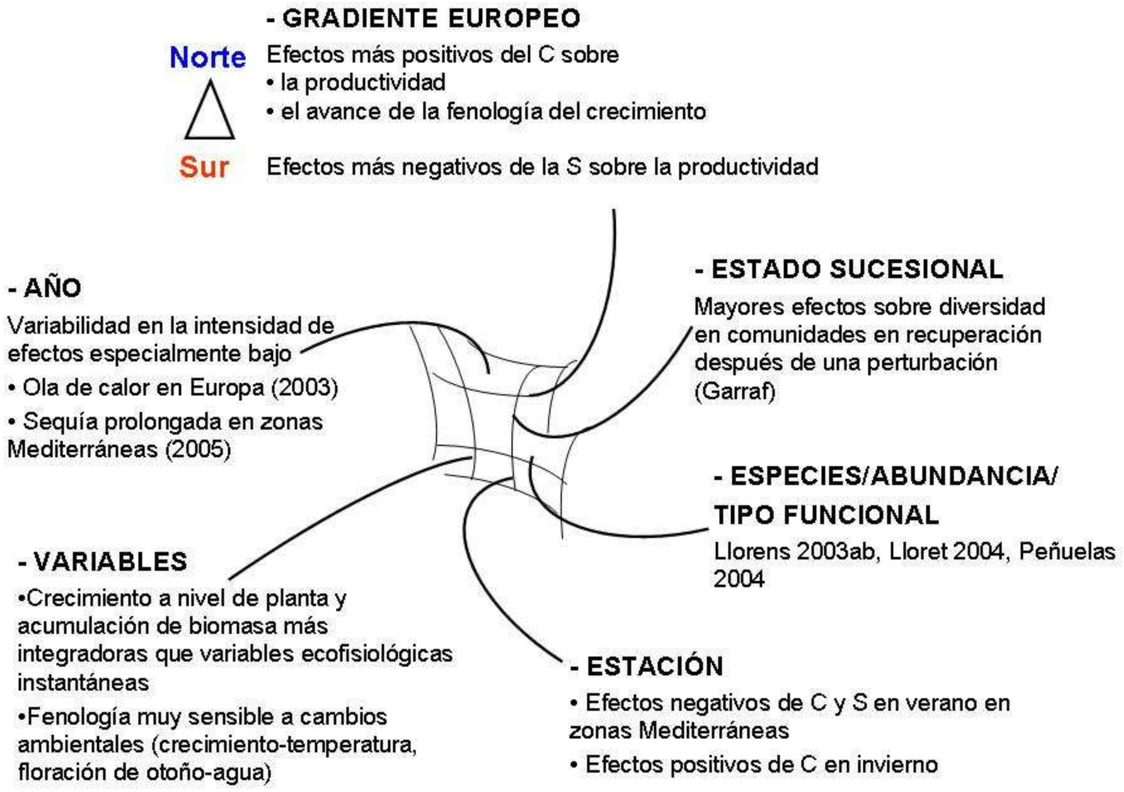
Sin embargo, la capacidad de **resiliencia** de los ecosistemas mediterráneos frente a una perturbación (la capacidad de volver a las condiciones iniciales) puede verse comprometida bajo unas condiciones más cálidas y secas, si estas afectan por ejemplo, al reclutamiento de plántulas, la capacidad de rebrote o la *performance* (tasa de crecimiento, floración) de los individuos establecidos (Glenn-Lewin *et al.*, 1992; Bazzaz, 1996, Riba, 1997; Lloret *et al.*, 1999; Llorens *et al.*, 2004; Lloret *et al.*, 2004; Llorens *et al.*, 2005). Como consecuencia, bajo unas condiciones climáticas cambiantes la colonización de especies justo después de la perturbación y el **proceso sucesional** posterior puede dar lugar al ensamblaje de una comunidad diferente.

Se han publicado muchos estudios sobre los efectos del cambio climático sobre la diversidad en diferentes tipos de comunidades (Kutiel *et al.*,. 1998; Stenberg *et al.*, 1999; Van Peer *et al.*, 2001; Van der Meer *et al.*, 2002; Thuiller, 2003; Hollister *et al.*, 2005) y otros tantos sobre los patrones de diversidad de plantas después del fuego (Moreno & Oechel, 1991; Ojeda *et al.*, 1996; Kutiel, 1997; Tárrega *et al.*, 1997; Ne'eman & Izhaki, 1999; Lloret *et al.*, 2003; Daskalakou & Thanos, 2004). En el **quinto capítulo** de esta tesis he aunado estas dos cuestiones y me he planteado qué efectos podrían tener el calentamiento y la sequía sobre la diversidad de especies vegetales pero bajo la perspectiva de sucesión vegetal después de una perturbación.

Las respuestas de las especies coexistentes a estos cambios ambientales pueden ser específicas, aunque algunos autores han observado que pueden ser respuestas ligadas a la **abundancia** de la especie (Tilman & El Haddi, 1992; Harte & Shaw, 1995; Smith & Knapp, 2003; Symstad *et al.*, 2003; Lloret *et al.*, 2004) o a la posesión de algún **carácter funcional** (Tilman & El Haddi 1992; Klanderud & Totland, 2005; Thuiller *et al.*, 2006).

- HIPÓTESIS

En cada uno de los capítulos se ha pormenorizado las hipótesis sobre los efectos de los tratamientos experimentales de calentamiento (C) y sequía (S) en los matorrales europeos y particularmente en el matorral mediterráneo de Garraf (Barcelona-España). A modo de resumen, se ha tenido en cuenta que los efectos pueden depender de los diversos factores que se exponen a continuación, así como de la interacción entre ellos.



- GRADIENTE EUROPEO

Norte Efectos más positivos del C sobre
- la productividad
- el avance de la fenología del crecimiento

Sur Efectos más negativos de la S sobre la productividad

- AÑO

Variabilidad en la intensidad de efectos especialmente bajo
- Ola de calor en Europa (2003)
- Sequía prolongada en zonas Mediterráneas (2005)

- ESTADO SUCESIONAL

Mayores efectos sobre diversidad en comunidades en recuperación después de una perturbación (Garraf)

- ESPECIES/ABUNDANCIA/ TIPO FUNCIONAL

Llorens 2003ab, Lloret 2004, Peñuelas 2004

- VARIABLES

- Crecimiento a nivel de planta y acumulación de biomasa más integradoras que variables ecofisiológicas instantáneas
- Fenología muy sensible a cambios ambientales (crecimiento-temperatura, floración de otoño-agua)

- ESTACIÓN

- Efectos negativos de C y S en verano en zonas Mediterráneas
- Efectos positivos de C en invierno

En el marco de la investigación presentada sobre los efectos del cambio climático en el matorral mediterráneo, también he participado en la realización de otros estudios:

Asensio D, Peñuelas J, Llusià J, **Prieto P**, Estiarte M, Filella I. *Interannual and seasonal changes in the soil Exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland* (enviado a International Journal of Soil Science).

Lloret F, Peñuelas J, **Prieto P**, Llorens L, Estiarte M. *Plant community changes induced by experimental climate change: seedling and adult species composition* (enviado a Journal of Ecology).

Llusià J, Peñuelas J, **Prieto P**, Estiarte M. *Seasonal contrasting changes of emission rates of isoprenoids in two dominant species of a Mediterranean shrubland submitted to a field experimental drought and warming* (enviado a Russian Journal of Plant Physiology).

Sardans, J, Peñuelas, J, **Prieto P**, Estiarte, M. *Changes in Ca, Fe, Mg, Mo, Na and S content in a Mediterranean shrubland submitted to an experimental drought and warming* (enviado a Environmental and Experimental Botany).

Sardans, J, Peñuelas, J, **Prieto P**, Estiarte, M. *Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland* (enviado a Global Change Biology).

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CHAPTER 1.

Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought. Reductions in primary productivity in the heat and drought year of 2003.

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ABSTRACT

We used a non-intrusive field experiment carried out at six sites -Wales (UK), Denmark (DK), the Netherlands (NL), Hungary (HU), Sardinia (Italy-IT) and Catalonia (Spain-SP)- along a climatic and latitudinal gradient to examine the response of plant species richness and primary productivity to warming and drought in shrubland ecosystems. The warming treatment raised the plot daily temperature by ca. 1 ºC, while the drought treatment led to a reduction in soil moisture at the peak of the growing season that ranged from 26 % at the Spanish site to 82 % in the Netherlands. During the seven years the experiment lasted (1999-2005), we used the pin-point method to measure the species composition of plant communities and plant biomass, litterfall, and shoot growth of the dominant plant species at each site.

A significantly lower increase in the number of species pin-pointed per transect was found in the warming plots and, especially, in the drought plots at the Spanish site, where the plant community was still in a process of recovering from a forest fire in 1994. No changes in species richness were found at the other sites, which were at a more mature and stable state of succession and thus less liable to recruitment of new species. The relationship between annual biomass accumulation and temperature of the growing season was positive at the coldest site and negative at the warmest site. The warming treatment tended to increase the aboveground net primary productivity (ANPP) at the northern sites. The relationship between annual biomass accumulation and soil moisture during the growing season was not significant at the wetter sites, but was positive at the drier sites. The drought treatment tended to reduce the ANPP in the NL, HU, IT, and SP sites. The responses to warming were very strongly related to the Gaussen aridity index (stronger responses the lower the aridity) whereas the responses to drought were not. Changes in the annual aboveground biomass accumulation, litterfall, and, thus, the ANPP, mirrored the inter-annual variation in climate conditions: the most outstanding change was a decrease in biomass accumulation and an increase in litterfall at most sites during the abnormally hot year of 2003. Species richness also tended to decrease in 2003 at all sites except the cold and wet UK site.

Species-specific responses to warming were found in shoot growth: at the Spanish site, *Globularia alypum* tended to grow less, while the other dominant species, *Erica multiflora*, grew 30 % more; at the UK site, *Calluna vulgaris* tended to grow more in the warming plots, while *Empetrum nigrum* tended to grow less. Drought treatment decreased plant growth in several studied species, although there were some species such as *Pinus halepensis* at the SP site or all the species at the UK site that were not affected.

The magnitude of responses to warming and drought thus depended greatly on the differences between sites, years, and species and these multiple plant responses may be expected to have consequences at ecosystem and community level. Decreases in biodiversity and the increase in *E. multiflora* growth at the Spanish site as a response to warming challenge the assumption that sensitivity to warming may be less well developed at more southerly latitudes; likewise, the fact that one of the studied shrublands presented negative ANPP as a response to the 2003 heat wave also challenges the hypothesis that future climate warming will lead to an enhancement of plant growth and carbon sequestration in temperate ecosystems. Extreme events may thus change the general trend of increased productivity in response to warming in the colder sites.

Key words: Biodiversity, climate change, drought, European gradient, forest-steppe, global warming, heathland, heat wave, litterfall, machia, net primary productivity, plant biomass, plant growth, shrubland, species richness.

INTRODUCTION

Global air temperatures have increased by 0.7 ºC during the twentieth century and are predicted to increase by between 1.1 and 6.4 ºC during the twenty-first century, with the greatest increases expected to occur at more northerly latitudes (IPCC, 2007). These increases will also be accompanied by changes in precipitation patterns that are much more difficult to forecast. However, many models predict that summer droughts in some central and southern European regions will become more prevalent (IPCC, 2007; Peñuelas *et al*., 2005).

Over the last decade the need for information regarding the physiological, ecological and evolutive response of organisms and ecosystems to climate change has been addressed by a growing number of observational and temperature- and precipitation-manipulation experiments around the world. As a result, a substantial body of data now exists that demonstrates the link between changes in regional climate and alterations in biological processes in ecosystems. Arft *et al*. (1999), Shaver *et al*. (2000), Peñuelas & Filella (2001), Rustad *et al*. (2001), Walther *et al*. (2002), Parmesan & Yohe (2003), Root *et al*. (2003) and others have reviewed many of these experiments. More recently we reported variable responses in plants and ecosystems to experimental warming and drought along a north-south gradient in European shrublands (Beier *et al*., 2004; Emmet *et al*., 2004; Llorens *et al*., 2004; Peñuelas *et al*., 2004).

The responses of plant productivity and other ecosystem processes to climate change are quite variable and increases, decreases, or no change at all have all been reported (see references in Rustad *et al*., 2001; Peñuelas *et al*., 2004). However, there seem to be some regularities such as a greater positive response of aboveground plant productivity to warming in colder ecosystems (Rustad *et al*., 2001). Furthermore, Antle *et al*. (2001) assembled a database of more than 2,500 studies that address climate and related physical or biological processes and found that 80 % of the studied species undergoing change were shifting in response to warming in the direction expected on the basis of known physiological constraints of species, while 20 % were changing in the opposite direction. There is also a large body of observational, satellite, and atmospheric data regarding plant species and ecosystems that shows clear biological responses to warming such as extended growing seasons and altitudinal and northward movement of species' distributions in both northern and southern, cold-wet and warm-dry ecosystems (Myneni *et al.*, 1997; Peñuelas & Filella, 2001; Peñuelas *et al.*, 2001, 2002; Walther *et al*., 2002; Parmesan & Yohe, 2003; Peñuelas & Boada, 2003; Root *et al.*, 2003, Menzel *et al*., 2006). Ecosystem processes and biological species may respond asymmetrically to climatic changes (Walther *et al*., 2002) and the overall effect on ecosystem functioning and species richness is therefore often highly complex and determined by the relative sensitivity of the different processes to climate change.

We conducted a non-intrusive field experiment (Beier *et al*., 2004; Peñuelas *et al*., 2004) for seven years at six sites from northern to southern Europe (UK, Denmark, Netherlands, Hungary, Italy, and Spain) to examine the response of plant species richness and aboveground primary productivity to warming and drought along a climatic and latitudinal gradient of shrubland ecosystems. The aim was to study the effects of the climatic changes expected to occur over the coming decades (IPCC, 2007; Peñuelas *et al*., 2005) and, additionally, we analyzed the effects of the 2003 heat wave. Our initial hypotheses were that plant primary productivity responses to warming would be greater in colder northern latitudes and the responses to drought greater in drier southern latitudes, since higher latitude ecosystems are typically limited by temperature (Chapin *et al*., 1995) and lower latitude Mediterranean and continental ecosystems by drought (Mitrakos, 1980; Peñuelas *et al*., 2001, Terradas, 2001, Kovács-Láng *et al.*, 2000). We also hypothesized that effects such as species-specific responses and the reduced number of recruited seedlings in warming and, above all, in drought plots found at some sites in a previous study (Lloret *et al*., 2004) would thus tend to lead to decreased species richness, especially at those sites still recovering their species-richness after a severe disturbance (for example, the serious fire that occurred five years prior to the start of the experiment at the Spanish site) by progressively recruiting new species. The experimental approach was specifically chosen to overcome some of the main drawbacks found in previous studies such as the over-intrusiveness of some of the methodologies used, the constant or diurnal rather than nocturnal manipulation of warming, the predominance of studies in cold and wet temperate and Arctic ecosystems, the lack of across-the-board protocols, and temporal (our results are the fruit of seven-years study instead of the two years in our previous study) limitations (Beier *et al*., 2004; Peñuelas *et al*., 2004). To approach vegetation (and ecosystem) response at larger spatial and temporal scales, we examined local studies across a natural climatic and environmental gradient along a transect from northern to southern Europe. With this geographical approach, we aimed to take into account long-term responses of vegetation to environmental conditions (Chapin *et al*., 1995; Shaver *et al*., 2000; Rustad *et al*., 2001). Long-term studies allow the natural inter-annual variability as a result of temporal variability in climatic conditions to be analyzed, thereby avoiding the often initially stronger transient response to treatments (Arft *et al*, 1999).

MATERIAL AND METHODS

Sites and manipulations

Experimental manipulations were carried out in shrublands at six European sites, in Wales (UK), in an atlantic heathland, Denmark (DK), in an atlantic heathland/grassland, the Netherlands (NL), in an atlantic heathland, Hungary (HU), in a continental forest-steppe, Sardinia (Italy-IT), in a mediterranean shrubland, and in Catalonia (Spain-SP) also in a mediterranean shrubland, spanning both a temperature and precipitation gradient, and an aridity gradient (Fig. 1) (Beier *et al*., 2004).

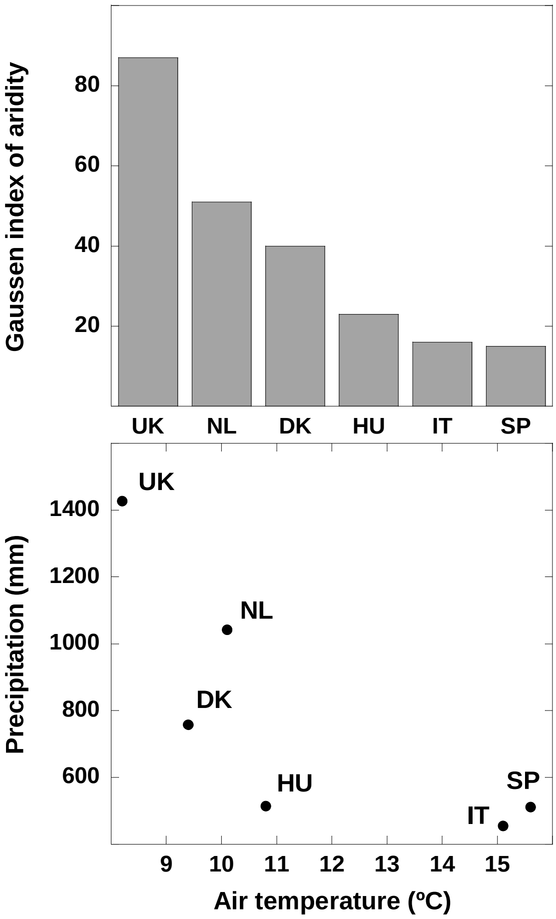

Figure 1. Gaussen index of aridity (precipitation/2temperature), mean annual precipitation, and mean annual air temperature at the studied shrubland study sites in Wales (UK), Netherlands (NL), Denmark (DK), Hungary (HU), Sardinia-Italy (IT), and Catalonia- Spain (SP).

At each site, we imposed field-scale nighttime warming, drought and control treatments and the response to the treatments was compared to control plots. Plots were 4x5 m^2, allowing for a buffer strip of 0.5 m at the perimeter. Each type of manipulation was replicated three times at each site. Pre-treatment measurements were conducted in order to identify variability between sites and plots.

Warming treatment

The warming treatment consisted of nighttime warming provided by reflective curtains covering the vegetation at night (Beier *et al.*, 2004). Solar energy is accumulated in the ecosystem during the day and part of this energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem with reflective aluminum foil curtains (ILS ALU, AB Ludvig Svensson, Sweden) reduces the loss of IR radiation. These curtains reflect 97 % of the direct and 96 % of the diffuse radiation. The warming plots are 20 m^2 (5x4 m) and are framed by light scaffolding that supports the reflective aluminum curtain approximately 20 cm above the top of canopy. This curtain is activated automatically by pre-established light (< 200 lux), rain, and wind (< 10 m s^{-1}) conditions (Beier *et al.*, 2004). The curtains reduced the heat loss during night by 64 %, from 33 W m^{-2} to 12 W m^{-2}, and increased the temperature of the soil and plants by 0.5-1.5 ºC (DK), 0-2 ºC (UK), 0-1 ºC (NL), 1-2 ºC (SP), 0.3-1.3 (HU), and 0.2-0.6 °C (IT). These temperature increases are in the range predicted by the next 20-30 years by Global Circulation Models (IPCC 2007). The magnitude of the warming effect depended on the site, time of the year, and meteorological conditions (Beier *et al.*, 2004). These moderate increases in temperature raised the average annual growth potential (Growing Degree Days - GDD) by 3-16 % at the non-Mediterranean sites. Overall at the six sites the number of days with frost was reduced by 19-44 % (Beier *et* *al.,* 2004). In order to avoid unwanted effects on the hydrological cycle, rain sensors automatically trigger curtain removal during rainfall. This warming treatment has been applied since spring 1999 (since 2001 in HU and IT).

This method has the advantage that unintended edge effects and artifacts are minimized. Measurements of curtain movements, temperatures, precipitation, water input into the plots, radiation balance during campaigns, relative humidity, and wind speed showed that the edge effects on the temperature increase, as well as unintended effects on wind and moisture conditions, were minimal. Since nighttime warming implies leaving the plots open during daytime, the effect on light conditions was negligible (Beier *et al.*, 2004).

Drought treatment

The drought treatment, which consisted of covering the vegetation with transparent waterproof covers, was put into practice for two months every year between 1999 to 2005 in the spring/summer growing seasons (in the Spanish and Italian sites an additional drought period was established in the autumn growing season; Beier *et al.*, 2004). The drought plots are constructed in a similar way to the warming plots except for the fact that the curtains are made of transparent plastic and are only drawn in case of rain and wind. During the drought period the rain sensors activate the curtains whenever it rains and remove them again once the rain stops. The curtains are also removed automatically if the wind speed exceeds 10 m s^{-1}. For the part of the year without the drought treatment, the drought plots were managed in the same way as the control plots. Decreases in soil moisture during the drought treatment ranged from 82 % in the Netherlands to 26 % in the Spanish site; air and soil temperatures were not affected. For the rest of the year, the drought treatment was not applied and so the received precipitation was not directly affected (Beier *et al*., 2004). Despite this soil moisture in the drought plots never completely recovered to the control levels.

Untreated control

Three untreated plots with similar light scaffolding as in the warming and drought plots but without curtains were set up as controls.

Plant response

We conducted yearly monitoring of plant responses to the above-described climate manipulation treatments and measured the following variables in all the experimental plots: plant-community species richness, plant-community cover and biomass, litterfall, aboveground net primary productivity, and individual plant growth. The objective was to assess relative changes in plant performance between treatment types for the different sites, years, and species.

Species richness, plant cover and biomass (pin-point method), litterfall, and aboveground net primary productivity

The pin-point method was used for measuring plant frequency and for indirectly estimating plant cover and biomass. Pin-point measurements were conducted annually at each site in July-August, although in Italy measurements were taken at the end of the growing season (September-May), at the beginning of June. Vegetation response is assessed by noting species types and calculating plant contacts and heights at a large number of points in each plot. This method enables, moreover, the assessment of changes in species composition and species-specific growth to be recorded. A sharpened

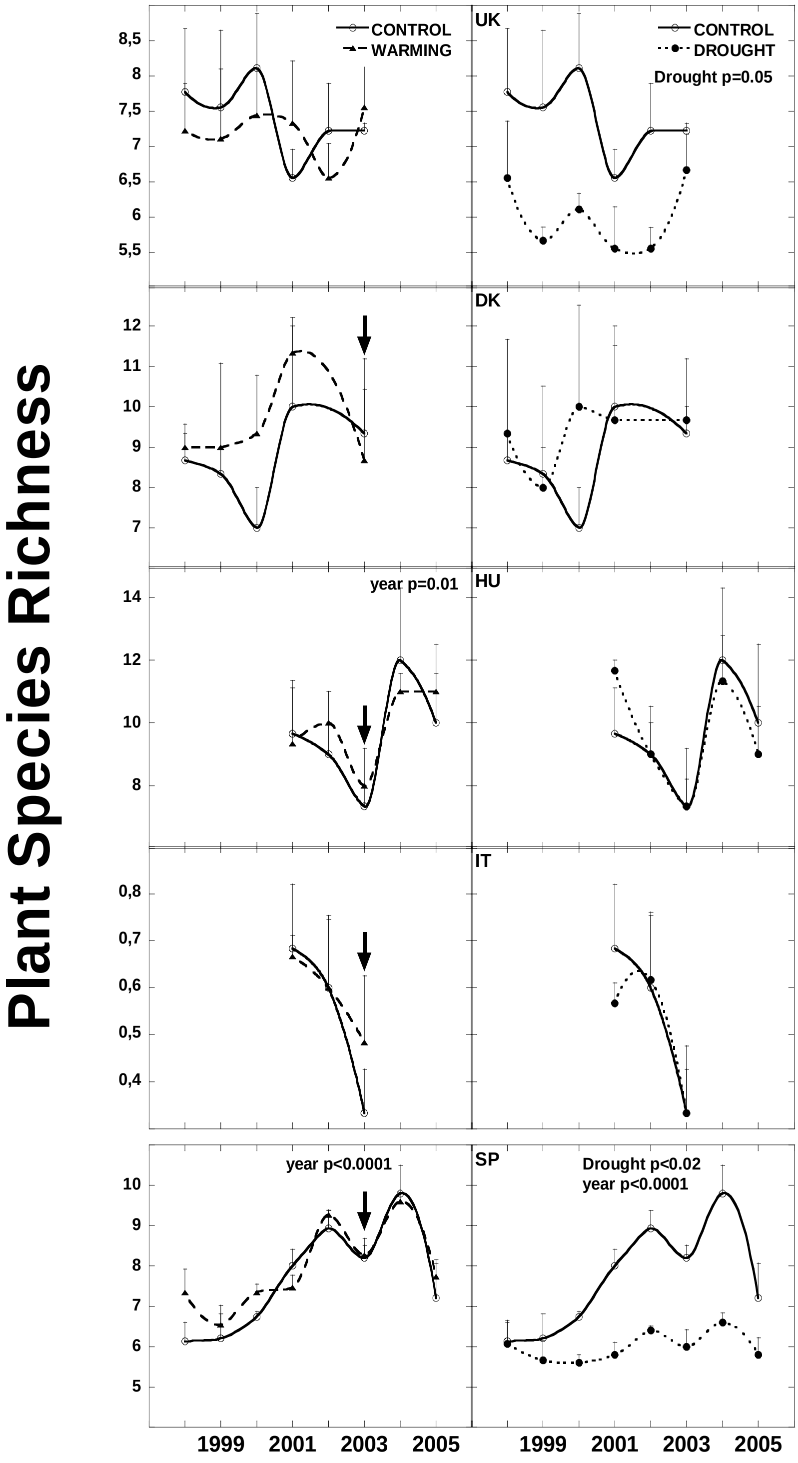

Figure 2. Changes in plant species richness - number of species per 3-m transect (SP and HU), per m^2 (IT), or per 0.5x0.5m subplot (UK and DK)- in response to warming and drought treatments in the period between 1998 (before the experimental treatments were applied) and the years of the experiment (1999-2005) in well developed shrubland sites in UK, DK, HU, and IT, and in a typical shrubland recovering from 1994 fire (SP). Whenever significant (repeated measures ANOVA), the effects of the studied factor or interaction are depicted in the corresponding panel. An arrow signals the decreases in 2003 in all sites but the UK site.

pin was lowered through the vegetation with a minimum of 300 measurement points for each experimental plot. These points were arranged at 5 cm intervals along replicated five 3-m long transect lines (in Spain and Hungary) or four 4-m long transect lines (in the Netherlands and Italy) or in 0.5x0.5 m subplots (in the UK and Denmark). Each plant hit with the pin was counted and the plant species (or species group) recorded. At some of the sites the height above the ground was also recorded for each hit by means of 1-cm marks along the pin or along a vertical rod held behind the pin in a way that did not disturb the vegetation. We recorded the total number of hits for each species (and for each plant part of each species, if relevant) per transect and also the total number of hits as a proportion of all pin hits per transect. We also calibrated the pin-point measurements against absolute biomass using destructive sampling outside the plots. In each site biomass was regressed against hit numbers (or proportions) or, if hit heights were recorded, against heights.

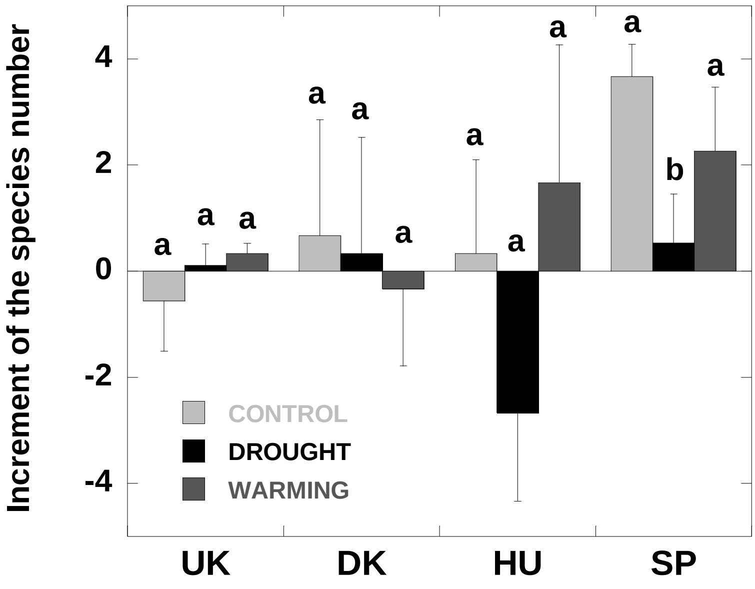

Figure 3. Change in number of species per transect or subplot (see Fig. 2 caption), in control, drought, and warming treatments at the UK, DK, HU, and SP sites after 5-7 years of experiment in the period 1998-2005. Bars indicate the standard errors of the mean (n=3 plot means).

In each plot we placed litterfall collectors (between 5 and 30 -depending on the site and the traits of the corresponding vegetation- small pots with small holes allowing for drainage). These collectors ranged in diameter from 15 to 4.4 cm and were randomly placed under the plant canopy (or below *E. multiflora, G. alypum,* and *Populus alba* plants in the scattered vegetation of Spain and Hungary). We retrieved the litter monthly (or every two or six months, depending on the site) and then oven-dried it at 60 ºC until constant weight. Litterfall from grasses (DK) was estimated by harvesting the standing dead biomass once a year from a 0.5 m^2 plot since the litter is not shed from the grasses which were a dominant part of the vegetation.

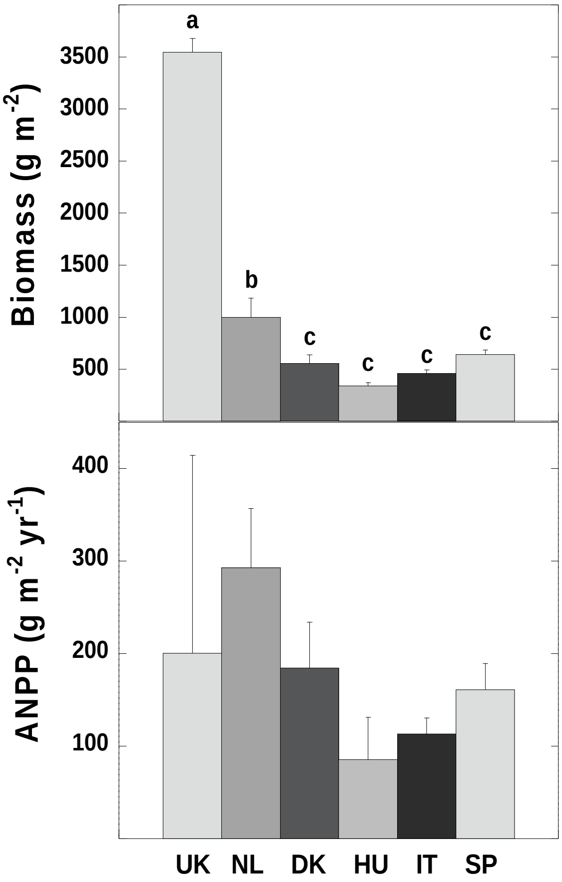

Figure 4. Total aboveground plant biomass and annual aboveground net primary productivity in the control plots at the UK, NL, DK, SP, HU, and IT sites. Data are from control plots in 2002. Bars indicate the standard errors of the mean (n=3 plots). Different letters indicate significantly different means (Bonferroni post-hoc test of the ANOVA).

Aboveground net primary productivity (ANPP) was estimated as the sum of the change in total aboveground biomass and litterfall. In DK, the aboveground net primary production is equal to the litterfall as the aboveground biomass of grasses and herbs senesce during the autumn.

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Individual plant growth

The pin-point method does not provide a direct calculation of yearly plant growth at the species or individual level (although the frequency of hits is affected by plant growth and was also used to indirectly assess net aboveground biomass change). Additional direct measurement of individual plant growth was obtained in the UK, HU, IT and SP sites (United Kingdom, Hungary, Italy, and Spain) by marking shoots and measuring them at intervals. A sample of 20-30 terminal shoots of the dominant site species were permanently marked in each plot (in the Hungarian site all shoots of 10 randomly selected shrubs per plot were measured). The length of each shoot was measured and plant growth estimated by using allometric relationships between biomass and shoot lengths obtained from a destructive sampling of plants from outside the plots.

Statistical analyses

We first conducted repeated measures ANOVAs and Bonferroni post-hoc comparison tests with each measured plant variable as a dependent variable and the country and treatment as fixed factors. We used only the mean value of each variable for each plot, so that n = 3 per treatment, because we considered the roof/plot as the smallest independent unit. We used as many sub-plots/replicate samples as possible (usually three or more with a maximum of 30, depending on the variable being measured) to provide a good mean estimate for each plot.

General regression models were built with plant biomass, litterfall, and individual plant growth as dependent variables, site, year, and species as fixed factors, and temperature, soil moisture, and precipitation during the growing season (i.e., the final three months, except in Italy, where we took into account the whole growing season from September to May) as independent variables. Differences with a p-value < 0.05 were considered as statistically different. Analyses were performed with the Statview software package (Abacus Concepts Inc., Cary, North Carolina, USA) and the *Statistica* software package (StatSoft, Inc. Tulsa, Oklahoma).

RESULTS

Species richness

The number of species pin-pointed per transect was lower in the drought plots than in the control plots (it was also lower in the warming plots than in the control plots although the difference was statistically not significant) at the SP site (Fig. 2 & 3). At this site, the plant community was recovering from a forest fire in 1994. During the six-year study period between 1999 and 2004, drought plots recruited less than one new species per transect and warming plots a little more than 2 new species, whereas control plots recruited more than three new species per transect (Fig 2 & 3). No significant changes due to warming or drought were found either at the other Mediterranean site (IT) or at the continental and northern sites (Fig. 2 & 3), which were already in a more stable state of succession.

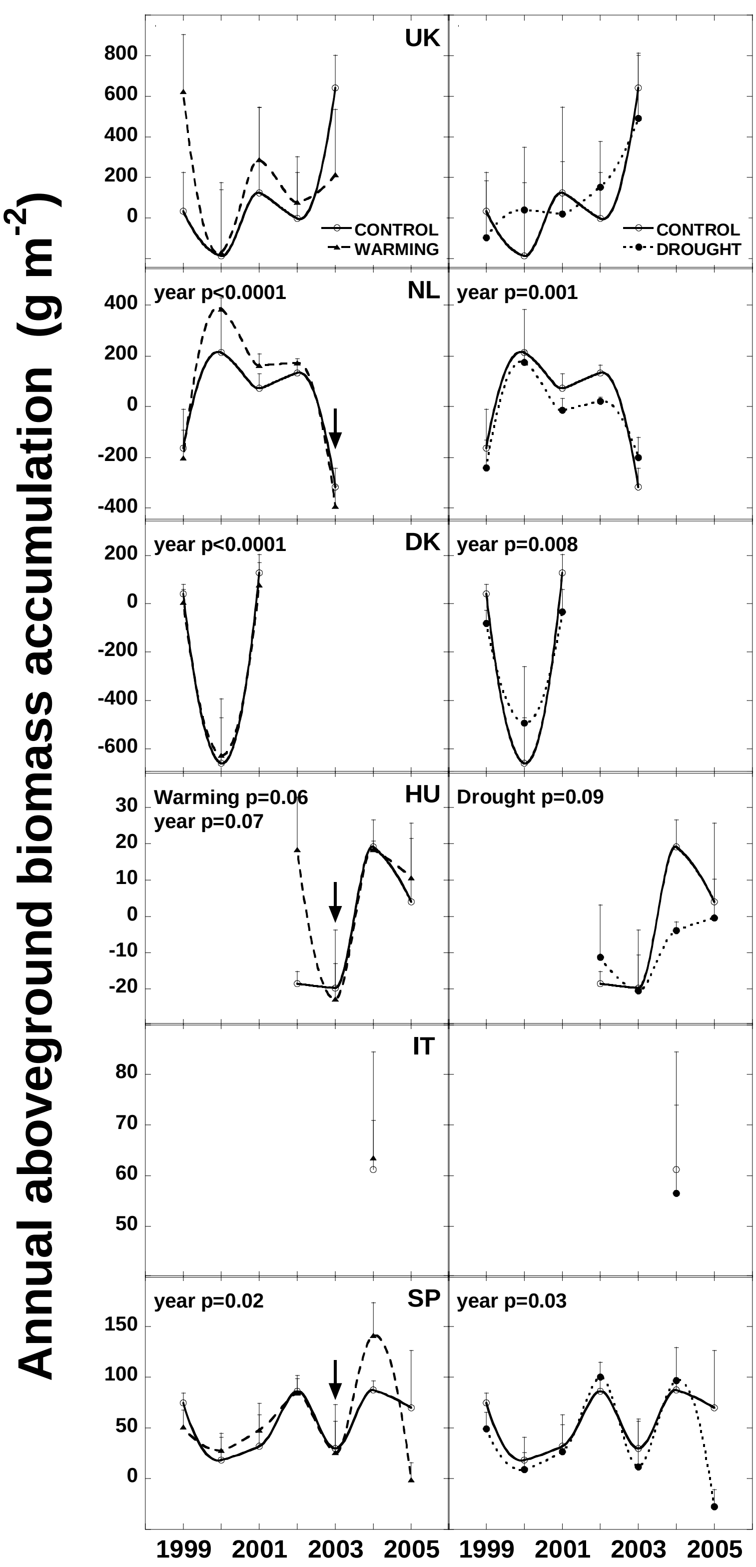

Figure 5. Changes induced by the warming and drought treatments in the annual plant aboveground biomass accumulation throughout the study period (1999-2005). Bars indicate the standard errors of the mean (n=3 plots). In DK site there was a heather beetle attack in 1999-2000 (Peñuelas et al 2004). When significant, the effects of the studied factor or the interaction are depicted in the corresponding panel. An arrow signals the decreases in 2003 in all sites but the UK site.

There was also a decrease in the number of species detected in 2003 at all sites apart from the UK (Fig. 2), a consequence of the especially hot and dry weather conditions of that year. A similar decrease was also noted in another very dry year in SP, 2005 (Fig. 2).

Community plant biomass

Plant biomass calculated from allometric relationships between pin-point measurements and biomass was significantly greater at the UK site (3500 g m^{-2}) than at the Dutch site (ca. 1000 g m^{-2}) and all the other sites (ca. 500 g m^{-2}) (Fig. 4).

The ca. 1 ºC experimental warming led to an increase of 69 % in plant aboveground biomass accumulation during the five years of experimentation at the UK site and of 72 % during the three years (2000-2002) with positive biomass accumulation at the Dutch site (Fig 5). The warming treatment increased the total plant biomass at the northern sites, UK and NL, and at the continental site, HU, but when the responses during the unusually hot year of 2003 were included, the global effect of warming was not significant except slightly in HU (Fig. 5). The drought treatment tended to reduce biomass accumulation at the NL, HU, and SP sites, but again, if the whole study period is taken into account, the overall reduction was only slightly significant in HU (Fig. 5).

The annual aboveground biomass accumulation (Fig. 5) followed the inter-annual variation in climate conditions and moreover presented a significant interaction for site x year ($F=3.89$, $p<0.01$). However, the most outstanding change was the decrease in biomass accumulation at all sites except for the UK in the hot year of 2003, with negative accumulation values at the NL and HU sites. The UK site, on the other hand, accumulated a greater amount of biomass than usual (Fig. 7) as a likely result of increased nutrient availability as soil microbes are released from oxygen limitation caused by excess of water.

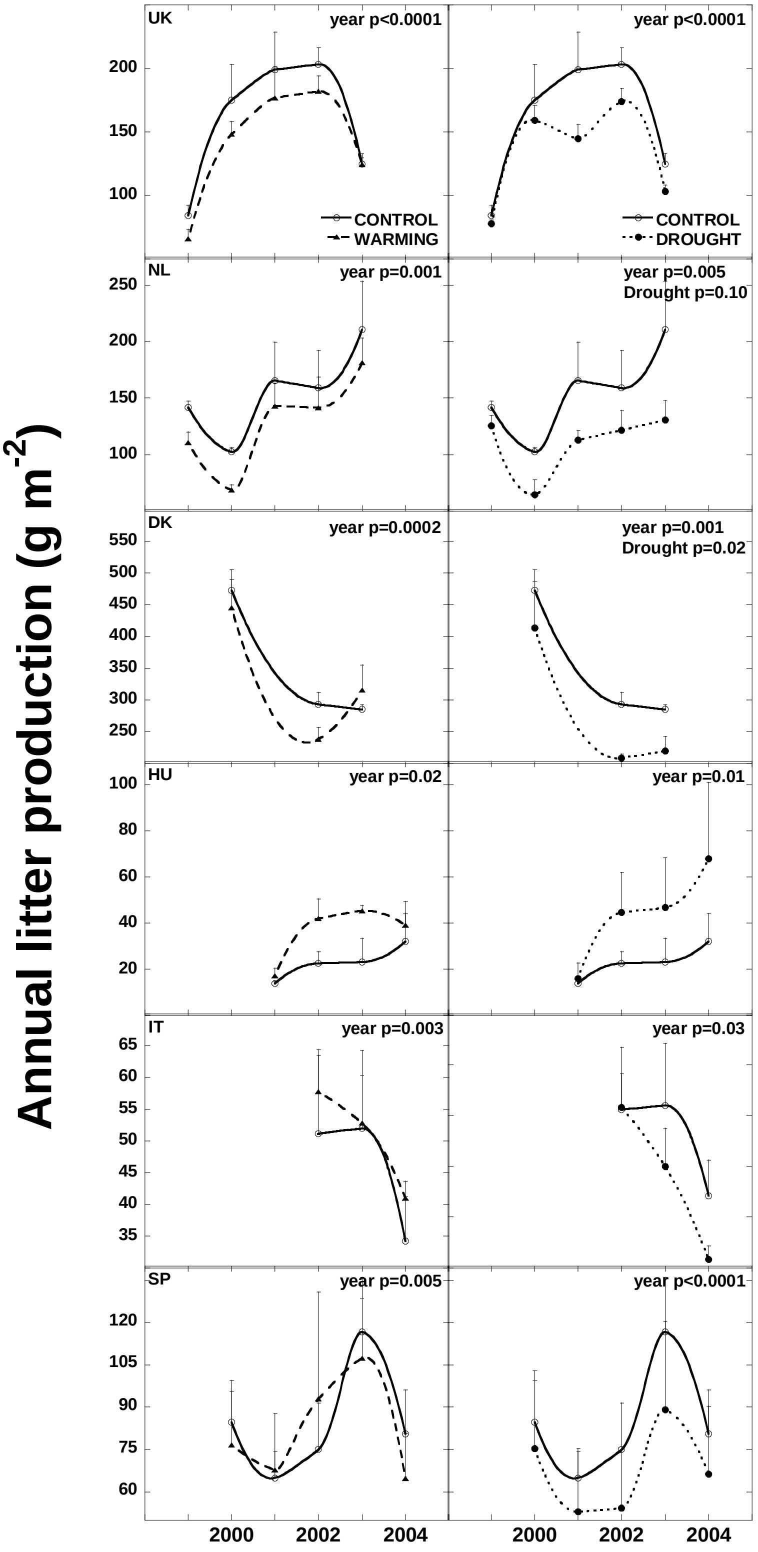

Figure 6. Changes induced by the warming and drought treatments on amounts of annual litterfall throughout the study period (1999-2005). Bars indicate the standard errors of the mean (n=3 plots). In DK site there was an attack by heather beetles in 1999-2000 (Peñuelas *et al.* 2004). Whenever significant, the effects of the studied factor or interaction are depicted in the corresponding panel.

Litterfall

There was less plant litterfall at the southern sites (ca. 50 g m^{-2} at the IT site and ca. 100 g m^{-2} in the SP site) than at the northern sites (ca. 150 g m^{-2} at the NL and the UK sites) (Fig.6), a logical consequence of their smaller plant biomass (Fig. 4).

The warming treatment tended to decrease (ca. 15 %) the amount of litterfall at the northern sites, tended to increase it at the HU site, and had no effect at the Mediterranean sites (Fig. 6). The drought treatment tended to decrease litterfall at all sites by ca. 25 %, the only exception being the HU site, where the drought treatment tended to increase litterfall (Fig. 6). However, the HU litter data are not comparable because of the deciduous character and the special dynamics of the clonal poplar shrubs that grow there: the amount of cover (and thus litter fall) of the poplars changed at different rates in different plots, that is, independently of the treatment type. In some plots shrub cover spread, but died back in others.

In line with the plant accumulation response, there was also an inter-annual variation in litterfall: an increase in the litterfall amount was recorded in the hot year of 2003 at the SP and the NL sites, whereas a decrease was recorded at the UK site (Fig 6).

Net primary productivity

In a typical year such as 2002 the aboveground net primary productivity (ANPP), calculated as the accumulated aboveground biomass plus litterfall, ranged between 100 and 200 g m^{-2} at the southern and continental sites and between 200 and 300 g m^{-2} at the northern sites (Fig. 4).

The warming treatment increased the ANPP, especially at the northern sites (the UK and the Netherlands) and at the Hungarian site, but when the responses to the unusually hot year of 2003 are included, the overall effect of warming throughout the whole experiment was not significant in the northern sites (see Figs. 5-8). Drought treatment reduced the ANPP at the NL, HU, IT and SP sites, but again, after taking the whole study period into account using a repeated measures analysis including all years, this reduction was nowhere significant (see Figs 5- 8).

The ANPP followed the inter-annual variation in climate conditions and the effect was different depending on the site as indicated by the significant interaction found for site x year ($F=7.28$, $p<0.001$). However, the most outstanding change was the decrease in ANPP in 2003 in all sites except for the UK site.

Individual plant growth

Plant growth measured as shoot length was not significantly affected by the warming treatment in the studied species at the HU (*Populus alba*) and IT (*Cistus monspeliensis)* sites, or in two of the three dominant species studied in Spain, *Globularia alypum* and *Pinus halepensis*. However, at the SP site, the growth of the other dominant species, *Erica multiflora*, was significantly enhanced (30 %) by the warming treatment (Fig. 8). There was also a contrasting response to warming at the UK site: *Calluna vulgaris* tended to respond positively to the warming treatment, while *Empetrum nigrum* tended to respond negatively (Fig. 8).

The drought treatment decreased growth in *G. alypum* (34 %) and *E. multiflora* (25 %) in Spain and in *C. monspeliensis* (21 %) in Italy, but had no significant effect either on *P. alba* at the HU site (Fig. 8) or on the two studied species in the UK (*C. vulgaris* and *E. nigrum*) that, however, also tended to grow less, especially *E. nigrum* (Fig. 8). There were also contrasting responses amongst the three studied species at the SP site, since the third dominant species in Spain, *P. halepensis,* was not significantly affected by the drought treatment (data not shown). Drought decreased plant growth in

D. flexuosa in DK, although in that country other more rhizomatous grasses did tend to increase growth in response to drought. Annual growth was significantly dependent not only on species and treatment, but also on the year and precipitation and soil moisture of the year (Figs. 7 and 8). Most studied plant species presented significantly higher growth rates in wet years (2002 and 2004) than in dry years (2001 and 2003) (Figs. 7 and 8)

Relationships between annual aboveground biomass accumulation and site temperature and soil moisture

Biomass accumulation and litterfall responses to warming and drought followed a gradient from wet/cold to dry/warm climatic environments. The responses to warming were very strongly related to the Gaussen aridity index with the maximum response occurring in the least arid site and with the minimum response in the most arid site (Fig. 9). The responses to the drought treatment were not significantly related to this aridity index (Fig. 9). Figure 10 highlights this gradient by contrasting the annual aboveground biomass accumulation with the temperature and soil moisture changes at the wettest and coldest (least arid) site (the UK) with those recorded at the driest and warmest (most arid) site (SP). The relationship with temperature was positive for annual biomass accumulation at the coldest site, whereas this relationship was negative for annual biomass accumulation at the warmest site (Fig. 10). The correlation with soil moisture was not significant at the wettest site, although annual biomass accumulation was positively related to soil moisture changes at the driest site (Fig. 10). The relationship between biomass accumulation and soil moisture did not significantly differ under the drought or warming treatments. Similarly, there were no significant differences between treatments in the response of biomass accumulation to temperature, except for a slight trend towards lower response of warming plots in the coldest site.

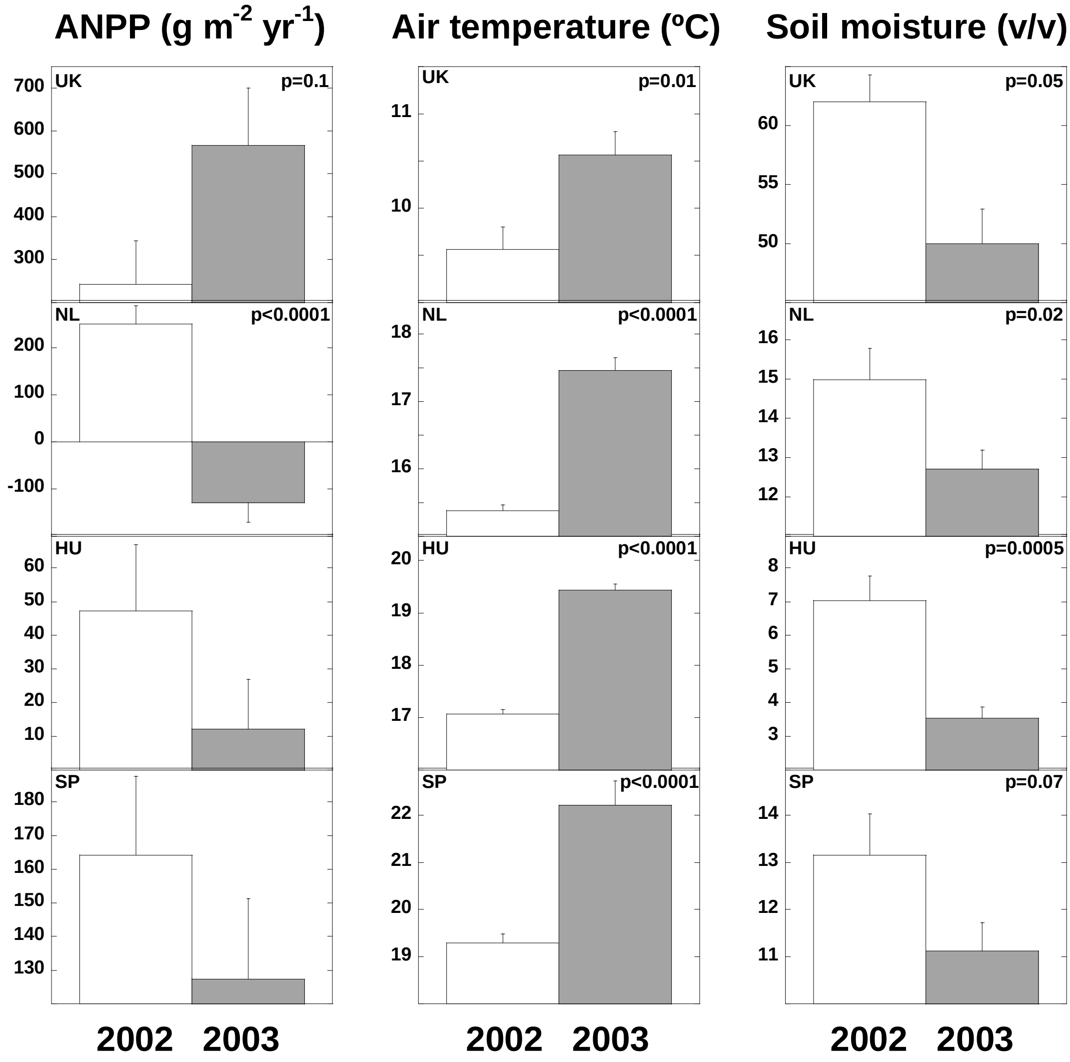

Figure 7. Annual ANPP (g m^{-2}) and mean temperature and soil moisture during the three months previous to sampling (growing season) in a typical year, 2002, and in an extremely hot and dry year, 2003. The DK and IT data are not presented because of the lack of data from 2002. Whenever significant, the *P* values of the differences between the two years are depicted in the corresponding panel. Bars indicate the standard errors of the mean (n=9 plot means).

DISCUSSION

The geographical gradient: responses in the wet/cold northern sites, but also in the dry/warm southern sites

Species richness

As a result of moderate warming (ca. 1 ºC) and drought (on average ca. 19 % lower soil moisture) there was a lower increase in species richness in the seven years study in treated plots than in control plots at the Spanish site, which had not reached a mature successional stage after the 1994 forest fire (five years before the experiments started). The resulting decrease in species richness agrees with the decrease in seedling recruitment and seedling diversity observed at this Spanish site (Lloret *et al.*, 2004). This decrease in species richness shows that the climate change predicted for the coming decades will have significant implications for vegetation composition, biodiversity, and structure, especially in plant communities that have not reached a mature successional stage. Such decreases in species richness may also play an important role in controlling the response of the overall carbon balance in these terrestrial ecosystems. In the other Mediterranean site (IT), where the state of succession was more mature and stable, no significant changes in the species richness occurred as a result of the experimental treatments. In the IT site, the previous major disturbance occurred ten years before the beginning of the experiment and, as reported by Calvo *et al.* (2002), Mediterranean shrublands generally completely recover within 12 years of a disturbance.

Changes in species composition in response to warming and altered water availability have also been reported in colder ecosystems such as a Rocky Mountain meadow (Harte & Shaw, 1995) and temperate bogs and fens (Weltzin *et al.*, 2003). The results from our study extend species composition findings into the warm and dry Mediterranean zone, which like most warm areas is normally not considered to be temperature sensitive. On the other hand, our findings do agree with species and ecosystem changes in species composition observed in Spain over the past 50 years of progressive warming and aridification (Peñuelas *et al.*, 2001; Peñuelas & Boada, 2003; Sanz-Elorza *et al.*, 2003; Hodar *et al.*, 2003; Jump & Peñuelas, 2006).

No significant changes in response to the experimental warming and drought were found in the species richness of the other sites (Fig. 2). The DK and NL sites had thick covers of grass and heather respectively with no bare soil available for the establishment of new species and other sites such as those in the UK, HU, and IT were in more mature successional stage as shrublands and therefore were less prone to new species recruitment.

This slightly greater response in species richness to warming at the SP site does not necessarily contradict our hypothesis that responses to warming would be greater at colder temperature-limited northern latitudes (Chapin *et al.*, 1995), since the Spanish site was at a different successional stage and its sensitive communities were still recovering from a major disturbance. However, the response of this warm site highlights the need to re-examine the assumption that sensitivity to warming will not be as great at southern latitudes, in particular wherever successional processes are still at work and where increased evapotranspiration associated to warming is significant. The expected sensitivity of these Mediterranean areas to more serious and longer droughts, which could affect natural recovery and re-colonization processes, is further confirmed by the decreased species richness in the drought plots at this dry Mediterranean site.

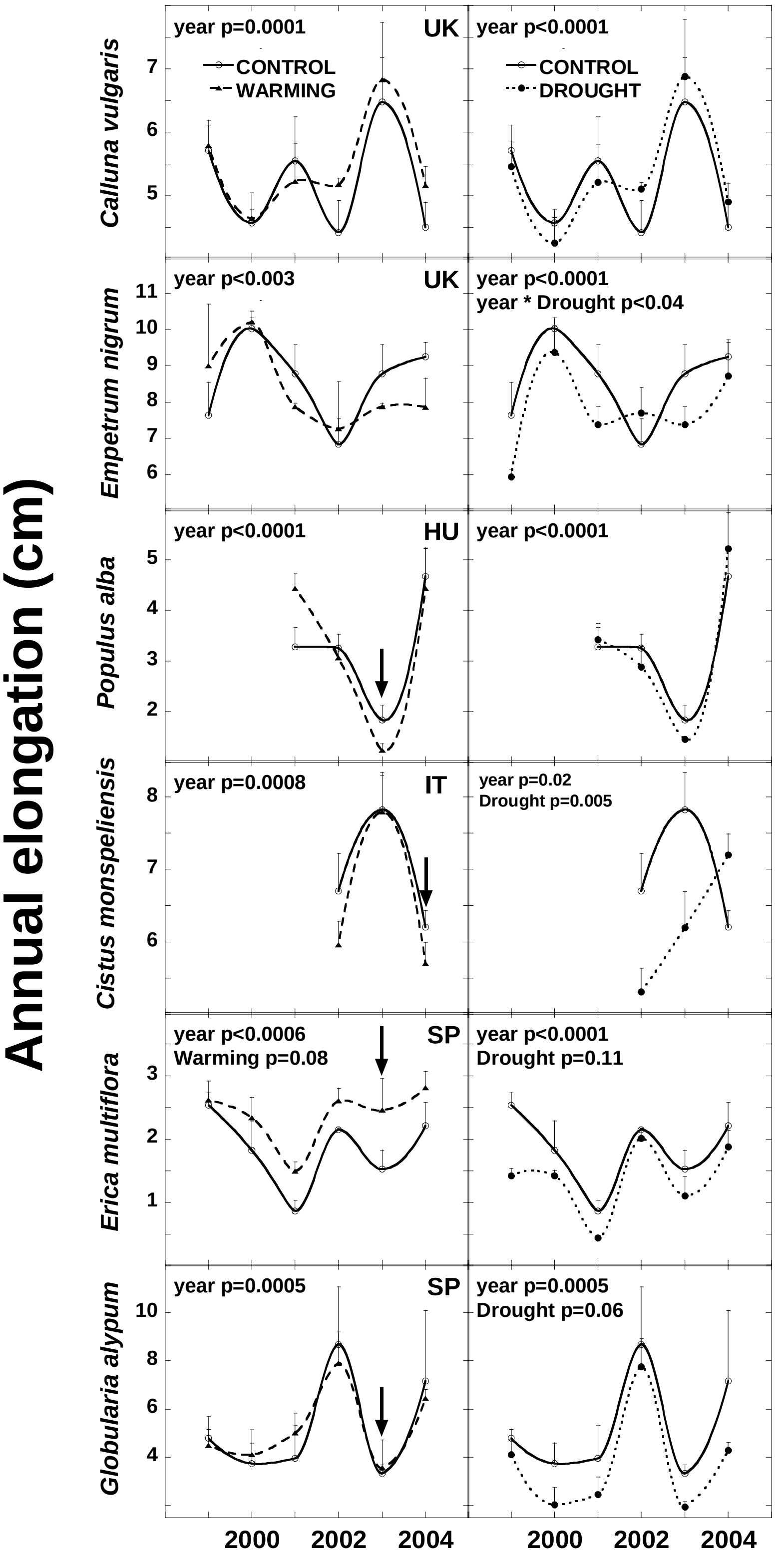

Figure 8. Changes produced by the warming and drought treatments in the annual growth of dominant plant species measured as the increases in shoot length at the HU (*Populus alba*), IT (*Cistus monspeliensis*), and SP (*Erica multiflora* and *Globularia alypum*) sites. Bars indicate the standard errors of the mean (n=3 plot means). Whenever significant, the effects of the studied factor or interaction are depicted in the corresponding panel. An arrow signals the decreases in 2003. In Italy the effects of the 2003 heat and drought were noticed in the growth measured in June 2004, at the end of the 2003-2004 growth period.

Net primary productivity

Trends in plant productivity confirmed the hypotheses that there would be more marked responses to warming at the northern sites (Fig. 9). Plant productivity was significantly affected by inter-annual climatic variation as the warming treatment tended to increase the total plant biomass at the northern (UK and NL) and continental sites (HU), although overall, and including therefore the hot year of 2003, changes were mostly not statistically significant.

These increases might be a direct result of increased photosynthesis rates (Körner & Larcher, 1988), although this does not seem to be the case here (see Llorens *et al.*, 2004). The direct effect of longer growth seasons (Peñuelas & Filella, 2001; Beier *et al.*, 2004; Prieto, 2007) seems more likely since Northern sites showed greater increases in growing degree-days (Beier *et al.*, 2004). An indirect effect of increased nutrient availability resulting from increased rates of N mineralization (Robinson *et al.,* 1997; Emmett *et al*., 2004; Schmidt *et al*., 2004) may also be a contributory factor and has been previously described in studies of plant growth and productivity in Arctic and sub-Arctic ecosystems that are generally limited by a low availability of N and/or P (Jonasson *et al*., 1999; Shaver & Chapin, 1995; Schmidt *et al*. 2002). In these ecosystems plant growth is affected by short-term temperature enhancement, partly due to the direct effects of warming and partly to the indirect effects of increased nutrient mineralization in the warmed soils (Callaghan & Jonasson, 1995). However, the lack of a very obvious and significant response in net primary productivity to warming at the colder sites in our experiment may be related to the age of the *Calluna vulgaris* plants, which are in a mature phase and therefore not accumulating much new biomass (Watt, 1947). These results also show that since warming did not decrease the total aboveground biomass, there were no overall negative effects of warming on plant growth as a result of increased frost damage in early phenological stages in any of the sites, as has been suggested by other studies (Ögren, 1996).

At the southern sites that are normally water-stressed, there was no general increase in plant productivity as a result of the warming treatment, since warming increases water loss (Beier *et al.*, 2004) and temperatures in these ecosystems are already close to the optimum for photosynthesis and growth (Peñuelas *et al.* 1998; Shaver *et al.*, 2000; Larcher, 2000). Photosynthetic rates may even decrease due to overheating in the warmest seasons or microenvironments (Peñuelas *et al.* 1998; Shaver *et al.*, 2000; Peñuelas & Llusià, 2002; Llorens *et al.*, 2003). Other reasons for the lack of response or a negative plant response to increasing temperature are the concomitant increases in evapotranspiration and decline in soil moisture often associated with warming, and the decreased availability of nutrient resources such as N and P (Rustad *et al.*, 2000; Sardans & Peñuelas 2004, 2005).

The drought treatment tended to reduce biomass accumulation at the NL, HU, IT, and SP sites, although after taking into consideration the whole study period, this reduction was not significant. In fact, in most southern semiarid areas drought already limits plant productivity (Mitrakos, 1980; Larcher, 2000; Peñuelas *et al.*, 2001; Terradas, 2001), as shown by the low ^{14}C recoveries (Gorissen *et al.,* 2004), photosynthetic rates (Llorens *et al.*, 2004), and standing plant cover and biomass in Mediterranean and continental shrublands (Fig. 4). Both the direct effects due to decreased photosynthetic rates and the indirect effects due to decreased nutrient availability of the drought treatment (Sardans & Peñuelas 2004; Sardans *et al.*, 2006) would tend to lead in the same direction towards decreased plant productivity.

The tendency of the warming treatment to decrease (ca. 15 %) the amount of litterfall only at the northern sites may be an effect of a longer leaf life-span at the temperature-limited northern sites. The drought treatment litterfall decrease, or tendency to decrease, at all sites (ca. 25 %) except for the HU site (where the data originating from the dynamic character of the poplar trees were not comparable) was unexpected since drought often encourages leaf fall (Harley *et al.*, 1987; Oliveira & Peñuelas, 2002; Ogaya & Peñuelas, 2006). These decreases might be related to lower precipitation levels diminishing litter production (Simmons *et al.* 1996). Further monitoring during the coming years is needed to be able to draw reliable conclusions regarding litterfall responses to warming and drought.

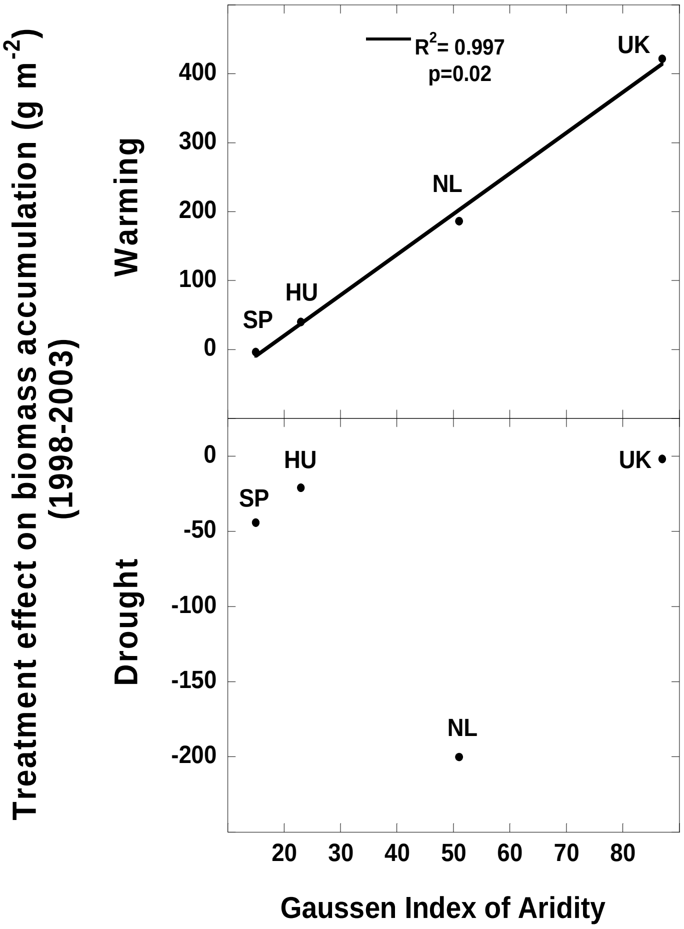

Figure 9. The relationship between the change of annual aboveground biomass accumulation (g m^{-2}) during the period 1998-2003 in response to warming and drought treatments and the Gaussen index of aridity (precipitation/2temperature) of each site. Data are from five years (1999-2003) for the UK, NL and SP sites and from four years (2001-2004) for the HU site. (DK site was not considered here because there was a heather beetle attack in 1999-2000 (Peñuelas et al 2004); the IT site was not either considered because we only had one year data available).

Both biomass accumulation and litterfall responses to warming and drought lie along a gradient from wet/cold to dry/warm environments that is highlighted in Figures 9-10. Figure 9 shows how aridity limits the response to warming. The response to the warming treatment increased the lower the aridity of the site (it was stronger in sites with high precipitation and cold temperatures). Figure 10 shows the trend towards a positive relationship between biomass accumulation and temperature at the coldest site, a negative relationship between these two factors at the warmest site, and a positive relationship between biomass accumulation and soil moisture in the driest site. Because the relationship between biomass and soil moisture was the same under control, drought, and warming, soil moisture alone will be a good predictor of biomass accumulation as temperatures increase due to climate change. Similarly, since the relationship between biomass accumulation and temperature was the same under all treatments, inter-annual temperature variation can be used to predict biomass accumulation response to warming. A slight trend towards lower response of warming plots in the coldest site can indicate a slight acclimation that deserves to be further studied.

Inter-annual differences. The 2003 heat wave and the decreases in species recruitment and ANPP.

Inter-annual changes in species richness, aboveground plant biomass accumulation, and litterfall occurred at all sites and mirrored inter-annual variation in climate conditions. The lower number of species detected in 2003 at all sites except the UK site, in line with the especially hot and dry weather conditions that year, was especially notable. There was also a decrease in biomass accumulation at all sites except the UK site in the unusually hot year of 2003. Temperatures at the UK site did not increase as much as at most other sites and this site was the most positively sensitive to warming as was to be expected from it being the coldest site. This finding is also consistent with the fact that the UK site is limited by excess water and thus drought can have neutral or positive effects (Fig. 7).There was also great inter-annual variation, with a significant increase in the amount of litterfall in 2003 at the SP and NL sites, contrasting with a decrease at the UK site, in consonance with their respective plant accumulations.

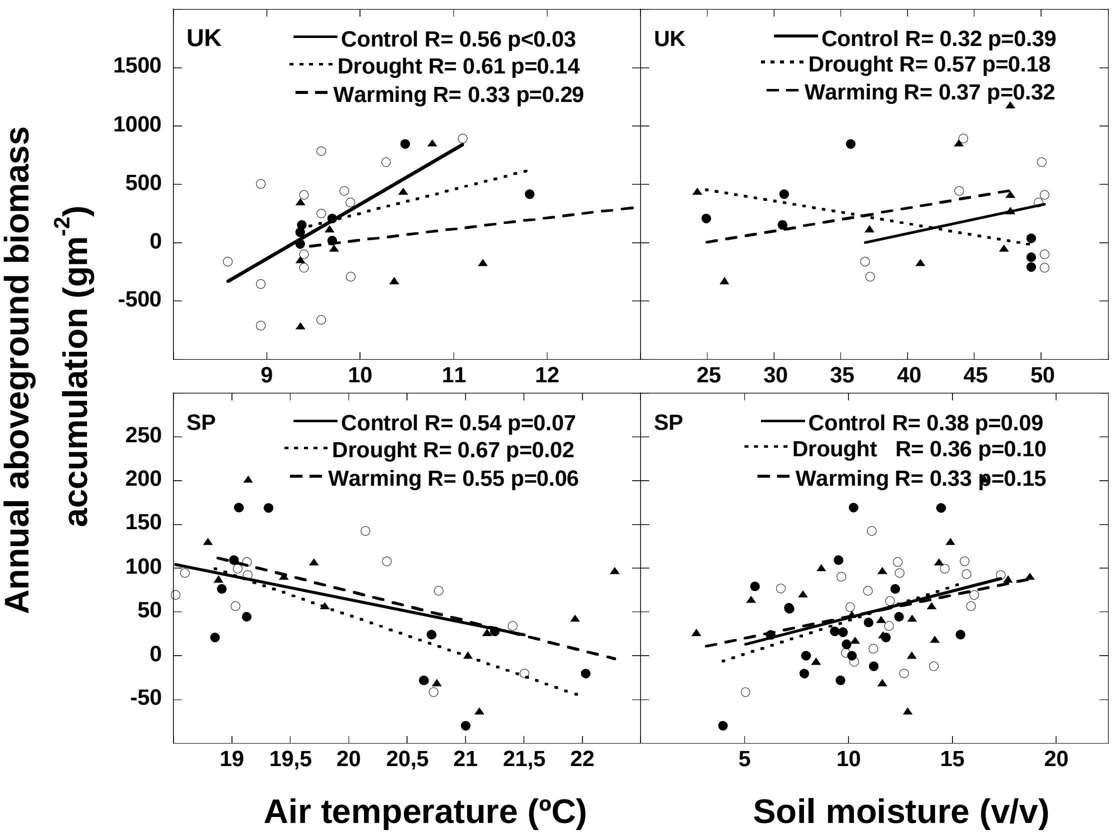

Figure 10. The relationship between annual aboveground biomass accumulation (g m^{-2}) in each treatment and the air temperature and soil moisture during the three months previous to sampling (growing season). Each point represents the data for one plot for one year. Data are from seven years (1999-2005) for the SP site, five years (1999-2003) for the UK site temperatures, and two years (2002-2003) for the UK site soil moistures.

Since severe regional heat waves such as the one that occurred in 2003 may become more frequent in a changing climate (Meehl & Tebaldi, 2004; Schär *et al.*, 2004), the expected future enhancement of plant growth and carbon sequestration resulting from climate warming in temperate ecosystems may not in fact occur, as has been suggested by Ciais *et al*., (2005). In fact, our results show an even stronger decrease in NPP than the 30 % reduction in gross primary productivity throughout Europe estimated by a recent study by Ciais *et al*., (2005), measuring ecosystem carbon fluxes, the remotely sensed radiation absorbed by plants, and crop yields at country level. The shrublands at the NL site even presented negative ANPP, contributing thus to positive carbon flux as previously anticipated by models (Cox *et al*., 2000; Dufresne *et al*., 2002).

Species-specific growth responses.

Our results show different responses to warming and drought not only between years and between sites, but also between species. Plant growth, measured as shoot length, was not significantly affected by the warming treatment in most studied species at the southern sites. On the other hand, *Deschampsia flexuosa* in DK and *Calluna vulgaris* in DK and UK tended to grow more in the warming plots. At the UK site *Calluna vulgaris* tended to grow more in the warming plots, whereas *Empetrum nigrum* tended to grow less as expected from its more northerly distribution. Plant growth also increased in one of the dominant species at the Spanish site, *E. multiflora,* but not in the other dominant species, *G. alypum.* This result, apart from the previous comments on possible effects resulting from different competitive abilities, also provides further evidence of the existence of a cold temperature limitation for plants in the Mediterranean winter (Mitrakos, 1980; Oliveira & Peñuelas, 2001, 2002, 2004) and highlights again that warm southern Mediterranean ecosystems may be more sensitive to warming than expected.

The warming treatment had a significant impact on extreme and seasonal climatic conditions and led to a large reduction in the number of frost days and an extended spring growing season (Beier *et al.*, 2004). The increased growth of some of these species may therefore be a direct result of a small constant temperature increase in annual mean values and/or the indirect effects of warming such as an extended growing season (Prieto, 2007).

The drought treatment, on the other hand, decreased plant growth by 25-35 % in the studied species at the SP site and by 21 % in *C. monspeliensis* at the IT site. Nevertheless, no decreased plant growth was recorded in the drought resistant tree species *P. halepensis* at the SP site, in *P. alba* at the HU site (as was to be expected, since these poplar shrubs get water support from their "mother tree" which has deep roots, and usually grows outside the plots), or at the UK site, where plenty of water was available usually in excess. In DK, the dominant grass *Deschampsia flexuosa* decreased plant growth by 30-40 %, as measured by the number of hits per pin. In contrast, rhizomatous grass species at the same site increased growth.

The species-specific responses to warming at the UK and SP sites or to drought at the SP and IT sites suggest that climate change may translate into changes in the species composition of communities in the near future as a consequence of different competitive abilities.

Ecosystem and community level responses and final remarks

If plant species composition in communities change in response to climate change, ecosystem responses to warming and drought will in turn be affected (Cornelissen *et al*., 1999), since species composition affects ecosystem properties such as plant growth rates or litter quantity and quality (and therefore microbial dynamics and litter decomposition), frost hardiness, and drought resistance. Most of these changes are expected to occur over the coming years and decades, although our study shows that they may occur in the space of just a few years as a result of a moderate change in climate. This is shown by the sensitive SP site, still recovering from a fire and in an early stage of succession and recruitment of new species.

This study is also a relatively long-term project, a fact that has enabled us to disregard certain changes such as the often measured increase in vegetative growth after warming in the early years of most experiments (Arft *et al*., 1999). As well, the length of the study period was sufficient for the complex interactions between environmental factors that limit plant growth to become apparent in the form of marked inter-annual variations. During the study period severely hot and/or dry years occurred and, given that severe regional heat waves such as those in 2003 may become more frequent in a changing climate (Meehl & Tebaldi**,** 2004; Schär *et al.,* 2004), the predicted enhancement of plant growth and carbon sequestration in temperate ecosystems in the future as a result of climate warming might in fact not take place, as suggested by our results and by those reported by Ciais *et al*. (2005). As commented above, these shrubs may even turn into carbon sources, contributing thus to positive carbon climate feeding.

On the other hand, the small overall differences in plant productivity in response to warming and drought treatments during the six years of study and the small differences between site responses along the climatic gradient suggests that the enhanced C fixation by plants induced by warming during the first two years of the experiment (Peñuelas *et al.*, 2004) will not be maintained in the longer term. This lack of maintenance of enhanced carbon fixation may occur as a consequence of extreme heat or drought events or due to progressive nutrient limitation (Luo *et al.*, 2004; Hungate *et al.*, 2004), as our results do not indicate any consistent increase in net N mineralization that might provide the additional inorganic-N required for continued enhanced plant growth. Limitations in other nutrients such as phosphorus may become progressively more important mechanisms controlling acclimation in these areas (Peñuelas *et al.*, 2004).

An additional interesting issue raised by this study is the fact that some of the studied sites, such as those in Denmark and Spain, experienced other disturbances apart from the experimental climate change. The attack by pests at the Danish site or the earlier forest fire at the Spanish site represent common natural occurrences that will interact with climate change, as is also the case for the P limitation at the NL site, where microbes are very strong competitors for P and thereby hamper any serious response by plants (Van Meeteren, 2005).

In summary, the magnitude of the response to warming and drought was very dependent on differences between sites, years, and plant species. The differences reported here, those reported in the literature (Shaver *et al.*, 2000; Rustad *et al.*, 2001), and the fact that plant responses seem transient and changeable over time (Arft *et al.*, 1999), illustrate the multiple responses to warming and drought and the complex interactions with other environmental factors that condition plant performance. The net response is still very difficult to predict. The prediction of plant responses to climate change must account for natural variability to avoid overestimation of the importance of warming and unrealistic projections of vegetation change. The prolongation of this study in the following years will help to further clarify some of these interactions and their longer term effects. Although the overall period responses to warming were very strongly related to the Gaussen aridity index of each site (stronger responses the lower the aridity), the decreases in biodiversity and the increased growth in *E. multiflora* in response to warming at the SP site (the most arid one) challenge the assumption that sensitivity to warming may be lower at more southerly latitudes. Finally, more frequent heat waves and droughts may further preclude predicted increases in plant productivity in response to warming and, instead, may even decrease productivity.

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CHAPTER 2.

Effects of experimental warming and drought on photosynthesis, shoot elongation and biomass accumulation in the dominant species of a Mediterranean shrubland.

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ABSTRACT

- We used a multi-dimensional approach to study the effects of experimental warming and drought on the plant carbon gain of a Mediterranean shrubland.
- We monitored photosynthesis, stomatal conductance and Fv/Fm at leaf level, growth at plant level and biomass accumulation at stand level. The experimentation period stretched over six years (1999-2005) and we focused on the two dominant shrub species, *Erica multiflora* L. and *Globularia alypum* L. and the tree *Pinus halepensis* L.
- The instantaneous ecophysiological traits most sensitive to the drought treatment were those of *P. halepensis*. Drought reduced overall photosynthesis and stomatal conductance and also reduced its Fv/Fm in summer. The most consistent effect of treatments was on seasonal growth of shrub species. Warming treatment increased shoot elongation in *E. multiflora,* drought treatment reduced shoot elongation in *G. alypum*, and growth of *P. halepensis* remained unaffected under both treatments. These effects were not yet significant on specific or total biomass accumulation at the stand level although we observed trends that may become significant in the longer term.
- We conclude that species-specific responses to treatments and inter-annual variability in the intensity of the responses may buffer a clear response in total biomass accumulation over the studied period. The results also suggest that under drier conditions larger accumulation of dead biomass may occur at stand level, which combined with higher temperatures, may thus increase fire risk in the Mediterranean area.

Keywords: *Erica multiflora*/ *Globularia alypum/ Pinus halepensis/* Mediterranean shrubland/ fluorescence/ photosynthesis/ stomatal conductance/ elongation/ biomass accumulation/ global warming/ drought

INTRODUCTION

The main conclusions of most General Circulation Models (GCMs) indicate that over this century, the mean global surface temperature will increase by 1.1 – 6.4 ºC depending on the socio-economic scenario and the resulting emissions of greenhouse gases (IPCC, 2007). Although there is no general agreement regarding future rainfall regimes, the increased potential evapo-transpiration linked to the warming would enhance the summer drought and water deficit in the Mediterranean ecosystems, as has already occurred in the last decades (Le Houérou, 1996; Piñol *et al.*, 1998; De Luís *et al.*, 2001; Peñuelas *et al.*, 2002, 2005; Peñuelas & Boada, 2003).

The predicted temperature rise could affect plant growth due to changes in photosynthetic rate, CO_2 losses by respiration, enhanced photorespiration and VOC production which present different responses depending on the temperature range (Long, 1991; Huntingford *et al.*, 2000; Peñuelas & Llusià, 2002). In the Mediterranean area, warming effects are expected to be dependent on the season. Increased temperatures in the warmest seasons in this area may decrease photosynthetic rates due to overheating (Peñuelas & Llusià, 2002; Llorens *et al.*, 2004), although warming also may alleviate minimum temperatures in colder seasons to which some Mediterranean species are sensitive (Mitrakos, 1980; Oliveira & Peñuelas, 2000, 2001, 2004; Ogaya & Peñuelas 2003a, b, 2007). Moreover, especially in Mediterranean ecosystems, warming effects may be related to the effect on water availability. For example, warming may enhance nutrient availability by accelerating decomposition and mineralization if water is not limiting (Sardans *et al.*, 2006, 2007).

Water stress has complex effects on plant functioning. One of the first consequences of reduced water availability is stomatal closure and hence the reduction of stomatal conductance and CO_2 uptake. Several studies have described restricted plant activity in the Mediterranean area in summer months due to heat and drought stress (Tenhunen *et al.*, 1990; Filella *et al.*, 1998; Peñuelas *et al.*, 1998, *in press*; Larcher, 2000; Llusià & Peñuelas, 2000; Yordanov *et al.*, 2000; Ogaya & Peñuelas, 2003a, b). Lower photosynthetic activity and reduced nutrient availability, due to reduced soil microbial activity (Sardans & Peñuelas, 2005), and reduced nutrient uptake by roots (Chapin, 1980) under low water availability, may conjunctly reduce growth.

Photosynthesis and stomatal conductance are instantaneous parameters widely used to study effects of environmental stresses on plant physiology. These studies often involve the use of chlorophyll a fluorescence, a non-invasive technique, for monitoring the functioning of the photosynthetic apparatus (Filella *et al.*, 1998; Peñuelas *et al.*, 1998; Llorens *et al.*, 2003a, b; Ogaya & Peñuelas, 2003a, b; Karavatas & Manetas, 1999: Loik *et al.*, 2000; Gratani *et al.*, 2000; Bellot *et al.*, 2004). The maximal photochemical efficiency, Fv/Fm ratio, is the most common parameter used in fluorescence; it is inversely proportional to damage in the PSII reaction centres (Farquhar *et al.*, 1989). Reductions in photochemical efficiency in Mediterranean species have been detected in summer and winter (Karavatas & Manetas, 1999; Gratani *et al.*, 2000; Larcher, 2000; Oliveira & Peñuelas, 2000, 2001, 2004; Llorens *et al.*, 2003a, b; Ogaya & Peñuelas, 2003a, b; Bellot *et al.*, 2004).

Several studies have examined the effects of changing environmental conditions on plant processes affecting carbon cycle, such as photosynthesis and growth, in field conditions. However, only a few have explored the corresponding effects on productivity or biomass accumulation at stand level and over medium-long term periods (Parson *et al.*, 1994; Chapin & Shaver, 1996; Kudo & Suzuki, 2003).

Here, we present the results of a study into the effects of experimental warming and drought on plant carbon gain in a Mediterranean shrubland using a multi-dimensional approach. We studied instantaneous ecophysiological responses at leaf level (photosynthesis, stomatal conductance and chlorophyll a fluorescence) together with other variables integrating carbon metabolism over time such as growth at the plant level and biomass accumulation at the stand level. The experimentation period covered six years (1999-2005) so as to include inter-annual variability in the plants' responses to treatments, and potential effects linked to changing species assemblage and competitive balance (the area studied is a post-fire shrubland still in the process of secondary succession towards a pine forest) (Hollister *et al.*, 2005; Peñuelas *et al.*, *in press*). Differential phenological, physiological and growth responses to the treatments have been found in previous short-term studies in *Erica multiflora* and *Globularia alypum*, the co-dominant shrub species, and *Pinus halepensis*, a tree that had gained dominance in the last years of the study (Llorens *et al*., 2003b, 2004; Llorens & Peñuelas, 2005). The ability of these species to respond to changed environmental conditions will determine their survival and performance and consequently their competitive ability, which may lead to longer-term changes in biomass accumulation at community level.

The aims of this study were to assess whether the experimentally simulated future warmer or drier conditions in the Mediterranean Basin could affect a) foliar instantaneous ecophysiological traits such as photosynthetic rates, stomatal conductances and Fv/Fm, b) growth and biomass accumulation of the dominant species of the shrubland, and c) total biomass accumulation at the stand level. Our hypotheses were that a) photosynthetic activity, stomatal conductance and Fv/Fm would present similar seasonal variations, with summer and winter as stressing periods, b) warming would affect these variables positively in colder seasons and warming and drought might reduce them in summer, c) responses of shoot elongation and biomass accumulation to the treatments would be species-specific as found in previous studies (Llorens *et al.*, 2004) and d) total aboveground biomass accumulation might be increased by warming and reduced by drought treatment although it would depend on inter-annual climate variability and the species-specific changes in growth.

MATERIAL AND METHODS

Study site and plant species

The study was carried out in a dry calcareous shrubland (*Rosmarino-Ericion*) at the Garraf Natural Park in North-East Spain (41º 18’N, 1º49’E), at 210 m above sea level. The climate is typically western Mediterranean with two rainy seasons (spring and especially autumn), mild winters and warm dry summers. The average annual precipitation during the period of the study was 592 mm with minimum monthly averages in January and June-July (20-25 mm). The mean annual air temperature was 15.2 ºC, with a maximum monthly mean of 27 ºC in the August of the 2003 heat wave and a minimum monthly mean of 5 ºC in January 2005.

The site is located in the south facing slope of a hill in old field terraces abandoned during last century. The substrate is composed of limestones and marls with a high presence of rock outcrops. In summer 1982 and spring 1994 the area suffered severe fires that destroyed *Pinus halepensis* forest and it subsequently converted to shrubland. The dominant species are *Erica multiflora* and *Globularia alypum*. Both species are resprouter shrubs distributed in dry calcareous and rocky places in the Mediterranean Basin (Bolòs & Vigo, 1995). *P. halepensis* was reintroduced by seeding after the last fire and is gaining dominance year on year. Other common species are *Dorycnium pentaphyllum, Pistacia lentiscus, Rosmarinus officinalis, Fumana ericoides, Fumana thymifolia* and *Helianthemum syriacum*. The plant community has an average annual aboveground net primary productivity of 160 g m^{-2} (Peñuelas *et al.*, *in press*) and the plant cover in 2005 reached values of up to 75 %.

Experimental design

We performed field-scale drought and night-time warming in treatment plots and compared them with untreated plots (controls). Plots were 4 m x5 m, allowing for a buffer strip of 0.5 m at the perimeter. Three replicate plots were prepared per treatment, giving a total of nine plots.

Warming treatment. The warming treatment consisted of night-time warming, using reflective curtains to cover the vegetation at night (Beier *et al.*, 2004). Solar energy is accumulated in the ecosystem during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem during the night with the reflective aluminium foil curtains (ILS ALU, AB Ludvig Svensson, Sweden) reduces the loss of IR radiation. The curtains reflected 97 % of the direct and 96 % of the diffuse radiation. The warming plots are covered by light scaffolding carrying the reflective aluminium curtain. The covering of the study plots is activated automatically according to a preset light level (< 200 lux), rain and wind (< 10 m s^{-1}) conditions (Beier *et al.*, 2004). In order to avoid influencing the hydrological cycle, rain sensors trigger the automatic removal of the covers during rain events. The warming treatment has been applied since spring 1999 with a warming effect on soil and plants of ca. 1 ºC with slight variations depending on the time of year and meteorological conditions (Beier *et al.*, 2004) (Fig 1).

Drought treatment. The drought treatment was carried out for 2 to 3 month periods in the spring and autumn growing seasons from 1999 to 2005 by covering the vegetation with waterproof, transparent covers. The drought plots are constructed similarly to the warming plots except that the curtain material is a transparent plastic and that the moving of the curtains is governed only by rain and wind. During drought periods the rain sensors activate the curtain to cover the plots whenever it rains and remove the curtains when the rain stops. The curtains are removed automatically if the wind speed exceeds 10 m s^{-1}. For the part of the year without drought treatment, the drought plots were run parallel to the control plots. The decrease of soil moisture during the applied drought treatment was 26 %, with variations depending on the season and year, while air and soil temperatures were not affected (Beier *et al.*, 2004) (Fig. 1).

"Untreated control". Three untreated control plots with a similar light scaffolding as for the warming and drought treatments, but without any curtain, were set up for comparison.

Precipitation was measured using three water collectors per plot. Soil moisture was also measured weekly using three TDR (time domain reflectometry) probes installed per plot. Air (+20 cm) and soil temperatures (-5 and -20 cm), recorded with Pt 100 sensors, and treatment functioning (curtain closure and removal detected by magnetic sensors installed at the end of the curtain movement) were recorded in a data-logger (Campbell Scientific, Inc. Logan, Utah, USA).

Fluorescence measurements

Leaf Chl fluorescence was measured every season from winter 2002-03 to winter 2003-04 and in autumn and winter 2004-2005. These measurements were taken from three plants of *E. multiflora*, *G. alypum* and *P. halepensis* per plot. The maximum photochemical efficiency of PSII (Fv/Fm) was measured with a modulated fluorometer PAM-2000 and leaf clip holder 2030-B (Walz, Effetrich, Germany). Fluorescence was measured at midday (11:00 – 13:00 h, solar time). The minimum and maximum dark adapted fluorescence (F0, Fm) and Fv/Fm, (where Fv=Fm-F0) were obtained after dark adaptation of the leaves for at least 20-25 minutes.

Leaf gas Exchange measurements

Leaf net photosynthetic rates and stomatal conductance were measured every season from winter 2002-03 to winter 2003-04 and from autumn 2004 to summer 2005. The measurements were taken from three plants of *E. multiflora*, *G. alypum* and *P. halepensis* per plot under saturating photosynthetic photon flux density conditions (PPFD = 1500 µmol m^{-2} s^{-1}) using a portable gas CO_2 and H_2O exchange system IRGA porometer (LCA-4, ADC, Hoddeson, Hertfordshire, UK). A terminal twig with intact leaves (the last 8 cm) was clamped in a PLC-2 ADC cuvette of 90 cm^3 connected to the ADC-LCA-4. All results are expressed on the corresponding projected leaf area basis measured with a Li-Cor 3100 Area Meter (Li-Cor Inc., Nebraska, USA).

Shoot elongation

The vegetative growth of three dominant species, *E. multiflora*, *G. alypum* and *P. halepensis,* was measured over three years (2002-2005) in winter and summer, as shoot elongation increments. A minimum of five plants per plot for each species (15 per treatment) were monitored season by season. For *E. multiflora* and *G. alypum*, species with several dominant stems, a minimum of five branches per plant were permanently marked with coloured wires. For each marked plant of *P. halepensis,* we measured its maximum height between the ground and the top of the dominant apical stem. For the growth calculations, only the same twigs or plants were compared in succeeding seasons. We calculated spring and autumn growth as the difference between measurements in consecutive non-growing seasons.

Pin Point Method

The pin point method was used to measure plant frequency, and indirectly estimate plant cover, biomass and growth (Peñuelas *et al*., 2004, *in press*). We measured all the vegetation contacts in the vertical at 305 points per plot with a long thin steel pin (1 mm diameter) incorporating a vertical measuring device. The points were equally distributed along 5 fixed and parallel transects per plot separated by 0.80 m (61 points per transect at 5 cm intervals). For each hit, the height, the species, the organ (leaf, stem or reproductive structure) and the state (dead or alive) were recorded. Measurements were taken in the non-growing season, summer, from 1998 to 2005.

Calibrations of the pin point measurements against biomass of the dominant shrubs and total biomass were obtained using destructive sampling outside the plots. Aboveground biomass was regressed against hit heights and expressed as g m^{-2}. For *P. halepensis,* we also made calibrations with destructive sampling of individuals outside the plots. The linear regressions that best related the shrub and total biomass by pin-pointing six calibration plots outside the treatment plots, and the linear regression that related pine biomass and maximal height per pine were the following:

Erica multiflora ($g\ m^{-2}$)	35.59 * MMH	$R^2 = 0.91$, $P < 0.001$
Globularia alypum ($g\ m^{-2}$)	44.38 + 33.61 * MMH	$R^2 = 0.98$, $P < 0.001$
Pinus halepensis ($g\ m^{-2}$)	3.45 * LN (MMH) - 10.38	$R^2 = 0.75$, $P = 0.0001$
Total biomass ($g\ m^{-2}$)	181.48 + 24.71 * MMH	$R^2 = 0.75$, $P = 0.0025$

where MMH indicates the mean maximal height per transect. The value of biomass accumulation per plot was estimated as the average of the 5 transects values.

We used the proportion between ‘alive’ and ‘dead’ contacts, and leaf and stem contacts (only ‘alive’) to study potential changes between years and treatments in structure and carbon allocation at stand level. The ratios per plot were estimated as the average of the 5 transects values.

Statistical analyses

All the statistical analyses were performed using one value per plot, obtained from averaging the plants measured per plot or the 5 transects per plot. Warming and drought treatments were always compared separately with control. Repeated measures ANOVAs were conducted to test global effects of experimental warming and drought on environmental conditions (monthly air temperature and soil moisture), instantaneous ecophysiological variables per species (Fv/Fm, photosynthesis, and stomatal conductance), shoot elongation per species, biomass accumulation per species and at stand level and ratios between leaf/stem and alive/dead. For shoot elongation, effects of treatment were tested considering overall year measurements and also spring and autumn growths. Repeated measures ANOVAs were also used to test the annual variability of ecophysiological parameters comparing analogous seasons of different years. In addition, analyses of variance (ANOVAs) were used to test the effect of the treatments in each season (for instantaneous ecophysiological variables and seasonal growth) or year (for biomass accumulation and leaf/stem and alive/dead ratios). Relationships between net photosynthetic rates and seasonal elongation and environmental conditions (water availability and temperature) were analysed using linear regressions. The analyses were performed with the Statview software package (Abacus Concepts Inc., 1998).

RESULTS

Environmental conditions

Temperatures through the year were typically Mediterranean with moderate cold winters and warm summers (Peñuelas *et al.*, 2005) (Fig. 1). The annual mean air temperature was 15.2 ºC. In 2003 it rose to 15.9 ºC and the mean temperature during July and August was the highest of the monitored period (27 ºC) (Fig. 1). The lowest monthly temperatures (5 ºC) were reached in January and February in 2005 (Fig. 1).

Soil moisture through the year was also typical for the Mediterranean Basin, with wet springs and, especially, autumns and dry summers (Fig. 1). The annual average soil moisture was 19 %, although it varied from year to year, between 15 % in 2005 and 20 % in 2002 and 2003. The soil moisture within each year was also variable. For example, 2003 showed the lowest mean soil moisture for July-August of all the years (6 % compared to the average of 8 %). The driest extended period corresponded to the dry autumn of 2004 and spring 2005. The mean soil moisture of the last trimester of 2004 and the first trimester of 2005 was 17 %, much lower than the average for this period in the five monitored years (23 %) (Fig. 1).

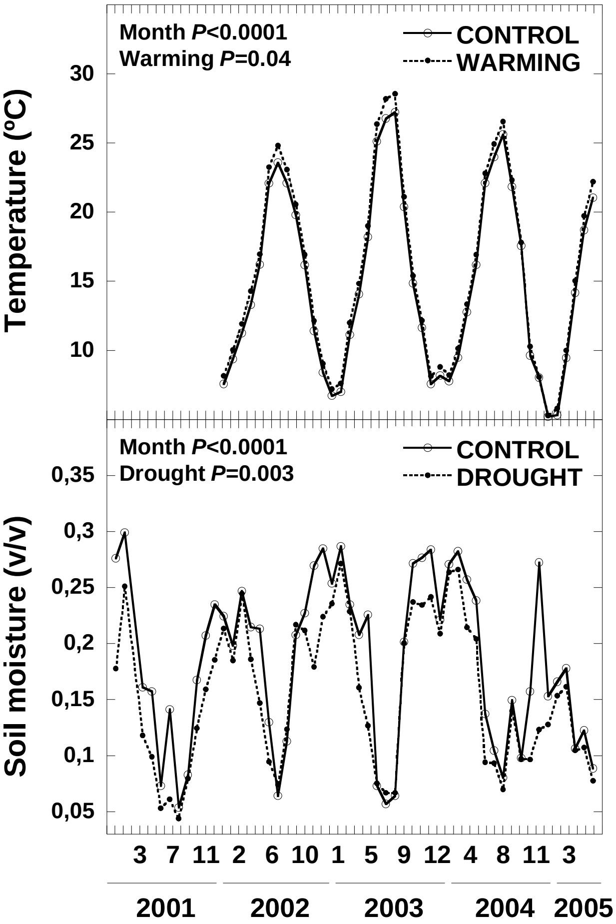

Figure 1. Monthly air temperature in control and warming plots and monthly soil moisture in control and drought plots in the study site. Statistical significance of the studied variables (RM-ANOVA) is depicted inside the panels.

Treatment effects on temperature and soil moisture

Warming treatment increased mean air temperature through the year by an average of 0.7 ºC with respect to control plots (RM-ANOVA, *P*= 0.04) (Fig. 1). The increase was greater for soil temperature (0.9 ºC), especially for the minimum temperature (1.5 ºC). Drought treatment did not significantly affect mean air temperature (Fig. 1) (Beier *et al.*, 2004; Peñuelas *et al.*, 2004).

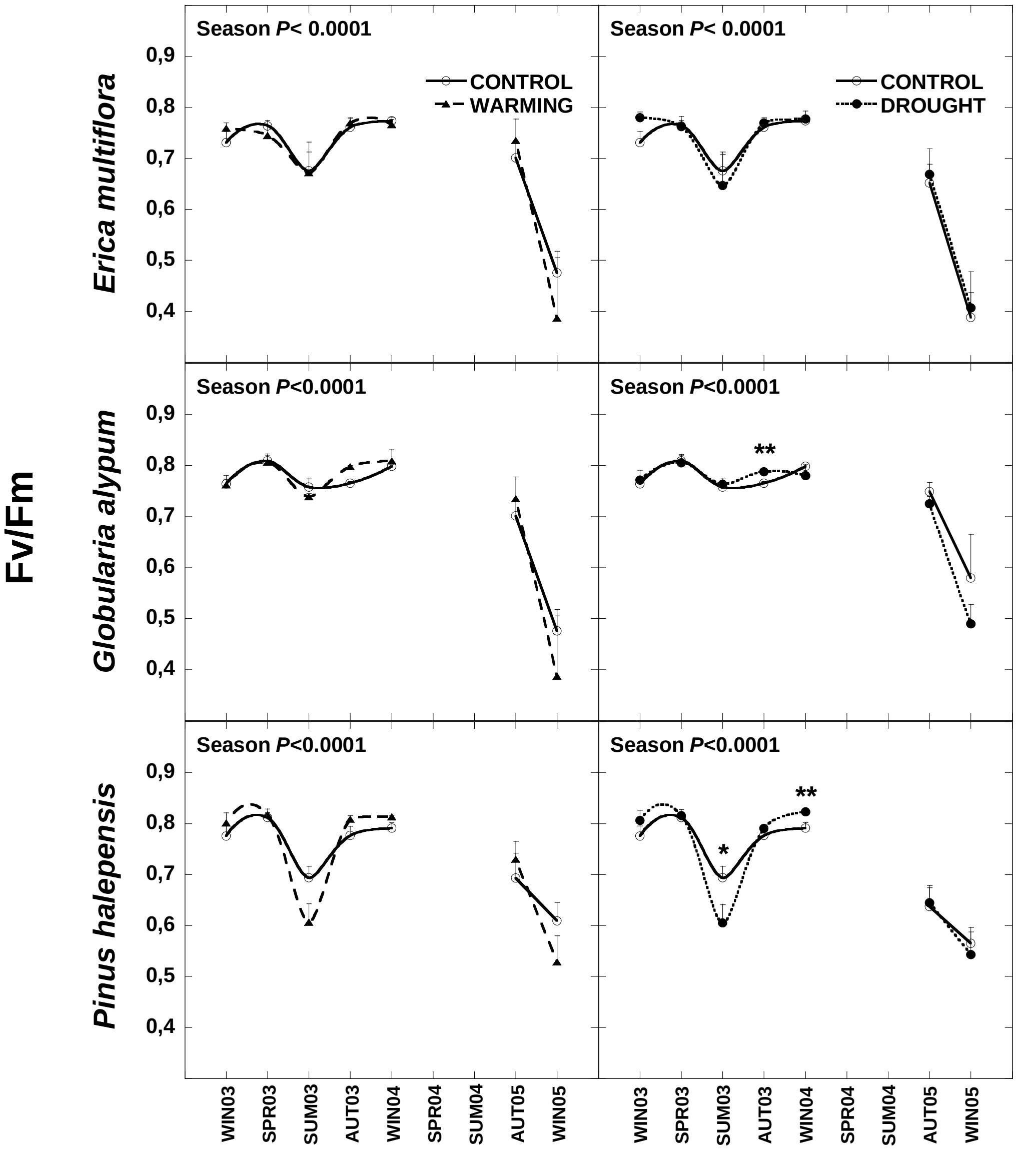

Figure 2. Seasonal maximal photochemical efficiency (Fv/Fm) for the three studied species between winter 2003 and winter 2005 for control, drought and warming plants. Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each season) is depicted inside the panels. "Season" indicates the significance for the seasonal variation of Fv/Fm over the study period. Asterisk indicates significant treatment effect (* if *P*<0.1 and ** if *P*<0.05). Error bars indicate the standard error of the mean (n=3 plot means of 3 measurements per plot).

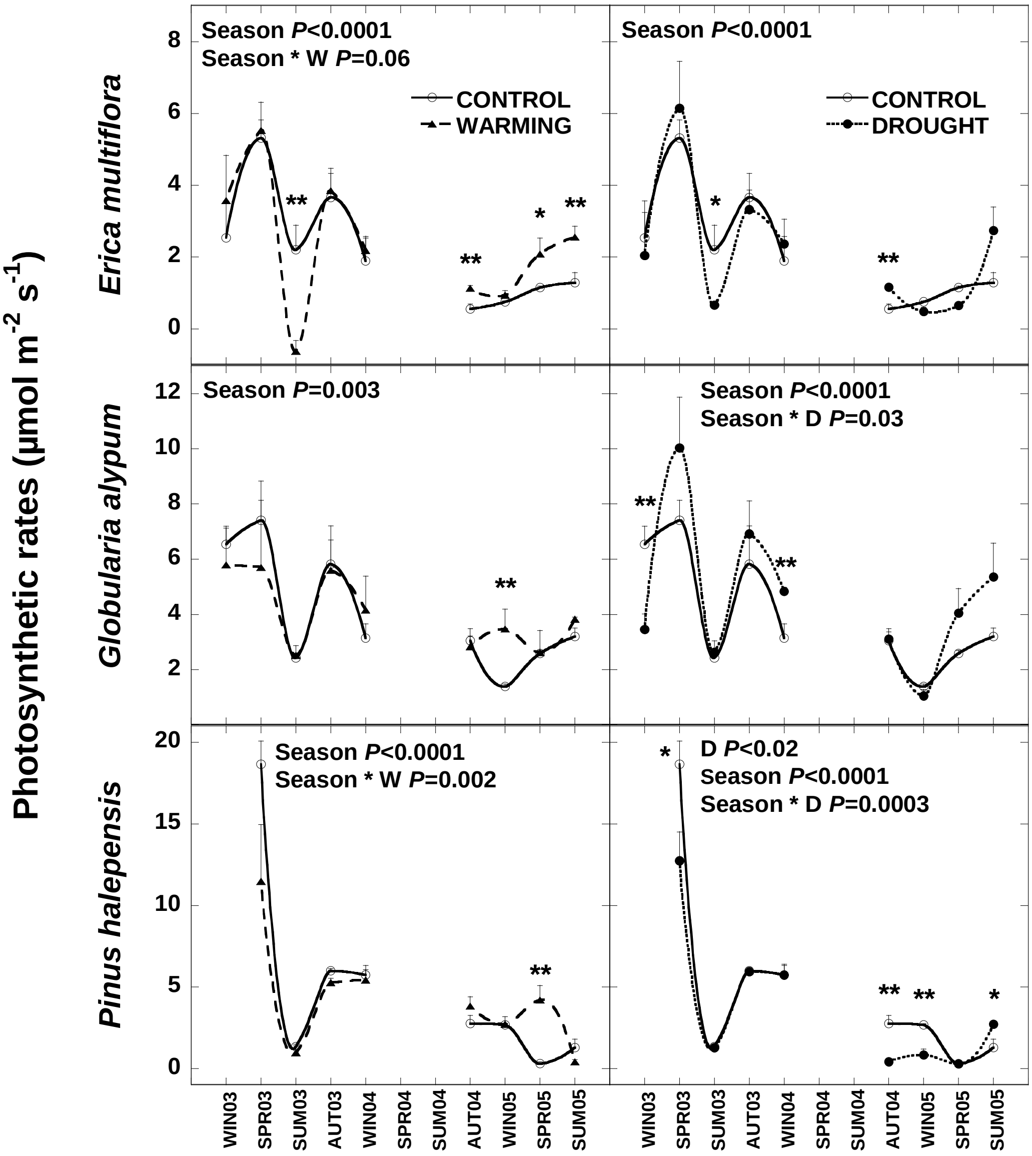

Figure 3. Seasonal photosynthetic rates for the three studied species between winter 2003 and mmer 2005 for control, drought (D) and warming (W) plants. Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each season) is depicted inside the panels. "Season" indicates the significance for the seasonal variation of photosynthetic rate over the study period and Season * D or W indicates effects season dependent. "*" indicates $P<0.1$ and "**"$P<0.05$. Error bars indicate the standard error of the mean (n=3 plot means of 3 measurements per plots).

Drought treatment reduced soil moisture through the year by an average of 19 % with respect to control plots (RM-ANOVA, *P*= 0.003) (Fig. 1), but this effect varied depending on the season. When the drought treatment was operating in spring, soil moisture in drought plots was 21 % lower than in control plots whereas the difference reached 30 % in autumn. Warming treatment did not significantly affect soil moisture (Fig. 1) (Beier *et al*., 2004; Peñuelas *et al*., 2004).

Fluorescence

The values for maximum photochemical efficiency of PSII (Fv/Fm) showed seasonal variability within the year and annual variability comparing analogous seasons for the studied species (Fig. 2). Fv/Fm ranged between 0.4 and 0.8. Fv/Fm fell in summer 2003 in *E. multiflora* and *P. halepensis* (0.67 and 0.69 respectively) although the absolute minimum values of Fv/Fm in *E. multiflora, G. alypum* and *P. halepensis* were reached in the dry and cold winter 2005 (0.48, 0.58 and 0.56 respectively) (Fig. 2).

Fv/Fm was more sensitive to drought treatment than to warming treatment (Fig. 2). Drought had positive effects in autumn 2003 for *G. alypum* and in winter 2004 for *P. halepensis* (*P*=0.005 and *P*=0.04 respectively). Fluorescence of *P. halepensis* was negatively affected (roughly significant *P*=0.10) only in summer 2003.

Photosynthesis and stomatal conductance

Photosynthesis also showed seasonal variability within the year and annual variability comparing analogous seasons for the studied species (Fig. 3). The highest photosynthetic rates were reached in spring 2003 for *E. multiflora, G. alypum* and *P. halepensis* (7.4, 5.3 and 18.6 µmol m^{-2} s^{-1} respectively). Autumn 2004, winter 2005 and spring 2005 were the seasons with lowest net photosynthetic rates for *E. multiflora*, *G. alypum* and *P. halepensis* respectively (0.53, 0.35 and 0.26 µmol m^{-2} s^{-1} respectively).

Increased temperatures in warming plots tended to increase photosynthetic rates in *E. multiflora* although the effect was season-dependent (Season * Warming, *P*=0.06) (Fig. 3). Photosynthetic rates in *G. alypum* and *P. halepensis* were also positively affected by warming treatment in winter and spring 2005 respectively (*P*<0.05 and *P*=0.01) (Fig. 3). A negative effect of warming was only detected for *E. multiflora* in summer 2003 (*P*=0.02) (Fig. 3).

Net photosynthetic rates in drought plots were both positively and negatively affected (Fig. 3). Drought treatment increased photosynthetic rates of *E. multiflora,* in autumn 2004 (*P*=0.02) and tended to reduce them in summer 2003 (*P*=0.09). Net photosynthetic rates for *G. alypum* were reduced and increased in winters 2003 (*P*=0.02) and 2004 (*P*=0.03) respectively. Drought treatment affected the photosynthetic rates in *P. halepensis* although the effects were season-dependent (Season * Drought, *P*=0.0003) (Fig. 3). Drought treatment reduced photosynthesis in this species in spring 2003 (*P*=0.06), autumn 2004 (*P*=0.01) and winter 2005 (*P*=0.007) and tended to increase it in summer 2005 (*P*=0.06).

There were positive statistically significant correlations between the photosynthetic rates in different seasons for *E. multiflora*, *G. alypum* and *P. halepensis* and the soil moisture of the week prior to measurements (R^2 ranged between 0.16 and 0.23) (Fig. 4).

Stomatal conductance paralleled to photosynthesis for the three studied species, displaying seasonal variability within the year and annual variability comparing analogous seasons (Fig. 5). *E. multiflora* and *G. alypum* presented the minimum stomatal conductance in winter 2005 (10 and 20 mmol m^{-2} s^{-1} respectively) and the maximum in spring 2003 (80 and 130 mmol m^{-2} s^{-1} respectively). *P. halepensis* exhibited more variable values of stomatal conductance, that ranged between 10 mmol m^{-2} s^{-1} in spring 2005 and 280 mmol m^{-2} s^{-1} in spring 2003.

Stomatal conductance of *E. multiflora* did not present a global response to any of the treatments (Fig. 5). For *G. alypum,* drought treatment had a season-dependent effect (Season * Drought, *P*=0.003) although the direction of the responses was highly variable (Fig. 5). A global negative effect of drought treatment on stomatal conductance was detected for *P. halepensis* (*P*=0.04). Reduced stomatal conductance was also found for this species in warming plots in spring 2003 (Fig. 5).

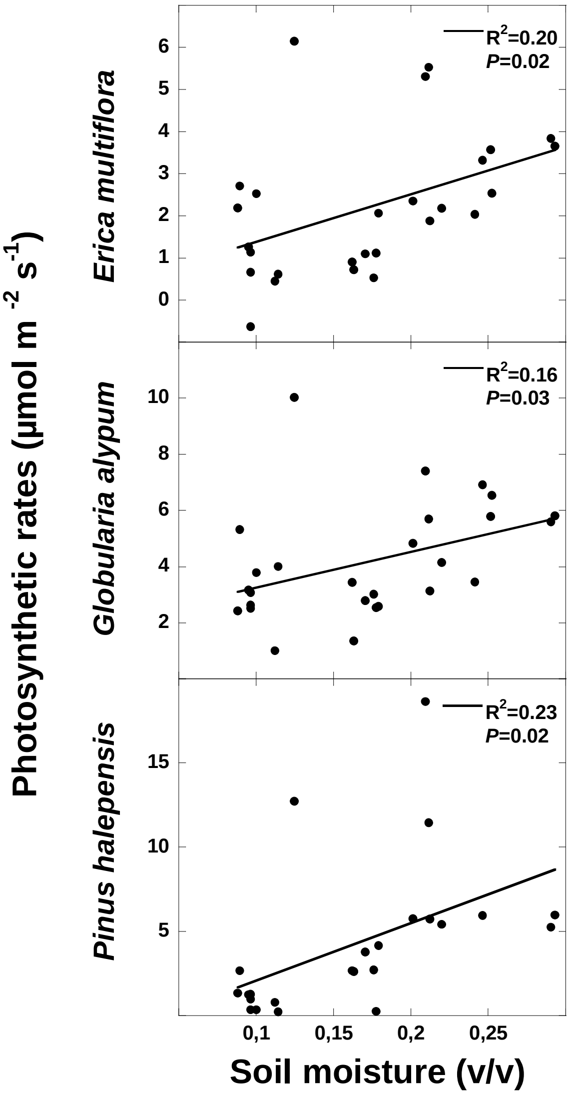

Figure 4. Relationships between the seasonal photosynthetic rates of *Erica multiflora, Globularia alypum* and *Pinus halepensis* and the mean soil moisture of the week previous to measurements. R^2 and significance of the correlation is depicted in each panel.

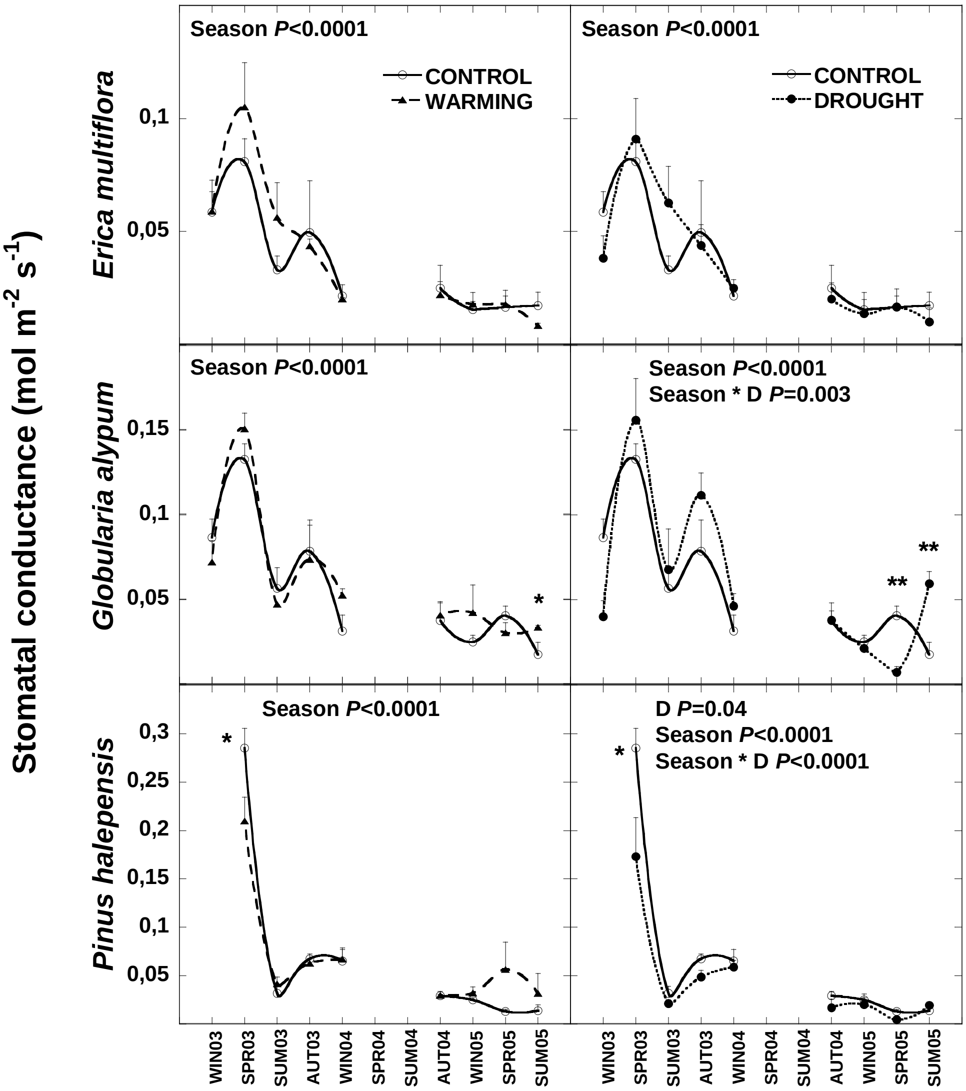

Figure 5. Seasonal stomatal conductance for the three studied species between winter 2003 and summer 2005 for control, drought (D) and warming (W) plants. Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each season) is depicted inside the panels. “Season” indicates the significance for the seasonal variation of stomatal conductance over the study period and Season * D indicates effects season dependent. “*” indicates $P<0.1$ and “**”$P<0.05$. Error bars indicate the standard error of the mean (n=3 plot means of 3 measurements per plot).

Shoot elongation

Shoot elongation in spring and autumn for the three studied species varied between years (Fig. 6). The greatest shoot elongation occurred in spring. In this season, *E. multiflora* grew less (1.6 cm) than *G. alypum* (6.8 cm) and *P. halepensis* (8 cm). *G. alypum* also exhibited a large shoot elongation in autumn (4.8 cm) compared to *E. multiflora* and *P. halepensis* (0.5 and 1 cm respectively).

Experimental warming increased shoot elongation of *E. multiflora* (P=0.05) (Fig. 6). A global significantly increased growth was found for autumn measurements (P=0.02) and longer shoots were also found in warming plots in spring 2002 and 2004 (both P<0.05). Conversely, warming tended to reduce growth of *G. alypum* (P=0.09), especially in spring (p=0.08), although only the reduction in spring 2004 was significant (P<0.05) (Fig. 6). Warming treatment affected shoot elongation of *P. halepensis* in spring, although the effect was year-dependent (Year * Warming, P= 0.006). Moreover, the direction of the response in this species was not the same in spring 2003 (positive) and springs 2004 and 2005 (negative).

Shoot elongation of *E. multiflora* and *P. halepensis* remained unaffected under drought treatment (Fig. 6). *G. alypum* plants in drought plots had smaller shoot elongation than plants in control plots (P=0.02) in both spring and autumn seasons (mean reduction of 39 % and 53 % respectively).

The spring shoot elongation of *E. multiflora*, *G. alypum* and *P. halepensis* were significantly correlated with the mean soil moisture in the first semester of the year (R^2= 0.77, P<0.0001; R^2= 0.62, P=0.0005 and R^2= 0.69, P=0.06 respectively) (Fig. 7). The autumn shoot elongation for *G. alypum* and *P. halepensis* were also significantly positively correlated with the soil moisture of the second semester of the year (R^2= 0.49, P=0.04) and with soil moisture in the period July-September (R^2= 0.59, P=0.02)

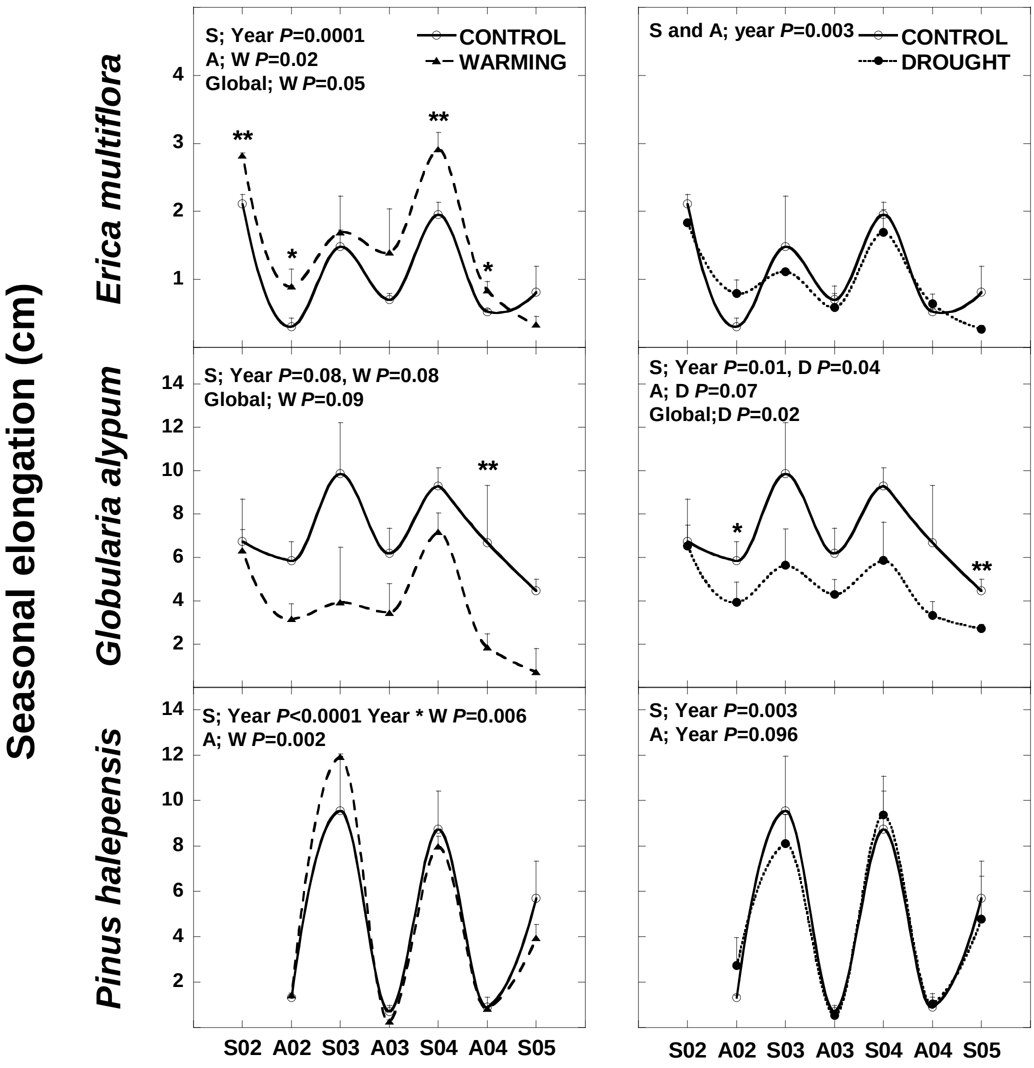

Figure 6. Seasonal shoot elongation (S: spring, A: autumn) for the three studied species between spring 2002 and summer 2005 for control, drought (D) and warming (W) plants. Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each season) is depicted inside the panels. Statistically significant effects are depicted in each panel separately for spring (S), autumn (A) or both growths (Global). "Year" indicates the significance for the annual variation of elongation over the study period and Year * W indicates effects year dependent. Seasonal treatment effect is depicted in each panel with "*" that indicates $P<0.1$ and "**" that indicates $P<0.05$. Error bars indicate the standard error of the mean (n=3 plots).

respectively. For *E. multiflora* it was positively correlated with the mean temperature of the second semester (R^2= 0.33, *P*=0.05) (Fig. 7). Autumn growth for *P. halepensis* presented a negative correlation with the mean temperature of the first semester (R^2= 0.61, *P*=0.01) (Fig. 7).

Biomass accumulation

The biomass accumulation of each of the studied species over the seven monitored years (2005-1998) is depicted in Fig. 8. In 1998 the estimated aboveground biomass for *E. multiflora* and *G. alypum* were 128 and 170 g m^{-2} respectively, and during the study period they doubled their biomass (241 and 344 g m^{-2} in 2005 respectively) (Fig. 8). Although *P. halepensis* had become more dominant towards the end of the study, its accumulated biomass up to 2005 was less than that of the dominant shrubs (73 g m^{-2}). *E. multiflora* had in average annual increment of 16 g m^{-2} although in favourable years, such as 2002, this species reached increments of almost 75 g m^{-2}. *G. alypum* accumulated an average of 25 g m^{-2} although in 1999 this reached almost 73 g m^{-2}.

The biomass accumulation for each species was not significantly affected by any of the treatments over the six years of experimentation. Neither did the relative accumulation between the last and first year of monitoring show statistically significant differences between treatments and control (Fig. 8).

The studied shrubland had an estimated aboveground biomass in control plots of 433 g m^{-2} in 1998 and after six years of experimentation, in 2005, it had risen to 827 g m^{-2} (Fig. 9). The shrubland had in average annual increase in biomass of 57 g m^{-2} although these yearly increases showed inter-annual variability. In favourable years such as 2002 and 2004, the annual aboveground biomass production was higher (86 and 87 g m^{-2} respectively) (Peñuelas *et al., in press*).

Although treatments did not significantly affect aboveground biomass accumulation at the stand level, after six years of experimentation the accumulated biomass in drought plots was 33 % less than in control plots (Fig. 9).

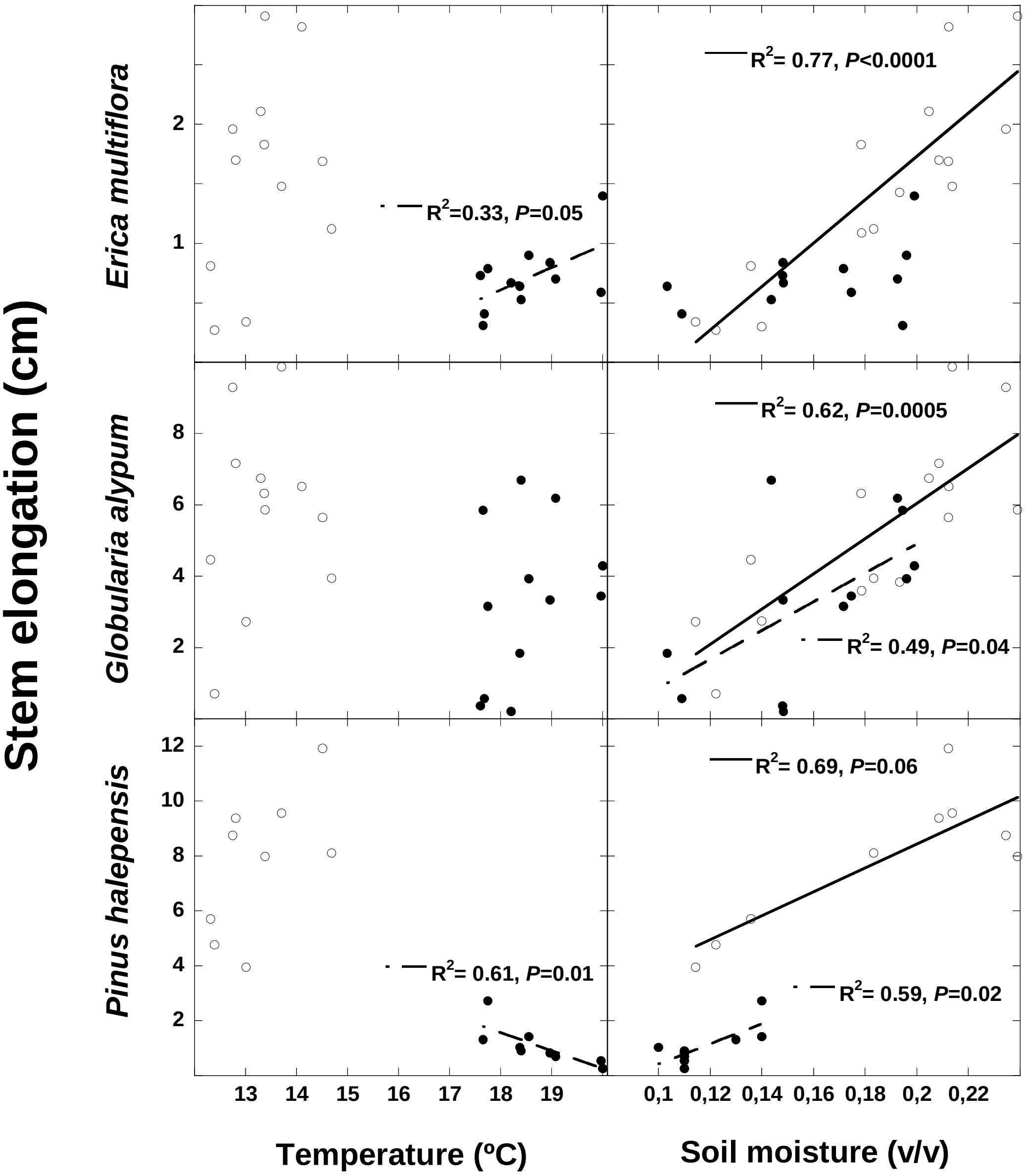

Figure 7. Relationships between the spring (empty circles and solid black line) and autumn (full cicles and discontinuous line) stem elongation of the three studied species and the mean temperature and soil moisture of the previous semester (except for autumn growth of *P. halepensis* for which we used the soil moisture in the period July-September). Each dot represents the mean value for a treatment each year. R^2 and significance of the correlation are depicted in each panel.

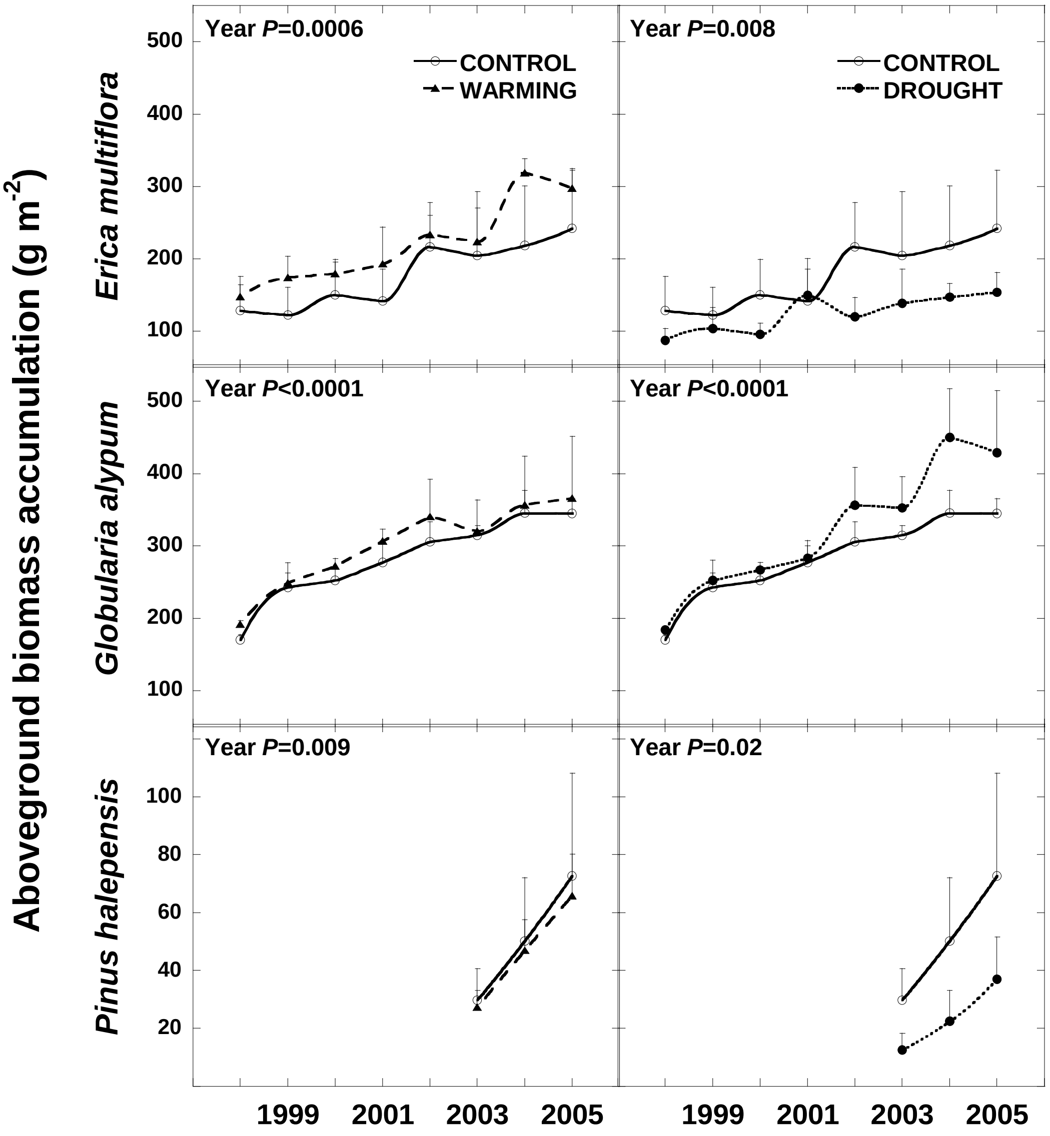

Figure 8. Annual aboveground biomass accumulation for the three studied species from 1998 (pre-treatment year) to 2005 for control, drought and warming treatments. Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each year) is depicted inside the panels. “Year” indicates the significance for the annual variation of aboveground biomass accumulation over the study period. Error bars indicate the standard error of the mean (n=3 plots).

Ratios between leaf/stem and alive/dead contacts

The ratio between leaf and stem contacts varied between years. The values ranged between 2 and 3 (Fig. 10). The maximum leaf/stem ratio in control plots was observed in 2002.

Neither of the treatments had a global effect on the leaf/stem ratio although in 2001 it was higher in drought plots than in control plots ($P<0.05$) (Fig. 10).

The ratio between alive and dead contacts also varied between years (Fig. 10). In 1999 alive and dead contacts had a ratio of about 5:1 although dead contacts were progressively increasing and in 2005 the proportion had decreased to 2:1. There was a positive correlation between this ratio of alive and dead contacts and the mean soil moisture in the period June-August ($R^2=0.68$, $P=0.002$) (Fig. 11).

The alive/dead contacts ratio in warming and drought treatments was similar that in control plots throughout the seven years of monitoring. However, treated plots showed a decreased ratio in 2001 with respect to the previous year, which was not observed in control plots. Drought treatment tended to reduce the alive/dead ratio although the effect was year-dependent (Year * Drought, $P=0.08$). The trend was broken in the dry year 2005 ($P<0.05$) (Fig. 10).

DISCUSSION

The range of values of Fv/Fm measured for the studied species and its seasonal variation were similar to those described in other studies also focusing on the ecophysiology of Mediterranean species (Karavatas & Manetas, 1999; Oliveira & Peñuelas, 2000, 2001, 2004; Llorens *et al.*, 2003a, b; Ogaya & Peñuelas, 2003a, b;

Bellot *et al*., 2004). The decreases in the maximal photochemical efficiency, especially in summer and winter, showed that *E. multiflora, G. alypum* and *P. halepensis* may suffer stress episodes during these seasons that could restrict their carbon gain.

For the three studied species, the lowest values of Fv/Fm were detected in winter 2005 (Fig. 2) coinciding with the lowest mean temperature of the week prior to measurements (mean of 5.5 and minimum temperature of 1.3 ºC) and with relatively low soil moisture (15 % in January). In some studies of Mediterranean plants 5º C has been used as a chilling temperature (Flexas *et al*., 1999; Vernieri *et al*., 2001). Some of these studies observed that after exposure to this chilling temperature, plants presented water-stress symptoms (Mcwilliam *et al*., 1982; Flexas *et al*., 1999; Melkonian *et al*., 2004). Consequently, photosynthesis may be reduced after chilling. In autumn 2004, the temperature during the week prior to measurements was less severe than in winter 2005 (mean of 11.7 and minimum temperature of 5.3 ºC). However, its combination with an extended period of low soil moisture (9.8 % in October) probably led to the observed drop in Fv/Fm, especially in *P. halepensis*. The low Fv/Fm values usually corresponded with low stomatal conductance rates suggesting that stress by low temperatures could be associated effectively to drought stress (Figs 2 & 5). Ogaya & Peñuelas (2003b) also found a strong decrease in Fv/Fm values in *Phillyrea latifolia* under the combination of drought and cold stress.

Low Fv/Fm values indicate photoinhibition, i.e. light-induced reduction of photosynthetic capacity (Farquhar *et al*., 1989). High levels of radiation or moderate levels combined with other environmental stresses may produce dynamic photoinhibition (reversible reductions of Fv/Fm) or chronic photoinbition (sustained reductions). Reduction in Fv/Fm reflects the action of photoprotective mechanism to dissipate excess energy (e. g. activation of the de-epoxidation of the xanthophyll cycle, up-regulation of Mehler reaction or enhanced photorespiration) although chronic photoinbition may be indication of permanent damage of the PSII reaction centre complex (Long *et al.*, 1994; Osmond, 1994, Oliveira & Peñuelas 2000; Peñuelas & Llusià 2002). Anyway, fotoinhibition reflects a potential reduction of photosynthesis and consequently a potential reduction of growth (Farquhar *et al.*, 1989; Oliveira & Peñuelas 2004). Our results showed that dry and cool winter 2005 was a period highly stressing for the activity of the studied species comparable to hot summer 2003, since the highest photoinhibition during that season was accompanied by low photosynthetic rates.

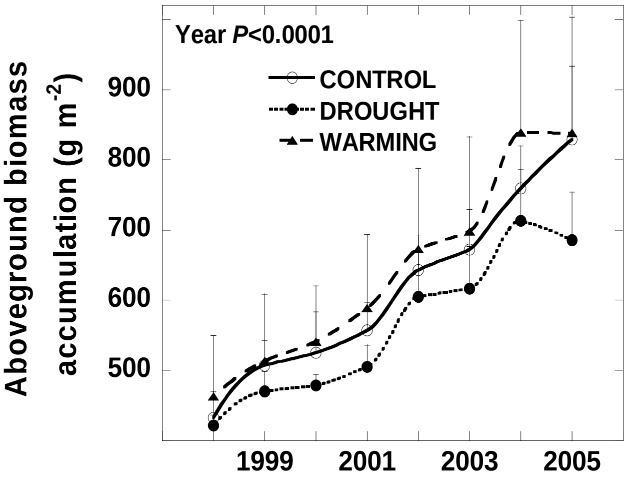

Figure 9. Total aboveground biomass accumulation from 1998 (pre-treatment year) to 2005 for control, drought and warming treatments. Statistical significance of the studied variable (RM-ANOVA for overall measurements and ANOVAs for each year) is depicted inside the panel. “Year” indicates the significance for the annual variation of aboveground biomass accumulation over the study period. Error bars indicate the standard error of the mean (n=3 plots).

We did not detect statistically significant effects of the warming treatment on Fv/Fm. However, increased temperatures may have year-dependent effects on this variable because a previous study, carried out in the same plots between 1999 and 2001, described positive effects of the warming treatment on Fv/Fm of *E. multiflora* and *G. alypum* in autumn and winter (Llorens *et al.*, 2003b). These positive effects were probably due to the warming treatment in association with the high soil moisture during the months previous to measurements (monthly soil moisture of January and October ranged between 20 and 27 %) (Llorens *et al.*, 2003b). Drought treatment only reduced Fv/Fm for *P. halepensis* in the hot summer 2003. Results of other studies with Mediterranean species under water stress suggest that the effects of water stress on photoinhibition are species-specific, year and season-dependent and also dependent on the type of leaf monitored (sunlit or shade) and the moment of the day (predawn or midday) (Valladares & Pearcy 1997; Llorens et al 2003b; Ogaya & Peñuelas, 2003a, b). Surprisingly, drought treatment enhanced Fv/Fm in *G. alypum* and *P. halepensis* in autumn 2003 and winter 2004. Some authors have shown that water deficit may increase the resistance of PSII photochemistry faced with other environmental constraints (Havaux, 1992; Epron, 1997; Valladares & Pearcy, 1997; Yordanow *et al.*, 2000; Ladjal *et al.*, 2000; Llorens *et al.*, 2003a).

Photosynthetic activity and stomatal conductance of the shrub species were in accordance with their different species-specific water use behaviour (Llorens *et al.*, 2003a). Higher photosynthetic rates and higher variation of rates and stomatal conductance were found in *G. alypum* (water spender species) than in *E. multiflora* (water conservative species) (Figs 3 & 5). The highest photosynthetic activity in spring 2003, the most favourable season of the studied period for the three species, was exhibited by *P. halepensis.* However, a high control of stomatal conductance reduced photosynthetic rates in other seasons to rates similar to the shrubs (Figs 3 & 5). Several studies have described a marked seasonal variation in photosynthetic rates and stomatal conductance in Mediterranean species with higher rates in spring and autumn and low rates in summer (Filella *et al.*, 1998; Llorens *et al.*, 2003ab; Ogaya & Peñuelas, 2003a, b). However in the present study, these variables varied significantly between analogous seasons of different years. Photosynthetic rates in the dry cool winter 2005 and spring

2005 were much lower than those in the same seasons of 2003 and 2004. The great variation in the photosynthetic rates was explained partly by soil moisture levels, in agreement with findings reported by other authors (Fig. 4) (Filella *et al.*, 1998;Llorens *et al.*, 2003a).

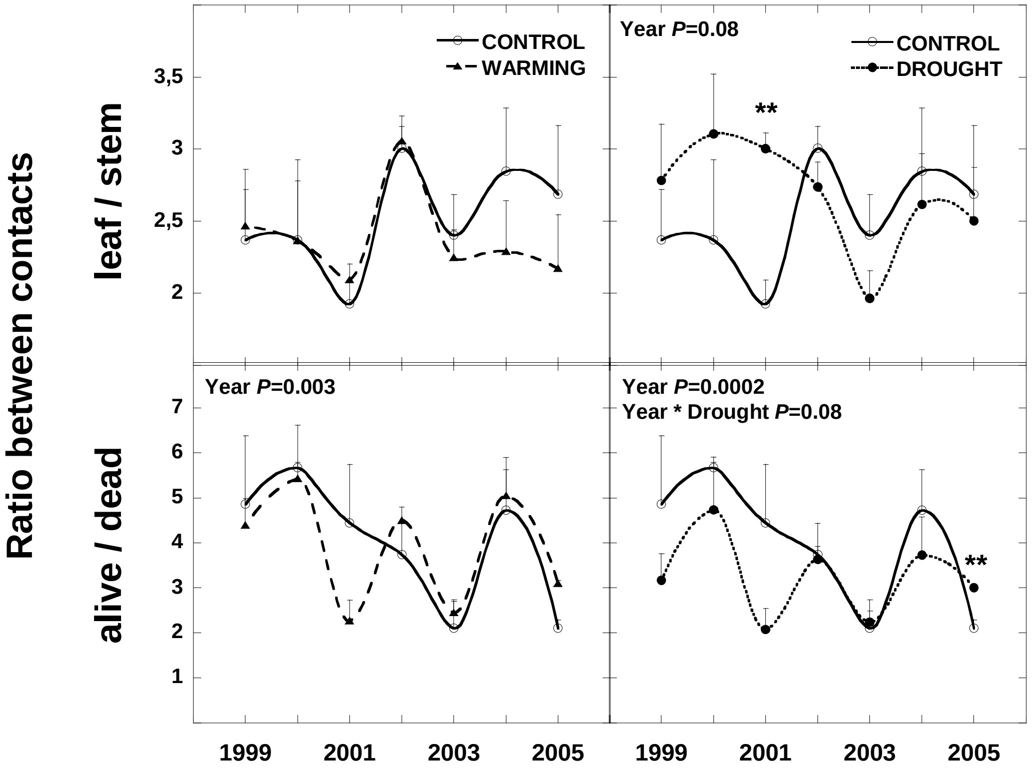

Figure 10. Ratio between leaf and stem contacts and alive and dead contacts at canopy level in control, warming and drought plots thorough seven years of study (1999-2005). Statistical significance of the studied variables (RM-ANOVA for overall measurements and ANOVAs for each year) is depicted inside the panels. “Year” indicates the significance for the annual variation of ratios over the study period and Season * Drought indicates effects season dependent. Error bars indicate the standard error of the mean (n=3 plots).

We found season-dependent effects of the warming treatment on the photosynthetic activity of *E. multiflora* and *P. halepensis* (Fig. 3) (Season * Warming P= 0.06 and P = 0.002 respectively). A large decrease in photosynthesis was observed in *E. multiflora* in warming treatment in summer 2003, during the European heat-wave, which also corresponded with a lower net ecosystem (plant + soil) CO_2 exchange at whole plant level (data not shown). Warming treatment also increased the photosynthetic rates of the studied species in the cold and dry second period of study, between autumn 2004 and summer 2005, suggesting a possible positive effect at lower temperatures in *G. alypum* and *P. halepensis* and in all seasons in *E. multiflora.*

Drought treatment had a global effect on the photosynthetic rates and stomatal conductance in *P. halepensis* supporting the idea that stomatal factors reduce photosynthetic activity in drought periods. Several studies have described negative effects of drought on these variables in Mediterranean species (Filella *et al*., 1998; Peñuelas *et al*., 1998; Llorens *et al*., 2003b). However, we did not find consistent effects of drought treatment on our shrub species. The lack of agreement could be related to differences in the intensity of the drought treatment (less in the last years than in the first years of the experiment) and to the specificities of the environmental conditions in each season.

Responses of photosynthesis to environmental changes did not always reflect changes in annual growth or production because growth is a long-term variable that integrates small short-term differences over time. It is consequently more sensitive to stress conditions than the instantaneous ecophysiological processes (Terradas, 2001). In the present study, the most sustained response to treatments in the dominant shrub species was the plant growth. Our findings based on the last three years of experimentation (2002-2005) were in agreement with a previous study based on the first three years (1999-2001) (Llorens *et al*., 2004).

Warming increased shoot elongation in *E. multiflora,* especially in autumn when elongation was in correlation with the mean temperature of the second semester of the year (Fig. 7). The global positive effect of warming on this species may be a consequence of advanced vegetative growth phenology in spring (Prieto, 2007) and higher growth rates (Llorens *et al.*, 2004; Farnsworth *et al.*, 1995; Harte & Shaw, 1995). For Mediterranean species, usually water limited, the interaction between advanced growth season and changes in precipitation patterns may not lead to increased plant growth. In our case, the advanced spring growth of *E. multiflora* in 2003 and 2004 in warming plots only corresponded with increased elongation in 2004, a year with high water availability (Prieto, 2007). At the stand level, warming did not significantly increase *E. multiflora* biomass accumulation, supporting the idea that biomass responses to warming are dependent on synergistic interactions between increased air temperature and water availability as well as on soil fertility (Chapin & Shaver, 1985; Parsons *et al.*, 1994).

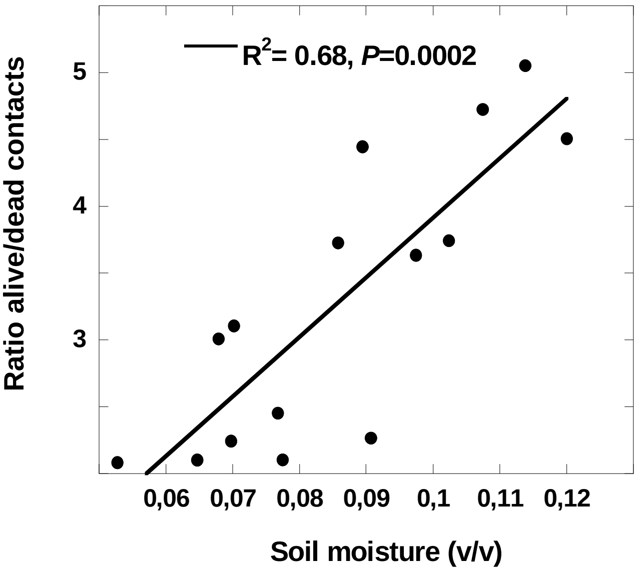

Figure 11. Relationship between the ratio alive/dead contacts at canopy level and the mean soil moisture of the period June-August. Each dot represents the mean value for a treatment each year. R^2 and significance of the correlation are depicted in the panel.

Spring stem elongation of the studied species were related to the mean soil moisture in the first semester of the year (Fig. 7) as are shoot length, leaf size or leaf area index in Mediterranean species (Kyparissis *et al.*, 1997; Kramer *et al.*, 2000; Mutke *et al.*, 2003). In adittion, autumn stem elongation of *G. alypum* and *P. halepensis*, was also related to soil moisture. However, only *G. alypum* was negatively affected by drought treatment. *G. alypum* is a water-spender species and it is able to extract water in dry conditions by sustaining very low water potentials (Llorens *et al*., 2003a) which may increase the risk of cavitation (Tyree & Sperry, 1989). After extended dry periods we observed that shoots of *G. alypum* began to dry from the apex to the base which was reflected in its lower elongation in drought treatment plots (Fig. 6). However, we did not find changes in the biomass accumulation of this species in drought plots (Fig. 8). The apparent competitive advantage of *G. alypum* in drought plots with respect to coexisting species (Prieto, 2007) could be partly related to its prodigal use of water that allows this species to use the water before the others do (Llorens *et al*., 2003a) and to its resprouting capacity. This allows plants to produce new shoots when water is available in spite of the drying of the older and longer ones after drought periods. Moreover, reductions in elongation may not reflect effects on root growth. In fact, uptake of belowground resources could be increased (e.g. through an increased ratio root/shoot) under drought conditions (Sharp & Davies 1985; Weltzin *et al*., 2000). Enhanced growth of roots has been suggested in water deficit conditions for *G. alypum* (Llorens *et al*., 2003b, 2004) which would help to sustain its productivity in drier conditions.

Autumn shoot elongation in *P. halepensis* depended on the soil moisture in summer and temperature in the second semester of the year. We found the greatest autumn growth in 2002 (that presented the wettest summer and mildest temperatures) and the smallest autumn growth in hot 2003 (Figs 1 & 7). In spite of these correlations and of the reductions in photosynthetic rates observed under drier conditions in *P. halepensis,* neither shoot elongation nor biomass accumulation were significantly affected by treatments (Figs 6 & 8). We monitored the effect of the treatments on the length of the dominant apical shoot of pine plants but we do not now know whether the consideration of the elongation of the lateral stems would have made significant the differences. Anyway, although treatments did not significantly affect individuals of *P.halepensis,* a parallel study focusing on the treatment effects on plant succession suggested that warmer and drier conditions may reduce competitive ability of this species against co-existing shrub species that growth earlier just after fire (Prieto, 2007). *P. halepensis* was the dominant species before fire, and how dry and warm conditions affect its recovery, the competitive relationships with co-existing shrub species and thus the vegetation structure, may have great implications in biomass accumulation at stand level in the long-term (Bellot et al., 2003).

At the stand level, temperature and soil moisture of the previous months determined the annual biomass production (Peñuelas *et al*., *in press*) but none of our treatments significantly affected aboveground biomass accumulation after six years of experimentation (Fig. 9). The balance between the patterns observed in biomass accumulation for the three studied species in drought plots (reduction in *E. multiflora* and *P. halepensis* and increase in *G. alypum*) resulted in a statistically insignificant reduction of 33 % in drought plots with respect to untreated plots which almost doubled their biomass from 1998 until 2005 (Fig. 9). Other studies have also described that the diferential response of species and life-forms to treatments may cancel out a clear global response (Harte & Shaw 1995; Weltzin *et al.*, 2000). The studied shrubland was a carbon sink throughout almost all the studied years. This accord with parallel studies that show how, eleven years after the last fire, this community is still in the process of recovery (Peñuelas *et al*., *in press*). However, this trend may change if extrem environmental conditions, such the heat wave in 2003, become more frecuent (Ciais *et al*., 2005; Peñuelas *et al*., *in press*).

The reduction in aboveground biomass accumulation in drought plots was accompanied by a smaller ratio of alive/dead contacts in these plots (marginally statistically significant, Year * Drought, P=0.08) (Fig. 10). In general, a higher proportion of dead contacts were also found both in treated and control plots in years with dry summers such as 2001, 2003 and 2005 (Fig. 11). This finding was likely in relation with the plant architecture of most of the coexisting shrubs species in the studied shrubland. They have several main branches with numerous thin stems that under dry conditions lose moisture content and die. Dead plant biomass is a good fuel and a greater accumulation of it increases fire risk (Terradas, 1996).

Final remarks

Instantaneous ecophysiological responses to the treatments did not always anticipate the effects on long-term variables. Effects on seasonal growth were species-specific but sustained throughout the experimentation period. But although the results point to gains of biomass in *E. multiflora* and *G. alypum* in warmer and drier conditions respectively, we did not find statistically significant changes for either species of the total biomass accumulation, at least in the medium term (6 years of experimentation). Biomass accumulation at the stand level in response to treatments reflects secondary responses (Hollister *et al*., 2005) that integrate short-term effects and longer-term changes in the competitive balance of species (growth and biomass allocation of previously established individuals and also changes in recruitment). Longer-term study or/and a greater number of experimental replicates may be necessary to detect significant changes.

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CHAPTER 3.

Acceleration of the onset of shrubland species spring growth in response to an experimental warming along a north-south gradient in Europe.

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ABSTRACT

Bud burst and shoot elongation were monitored in 8 shrub and grass species in six European sites to describe the onset of spring growth and its response to experimental warmer conditions. The sites spanned a broad gradient in average annual temperature (8.2 - 15.6 ºC) and precipitation (511 - 1427 mm). *Vaccinium myrtillus* and *Empetrum nigrum* in Wales, *Deschampsia flexuosa* in Denmark, *Calluna vulgaris* in Netherlands, *Populus alba* in Hungary and *Erica multiflora* in Spain were species responsive to increased temperatures. However, although the acceleration of spring growth was the commoner responses to warming treatment, the responses in each site were species-specific and year dependent. Our results highlight the difficulty of making general projections about the effects of climate change on plant phenology and also, the importance of taking into account the co-occurring environmental factors of a particular site and their interactions for each species.

Keywords: *Vaccinium myrtillus, Empetrum nigrum, Deschampsia flexuosa, Calluna vulgaris, Populus alba, Erica multiflora, Globularia alypum, Cistus monspeliensis,* climate warming, phenology, bud bursting, shrubland.

INTRODUCTION

Plant development and growth are strongly influenced by a series of environmental factors, in particular by temperature (Nizinski & Saugier 1988; Hari & Häkkinen 1991; Hughes 2000; Rötzer & Chmielewski 2001; Peñuelas *et al.*, 2002, 2004a). Temperature is globally changing and is projected to further change substantially as the climate changes (IPCC 2001, 2007). The major predictions of most general circulation models (GCMs) indicate that over this century the mean global surface temperature will increase by 1.1 – 6.4 ºC depending on the socio-economic scenarios and the resulting emissions of greenhouse gases (IPCC 2007). Greater increases in the mean temperature are expected in the coldest months at high latitudes producing milder winters and warmer springs (Hari & Häkkinen 1991; Ahas *et al.*, 2002; Schwartz *et al.*, 2006) and it has been suggested that plants from cold biomes will be especially sensitive to this climate warming (Aerts *et al.*, 2006). Although there is no general agreement regarding future rainfall regimes (Kutiel *et al.*, 2000), Mediterranean communities would be greatly affected at least by the increased potential evapotranspiration linked to the warming (Le Houérou 1996; Piñol *et al.*, 1998).

Plant phenology, e.g. bud burst, shoot elongation and flowering, has been recognized as an important instrument in the field of climate change research in the last decade (Schwartz 1999; Menzel 2000; Osborne *et al.*, 2000; Roetzer *et al.*, 2000; Peñuelas & Filella 2001; Piao *et al.*, 2006; Menzel *et al.*, 2006) because it is the major short-term responsive aspect of nature to warming and the simplest to record (Spark & Menzel 2002; Walther *et al.*, 2002; Linderholm 2006). An improved knowledge of the geographical distribution of the timings of phenological phases and their relationship to climate will improve predictions about the response of vegetation to climate change (Kramer *et al.*, 2000; Peñuelas & Filella 2001; Rötzer & Chmielewski 2001; Hollister *et al.,* 2005; Schwartz *et al.,* 2006).

Numerous studies have shown the clear effect of temperature on the onset, end, rate and duration of many developmental processes in plants (Warrington & Kanemasu 1983; Nizinski & Saugier 1988; Hari & Häkkinen 1991; Schultz 1992; Spano *et al.,* 1999; Pop *et al.,* 2000; Peñuelas & Filella 2001; Peñuelas *et al.,* 2002; Mutke *et al.,* 2003). Particularly, most studies have detected a clear relationship between the spring phenophases (budding, leafing, flowering) and the temperatures in the preceding months (Fitter *et al.,* 1995; Osborne *et al.,* 2000; Fitter & Fitter 2002; Peñuelas *et al.,* 2002; Walther *et al.,* 2002; Chen *et al.,* 2005; Crepinsek *et al.,* 2006; Piao *et al.,* 2006; Menzel *et al.,* 2006).

Bud burst is a character with high phenotypic plasticity and it is expected under a future warmer climate plants will flush earlier and profit from favorable spring conditions with high solar radiation and soil moisture (Kramer 1995; Pop *et al.,* 2000). Studies based on long phenological series, supported by field-based surveys or satellite remote sensing, have described how springtime phases from a wide range of taxa and across a wide range of geographic sites have occurred progressively earlier (Menzel & Fabian 1999; Peñuelas & Filella 2001; Rotzer & Chmielwski 2001; Parmesan & Yohe 2003; Menzel *et al.,* 2006). For Europe, the shift in the onset of growing season in relation to the increased temperature recorded in last decades differs depending on the sites, the species, and the periods considered. It has been estimated that an increase in temperature in winter or early spring of 1 °C corresponds to an advance in the onset of spring events of ca. 6 days (Menzel & Fabian 1999), and an increase of 1.4 ºC was reported to advance the onset by up to 16 days in a Mediterranean site (Peñuelas *et al.,* 2002). Recently, Menzel *et al.* (2006) reported an overall advancement of the spring/summer events of 2.5 days per 1 ºC on average for the whole Europe.

Different responses of plant phenology to climate change can be expected in diverse ecosystems since their environmental constraints are different (Kramer 1995; Peñuelas *et al.*, 2002; Sparks & Menzel 2002; Ogaya & Peñuelas, 2004; Rötzer *et al.*, 2004; Llorens & Peñuelas 2005; Crepinsek *et al.*, 2006). Moreover, species-specific responses may affect the structure and functioning of ecosystems (Peñuelas & Filella 2001) due to altered competitive relationships and loss of synchronization of development between species (Corlett & Lafrankie 1998; Hughes 2000; Peñuelas & Filella 2001; Fitter & Fitter 2002; Gordo & Sanz 2005). It has been suggested that plants able to take advantage of warmer and longer growing seasons are likely to dominate future communities (Pop *et al.*, 2000) and species lacking phenological adaptability might experience greater stress or even extinction during extended climate change (Bradley *et al.*, 1999).

In the present study, we took an integrative approach combining a wide geographical gradient and an experimental system in which temperature was manipulated to study its influence on phenology and the differences between site- and species- specific climate change impacts (Kramer 1995; Dunne *et al.*, 2004). The aims of this study were: (1) to characterize the spring growth phenology for dominant species of shrublands in different sites along a gradient of temperature and precipitation from Northern to Southern Europe (8.2 - 15.6 ºC and 1427 - 511 mm) and (2) to investigate the specific responses of sites and species to an experimental field warming simulating the future climate conditions forecasted by GCM and ecophysiological models (IPCC 2001, 2007; Peñuelas *et al.*, 2005). We hypothesized that (1) higher temperatures imposed by our warming treatment would lead to an earlier spring growth; and that (2) the magnitude of responses would depend on the geographical gradient, being major for species in places where cold seasons constrain plant activity.

MATERIALS AND METHODS

Study Sites

Experimental manipulations were carried out on shrubland ecosystems at six sites (Table 1); Wales-UK (UK) (53º03' N 3º28' W), Denmark (DK) (56º23'N 10º57'W), The Netherlands (NL) (52º24'N 5º55'W), Hungary (HU) (46º53' N, 19º23' E), Sardinia-Italy (IT) (40º 36' N, 8º 9' E) and Catalonia-Spain (SP) (41º18' N, 1º49' E) spanning a European gradient in temperature and precipitation (Beier *et al.,* 2004; Peñuelas *et al., in press*). The study sites are semi-natural shrublands. The three Northern sites have Atlantic wet (UK) and dry (DK and NL) climate respectively. The vegetation of these northern sites is dominated by mature *Calluna vulgaris* although in UK there is also *Vaccinium myrtillus* and *Empetrum nigrum,* with very sparse *Deschampsia flexuosa.* In DK, over the last 30 years the grass *Deschampsia flexuosa* has gained increasing dominance, probably as a consequence of the low level of management and the increasing atmospheric input of nutrients. The DK site was struck by heather beetle attacks in the summer of 1999 and especially in 2000. The beetle attacks killed the *Calluna* plants from large parts of the area and they had to regenerate from seeds. The NL site is a monoculture of old *Calluna vulgaris* plants. In the HU site, the climate is continental and arid, particularly in the summer months. The site has a specific type of vegetatively emerged sprouts of the clonal, winter deciduous, *Populus alba* that forms small shrubs which rarely grow to trees. The two Southern sites have a Mediterranean climate. The IT site is a short macchia dominated by *Cistus monspeliensis* and *Helichrysum italicum.* The SP site is an early successional garrigue, established in the year 1994 after the occurrence of a wildfire, dominated by *Erica multiflora* and *Globularia alypum*.

Experimental manipulations

On each site we performed field-scale night-time warming in treatment plots and compared them with untreated plots (controls). Plots were 4 m x 5 m, allowing for a buffer strip of 0.5 m at the perimeter. Three replicate plots were established per treatment, giving a total of six plots per site.

Warming treatment. This treatment involved night-time warming using reflective curtains covering the vegetation at night (Beier *et al.,* 2004). Solar energy is accumulated in the ecosystem during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem with the reflective aluminum foil curtains (ILS ALU, AB Ludvig Svensson, Sweden) reduces the loss of IR radiation. The foils reflect 97% of the direct and 96% of the diffuse radiation. The warming plots were covered by light scaffolding carrying the reflective aluminum curtain. The covering of the plots is activated automatically according to preset light (< 200 lux), rain and wind (< 10 m s^{-1}) conditions (Beier *et al.*, 2004). The curtains reduced the heat loss during night by 64% from 33 W m^{-2} to 12 W m^{-2}. To avoid the influence of warming treatment on water availability, rain sensors triggered removal of the covers during rain events. The warming treatment has been applied since spring 1999 with an effect of warming the air and soil in the order of 0.7 and 0.8 ºC respectively with variations depending on site (Table 1), time of the year and meteorological conditions (Beier *et al.,* 2004). The number of days with frost was reduced by 19-44% (Beier *et al.,* 2004).

Untreated control. Three untreated control plots, with a similar light scaffolding, as for the night-time warming treatment, but without the curtains, were established for comparison.

Environmental conditions were monitored in all plots. Precipitation entering the plots was measured using three water collectors. Soil moisture was also measured weekly by means of three time domain reflectometry (TDR) probes installed per plot. Air (+20 cm) and soil temperatures (-5 and -10 cm) and the functioning of the treatments, (curtain closure and removal detected by magnetic sensors installed at the end of the curtain movement), were recorded in a datalogger (Campbell Scientific, Inc. Logan, Utah, USA).

Table 1. Environmental conditions (mean annual temperature and mean annual precipitation) at the six European shrubland sites, effect of warming treatment on the mean annual air and soil temperatures and species, years and experimental periods studied.

Site	**Warming effect (ºC)** **Air**	**and Soil**	**Species**	**Years**	**Period of growth**
Wales (United Kingdom) (8.2 ºC, 1427 mm)	+ 0.5	+ 0.7	*Calluna vulgaris* *Empetrum nigrum* *Vaccinium myrtillus*	2000 2002 2004	April-August
Denmark (9.4 ºC, 758 mm)	+ 1.1	+ 0.7	*Calluna vulgaris* *Deschampsia flexuosa*	2003	April-September
The Netherlands (10.1 ºC, 1042 mm)	+ 0.5	+ 0.6	*Calluna vulgaris*	2003	April-September
Hungary (10.8 ºC, 515 mm)	+ 1.6	+ 1	*Populus alba*	2003 2004	March-May
Sardinia (Italy) (15.6 ºC, 511 mm)	+ 0.54	+ 0.12	*Cistus monspeliensis*	2002 2003	February
Catalonia (Spain) (15.1 ºC, 455 mm)	+ 0.73	+ 0.91	*Erica multiflora* *Globularia alypum*	2003 2004 2005	March-June

Phenology and growth measurements

Two procedures were followed to determine the timing of spring growth in the different studied species depending on the site. In UK and DK we monitored the shoot elongation until the maximum length was reached. In NL and HU we monitored the appearance of green buds with leaves starting to unfold, in IT the appearance of new leaves and in SP the appearance of new shoots. Measurements were taken weekly until the shoot elongation, or the appearance of new leaves or newly grown branches stopped. We defined the spring growth date as the date when half of the maximum growth was reached or the date when half of the new shoots/leaves had appeared, following a common approach to previous studies (Partanen *et al.,* 1998; Chen *et al.,* 2005). Previously, we had tested that there were no significant differences in the maximum length or the maximum percentage of new shoots/leaves between control and treated plots.

Statistical analyses

All the statistical analyses were performed using one value per plot obtained from the average of 5-10 plants except for linear regressions for which we used only the mean per treatment. In order to ensure the use of the date for 50 % growth to define phenology of growth, analyses of variance (ANOVAs) were previously conducted to check the annual effects of the treatments on the maximum elongation and maximum percentage of new shoots. Survival analyses were used for testing differences in the time for 50 % growth. Survival time was defined as the number of days from January 1st each year until the date for 50 % growth. We used the Kaplan-Meier nonparametric method for the computation of survival curves and log-rank (Mantel-Cox) statistics to test for differences between treatments and controls. Repeated measurements analyses of variance were used to test inter-annual differences in the dates for 50 % growth in control plots. Relationships between the timing of the spring growth, expressed as the date for 50 % growth, and the temperature and precipitation in previous months were tested using linear regressions for the SP species. Data values expressed in percentage were arcsine-square root transformed to homogenize variances. ANOVAs, repeated measures analyses of variance, survival analyses and regressions were performed with the Statview software package (Abacus Concepts Inc., Cary, North Carolina, USA).

RESULTS

Characterization of the spring growth phenology

Except *Empetrum nigrum* and *Globularia alypum*, all species monitored for more than one year showed interannual differences in the timing of the growth in control plots (Table 2). The earliest occurring spring event was the leafing of *Cistus monspeliensis* in the IT site in February. The growth date for this species occurred six days earlier in 2002 respect 2003 (Table 2). In the SP site, *Erica multiflora* and *Globularia alypum* began to grow in April and both reached the date for 50 % growth in May (Table 2, Fig 1). For these species, the dry late-winter and early-spring of 2005 had opposite effects on the timing of spring growth in control plots. This drought period delayed the growth date for *Globularia alypum* (not significantly) but coincided with the earliest spring growth of the three monitored years for *Erica multiflora* (Table 2). *Erica multiflora* and *Globularia alypum* presented statistically significant negative relationships between the date for 50 % growth and the temperature of the first four months of the year (minimum and mean temperatures respectively) (Fig 2). The date for 50 % growth in *Globularia alypum* also was correlated with the accumulated precipitation in the first four months across the years ($p<0.04$) (Fig 2). Bud break for *Populus alba* in HU began in mid-April and more than half of the plants

Table 2. Annual date for 50 % growth in control plots (expressed in day of year) and the annual effect of warming treatment (Control “C” minus Warming “W”) on the dominant species of the five studied sites. Precipitation and mean temperature in the previous three months $_{(1)}$ or four months $_{(2)}$ before growth date. For *Erica multiflora* we used the minimum temperature $_{(2m)}$. Bold type indicates significant inter-annual differences in the timing of growth in control plots (p<0.05) or significant effects in the warming plots (p<0.05). Different letters indicate significantly different means (Bonferroni post-hoc test of the ANOVA). Asterisk (*) indicates a significance of 0.05<p<0.1. Standard errors are between brackets.

Site	**Species**	**Years**	**Growth date (DOY)**	**Warming Advancements (days)**	**Previous Rain (mm)**	**Previous Temperature (ºC)**
UK	*Calluna vulgaris*	2000	**173 a**	4.3 (4.18)	$293.3_{(1)}$	$7.6_{(1)}$
		2002	**172 a**	-7 (2.08)	$185.1_{(1)}$	$11.15_{(1)}$
		2004	**152 b**	-1.3 (1.86)	$215.7_{(1)}$	$6.2_{(1)}$
	Empetrum nigrum	2000	192	2 (4.04)	$293.3_{(1)}$	$8.9_{(1)}$
		2002	186	-1.3 (12.47)	$185.1_{(1)}$	$11.15_{(1)}$
		2004	182	**-9.3 (1.86)**	$195.9_{(1)}$	$9.2_{(1)}$
	Vaccinium myrtillus	2000	**137 a**	1.3 (2.40)	$293.3_{(1)}$	$5.9_{(1)}$
		2002	**140 a**	**6* (2)**	$185.1_{(1)}$	$11.15_{(1)}$
		2004	**133 b**	1.3 (0.88)	$204.7_{(1)}$	$4.3_{(1)}$
DK	*Calluna vulgaris*	2003	157	4 (2.1)	$141_{(1)}$	$7.7_{(1)}$
	Deschampsia flexuosa	2003	130	**18.7 (0.7)**	$83_{(1)}$	$2.9_{(1)}$
NL	*Calluna vulgaris*	2003	**164**	**-28.7 (6.30)**	$169_{(1)}$	$7.7_{(1)}$
HU	*Populus alba*	2003	**116 a**	2.2 (0.9)	$39.7_{(1)}$	$17.4_{(1)}$
		2004	**110 b**	**1.6 (0.6)**	$235.7_{(1)}$	$15.9_{(1)}$
SP	*Erica multiflora*	2003	**134 a**	**18.3 (5.5)**	$214.4_{(2)}$	$3.4_{(2m)}$
		2004	**136 a**	**16.7 (5.4)**	$338.8_{(2)}$	$2_{(2m)}$
		2005	**125 b***	4.7 (1.3)	$97.4_{(2)}$	$2.4_{(2m)}$
	Globularia alypum	2003	125	4.0 (3.1)	$214.4_{(2)}$	$9.7_{(2)}$
		2004	124	3.0 (3.6)	$338.8_{(2)}$	$9.5_{(2)}$
		2005	134	10.0	$97.4_{(2)}$	$8.5_{(2)}$
IT	*Cistus monspeliensis*	2002	**44 a**	-1.67 (1.67)	$136_{(1)}$	$10_{(1)}$
		2003	**50 b**	0	$169.2_{(1)}$	$10.8_{(1)}$

had bud bursted less than one week later (Table 2, Fig 3). The earliest growth date for this species occurred in the rainy 2004. The appearance of new shoots of *Calluna vulgaris* in NL began at the end of March and continued until mid-August (Table 2, Fig 4). The date for 50 % growth for this species occurred in June. In the DK site, *Deschampsia flexuosa* and *Calluna vulgaris* began to grow in April but the former reached half of its maximum length a month earlier than the latter (May and June respectively) (Table 2, Fig 5). Growth of *Calluna vulgaris*, *Empetrum nigrum* and *Vaccinium myrtillus* in UK began between April and May and leveled out between July and August. The dates for 50 % growth for these species were in June, July and May respectively (Table 2, Fig 6). *Calluna vulgaris* and *Vaccinium myrtillus* presented an earlier growth date in 2004 respect previous years.

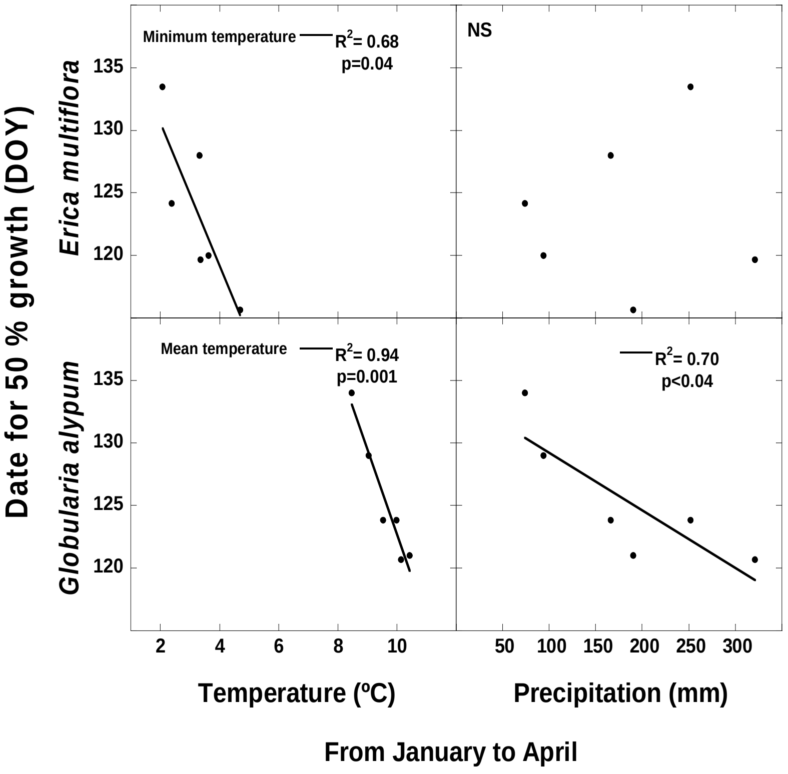

Figure. 1 Date for 50 % growth for the dominant species of the Spanish site as a function of temperature and accumulated precipitation in the previous four months. Each dot is the mean for either the control or the warming treatment in 2003, 2004 and 2005. For *Erica multiflora* we used the minimum temperature and for *Globularia alypum* the mean temperature. “NS” indicates no significant relationship.

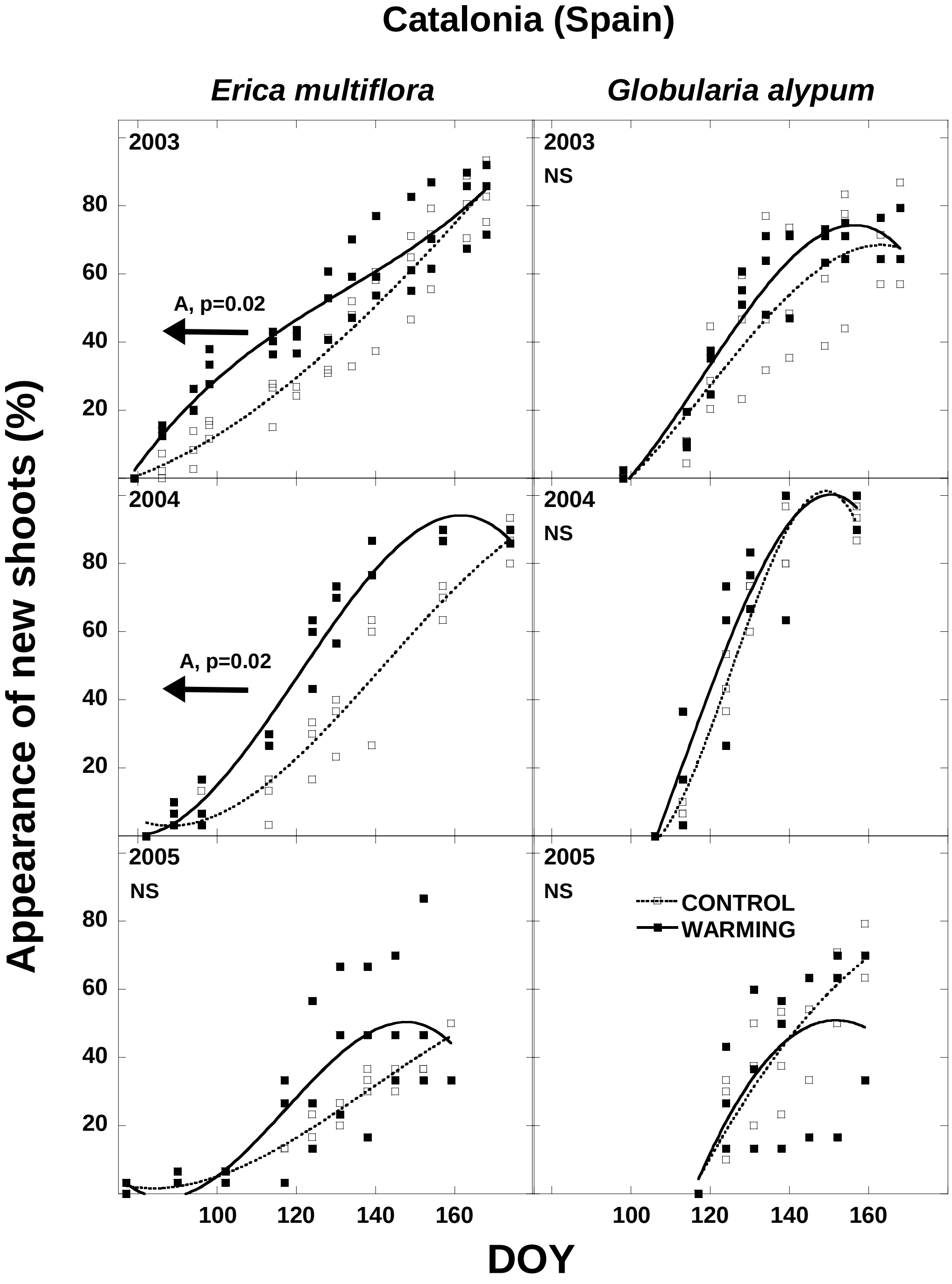

Figure. 2 Annual sprouting in *Erica multiflora* and *Globularia alypum* in control and warming plots at the Spanish site in 2003, 2004 and 2005. Each dot is the value of a plot. “A” indicates a significant advance of phenology in response to warming (Log-rank Mantel-Cox test). “NS” indicates no significant effect of the treatment.

Effects of warming on the timing of spring growth

The clearest effect of warming treatment was the acceleration of the onset of growth of some of the studied species (Table 2, Fig 1-3-5-6). From a total of 22 observation series, 15 exhibited growth acceleration (of which 5 were statistically significant) while 6 exhibited growth retardation (of which 2 were significant). However, we found differences in the responses depending on the site, the species and the year. Significantly earlier dates for 50 % growth were detected in SP, HU, DK and UK (Table 2, Fig 1-3-5-6). *Cistus monspeliensis* at the Italian site was not affected in either of the two studied years but warming treatment in this site was weaker than in the others sites (Table 2). In Spain, in 2003 and 2004, warming treatment sped up the onset of the growth in *Erica multiflora* 17.5 days on average (p=0.02 for both years) but there were no significant effects in 2005, or in any year for *Globularia alypum* (Table 2, Fig 1). In Hungary, we detected a significant acceleration of the buds' development in *Populus alba* in 2004 but not in 2003 (Table 2, Fig 3). In Netherlands, growth in *Calluna vulgaris* was delayed 28.7 days (p=0.02) (Table 2, Fig 4). In Denmark, *Deschampsia flexuosa* sped up its shoot growth by 19 days (p=0.02) but there was no significant change in *Calluna vulgaris* (Table 2, Fig 5). In Wales, the warming treatment resulted in 6 days earlier date for 50 % growth in *Vaccinium myrtillus* in 2002 (p=0.06). *Calluna vulgaris* and *Empetrum nigrum* exhibited delayed dates in warming plots, although only the difference detected in 2004 for the latter species was statistically significant (Table 2, Fig 6). Globally, we did not detect a geographical gradient in the magnitude of the accelerations of phenology in response to warming. However, the two statistically significant delays were detected in northern sites (Fig 7).

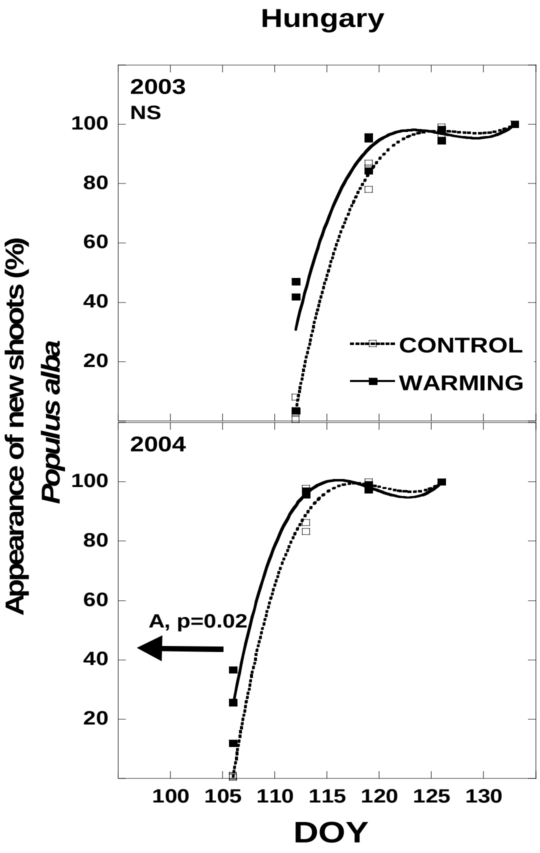

Figure. 3 Sprouting process in *Populus alba* in control and warming plots at the Hungarian site in 2003 and 2004. Each dot is the value of a plot. “A” indicates a significant advance of phenology in response to warming (Log-rank Mantel-Cox test). “NS” indicates no significant effect of the treatment.

DISCUSSION

Shifts in the date for 50 % growth in response to increased temperatures are in agreement with the findings of previous studies indicating important role of temperature over spring plant phenology in cold and temperate regions (Heide 1993; Ahas *et al.,* 2002; Peñuelas *et al.,* 2002; Jato *et al.,* 2004; Gordo & Sanz 2005; Crepinsek *et al.,* 2006; Schwartz *et al.,* 2006). Previous studies using long series field data have already revealed a trend towards earlier onset of growing season associated with increased temperatures during the last decades (Menzel & Fabian 1999; Menzel 2000; Peñuelas *et al.,* 2002; Schwartz *et al.,* 2006; Menzel *et al.,* 2006). In the present study our experimental warming affected spring growth in the expected way; 68 % of cases exhibited earlier onset of growing season (23 %

of the total were significant) and 27 % delayed onset of vegetative growth (9 % of the total were significant). We did not detect a geographical gradient in the experimental warming effects (Fig 7). The significant responses to this treatment and their magnitude had a great variability in each site depending on the species and the meteorological specificities of each year.

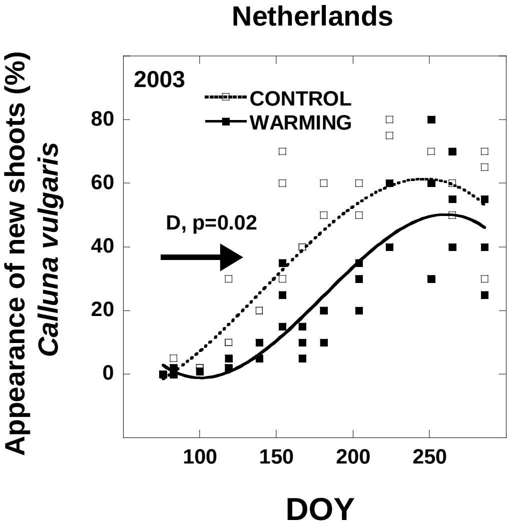

Figure. 4 Sprouting process in *Calluna vulgaris* in control and warming plots at the Dutch site in 2003. Each dot is the value of a plot. “D” indicates a significant delay of phenology in response to warming (Log-rank Mantel-Cox test).

In our experimental studies, the warming treatment accelerated the spring growth date in species distributed in north and south sites. *Vaccinium myrtillus* (UK), *Deschampsia flexuosa* (DK), *Populus alba* (HU) and *Erica multiflora* (SP) presented a flexible growth system capable of adjusting vegetative phenology to warmer conditions, accelerating their spring growth on average 6, 19.2, 1.6 and 17.5 days respectively. Some studies have reported acceleration of spring phenophases particularly in Central, Northern and Western European regions (Menzel 2000; Ahas *et al.,* 2002; Schwartz *et al.,* 2006). Many studies have also found that temperature has a strong influence on spring phenophases (leaf unfolding, shoot-growth, spring flowering) in Mediterranean species, especially when water is available. This suggests that the phenology in Mediterranean plants is at least as responsive as the phenology of plants in colder biomes (Osborne *et al.*, 2000; García-Mozo *et al.,* 2002; Peñuelas *et al.,* 2002; Mutke *et al.,* 2003; Gordo & Sanz 2005). Results published recently by Menzel *et al.* (2006) describe for some spring phenophases and species a stronger response to temperature in warmer than in colder countries. This agrees with findings of early spring phenophases being more responsive to warming than later spring events (Menzel 2000; Roetzer *et al.,* 2000; Ahas *et al.,* 2002; Menzel *et al.,* 2006).

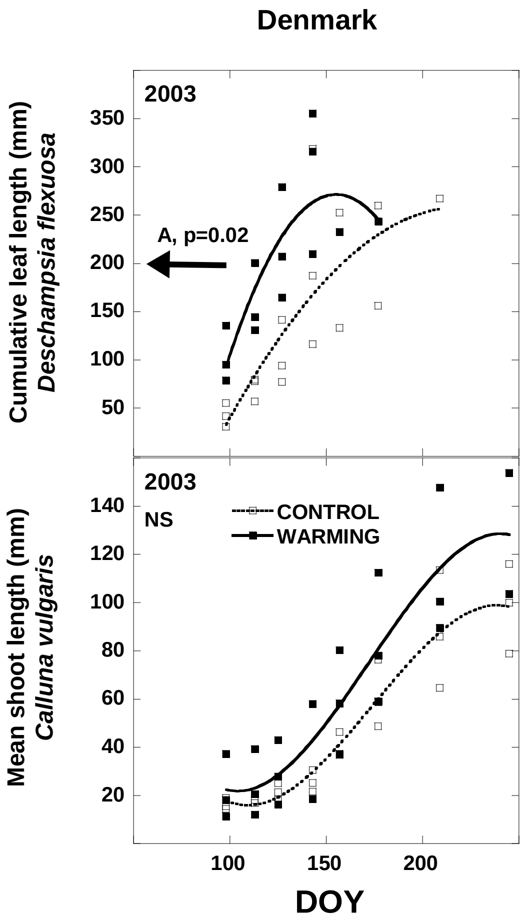

Figure. 5 Elongation process in *Deschampsia flexuosa* and *Calluna vulgaris* in control and warming plots at the Danish site in 2003. Each dot is the value of a plot. "A" indicates a significant advance of phenology in response to warming (Log-rank Mantel-Cox test). "NS" indicates no significant effect of the treatment. The *Calluna* shoots are unusually long as the 3 year old *Calluna* plants regenerated after a heather beetle attack in 2000 in dense grass vegetation.

Warmer temperatures were accompanied by delays in spring growth in Wales in 2004 for *Empetrum nigrum* and in *Calluna vulgaris* in Netherlands in 2003. This is an unexpected finding as winter temperatures are often limiting for growth in cold biomes, and it has been suggested that these environments are especially sensitive to climate warming (Menzel 2000; Aerts *et al.,* 2006). Shevtsova *et al.* (1997) described positive effects of increased temperatures on phenology and shoot growth of *Empetrum nigrum* in the Finnish Subarctic. However, our studied site corresponds with the southern distribution area of this species generally distributed in the cooler regions of the northern hemisphere (Bell & Tallis 1973). In fact, upward shifts in southern distribution of *Empetrum nigrum* have been attributed to increasing mean winter temperatures during the ninetieth and the first half twentieth centuries (see ref in Bell & Tallis 1973). Some authors have suggested that warming through milder winters and less fluctuating temperatures, may produce an inadequate chilling (needed for dormancy release) and consequently a delayed bud burst (Heide 1993; Partanen *et al.,* 1998) that may endanger *Empetrum nigrum* plants under unfavourable conditions.

Our results underscore the species-specific nature of the responsiveness of spring growth to temperature (Kramer 1994, 1995; Peñuelas *et al.,* 2002, 2004b; Hollister *et al.*, 2005). The acceleration of the growth of the Spanish species are particularly showy since in general, the phenology pattern in sites where water is seasonally limiting has been related to the period when water first becomes available to the plants (Corlett & Lafrankie 1998; Peñuelas *et al.,* 2004a). However, as described by Ogaya & Peñuelas (2004) in a Mediterranean forest, the influence of water availability and temperature in the control of leaf development and spring flowering may vary depending on the species. In the present study, the spring growth date for both Spanish species was associated with the mean temperature of the previous months (Fig 1), although only the growth of *Erica multiflora* was accelerated by warming treatment. The lack of significant acceleration in the growth of *Globularia alypum* in warming plots can be consequence of its stronger dependency to the soil water status described for some ecophysiological parameters (Llorens *et al.,* 2003) as well as for growth phenology in this study (Fig 1). For *Erica multiflora*, the relationship with water availability was not significant, although the dry period between late-winter and early-spring in 2005 accelerated the onset of growth in *Erica multiflora* in control plots as compared to 2003 and 2004 (Table 2). *Erica multiflora* is a species with a conservative strategy respect to water use (Llorens *et al.,* 2003) and, at the light of the warming effects described in this study, this earlier growth in 2005 might be a consequence of an increased leaf temperature resulting from reduced leaf transpiration under lower water availability. The lower stomatal conductances reached in *Erica multiflora* in 2005 (winter and spring) relative to the rates in 2003 and 2004 support this hypothesis (Prieto, 2007).

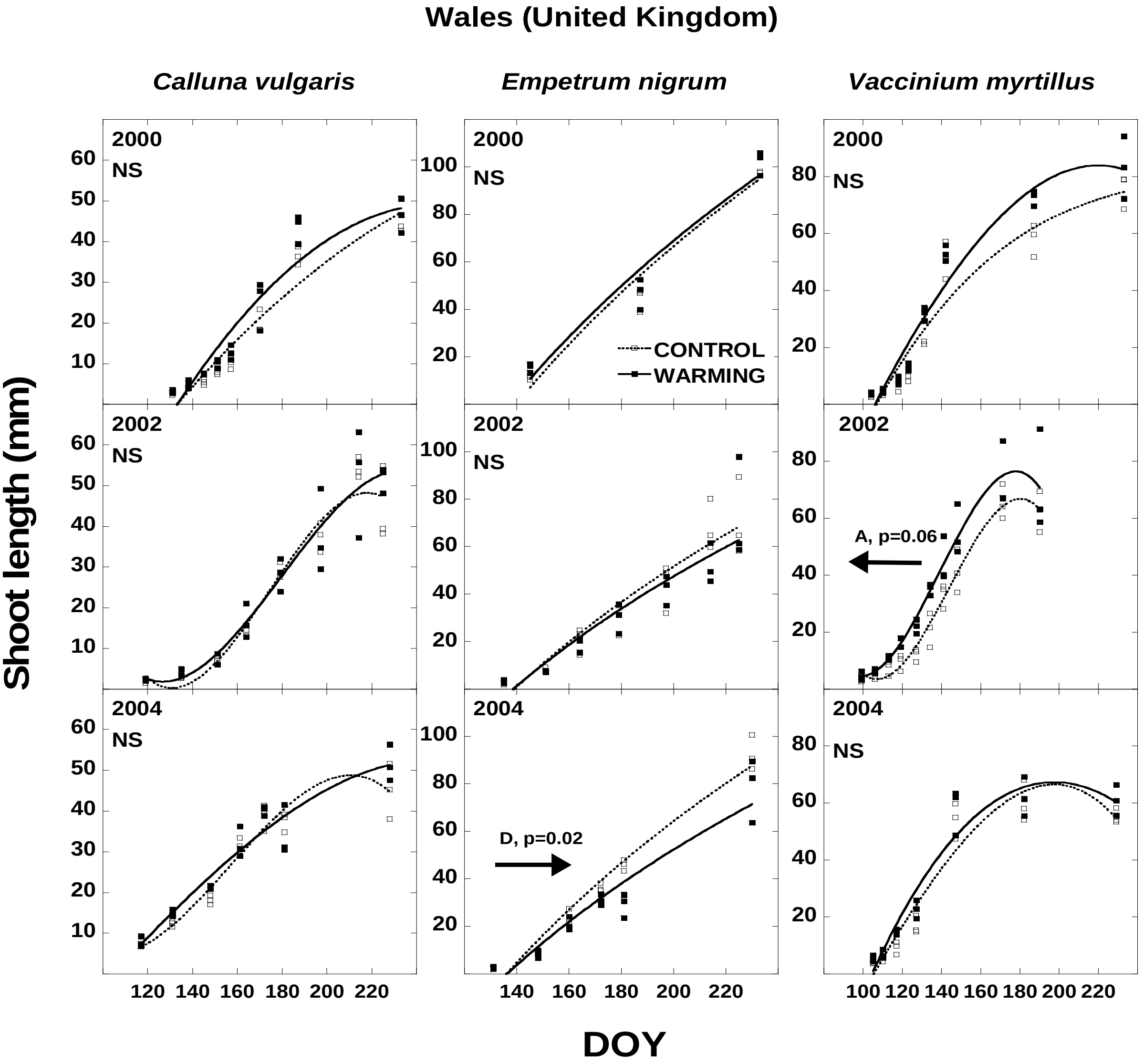

Figure. 6 Elongation process in *Calluna vulgaris. Empetrum nigrum* and *Vaccinium myrtillus* in control and warming plots at the Welsh site in 2000, 2002 and 2004. Each dot is the value of a plot. "A" and "D" indicates a significant advance or delay of phenology in response to warming respectively (Log-rank Mantel-Cox test). "NS" indicates no significant effect of the treatment.

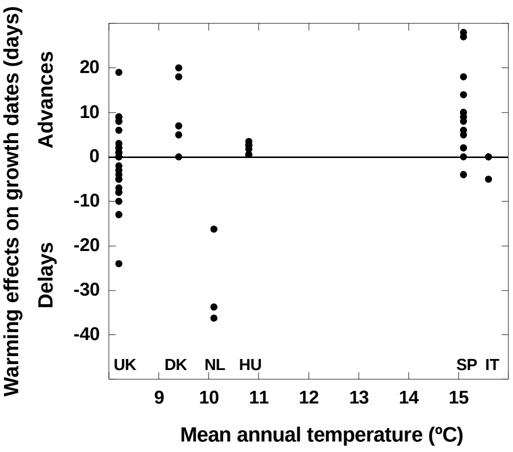

Figure. 7 Advances and delays of warming treatment on growth date (Control minus Warming) in relation to mean annual temperature of the site in different European sites and species. Each dot is the value of a plot. Table 1 provides information of the sites and studied species.

Plant responses to warming also depended on specific combinations of environmental drivers in particular years, for example, on the temperature or the amount and distribution of rainfall throughout the seasons and preceding years. The date for bud break of *Populus alba* occurred one week earlier in the cooler and rainy 2004 than in 2003. However, warming resulted in earlier bud break only in 2004, probably due to the temperatures being lower in the trimester previous to bud break date than in 2003 (15.9 ºC and 17.4 ºC respectively). In *Erica multiflora,* in spite of the clear acceleration of spring growth dates in warming plots in 2003 and 2004, we did not detect a significant change in 2005, which was the year with the driest late winter and spring during the seven recent years. Moreover, the earlier onset of the growth date in 2003 and 2004 was only accompanied by greater spring shoot elongation in 2004 (Prieto, 2007), probably due to the high temperatures reached during the European heat wave in 2003, which enhanced evapotranspiration and reduced water availability for shoot growth. Also in 2003, the warm and dry conditions of the previous trimester to growth were probably important to determine the significant delay in spring growth in *Calluna vulgaris* in Netherlands under the warming treatment. This species exhibited different responses to warming treatment in Wales and Denmark depending on the year, but none of them were significant. So, further studies including the inter-annual variability of the responses are necessary to understand the future effects of global warming in growth phenology of *Calluna vulgaris*.

At community level, statistically non-significant increases in biomass accumulation took place in warming plots in the Welsh, Danish, Dutch, Hungarian and Spanish sites (Peñuelas *et al., in press*; Prieto, 2007). So, although warming may imply a lengthening of the vegetative growth period, and consequently increased carbon fixation (Menzel & Fabian 1999; Schwartz 1999; Menzel 2000; Pop *et al.*, 2000; Peñuelas & Filella, 2001; Linderholm 2006), other limiting factors such as water availability, and species-specific and year-dependent responses may preclude significant changes in the community biomass accumulation.

Conclusions

Our results show the great potential effects of warmer temperatures over the phenology of the spring growth of dominant species in different European shrublands. Our study also shows overall difficulties of applying simple predictive relationships (Rötzer & Chmielewski 2001; Ahas *et al.*, 2002) to project global change effects on phenology. Various combinations of environmental factors co-occur at different European sites and the interactions between different drivers can importantly alter phenology. Although earlier onset of the vegetative growth may lengthen the growing season, the great variability in the responses between sites, species and years point out the necessity for studies at longer temporal and spatial scales to enable more advanced estimates of the effects on the net carbon uptake.

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CHAPTER 4.

Flowering of *Globularia alypum* and *Erica multiflora* in Mediterranean garrigue, and its response to precipitation and to experimental drought and warming.

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ABSTRACT

- *Background and Aims* The relationships between the autumn flowering of two dominant and co-occurring species of Mediterranean vegetation, *Globularia alypum* L. and *Erica multiflora* L. and precipitation and experimental drought and warming was analysed to improve predictions about the response and adaptation of Mediterranean vegetation to climate change.
- *Methods* Beginning of anthesis and peak date (date of maximum flowering intensity) were monitored over four years (2001-2004) in a garrigue in the NE of the Iberian Peninsula. Two experimental treatments were applied, increased temperatures (+ 0.73 ºC) and reduced water availability (-17 %) with respect to untreated plots.
- *Key Results* Flowering of *Globularia alypum* and *Erica multiflora* differed greatly between years depending on the precipitation of the previous months and the date of the last substantial rain (above ten mm). *Globularia alypum* also flowered once or twice (unimodal or bimodal) resulting from differences in the distribution and magnitude of the precipitation in late-spring and summer (when floral buds develop). The drought treatment delayed and decreased flowering of *Globularia alypum* in 2001 and tended to delay flowering in 2002. However, warming had little effect: it extended autumn flowering in 2001 and it tended to increase peak intensity in 2002. Flowering of *Erica multiflora* was unaffected by both treatments.
- *Conclusions* Autumn flowering of *Globularia alypum* and *Erica multiflora* is more dependent on annual changes of water availability than on those of temperature. Considerable interannual plasticity in the beginning of anthesis and peak date and on the unimodal or bimodal flowering constitutes a safe strategy for both species in front of precipitation and temperature changes in this area. However, severe changes in

precipitation in spring and summer may severely affect flowering of *Globularia alypum.*

Key words: *Globularia alypum*, *Erica multiflora*, autumn flowering, drought, global warming.

INTRODUCTION

The study of plant phenology (the timing of life cycle stages) is important in understanding responses of vegetation to climate change, because improved knowledge of the geographical distribution of the timing of phenological phases and their relationship to climate will improve predictions of effects (Schwartz, 1999; Kramer *et al*., 2000; Peñuelas and Filella, 2001; Hollister *et al*., 2005; Menzel *et al*., 2006). Studies based on long phenological series, supported by field-based surveys or satellite remote sensing, have shown that particular phases in plant development (budding, leafing, flowering) are particularly sensitive to temperature and that they have been occurring progressively earlier (Menzel, 2000; Osborne *et al*., 2000; Rötzer and Chmielwski, 2001; Ahas *et al*., 2002; Walther *et al*., 2002; Parmesan and Yohe, 2003; Gordo and Sanz, 2005; Piao *et al*., 2006; Schwartz *et al*., 2006). For Europe, it has been estimated that an increase in temperature in winter or early spring of 1 °C corresponds to an advance in the onset of spring events of ca. 6 days (Menzel & Fabian 1999), and an increase of 1.4 ºC was reported to advance the onset by up to 16 days in a Mediterranean site (Peñuelas *et al*., 2002). Recently, Menzel *et al.* (2006) reported an overall advancement of the spring/summer events of 2.5 days per 1 ºC on average for the whole Europe. Since bud-bursting and flowering are not generally autumn phenophases fewer studies have focused on autumn events and in consequence, changes in phenophases occurring in autumn are not as clear nor as well known as in spring. There is some evidence of later onset of autumn phenological phases but there is no general agreement about the patterns (Menzel and Fabian, 1999; Menzel, 2000; Peñuelas *et al*., 2002; Walther *et al*., 2002; Chen *et al*., 2005; Gordo and Sanz, 2005).

The climate of the Western Mediterranean region is characterized by a pronounced seasonality: hot dry summers, cool winters and mild rainy, springs and autumns. Species grow and flower mainly in spring, thus avoiding winter cold and summer drought. *Globularia alypum* and *Erica multiflora* are co-occurring dominant species in some coastal Mediterranean shrublands, which also grow (Llorens *et al*., 2004) and flower (Llorens and Peñuelas, 2005) in the autumn, which is exceptional in Mediterranean shrubland communities (Orshan, 1989; Castro-Díez and Montserrat-Martí, 1998; Picó and Retana, 2001; Tèbar *et al*., 2004). Studies of Mediterranean plants have shown variable environmental control of non-spring flowering, being temperature one of the most important factors (Nilsen and Muller, 1981; Picó and Retana, 2001, Peñuelas *et al*., 2002; Jato *et al*., 2004; Ogaya and Peñuelas, 2004). Restricted water availability is another important factor associated with delayed flowering and decreased flower and fruit production (Gordon *et al*., 1999; Ogaya and Peñuelas, 2004; Peñuelas *et al*., 2004*ab*; Llorens and Peñuelas, 2005; Giménez-Benavides *et al*., 2007).

General conclusions of most of the General Circulation Models (GCMs) are that, over this century, mean global surface temperature will increase by 1.1 – 6.4 ºC depending on the socio-economic scenarios and the resulting emissions of greenhouse gases (IPCC 2007). Models also predict decreased rainfall and increased variability in its distribution (De Luís *et al*., 2001). Although there is no general agreement regarding future amount and timing of precipitation in the Mediterranean area, plant communities are likely to be greatly affected by the increased potential evapo-transpiration linked to the warming (Le Houérou, 1996; Piñol *et al*., 1998). These expected climatic changes may pose particular problems to plants with growth, e.g. leaf appearance, and reproduction, e.g. flowering, triggered by environmental conditions such as temperature or water availability. Such conditions act as cues regulating plant growth and development and specifically, may critically affect plant reproduction and flowering (Peñuelas and Filella; 2001; Peñuelas *et al*., 2004*a*; Llorens and Peñuelas, 2005; Sherry *et al*., 2007).

Flowering responses to climate change seem to be species-specific since co-existing species may have different environmental constraints (Peñuelas *et al*., 2002; Ogaya and Peñuelas, 2004; Llorens and Peñuelas, 2005). The consequences of a change (advance or delay) of flowering phenology might be a decoupling of species interaction, such as between plants and their pollinators, altered competitive relationships and different abundances ranges of species, for example by changed seedling recruitment (Bond, 1995; Gordo and Sanz, 2005). Together, all these readjustments may lead to important changes in the structure, composition and functioning of the ecosystem (Peñuelas and Filella, 2001; Hollister *et al*., 2005) and therefore, changes in phenological processes may have huge socio-economic consequences around the world.

The aims of this study were (i) to describe the flowering of two common Mediterranean species, *Globularia alypum* and *Erica multiflora,* that flower in autumn, (ii) to explain the relations between the flowering phenology and the environmental conditions which vary interannually (mostly precipitation), and (iii) to study the responses to experimental field warming and drought that simulate the future climate conditions forecasted by GCM and ecophysiological models (IPCC, 2001, 2007;

Peñuelas *et al*., 2005). We hypothesized that: (a) the beginning of anthesis and peak date (date of maximum flowering intensity) will vary between years according to the precipitation, since these phenophases occur after the summer drought and therefore under water deficit conditions, and (b) flowering would be delayed and/or the intensity of the flowering peak would be reduced in response to projected future drier conditions, especially in *Globularia alypum* which begins to flower in late-summer, and warming would enhance flowering, especially in *Erica multiflora* that flowers later than *Globularia alypum,* in wet and colder periods.

MATERIALS AND METHODS

Study site and plant species

The study was carried out in a dry calcareous shrubland (*Rosmarino-Ericion*) in the Garraf Natural Park in North-East Spain (41º 18'N, 1º49'E), at 210 m above sea level. The climate is typically Mediterranean with two rainy seasons (spring and autumn), mild winters and hot, dry summers. During a seven-year study period (1998 to 2005), the mean annual precipitation was 592 mm with least in January and June-July (20-25 mm). The mean average annual air temperature was 15.2 ºC and the monthly mean maximum was 27 º C in August 2003 and the minimum 5 º C in January 2005.

The site is a south facing slope in abandoned old field terraces. The substrate consists of limestones and marls, with a many rock outcrops. In summer 1982 and spring 1994 the area suffered severe fires that destroyed *Pinus halepensis* forest and converted it into a garrigue. The dominant species are *Erica multiflora* and *Globularia alypum*. Both are re-sprouter shrubs distributed in dry calcareous and rocky places in the Mediterranean

Basin (Bolòs and Vigo, 1995). *Pinus halepensis* was reintroduced by seeding after the last fire and is gaining dominance. Other common species are *Dorycnium pentaphyllum, Pistacia lentiscus, Rosmarinus officinalis, Fumana ericoides, Fumana thymifolia* and *Helianthemum syriacum*. The plant community has an average annual aboveground net primary productivity of 160 g m^{-2} (Peñuelas *et al*., *in review*) and the plant cover in 2005 reached 75 %.

Diagram 1. Distribution of the experimental plots (4 m x5 m) with night-time warming (in red) or drought (in blue) treatments and controls (in grey).

Experimental treatments manipulations

We imposed three separated experimental treatments; field-scale nighttime warming, drought and control, and the response to warming and drought were compared separately to the response of control plots. Plots were 4 m x 5 m, allowing for a buffer strip of 0.5 m at the perimeter. Each type of manipulation was replicated three times, giving a total of nine plots (three warming, three drought and three controls) (Diagram 1). The experimental treatments were applied continuously from 1999 to 2005.

Drought treatment. The drought treatment was applied for 2 to 3 month periods in the spring and autumn growing seasons by covering the vegetation with waterproof, transparent covers. For the rest of the year the drought treatment was off. The drought plots are constructed similarly to the warming plots except that the curtain material is a transparent plastic and that the moving of the curtains is governed only by rain and wind. When the drought treatment is operating, the rain sensors activate the curtain to cover the plots whenever it rains and remove the curtains when the rain stops. The curtains are removed automatically if the wind speed exceeds ten m s^{-1}.

Warming treatment. The warming treatment consisted of covering the vegetation during the night-time with reflective aluminium foil curtains (ILS ALU, AB Ludvig Svensson, Sweden) (Beier *et al.*, 2004). Solar energy is accumulated in the plots during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. Covering reduces the loss of IR radiation. The curtains reflected 97 % of the direct and 96 % of the diffuse radiation from vegetation to night-sky. The warming plots are covered by light scaffolding carrying the reflective aluminium curtain. The covering of the study plots is activated automatically when solar radiation is less than 0.4 Wm^{-2} according to a preset light level. The curtains are removed automatically if wind speed exceeded 10 m s^{-1} (Beier *et al.*, 2004). In order to avoid influencing the hydrological cycle, rain sensors trigger the automatic removal of the covers when it rained at night.

This method has the advantage that unintended edge effects and artifacts are minimized. Measurements of curtain movements, temperatures, precipitation, water input into the plots, radiation balance during campaigns, relative humidity, and wind speed showed that the edge effects on the temperature increase, as well as unintended effects on wind and moisture conditions, were minimal. Since nighttime warming implies leaving the plots open during daytime, the effect on light conditions was negligible (Beier *et al.*, 2004).

"Untreated control". Three untreated control plots were set up for comparison, with a similar light scaffolding as for the warming and drought treatments, but without any curtains.

Environmental conditions were monitored in all plots. Precipitation within the plots was measured using three water collectors. Soil moisture was also measured weekly by means of three time domain reflectometry (TDR) probes installed per plot. Air (+20 cm) and soil temperatures (at 5 and 10 cm depth) and the functioning of the treatments, (curtain closure and removal detected by magnetic sensors, were recorded (Datalogger, Campbell Scientific Inc. Logan, Utah, USA).

Data collection and analysis

We monitored the timing of the autumn flowering of *Erica multiflora* and *Globularia alypum* from observations made biweekly during the less active flowering period and weekly during the most active flowering period, over four consecutive years (2001-2004). Four-12 *Erica multiflora* plants and 11-15 *Globularia alypum* plants were monitored in each plot. For each plant we estimated the flowering potential, as the quantity of new shoots where floral buds could potentially develop and then, the percentage of shoots with functional flowers (live flowers with at least a petal and stamen) relative to its flowering potential. Four flowering categories were established depending on the percentage of shoots with functional flowers: one (1-25%), two (26-50%), three (51-75%) and four (76-100 %). We computed the percentage of branches with functional flowers per plot for each census occasion as the mean of the values of all plants in the plot. We followed this procedure until no new flowers opened. We determined three traits to characterize the flowering process: beginning date (date at which flowering was greater than 1%), peak date (date when flowering per plot reached its maximum intensity), and the intensity of the peak.

Statistical analyses

All the statistical analyses were performed using one value per plot, obtained from averaging the plants measured per plot. Drought and warming treatments were always compared separately with control. Data values expressed in percentage were arcsine-square root transformed to homogenize variances. RM-ANOVAs were conducted to test inter-annual differences in the beginning and peak dates and intensities in control plots and also to check the global effects of the warming and drought treatments on the flowering. ANOVAs were used to test potential effects of the treatments on the peak intensity. Post-hoc (Fisher PLSD) tests were performed for comparisons between years for each species. Survival analyses were used to test differences in the beginning and peak dates between treatments and controls. Survival time was defined as the number of days from January 1 until the beginning of flowering or peak day occurred. We used the Kaplan-Meier non-parametric method for the computation of survival curves and log-rank (Mantel-Cox) statistics to test for differences between treatments and controls. Correlation analyses were conducted in order to examine the relationship of the accumulated precipitation and the average temperature (from different periods prior to flowering) with the flowering traits. We also tested the relationship with the date when the last important rain event (more than 10 mm) just before peak date took place. The correlations were conducted with treatment mean values, and therefore with values having known associated variance. Thus a model II regression by means of the reduced major axis method was used (Sokal and Rohlf, 1995). For *Globularia alypum*, in cases of bimodal flowering pattern, we always used the most prolific peak (the second one in 2001 and the first one in 2003). Survival analyses, ANOVAs, Fisher PLSD test, RM-ANOVAs, and correlation and regression analyses were performed with the Statview software package (Abacus Concepts Inc., Cary, North Carolina, USA).

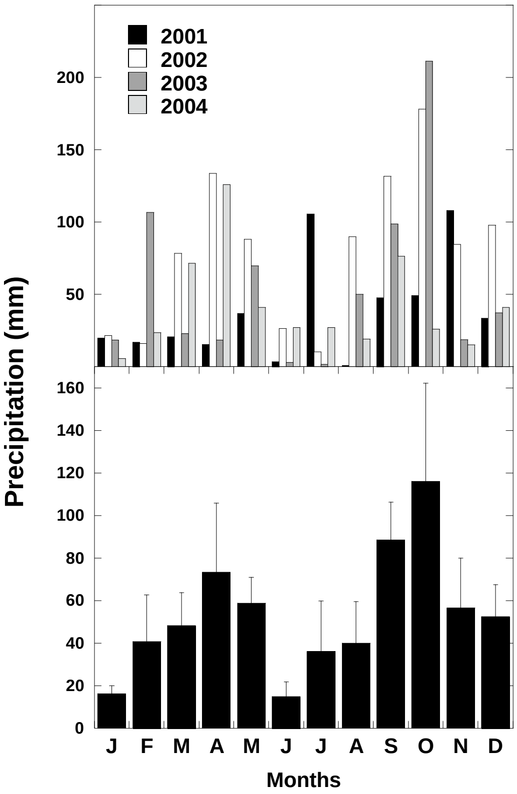

Figure 1. a) Monthly precipitation and b) monthly average precipitation based on the four years-period of study (2001-2004). Bars indicate the standard errors of the mean (n=4 years means).

RESULTS

Environmental conditions

Precipitation during the four years of the study (2001-2004) showed great inter-annual variability from 458 mm in 2001 to 956 mm in 2002. The distribution throughout the year was typically Mediterranean, although variable, with spring, and especially autumn, rain (Fig 1). The driest periods were in summer, June, July and August, which together had on average 80 mm, and mid-winter, January, with 20 mm. Temperature through the year was typically Mediterranean with moderately cold winters and hot summers. The annual mean air temperature was 15.2 ºC.

Drought treatment by covering with transparent sheet, reduced mean annual soil moisture by an average of 17 % with respect to control plots (2001-2004 period). But this effect varied between periods and years (Table 1). During the period of flower development (spring and summer), the soil moisture was reduced by an average of 13 % with respect to control plots although in 2001 the reduction in spring reached the 44 % (Table 1). The average soil moisture both in summer and the whole spring-summer periods presented statistically significant interannual variability ($p<0.001$). The experimental drought reduced, but not statistically significantly ($p=0.11$), the average soil moisture in spring-summer period (Table 1). The warming treatment did not affect soil moisture (Beier *et al.,* 2004; Peñuelas *et al.,* 2004*b*).

Covering at night warmed the plots, increasing mean air temperature through the year by an average of 0.73 ºC compared to control plots. The increase was greater for soil mean temperature (0.9 ºC) and especially for the minimum temperature (1.5 ºC). Drought treatment affected neither mean nor minimum air temperature or soil temperature (Beier *et al.,* 2004; Peñuelas *et al.,* 2004*b*).

Table 1. Average soil moisture (%) in summer and in spring-summer periods in control (C) and drought (D) plots for the studied years (2001-2004). Mean (+- standard errors: n=3). Significant differences (Fisher post-hoc test) among years are indicated by different letters and different letters between brackets indicates significant effect of drought treatment on soil moisture (ANOVA).

	Summer (from June 1 to August 31)		**Spring – Summer** (from April 1 to August 31)	
Year	**C**	**D**	**C**	**D**
2001	9 (1.2) ab (a)	5 (0.4) a (b)	11 (1.2) a (a)	8 (0.4) a (b)
2002	10 (1.3) a	10 (0.8) b	16 (1.4) b	14 (0.6) b
2003	6.5 (0.7) b	7 (0.7) c	14 (1) ab	12 (0.1) c
2004	11 (0.9) a	9 (0.6) bc	16 (1.2) b	14 (0.9) b

Characterization of the flowering and treatment effects

Globularia alypum presented two flowering patterns depending on the year (Fig 2 and 3). In 2001 and 2003 two peaks occurred, whereas in 2002 and 2004 only one peak was noted. Flowering features for this species showed a significant inter-annual variability (RM-ANOVA; beginning and first peak date: $p<0.0001$; first peak intensity: $p=0.01$ and second peak date: $p=0.0002$) (Table 2) in correlation with the precipitation as described below.

Drought treatment affected flowering of *Globularia alypum* in 2001 (RM-ANOVA; $p=0.004$). The intensity of the first peak was reduced by 13 % (ANOVA; $p=0.03$) and drier conditions retarded the second peak date by almost ten days (Log-rank; $p=0.02$) (Fig 3). Experimental drought also tended to increase the percentage of open flowers at the end of the flowering period (ANOVA; $p=0.09$) (Fig 3). In the next three monitored years, we only observed a slow down of 11 days in the appearance of the earlier open flowers of *Globularia alypum* in 2002 (Log-rank; $p=0.06$) (Fig 3). In warming plots we detected a longer period between the beginning of flowering and the end of the second peak (ANOVA; $p=0.04$) in 2001 that included a reduced flowering at the end of the late-summer peak and an enhanced flowering at the end of the autumn peak. In 2002, an increased peak intensity of 6% was almost significant (ANOVA; p=0.06) (Fig 3).

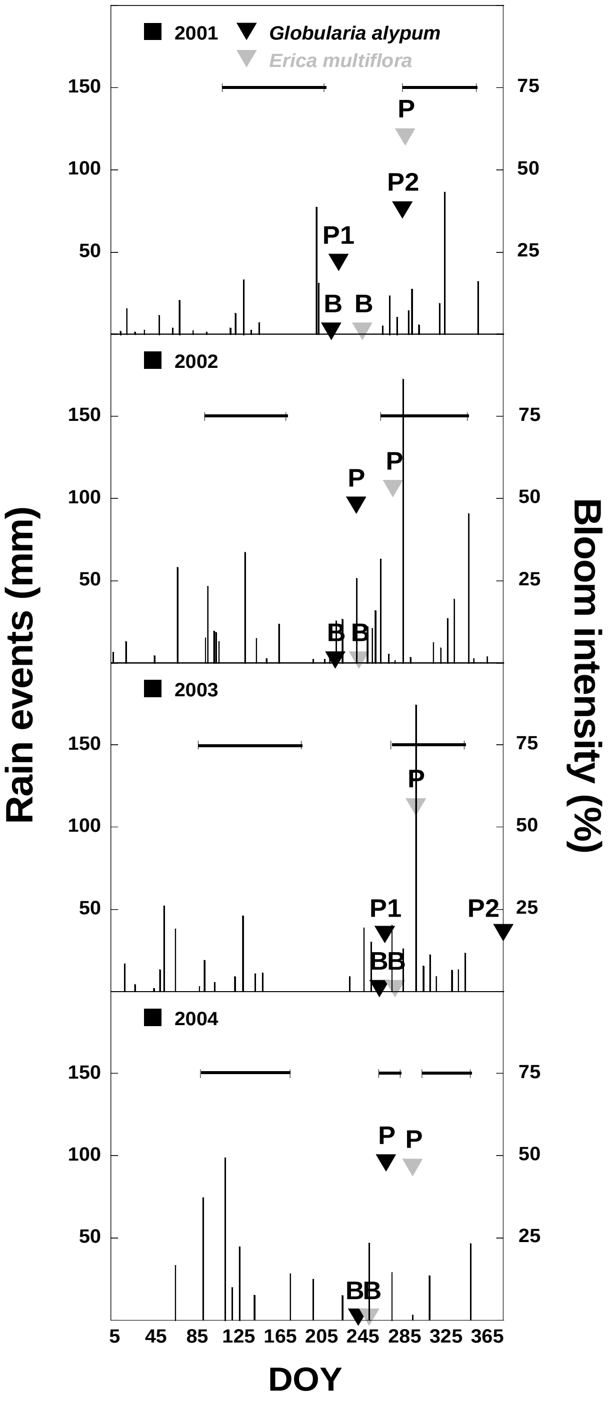

Figure 2. Distribution and magnitude of rainfall and beginning of anthesis “B” and peak date “P” (date of maximum flowering intensity) for each year of the study (2001-2004) for *Globularia alypum* and *Erica multiflora* in control plots. For *Globularia alypum* “P1” indicates the first peak and “P2” the second peak. Horizontal bars show the period of functioning of drought treatment.

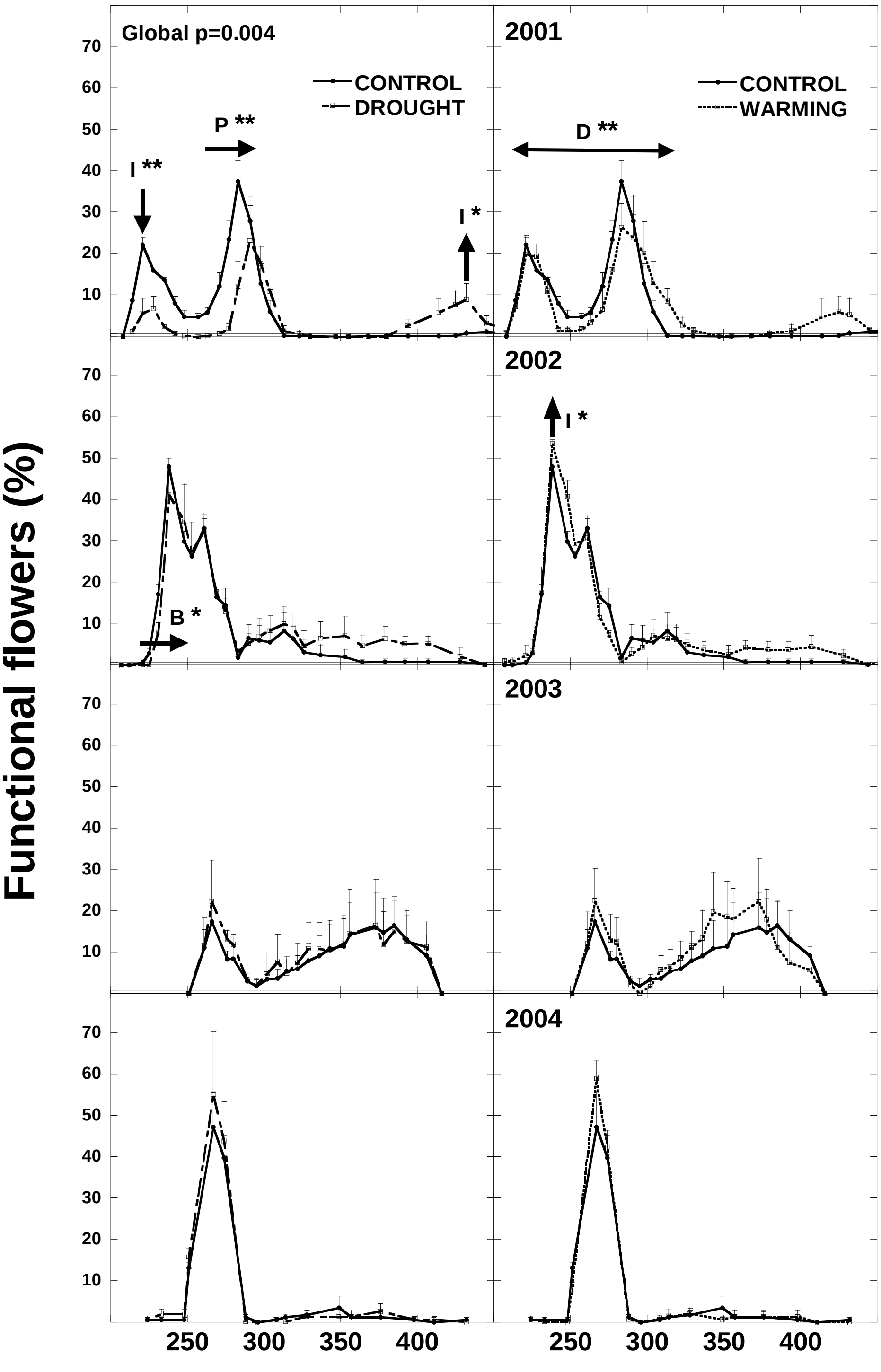

Figure 3. Annual flowering patterns for *Globularia alypum* in control, drought and warming treatments (2001-2004). Letters indicate the significant treatment effects on beginning date (B), peak date (P), peak intensity (I) and flowering duration (D). "**" indicates p <0.05. "*" indicates p <0.1. Arrows indicate the direction of the change.

Table 2. Annual mean dates of the beginning of flowering and peaks, expressed in days of the year (DOY), and peak intensity (%) for *Globularia alypum* and *Erica multiflora*. Each value is the mean of the variable in control and treated plots if treatments did not affect it. Bold type indicates significant inter-annual variability (RM-ANOVAs; p<0.001). Different letters indicate significantly different means (Fisher post-hoc test of the ANOVA; p<0.05).

Species	**Year**	**Beginning**	**First bloom**		**Second bloom**	
		DOY	**DOY**	**Intensity (%)**	**DOY**	**Intensity (%)**
Globularia alypum	2001	**212 a**	**223 a**	**22 a**	**285 a**	32
	2002	**219 a**	**238 b**	**48 b**		
	2003	**261 b**	**268 bc**	**17 a**	**372 b**	21
	2004	**241 bc**	**267 bc**	**48 b**		
Erica multiflora	2001	**248 a**	**293 a**	57		
	2002	**241 a**	**271 b**	56		
	2003	**276 b**	**299 a**	56		
	2004	**251 a**	**296 a**	53		

Erica multiflora exhibited a common flowering pattern throughout the 4 studied years with a single peak in the flowering period (they flowered only once) (Fig 2 and 4). The mean dates of beginning of flowering and peak differed significantly between years (RM-ANOVA; p<0.0001) (Table 2) and correlated with the precipitation. The beginning of flowering in 2003 occurred later, and the peak date in 2002 was earlier than in other years. Peak intensity was about 56% and did not vary between years (Table 2). For *Erica multiflora* neither drought treatment nor warming treatment affected flowering (Fig 4).

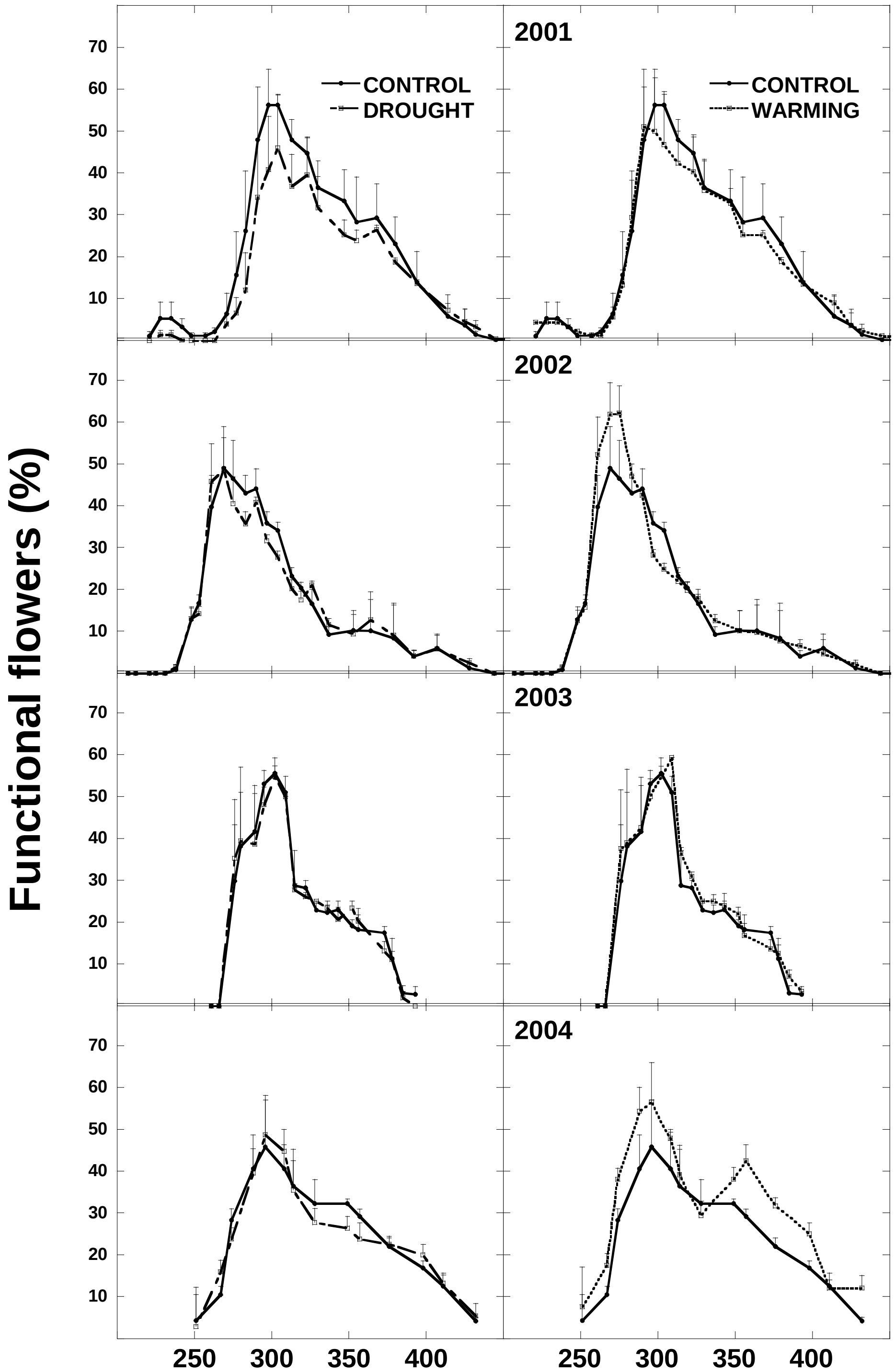

Figure 4. Annual flowering patterns for *Erica multiflora* in control and drought and warming treatments (2001-2004). There was no significant effect of any treatment

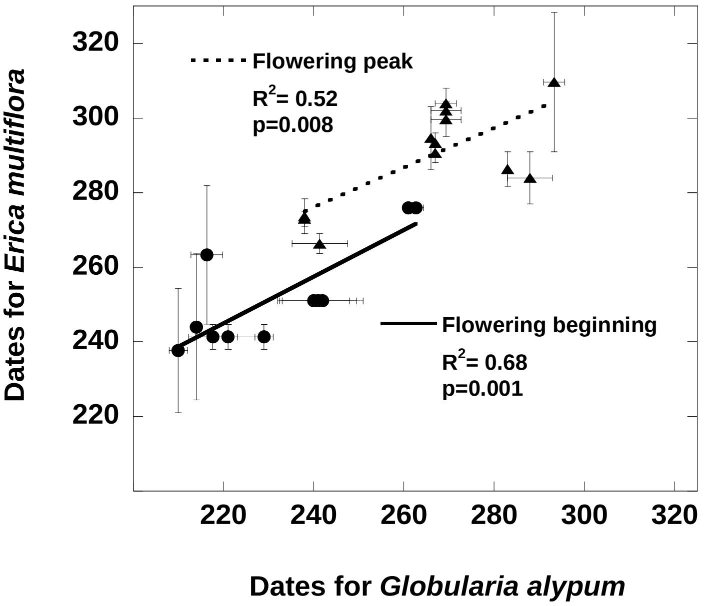

Figure 5. Synchronicity between the beginning of flowering and peak dates for *Globularia alypum* and *Erica multiflora.* Each dot corresponds to a mean value per treatment (n=12). Bars indicate the standard errors of the mean (n=3 plots).

Rainfall and flowering

The beginning of flowering and peak date for both species varied synchronously within years (beginning; R^2=0.68, p=0.001 and peak; R^2=0.52, p=0.008) (Fig 5). So delayed or advanced beginning and peak dates in the different years for *Globularia alypum* corresponded with delayed or advanced dates for *Erica multiflora,* indicating that flowering in both species is affected similarly by environmental variables. Precipitation was more closely correlated with flowering than was temperature. We found a negative, significant relationship between the beginning date of *Globularia alypum* and *Erica multiflora* and the accumulated precipitation in June-July (R^2=0.36, $p<0.04$) and June-July-August (R^2=0.52, p=0.008) respectively (Fig 6).

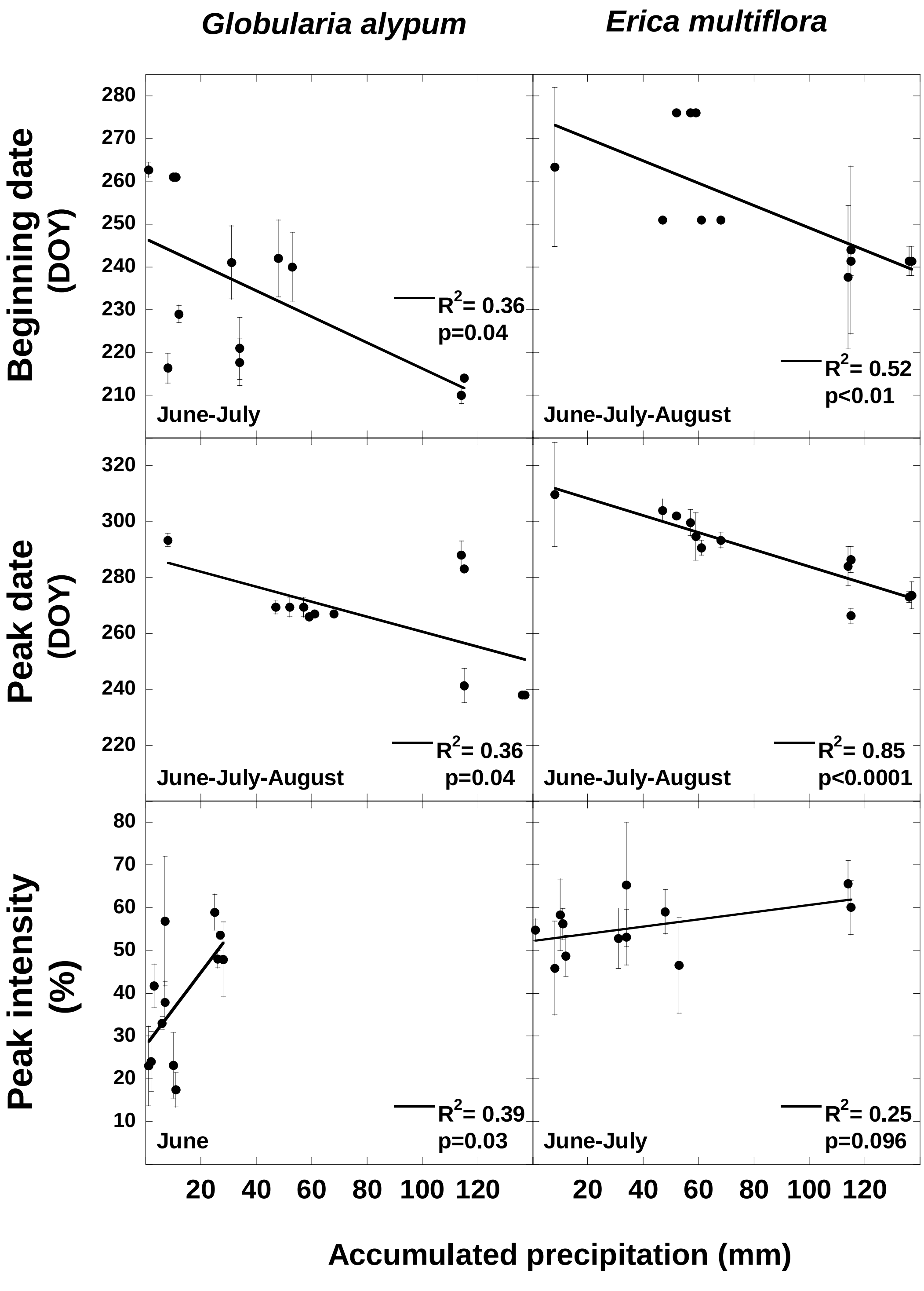

Figure 6. Relationships between the beginning of the flowering, the peak date and peak intensity for *Globularia alypum* and *Erica multiflora* and the accumulated precipitation in previous months (period depicted in each panel). Each dot corresponds to a mean value per treatment (n=12). Bars indicate the standard errors of the mean (n=3 plots).

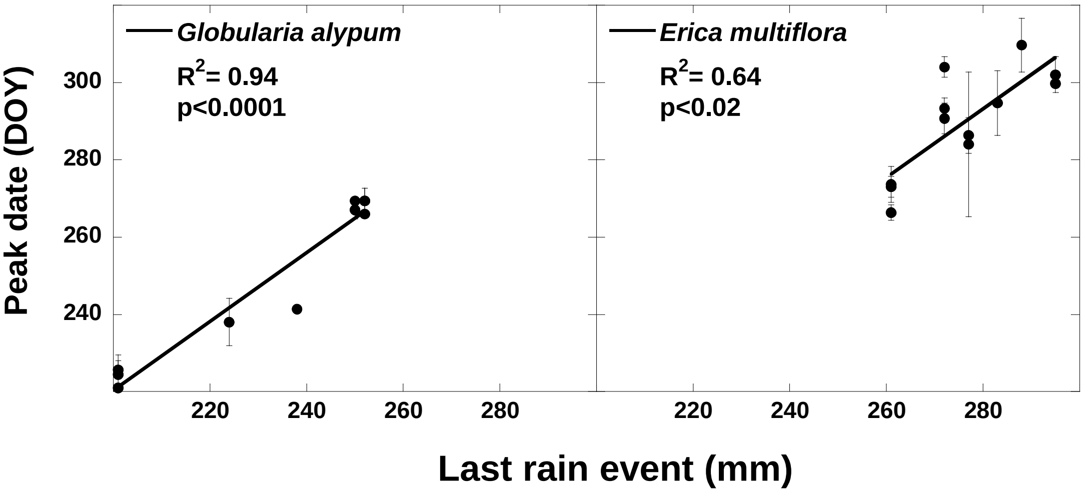

Figure 7. Relationships between the peak date for *Globularia alypum* and *Erica multiflora* and the date of the last rain exceeding 10 mm before the peak date. Each dot corresponds to a mean value per treatment (n=12). Bars indicate the standard errors of the mean (n=3 plots).

There was also negative correlations between the peak dates for *Globularia alypum* and *Erica multiflora* and the accumulated precipitation in June-July-August (R^2=0.36 p=0.04 and R^2=0.85 p<0.0001 respectively) (Fig 6). A positive, significant relationship was found between the peak intensity of *Globularia alypum* and the accumulated precipitation in June (R^2=0.39, p=0.03) (Fig 6). The peak intensity for *Erica multiflora* was marginally significant correlated with the accumulated precipitation in June and July (R^2=0.25, p=0.096) (Fig 6). The control of the precipitation over the peak date for both species was also manifested in the significant relationship between the peak date and the date of the last important rain event (more than 10 mm) just before peak date (*Globularia alypum;* R^2=0.94, p<0.0001; *Erica multiflora;* R^2=0.64, p<0.02) (Fig 7). In general, the beginning of the flowering of both species was advanced in years with a rainy summer. In those conditions, the peak date for *Erica multiflora* was also advanced but the peak date for *Globularia alypum* seemed mostly triggered by the close occurrence of a relatively important rain event (water availability in short-period).

DISCUSSION

Similar inter-annual behaviour was observed in terms of synchronization of flowering for *Globularia alypum* and *Erica multiflora,* suggesting that they were influenced in the same way by the environment (Fig 5). As described for other species in the Iberian Peninsula (Peñuelas *et al*., 2004*a*), advanced and increased flowering was found in response to increased precipitation during the previous months (Fig 6). Our results show that the date of maximum flowering intensity of both species responded to the proximity of an important rain event especially in *Globularia alypum,* which uses water less conservatively than *Erica multiflora* (Llorens *et al*., 2003). Our findings agree with Llorens and Peñuelas (2005) about the important role of water availability in determining year-to-year shifts in flowering for these species. Similar conclusions were reached from a study in a tropical forest in Costa Rica that undergoes periods of drought (Opler *et al*., 1976). Their results suggested that precipitation triggered anthesis and synchronized flowering of trees and shrubs. An opportunistic response to water availability has been considered the simplest explanation for patterns in sites where water is seasonally limiting and it is considered a safe strategy for the control of flowering (Friedel *et al*., 1993; Castro-Díez and Montserrat-Martí, 1998; Corlett and Lafrankie, 1998).

Besides the inter-annual variability in the beginning and peak date, *Globularia alypum* presented a multiple flowering (flowered once or twice) depending on the year (Fig 2 and 3). Multiple flowering patterns have also been described for other species (Opler *et al*., 1976; Picó and Retana, 2001) and it has been considered an adaptive response to the unpredictability of drought intensity and duration (Llorens and Peñuelas, 2005). The majority of the flower head buds of *Globularia alypum* develop in late-spring and early-summer, just in the apex of the new shoot. In years such 2002 and 2004, with relatively wet springs and summers (Fig 1 and 2), flower head buds opened in a unique and large peak in late-summer or autumn (Fig 3). In 2001 and 2003 the flowering pattern in *Globularia alypum* was bimodal. For both years, we detected longer dry periods without any significant precipitation than in the other studied years, 2002 and 2004; 70 days from mid-spring to early-summer in 2001 and 98 days from late-spring to mid-summer in 2003 whereas dry period lasted only 55 days in 2002 and 63 days in 2004 (Fig 2). Moreover, high temperatures in summer 2003 increased the loss of water by evapotranspiration (mean temperature during June-August in 2003 was 26.4 ºC compared to the average of 24.3 ºC). The bimodal flowering patterns observed in 2001 and 2003 exhibited differences, probably associated to the variable distribution and magnitude of the rain events in summer-autumn. In 2001, after the long dry period, the rain events in early summer released only a small fraction of the floral head buds, which corresponded with the late-summer peak. The increase in water availability was probably insufficient and later rains (33 mm collected in one week between 270 and 277 DOY) were needed to stimulate a second peak in autumn, which was bigger than the first (Fig 2). In 2003, extended drought periods caused a high mortality of new shoots and consequently of flower head buds (field observation) which resulted in the decimation of the first peak in late summer. In this year, other flower head buds were formed on top of shoots grown in autumn and opened in a second peak in winter. Conversely, flower buds of *Erica multiflora* appear in August-September and flowers open gradually in autumn or even in winter in a single and lengthened peak, independently of the meteorological specificities of the year, as we described in the four years of study (Fig 4). This unimodal flowering pattern and the lack of inter-annual variability in the peak intensity in *Erica multiflora* may be linked with its conservative use of water (Llorens *et al*., 2003).

We detected some significant changes in the flowering process in drought plots that consisted in delays in the beginning of flowering and peak date, and reductions in the peak intensity. But, as observed in other studies with Mediterranean species, the effects were clearly species-specific and depended on the conditions in each year (Ogaya and Peñuelas, 2004; Peñuelas *et al.*, 2004*a*; Llorens and Peñuelas, 2005). In spite of the clear relationship between the timing of flowering and water availability, drought treatment did not have global effects throughout the four flowering annual series data. *Erica multiflora* remained unaffected by drought, a treatment that only had clearly significant effects over the flowering of *Globularia alypum* in 2001. In this year, the reduced peak intensity in flowering of *Globularia alypum* in drought treatment was followed by an increased percentage of functional flowers at the end of the flowering period (when drought treatment was off). This result suggest a great ability of *Globularia alypum* to adequate the anthesis to the favourable periods (mostly linked to water availability). The direction of the effects of drought treatment in the present study was in agreement with a previous study carried out in 1999 and 2000 (Peñuelas *et al.*, 2004*a*, Llorens and Peñuelas, 2005). However, more marked changes in flowering for this species were found during the first three years of experimentation (1999-2001), probably related to a combination of the particular environmental conditions of each year and the higher effect of drought treatment on soil moisture during the first three years (29 % soil moisture decrease vs. 14 % decrease in 2002-2004). The apparent paradox involving the significant relationships between flowering and water availability and the absence of a significant effect of our experimental drought treatment on flowering of *Erica multiflora,* is explained by the natural interannual variability of soil moisture in summer and spring-summer periods being greater than the reduction of soil moisture in drought plots (Table 1).

Complementary studies at community level showed that after seven years of experimental drought, neither biomass accumulation nor relative abundances of *Globularia alypum* and *Erica multiflora* were negatively affected (Prieto 2007). In fact, *Globularia alypum* seems to have a competitive advantage over co-existing species. Drier conditions may not reduce established plant performance, in part thanks to its capacity to re-sprout, but they may affect the recruitment of young plants. In 2001, when flowering of *Globularia alypum* was delayed by drought treatment, there was also a reduction in its seedling recruitment probably due to a reduction in pollinators, injuries caused by late frost and indirect effects on germination and survival (Peñuelas *et al.*, 2004*a*). Longer term studies are required to better understand the potential effects of drought on Mediterranean shrubland features, and the future distribution of these species.

Many observational studies have reported advancements in the flowering phenology as a consequence of increased temperatures (Farnsworth *et al.*, 1995; Suzuki and Kudo, 1997; Menzel and Fabian, 1999; Sandvik and Totland, 2000; Abu-Asab *et al.*, 2001; Peñuelas and Filella, 2001; Fitter and Fitter, 2002; Walther *et al.*, 2002; Sherry *et al.*, 2007). Peñuelas *et al.*, (2002) estimated that plants in the Mediterranean region flower on average 6 days earlier than in 1952. However, the warming effect on flowering seems to be species-specific (Peñuelas and Filella, 2001; Peñuelas *et al.*, 2002) and moreover seems to depend on the local and regional variability of climatic conditions (Kudo and Suzuki, 2003), the life form (Farnsworth *et al.*, 1995), the time when it occurs and the specificities of the environmental conditions occurring at each moment of the year (Fitter and Fitter, 2002; Gordo and Sanz, 2005; Sherry *et al.*, 2007). In the present study, the flowering of neither species was sensitive to increased temperatures, a reasonable result since the flower head buds of *Globularia alypum* and the flower buds of *Erica multiflora* develop in late spring-summer when temperatures are high. Previous studies have shown that the timing of vegetative growth of *Erica multiflora* is very responsive to increased temperature (Peñuelas *et al.*, 2004*b*, in review; Prieto 2007**)**. However, this process takes place in late-winter and spring when temperatures are lower and warming treatment alleviates them. Neither did we find any negative effects of the warming treatment due to accentuated summer temperatures and drought in either of the studied species.

In summary, our results show that beginning of anthesis and peak date (date of maximum flowering intensity) vary in these two species depending on the precipitation during the previous months and the date of last significant rain event (>10mm), especially in *Globularia alypum*. The plasticity in flowering of this species in response to water availability was reflected also in its multiple flowering patterns. Our results suggest that precipitation-dependent autumn flowering for both species is a safe strategy when faced with the unpredictable water availability in the Mediterranean area. However, extreme changes in rainfall pattern in spring and summer may seriously affect the phenology of flowering of *Globularia alypum* whereas the phenology of *Erica multiflora* seems much less sensitive.

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CHAPTER 5.

Experimental drought and warming decrease diversity and slow down post-fire succession in a Mediterranean shrubland.

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ABSTRACT

Plant community recovery (species richness, diversity and composition) of a post-fire Mediterranean shrubland was monitored over a seven year period (1998-2005) under experimental drought and warming that simulated the environmental conditions forecast for this area in the coming decades. Species richness and Shannon's Index were positively correlated with accumulated precipitation in the growing season and both variables were negatively affected by reduced water availability in drought plots. The relative abundance of the different species in both treatments was linearly correlated with their relative abundance in control plots. Moreover, we found species-specific responses to treatments. Drought and warming treatment reduced the competitive ability of the obligate seeder tree *Pinus halepensis* against native resprouter shrubs and consequently, the transformation from shrub to pine tree dominated vegetation was slowed down. Conversely, the water spender strategy of *Globularia alypum* may allow this species to maintain a dominant position in drought plots. Therefore, future drier and warmer conditions in Mediterranean areas may severely affect plant community recovery after a disturbance, due to the existence of both abundance-dependent and species-specific responses that may change inter-specific competitive relationships.

Keywords: diversity, species richness, Mediterranean shrubland, post-fire succession, *Erica multiflora, Globularia alypum, Pinus halepensis*.

INTRODUCTION

A number of experiments have studied the potential effects of climate change on diversity of plant species in different types of plant community (Kutiel et al. 1998, Sternberg et al. 1999, van Peer et al. 2001, van der Meer et al. 2002, Thuiller 2003, Hollister et al. 2005). This experimental approach can be addressed by temperature or rainfall manipulations at stand level where the interactions between species assemblies and climate occur (Harte and Show 1995, Robinson et al. 1998, Sternberg et al. 1999, Weltzin et al. 2000, Buckland et al. 2001, Morecroft et al. 2004). However, the effects of climate change on diversity have been more rarely considered in relation to the successional process given the necessity for long-term field experiments involving climate manipulation.

In plant communities, the successional trend following disturbance may be sensitive to environmental fluctuations, particularly year to year climatic variability that may affect the establishment and growth rates of the species (Glenn-Lewin et al. 1992, Bazzaz 1996). It has been proposed that while short-term climate fluctuations would have little effect on long-term successions, climate changes persisting over decades to centuries would modify the direction of succession (Prentice 1992). In recent decades, climatic variability has shown directional tendencies that at global scale represent a significant warming and at local scale may also involve changes in the precipitation range (IPCC 2007). At medium to long time-scales and in the absence of other disturbances, new communities may arise as a result of the fitting between the species niche and the new climatic conditions (Prentice 1992, Iverson and Prasad 2001, Bakkenes et al. 2002, Hamann and Wang 2006, Thuillier et al. 2006). However, climatic trends have been more rarely addressed in studies of vegetation dynamics at time-scales of a few years or decades, in which establishment and decline of populations occur (Brown et al. 1997, Grime et al. 2000, Morecroft et al. 2004, Klanderud and Totland 2005). Particularly, if conditions become less productive, biomass accumulation, typical of late successional stages, is expected to slow down. Patterns of species replacements, however, may become more complex as recruitment and biological interactions are often species-specific and sensitive to climate variability (Choler et al. 2001, Greenlee and Callaway 1996, Lloret et al. 2005). So the resulting pattern of plant diversity may be quite different from that expected under non-directional climate fluctuations. For instance, species coexistence may be enhanced by climate fluctuation preventing the dominance of some species, and eventual competitive exclusion, and by temporal fluctuation in colonization (Fernández-Illescas and Rodríguez-Iturbe 2003, Takenaka 2005).

In the eastern Iberian Peninsula early successional shrublands have become dominant in the last few decades as a result of two main processes. First, secondary succession occurs after the abandonment of croplands and pastures (Baeza et al. 2002). Second, there is a regressive impact on forest ecosystems as a consequence of the increase in wildfires resulting from changes in climate and also in human socio-economic activities involving land use (Lloret et al. 2002). Several studies have been addressed on post-fire time-course regeneration patterns, focusing on species richness and diversity (Moreno and Oechel 1991, Ojeda et al. 1996, Kutiel 1997, Tárrega et al. 1997, Ne’eman and Izhaki 1999, Lloret et al. 2003, Daskalakou and Thanos 2004). However climatic variability associated to global climate change has not been included in these analyses.

General conclusions of most of the General Circulation Models (GCMs) indicate that over this century, the mean global surface temperature will increase by 1.1 – 6.4 ºC depending on the socio-economic scenarios and the resulting emissions of greenhouse gases (IPCC 2007). Although there is no general agreement regarding future rainfall regimes, there is evidence of increased variability in rainfall distribution, and particularly decreasing precipitation during summer in the Mediterranean area (Kutiel et al. 2000, De Luís et al. 2001, Gibelin and Deque 2003). Moreover, the increased potential evapo-transpiration linked to the warming would enhance the summer drought and the water deficit in the Mediterranean ecosystems as has already occurred in the last decades (Le Houérou 1996, Piñol et al. 1998, Peñuelas et al. 2002, 2005, Peñuelas and Boada 2003). The impact of climate change may be particularly relevant in Mediterranean post-fire shrublands where a strong relationship between species richness and water availability has been described (Kutiel et al. 1997, 2000, Keeley et al. 2005). Particularly, changes in climate conditions may affect the recovery ability after disturbance because seedling emergence and recruitment, as well as resprouting capacity depend largely on rain events and on summer water scarcity (Riba 1997, García-Fayos and Verdú 1998, Lloret et al. 1999).

Species response to climate directional change is likely to be non-random. Rare and uncommon species seem more susceptible to being lost as a consequence of widespread stress factors, such as drought, warming or fragmentation whereas abundant species are rarely lost (Tilman and El Haddi 1992, Harte and Shaw 1995, Smith and Knapp 2003, Symstad et al. 2003, Lloret et al. 2004). Species responses can be related to biological attributes but also to interactions with other species, such as predators or pathogens (Tilman and El Haddi 1992, Klanderud and Totland 2005, Thuiller et al. 2006). Plant functional types (PFTs) have been introduced as sets of species sharing biological attributes that reflect ecological constraints at community level (Lavorel et al. 1997). Recently, Thuiller et al. (2006) found common responses to environmental changes in different species from Namibia grouped by life-forms: perennial herbs, geophytes and trees were predicted to be negatively impacted, whereas annual herbs and succulent species would remain relatively stable.

In the present study we report the results from an *in situ* climate manipulation performed in an early successional Mediterranean shrubland established in 1994 after a wildfire. We used a non-intrusive procedure in which precipitation and temperature were manipulated for six consecutive years since 1999. The aim of this study was to detect the impact of experimental drought and warming treatments on secondary succession through changes in species richness, diversity and composition, and life-forms abundance. Particularly, we focused on changes in common and dominant species (*Pinus halepensis*, *Erica multiflora* and *Globularia alypum*) that have great influence over the structure and functioning of the plant community, and that show differential phenological, physiological and growth responses to the treatments (Llorens et al. 2003, 2004, Peñuelas et al. 2004, Llorens and Peñuelas 2005, Peñuelas et al. *in press*). Since in Mediterranean-type ecosystems water represents a major limiting factor, we hypothesized that drier or warmer (causing increased evapotranspiration) conditions would result in decreased species richness because of insufficient growth rates and because of a slower species replacement due to limited colonization. The result would be the maintenance of the community at an early, less diverse successional stage (Bazzaz 1996). Following previous observations on seedlings from the same experimental plots (Lloret et al. 2004), we also hypothesized that species losses in drought or warming treatments would be inversely related to their relative abundance in untreated (control) plots, although extreme conditions might also result in species-idiosyncratic responses.

MATERIAL AND METHODS

Study site and plant species

The study was carried out in dry calcareous shrubland (*Rosmarino-Ericion*) at the Garraf Natural Park in North-East Spain (41º 18'N, 1º49'E), at 210 m above sea level. The climate is typically Mediterranean with two rainy seasons (spring and autumn), mild winters and hot dry summers. During the seven-year study period (1998 to 2005), the mean annual precipitation was 592 mm with minimum monthly averages in January and June-July (20-25 mm). The mean annual air temperature was 15.2 ºC and the maximum monthly mean was 27 ºC in the August 2003 heat wave, and the minimum monthly mean was 5 ºC in January 2005.

The site is located on the south facing slope of a hill in old field terraces abandoned during the last century. The substrate consists of limestones and marls, with a high presence of rock outcrops. In summer 1982 and spring 1994 the area suffered severe fires that wiped out *Pinus halepensis* forest and converted it into a shrubland. The dominant species are *Erica multiflora* and *Globularia alypum*. Both species are resprouter shrubs distributed in dry calcareous and rocky places in the Mediterranean Basin (Bolòs and Vigo 1995). *Pinus halepensis* was reintroduced by seeding after the last fire and is gaining dominance year on year. Other common species are *Dorycnium pentaphyllum, Pistacia lentiscus, Rosmarinus officinalis, Fumana ericoides, Fumana thymifolia* and *Helianthemum syriacum*. The plant community has an average annual aboveground net primary productivity of 160 g m^{-2} (Peñuelas et al. *in press*) and the plant cover in 2005 reached values of up to 75 %.

Experimental design

We performed field-scale drought and night-time warming in treatment plots and compared them with untreated plots (controls). Plots were 4 m x 5 m, allowing for a buffer strip of 0.5 m at the perimeter. Three replicate plots were prepared per treatment, giving a total of nine plots.

Warming treatment. The warming treatment consisted of covering the vegetation at night with reflective curtains (Beier et al. 2004). Solar energy is accumulated in the ecosystem during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem during the night with the reflective aluminium foil curtains (ILS ALU, AB Ludvig Svensson, Sweden) reduces the loss of IR radiation. The curtains reflected 97 % of the direct and 96 % of the diffuse radiation. The warming plots are covered by light scaffolding carrying the reflective aluminium curtain. The covering of the study plots is activated automatically according to a preset light level (< 200 lux). The curtains are removed automatically if wind speed exceeds 10 m s^{-1} (Beier et al. 2004). In order to avoid influencing the hydrological cycle, rain sensors trigger the automatic removal of the covers during rain events. The warming treatment has been applied since spring 1999 with a warming effect on soil and plants of ca. 1 ºC with slight variations depending on time of the year and meteorological conditions (Beier et al. 2004).

Drought treatment. The drought treatment was applied for 2 to 3 month periods in the spring and autumn growing seasons from 1999 to 2005 by covering the vegetation with waterproof, transparent covers. The drought plots are constructed similarly to the warming plots except that the curtain material is a transparent plastic and the moving of the curtains is governed only by rain and wind. When the drought treatment is operating, the rain sensors activate the curtain to cover the plots whenever it rains and remove the curtains when the rain stops. The curtains are removed automatically if the wind speed exceeds 10 m s^{-1}. The maximum decrease in soil moisture during the applied drought treatment was 33 % while air and soil temperatures were not affected. For the rest of the year, the drought treatment was not applied and so the received precipitation was not directly affected (Beier et al, 2004). Despite this soil moisture in the drought plots never completely recovered to the control levels.

Untreated control. Three untreated control plots were set up for comparison, with a similar light scaffolding as for the warming and drought treatments but without any curtains.

Environmental conditions were monitored in all plots. Precipitation within the plots was measured using three water collectors. Soil moisture was also measured weekly by means of three time domain reflectometry (TDR) probes installed per plot. Air (+20 cm) and soil temperatures (-5 and -10 cm) and the functioning of the treatments, (curtain closure and removal detected by magnetic sensors installed at the end of the curtain movement), were recorded in a datalogger (Campbell Scientific, Inc. Logan, Utah, USA).

Data collection; Pin point method

The pin point method provides a record of species composition and abundance in each treatment, and indirectly an estimation of species richness and diversity. We measured all the vegetation contacts in the vertical of 305 points per plot by a thin and long steel pin (1 mm diameter) incorporating a vertical measuring device. The points were equally distributed in 5 fixed and parallel transects per plot separated by a distance of 0.80 m (61 points per transect separated by 5 cm). For each hit, the height, the organ (leaf, stem or reproductive structure) and the state (dead or alive) were recorded. Plants were identified to species level, although Gramineae were pooled. Sampling was always done in July, during the non-growing season, from 1998 to 2005. In 1998 pre-treatment measurements were taken in order to identify variability between plots.

Data analysis

We focused on the following components to describe diversity in the studied community: number of species (species richness), species relative abundance, the particular species present (species composition) and the diversity (Shannon Index). Species richness was expressed as the number of different species, with the Gramineae being pooled. The variables were estimated per transect and then we averaged the five transect values to obtain a mean value per plot. That is, transects were considered as the sampling unit (alpha diversity). This made the analysis more sensitive, given that the summation of the five transects per plot would correspond to the asympthotic part of the species-area relationship, where differences among treatments are expected to be lower. Results obtained in 2004 were compared with those from 1998 to evaluate the medium-term responses of the number of species to the drought and warming treatments. We did not use the last year (2005) because the extremely dry spring of that year and previous autumn (100 % less precipitation that an average year) did not represent the standard effects observed during the seven years of study. The relative abundance of the species was calculated as the proportion of alive contacts of each species relative to the total number of alive contacts per transect. We used the alive contact because most of the studied species were perennials and dead plants or plant parts can persist. Relative abundances were chosen instead of cover in order to take into account the vertical structure of the community. We used the relative abundances in three complementary ways:

a) to calculate the Diversity Shannon's Index (H) (see equation in Begon et al. 1996),

b) to detect changes in composition linked to abundance-dependent responses to treatments,

c) to monitor the evolution of each dominant and common species with respect to the other species along the plant succession, and

d) to study changes in relative abundance of the main life forms: herbs, shrubs (or woody small plants) and trees.

Statistical analyses

Regression analyses were conducted in order to examine the influence of the precipitation (from different periods previous to the vegetation samplings) on the species richness, the Shannon's Index, and the abundance of life forms and dominant, common and uncommon species. We chose the accumulated precipitation during the four months previous to sampling (growing season) as explicative variable. We used as dependent variables the species number, the annual increment in the number of contacts for life forms and for dominant and common species (calculated as the difference between the numbers of contacts between two consecutive years) and the annual number of transects with presence of uncommon species. We also used regressions analyses to test the annual relationship between the relative abundance (transformed in logarithms) of the different species in treated and control plots.

The effect of treatments was analyzed using one value per plot obtained from the average of 5 transects. Drought and warming treatments were always compared separately with control. Repeated measures ANOVAs were conducted to check treatment effects on species richness, Shannon's Index and percentage of the relative abundance of common and dominant species throughout the experimentation period from 1999 to 2005. Relative abundances were arcsine transformed to reach the normality assumptions of the ANOVA.

All analyses were performed with the *Statview* software package (Abacus Concepts Inc., Cary, North Carolina) and the *Statistica* software package (StatSoft, Inc. Tulsa, Oklahoma).

RESULTS

Environmental conditions

Precipitation during the seven-year study period showed high inter-annual variability. The annual average accumulated precipitation was 592 mm, with annual variations between 421 mm in 1999 and 956 mm in 2002. The distribution within each year was also variable. For example, 2005 had an accumulated precipitation of 610 mm but with a very dry first semester (140 mm). Distribution of precipitation through the year was typical of the Mediterranean Basin, with rainy springs and autumns and dry summers. The three-month summer period, June, July and August, had, on average, 80 mm of precipitation (Fig 1).

Temperature through the year was also typically Mediterranean with moderate cold winters and warm summers (Peñuelas et al. 2005). The annual mean air temperature was 15.2 ºC. In 2001 and 2003 it rose to 15.7 ºC and 15.5 ºC respectively (Fig 2).

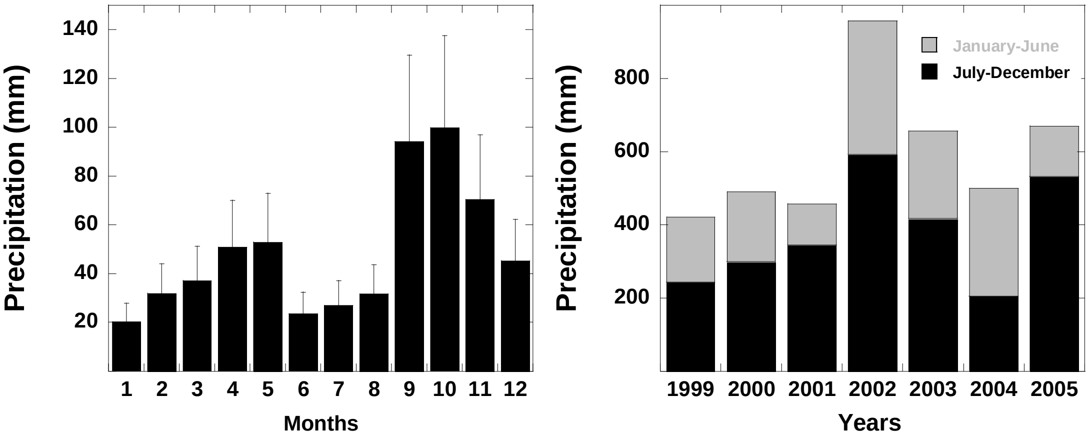

Figure 1. Monthly average precipitation in the study site in the period 1999-2005 and accumulated precipitation by semesters for each year in the same period. Bars indicate the standard errors of the mean (n=7 year means).

Treatments effects on environmental conditions

Drought treatment reduced soil moisture through the year by an average of 19 % with respect to control plots (RM-ANOVA, *P*=0.005) (Fig 2), but this effect varied depending on the season. When the drought treatment was operating in spring, soil moisture in drought plots was 21 % lower than in control plots whereas the difference reached 30 % in autumn. Warming treatment did not significantly affect soil moisture (Beier et al. 2004, Peñuelas et al. 2004) (Fig 2).

Warming treatment increased mean air temperature through the year an average of 0.7 ºC with respect to control plots (RM-ANOVA, *P*=0.03) (Fig 2). This increase was greater in soil temperature (0.9 ºC) and especially for the minimum temperature (1.5 ºC). Drought treatment did not significantly affect mean air temperature (Beier et al. 2004, Peñuelas et al. 2004) (Fig 2).

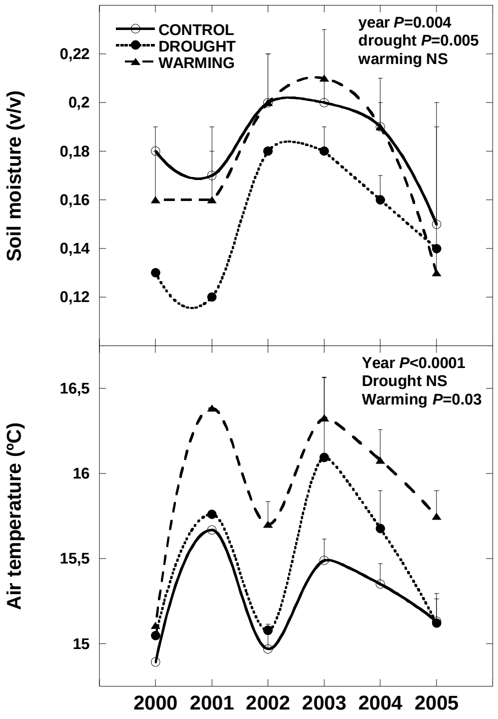

Figure 2. Effects of the treatments on the average annual soil moisture and air temperature. Bars indicate the standard errors of the mean (n=3 plot means). Significance of the treatments (RM-ANOVA) is depicted inside each panel.

Species composition, richness and Shannon's Index

We found a total of 29 species and the Gramineae group (represented mainly by *Brachypodium phoenicoides* and also *Hyparrhenia hirta* and *Ampelodesmos mauritanica).* Most of species are perennials and belong to 20 plant families (Table 1). The dominant (*Erica mutliflora* and *Globularia alypum*) and common species (*Dorycnium pentaphyllum, Fumana ericoides, Fumana thymifolia,* Gramineae group, *Pinus halepensis, Pistacia lentiscus* and *Helianthemum syriacum*) were present in all treatments throughout the study period. Several species were uncommon and their presence varied from year to year; this was the case, for instance, with *Galium lucidum* which only appeared in 2002 and 2004 in all treatments (years with wet springs). Some species were only observed in a certain plot (*Eryngium campestre, Sedum sediforme, Quercus coccifera, Juniperus oxycedrus, Lithospermum fruticosum, Asperula cynanchica* and *Bupleurum fruticosum*), where they probably became established after the wildfire from seeds already present in these plots or from seeds dispersed from nearby patches (Tárrega et al. 1997).

Table 1. Species detected in the shrubland and their plant families, life form (H; herb S; shrub, T; tree, P; parasite), and abundance (U; uncommon, C; common, D; dominant). * indicates that the abundance depended on the treatment. Uncommon species "U", were those with heterogeneous distribution among plots (not present at least in 2 out of the 3 plots) and relative abundances lower than 1 %. Common species "C", were those with homogeneous distribution (present at least in 2 out of the 3 plots) and with relative abundances higher than 1 %. Dominant species "D" were those that presented also a homogeneous distribution and their relative abundance was higher than 20 %.

Species	**Family**	**Life form**	**Abundance**
Asperula cynanchica	Rubiaceae	H	U
Argyrolobium zanonii	Leguminosae	S	U
Bupleurum fruticosum	Umbelliferae	S	U
Coris monspeliensis	Primulaceae	S	U
Cuscuta epithymum	Convolvulaceae	P	U
Dorycnium pentaphyllum	Leguminosae	S	C
Eryngium campestre	Umbelliferae	H	U
Erica multiflora	Ericaceae	S	D
Euphorbia serrata	Euphorbiaceae	H	U
Fumana ericoides	Cistaceae	S	C
Fumana thymifolia	Cistaceae	S	C
Globularia alypum	Globulariaceae	S	D
Gramineae	Gramiaceae	H	C*
Galium lucidum	Rubiaceae	H	U
Helichrysum stoechas	Asteraceae	S	U
Helianthemum syriacum	Cistaceae	S	C*
Juniperus oxicedrus	Cupressaceae	S	U
Lithospermum fruticosum	Boraginaceae	S	U
Odontites lutea	Scrophulariaceae	P	U
Ononis minutisima	Leguminosae	S	U
Psoralea bituminosa	Leguminosae	H	U
Pinus halepensis	Pinaceae	T	C
Pistacia lentiscus	Anarcadiaceae	S	C
Polygala rupestris	Polygalaceae	S	C*
Quercus coccifera	Fagaceae	S	U
Rosmarinus officinalis	Labiatae	S	C
Rubia peregrina	Rubiaceae	H	U
Sedum sediforme	Crassulaceae	S	U
Teucrium polium	Labiatae	S	U
Ulex parviflorus	Leguminosae	S	U

In control plots, the mean species richness per transect tended to increase throughout the study period from 6.1 in 1998 to 9.8 in 2004 (Fig 3) although it showed a significant inter-annual variability with important decreases in 2003 (when very high summer temperatures were reached) and 2005 (after a dry autumn 2004 and spring 2005) (Fig 3). Shannon‘s Index followed a similar trend to species richness, rising from 1.3 in 1998 to 1.7 in 2004 in controls (Fig 3).

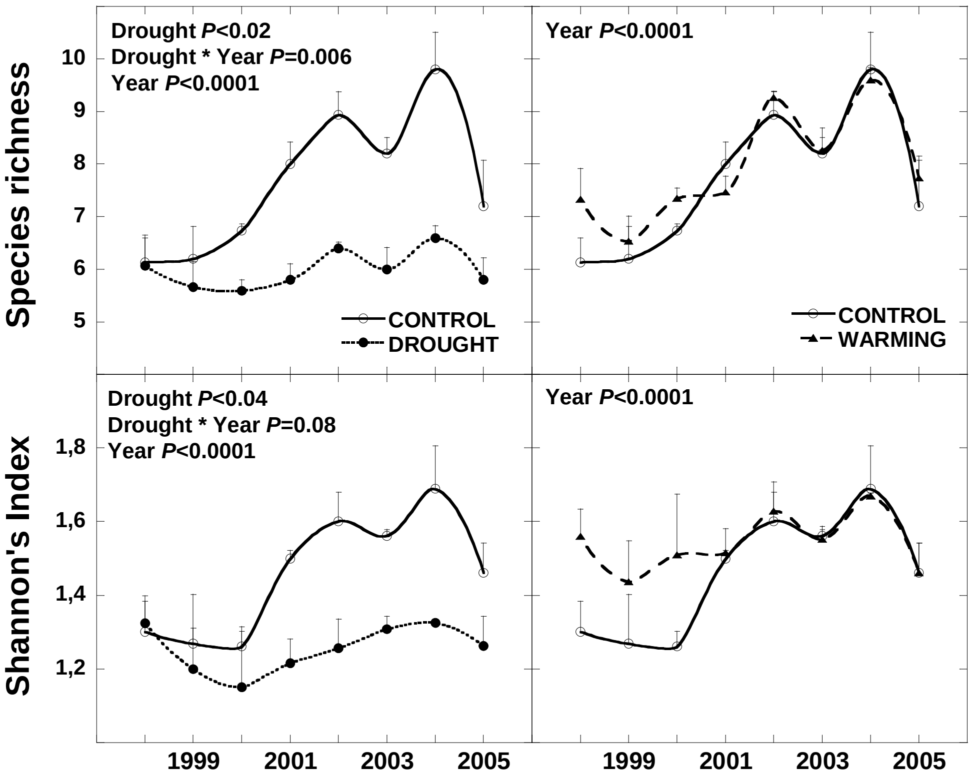

Figure 3. Number of species and Shannon’s Index per 3-m transect in response to warming and drought treatments in 1998 (pre-treatment year) and in the years of the experiment (1999-2005) in a shrubland recovering from a fire in 1994. Whenever significant (RM-ANOVA), the effects of the studied factor are depicted in the corresponding panel. Bars indicate the standard errors of the mean (n=3 plots means).

A significantly lower species richness and Shannon‘s Index per transect was found in the drought plots than in the controls over the course of the experiment (*P* <0.02 and *P* <0.05 respectively) (Fig 3) and the effect of drought was significant for the number of uncommon species although it depended on the year (drought * year interaction, *P*=0.0002) (Fig 4). Uncommon species were clearly less represented in years such 2003 and 2005, with warm and dry periods, compared to more favourable years (Fig 4 and 5). The relative increase in the number of species in 2004 with respect to the pre-treatment year (a six year period) was significantly less in drought plots compared to control plots (0.5 compared with 3.5 additional species per transect respectively) (*P*=0.01). Although the increase in the number of species was also smaller in the warming plots than in control plots, the difference was not significant (Fig 6).

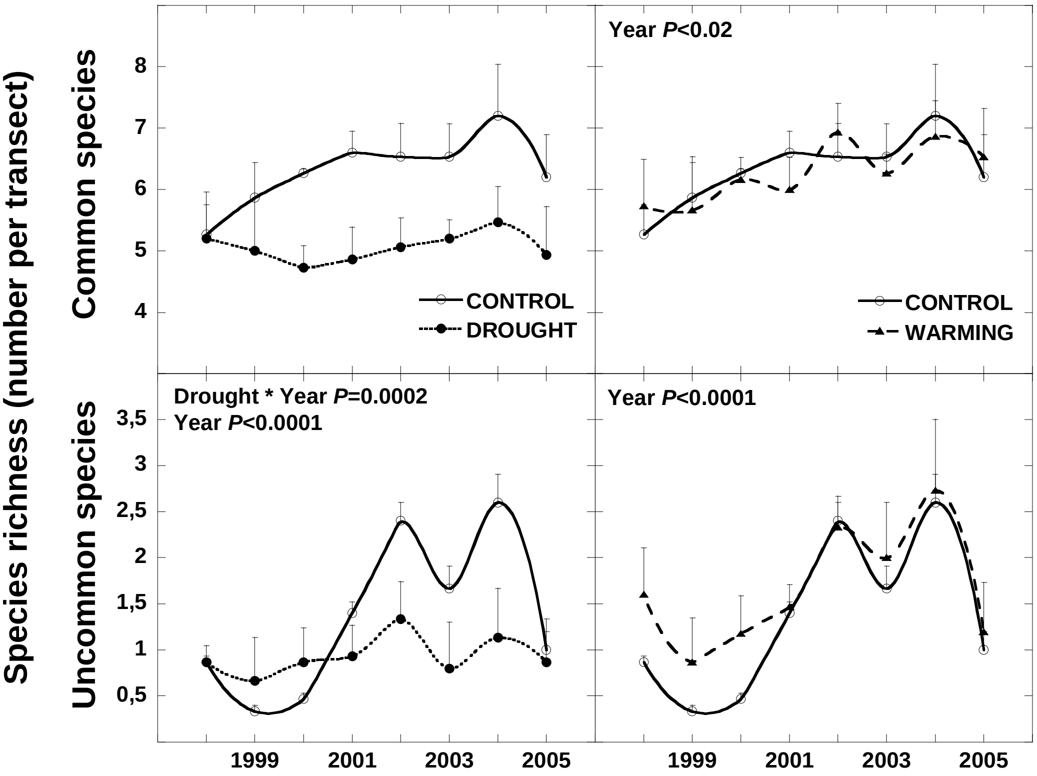

Figure 4. Number of common and uncommon species per 3-m transect in response to warming and drought treatments in 1998 (pre-treatment year) and in the years of the experiment (1999-2005) in a shrubland recovering from a fire in 1994. Whenever significant (RM-ANOVA), the effects of the studied factor are depicted in the corresponding panel. Bars indicate the standard errors of the mean (n=3 plots means).

We found a significant relationship between both the mean species richness and the mean Shannon's Index and the accumulated precipitation in the 4 months previous to measurements (R^2=0.80, P<0.0001 and R^2=0.67, P<0.0001 respectively) (Fig 7).

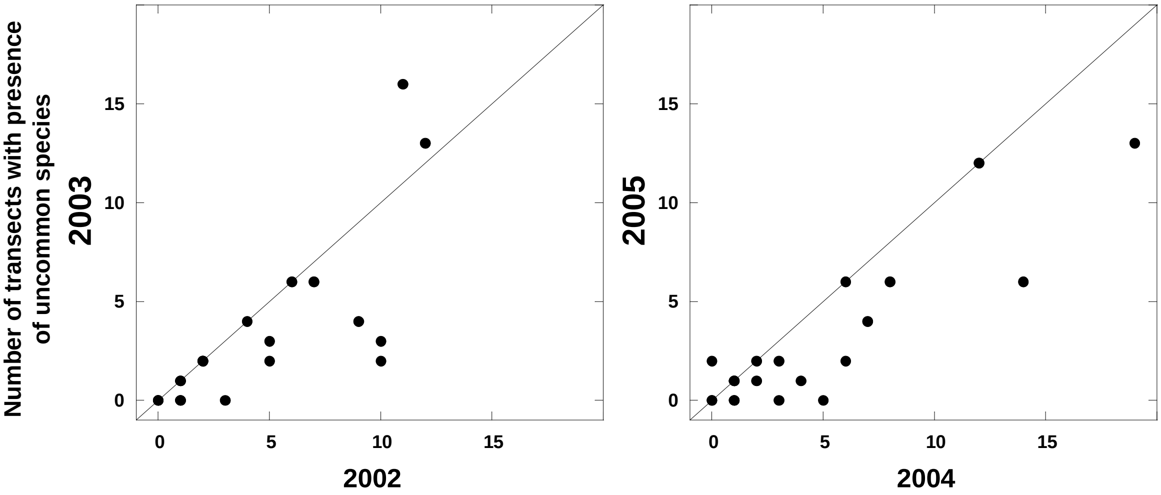

Figure 5. Relationship between the number of transects with presence of uncommon species (for the 9 plots) in the hot 2003 and dry 2005 (January-June precipitation; 239 mm and 138 mm respectively) and the relatively wet 2002 and 2004 (January-June precipitation; 364 and 295 mm respectively). Each dot represents the data for each uncommon species.

Significant positive linear relationships were also found between the annual increase in the number of contacts of some dominant and common species (*Globularia alypum*, *Erica multiflora*, *Dorycnium pentaphyllum*, *Pinus halepensis* and Gramineae group) and the accumulated precipitation in the four previous months (Table 2). The presence of some uncommon species (*Argyrolobium zanonii, Coris monspeliensis, Galium lucidum, Helichrysum stoechas, Lithospermum fruticosum, Odontites lutea, Ononis minutissima* and *Polygala rupestris),* expressed as number of transects with presence, was also related to the accumulated precipitation during the four months previous to measurements (Table 2).

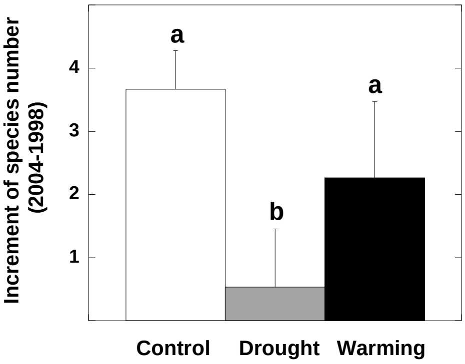

Figure 6. Change in the total number of species per transect in control, drought, and warming treatments after 7 years of monitoring in the period 1998-2004 (1998 was the pre-treatment year). Bars indicate the standard errors of the mean (n=3 plot means). Different letters indicate significantly different means (Bonferroni post-hoc test of the ANOVA).

Life-form changes during succession and responses to treatments

Shrubs were the most abundant life form in all plots, although in control plots their relative abundance was reduced from 95 % to 78 % over the study period. Simultaneously, in control plots the relative abundance of trees (*Pinus halepensis*) increased from less than 1 % to 18 %, whereas the abundance of herbs fluctuated, although they always showed low relative abundance (between 2 and 5 %) (Fig 8).

Drought treatment reduced herb and tree abundance over the experimentation period (1999-2005) relative to control plots (RM-ANOVA; drought * year interaction, *P*=0.06 and *P*=0.04, respectively) and increased shrub abundance (RM-ANOVA; drought * year interaction, *P*=0.0004), although this effect varied between years. Shrub and tree abundance also was different in warming treatment and control plots over the experimentation period (RM-ANOVA; drought * year interaction, *P*=0.005 and *P*=0.0003, respectively), but herb abundance was not (Fig 8).

For the three main life forms into which species were grouped (herbs, shrubs and trees), positive linear relationships between annual increase in the number of contacts and the accumulated precipitation in the 4 months previous to sampling were found (Table 2).

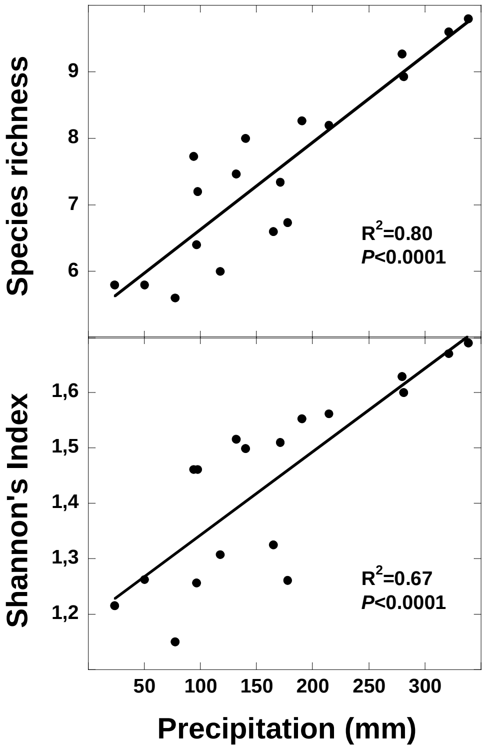

Figure 7. Relationship between the species number and the Shannon's diversity Index and the accumulated precipitation for each treatment during the four months previous to sampling (growing season). Each point represents a mean value per treatment for each year (2000-2005).

Abundance-dependent responses to treatments

We found significant positive linear correlations between the relative abundances of the different species in treated and control plots for the different years (Fig 9, 10). These comparisons showed that the less abundant species in treated plots were also less abundant species in control plots. We transformed relative abundances into logarithms to visualize the less abundant species. The relationship between the relative abundance in treated and control plots tended to become weaker with increasing number of years of experimental treatments. This decrease in the relationship was statistically significant for the drought treatment, indicating a shift in the community in relation to control plots (Fig 11).

Table 2. Linear regression analyses per species and life forms between the annual increment in the number of contacts (for the dominant or common species and life forms) or the annual number of transects with presence (for the uncommon species), and the accumulated precipitation (mm) during the four months previous to sampling (growing season). Whenever significant, *P* values are highlighted in bold type.

Dominant or Common Species	**R^2**	*P*	**n**	**F**
Dorycnium pentaphyllum	0.35	**0.009**	18	8.77
Erica multiflora	0.21	**0.05**	18	4.30
Fumana ericoides	0.12	0.16	18	2.19
Fumana thymifolia	0.01	0.65	18	0.22
Globularia alypum	0.23	**0.04**	18	4.78
Gramineae	0.46	**0.002**	18	13.55
Pinus halepensis	0.47	**0.001**	18	14.50
Pistacia lentiscus	0.01	0.67	18	0.19
Rosmarinus officinalis	0.19	**0.07**	18	3.73
Helianthemum syriacum	0.05	0.40	18	0.85
Uncommon Species	**R^2**	*P*	**n**	**F**
Argyrolobium zanonii	0.44	**0.003**	18	12.43
Coris monspeliensis	0.60	**0.0002**	18	23.92
Eryngium campestre	0.14	0.13	18	2.59
Euphorbia serrata	0.13	0.15	18	2.30
Galium lucidum	0.56	**0.004**	18	20.28
Helichrysum stoechas	0.41	**0.004**	18	11.02
Lithospermum fruticosum	0.24	**0.04**	18	5.18
Odontites lutea	0.56	**0.0003**	18	20.48
Ononis minutissima	0.31	**0.02**	18	7.15
Psoralea bituminosa	0.13	0.14	18	2.45
Polygala rupestris	0.41	**0.004**	18	10.94
Rubia peregrina	0.05	0.39	18	0.80
Cuscuta epithymum	0.0005	0.93	18	0.008
Ulex parviflorus	0.04	0.42	18	0.69
Life form	**R^2**	*P*	**n**	**F**
Herb	0.30	**0.02**	18	6.77
Shrub	0.22	**0.002**	18	4.47
Tree	0.46	**0.05**	18	13.55

Species-specific responses of common and dominant species to treatments

The sequential changes in the relative abundances of the dominant and common species reflected the dynamic stage of the studied shrubland after the last fire in 1994 (Fig 12). In control plots, some common species, such as *Rosmarinus officinalis* and *Pinus* *halepensis*, tended to increase in relative abundance, while others tended to diminish (*Globularia alypum*, *Fumana ericoides* and *Dorycnium pentaphyllum*) or showed no clear trend (Gramineae group*, Fumana thymifolia* and *Erica multiflora*).

The relative abundance of common and dominant species was more sensitive to the drought treatment than to the warming one and the effects of both treatments depended on the year. Drought treatment significantly reduced the relative abundance of the Gramineae group and *Pinus halepensis* over the experimentation period although these effects were dependent on the year (for both; ANOVA, drought * year interaction, *P*=0.04) (Fig 12). In 2005 the Gramineae group in control plots represented 2 % of the total contacts while it had almost disappeared in drought plots. The relative abundance of *Pinus halepensis* in 2005 rose to almost 20 % of the total contacts in control plots while in drought plots the figure was only 8 %. For *Globularia alypum*, the drought treatment had the opposite effect (ANOVA, drought * year interaction, *P*=0.09) and in the last sampled year, the relative abundance of this species in drought plots was 80 % higher than in the controls (Fig 12). The relative abundance of *Fumana ericoides* tended also to be affected by drought treatment over the experimental period although the effects were dependent on the year (ANOVA, drought * year interaction, *P*=0.07) (Fig 12). Warming only reduced the relative abundance of *Pinus halepensis,* although the effect of this treatment varied depending on the year (warming * year interaction, *P*=0.0003) and in 2005 this species was 56 % less abundant in warming plots than in control ones (Fig 12).

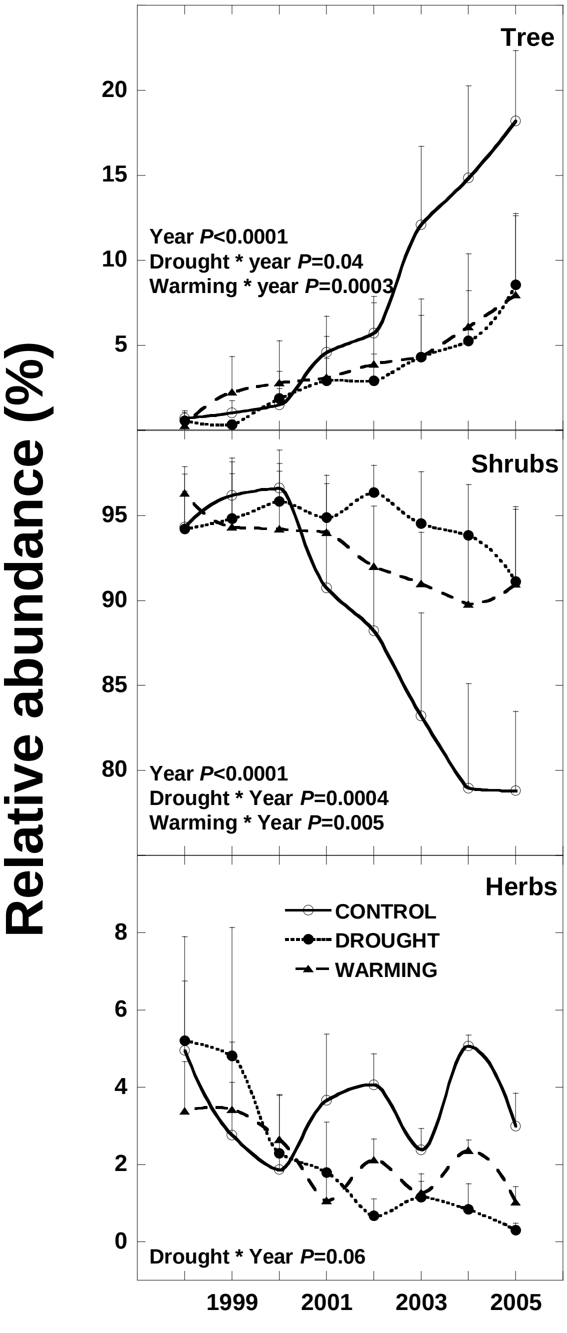

Figure 8. Pattern of relative abundances of life-forms (Herbs, shrubs, trees) during the seven-year study period in control, drought and warming treatments. Whenever significant (RM-ANOVA), the effects of the studied factor or interaction are depicted in the corresponding panel. Bars indicate the standard errors of the mean (n=3 plots means).

DISCUSSION

Plant species richness and diversity increased during the study period (1998-2005), which indicates that the studied shrubland was still in the process of successional recovery eleven years after a fire. This is in accordance with the parallel increases that we found in plant cover (from 52 % in 1998 to 76 % in 2005) and aboveground biomass (from 433 gm^2 in 1998 to 829 gm^2 in 2005) in control plots (Prieto 2007).

Plant species richness and diversity were very sensitive to reductions in water availability from the first year of experimentation. In 2004, after six years of monitoring, drought plots only showed a mean increment of 0.5 species per transect whereas control plots showed an increment of 3.5 species per transect. In the Mediterranean Basin, water availability is generally considered the main climatic constraint for plant activity (Larcher 2000). In the present study, the number of species and the Shannon's Index were significantly correlated with previous precipitation in accordance with other studies that have also described these positive correlations in post-fire successions under Mediterranean conditions (Kutiel et al. 1997, Keeley et al. 2005). Other studies in temperate and tropical grasslands have also shown reductions in the species richness under drier conditions (Tilman and El Haddi 1992, Kennedy et al. 2003) or increases if summer rainfall was supplemented (Sternberg et al. 1999). Our results show the sensitivity to drought of a woody community under a moderate reduction of water availability during a relatively short period of time. Since our information about diversity comes from transect intercepts that are influenced by plant structure we can expect that the observed patterns of decreased species richness are partially related to plant growth.

The appearance of uncommon species (*Argirolobium zanonii, Coris monspeliensis, Galium lucidum, Helichrysum stoechas, Odontites lutea, Ononis minutissima*) that led to the continuous increase in species richness estimated from transect contacts was reduced in drier conditions (drought plots and dry years). A large effect of water availability has been widely described for the recruitment of annuals (Fowler 1986, Shoshany et al. 1996). However, in our study almost all the described species were perennials (except *Odontites lutea* and *Cuscuta epithymum).* The positive significant correlations of the annual increase in the number of contacts of some dominant and common species and the presence of uncommon species with the accumulated precipitation in the 4 months previous to the measurements could indicate the effects that of water availability has on different phases of the plant life cycles such as seedling recruitment, reproduction, and growth, thus resulting in biomass accumulation. In particular, the lower species richness and diversity values observed in drought plots compared to controls could be in part a result of the effects of treatments on the establishment of the different species. Lloret et al. (2004) reported that drought treatment decreased species richness and the number of emerging seedlings. Some authors have pointed out that seedlings of different species can respond differently in conditions of water competition for example, as a result of their ability to use below-ground resources (Brown and Archer 1999, Lloret et al. 1999, Gasque and García-Fayos 2004). Similarly, the warming treatment could increase abiotic stress on seedlings by increasing evaporative demand (Lloret et al. 2005), a factor less relevant for established plants with a developed root system.

Several studies carried out in northern communities, where climate warming is expected to have a strong effect (Corlett and Lafrankie 1998), did not report the expected positive effects of warming over species richness or even described negative effects, suggesting that the potential effects of climate warming depend on the plant community studied (the identity and responsiveness of the dominant species) or the duration of the experiment (short and long- term responses) (Chapin et al. 1995, Farnsworth et al. 1995, Sandvik et al. 2004, Hollister et al. 2005, Jónsdóttir et al. 2005). Even though winter temperatures have been recognized as an important factor controlling plant structure, functioning, distribution and phenology in the Mediterranean region (Mitrakos 1980, Tretiach 1993, Oliveira and Peñuelas 2000, Ogaya and Peñuelas 2003), our warming treatment did not significantly affect the species richness and diversity during the studied period.

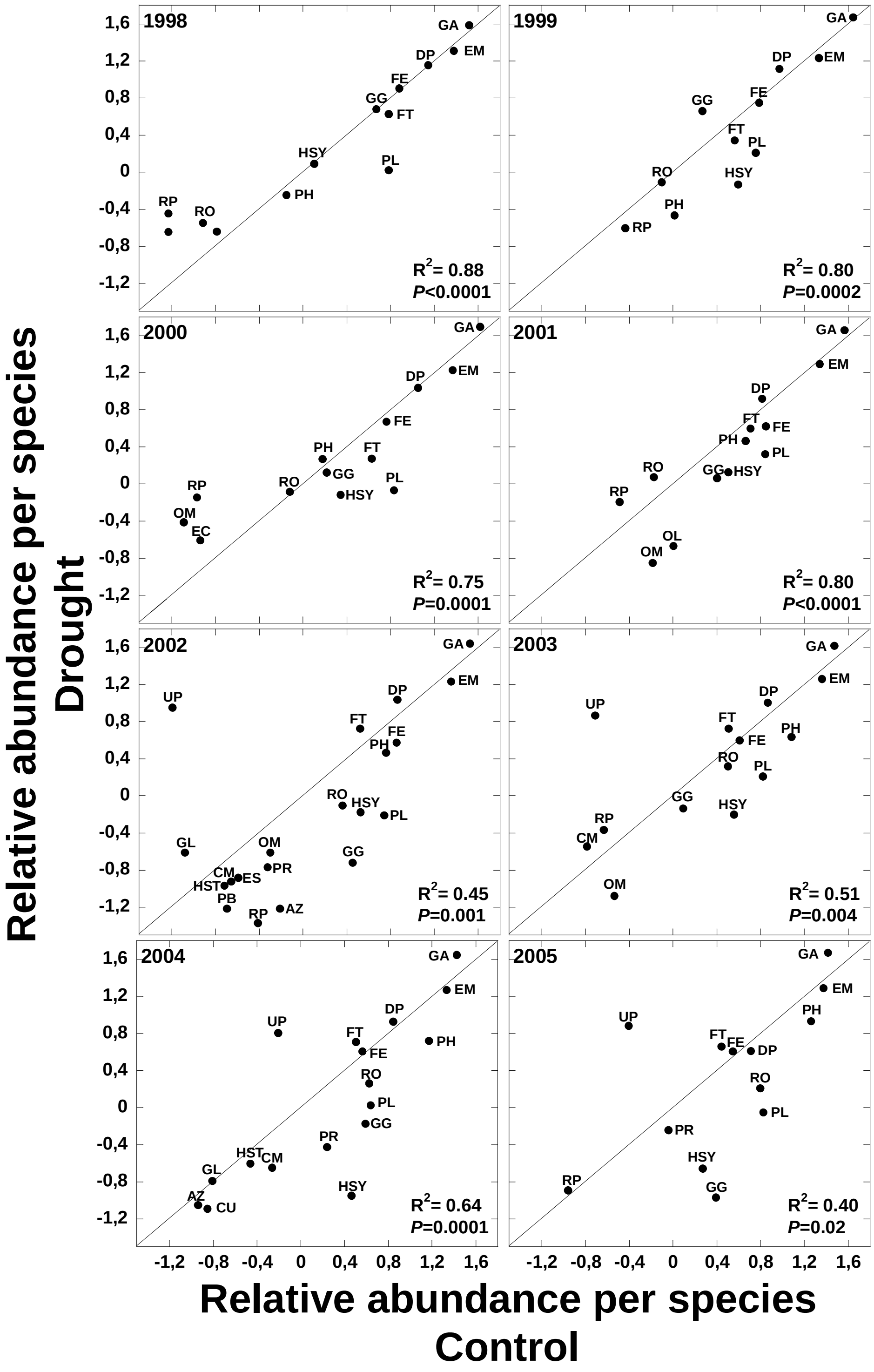

Figure 9. Annual relationship between the relative abundances (in logarithms) of the different species in drought and control plots for the studied eight-year period (1998-2005). Each point represents the data for each species present in both drought and control treatments. The acronyms of the species present in treated and control plots are included. AZ; *Argyrolobium zanonii,* CM; *Coris monspeliensis,* CU; *Cuscuta epithymum.,* DP; *Dorycnium pentaphyllum,* EC; *Eryngium campestre,* EM; *Erica multiflora,* ES*;*

Euphorbia serrata, FE*; Fumana ericoides,* FT; *Fumana thymifolia, GA; Globularia alypum,* GG; Gramineae group*, GL; Galium lucidum,* HSY*; Helianthemum syriacum,* HST*; Helichrysum stoechas,* LF*; Lithospermum fruticosum,* OL*; Odontites lutea,* OM*; Ononis minutissima,* PB*; Psoralea bituminosa,* PH*; Pinus halepensis,* PL*; Pistacia lentiscus,* PR; *Polygala rupestris,* RO; *Rosmarinus officinalis,* RP*; Rubia peregrina,* UP*; Ulex parviflorus.*

In Mediterranean areas, plant regeneration after fire is considered a process of “autosucession”. Accordingly, the species that existed before fire have mechanisms of regeneration, such as resprouting and dormant seed banks, which allow a rapid recovery of the structure and the species composition (Ojeda et al. 1996, Tárrega et al. 1997, Lloret et al. 2002, Pausas and Verdú 2005). The colonization process after fire is determinant in defining the future composition and structure of the community (Lloret 1998, Keeley 2005). Throughout the study period, we found co-dominance between species with sprouting and seeding strategies. Relative abundances in 1998 show that resprouting shrubs (*Globularia alypum, Erica multiflora* and *Dorycnium pentaphyllum*) and short-lived seeder shrubs with germination stimulated by fire (*Fumana* sp.) (Lloret 1998) had a great ability to take advantage of the low plant density and the greater availability of resources after fire. The fast growth of these species in the first years probably gave them an advantage over other long-lived obligate seeders such as *Rosmarinus officinalis* and *Pinus halepensis* which took longer to expand their distribution within control plots. Other workers have also pointed to competition between the rapidly developing shrubs and *Pinus halepensis* as the factor delaying regeneration in a post-fire succession in Algeria (Moravec 1990). In our study site, the recruitment of *Rosmarinus officinalis* and *Pinus halepensis* after the fire in 1994 was probably dependent on the arrival of seeds from surrounding areas and on the reintroduction of seeds respectively. Since this wildfire occurred in spring only twelve years after a previous fire, the seed bank of these species was probably depleted (Lloret

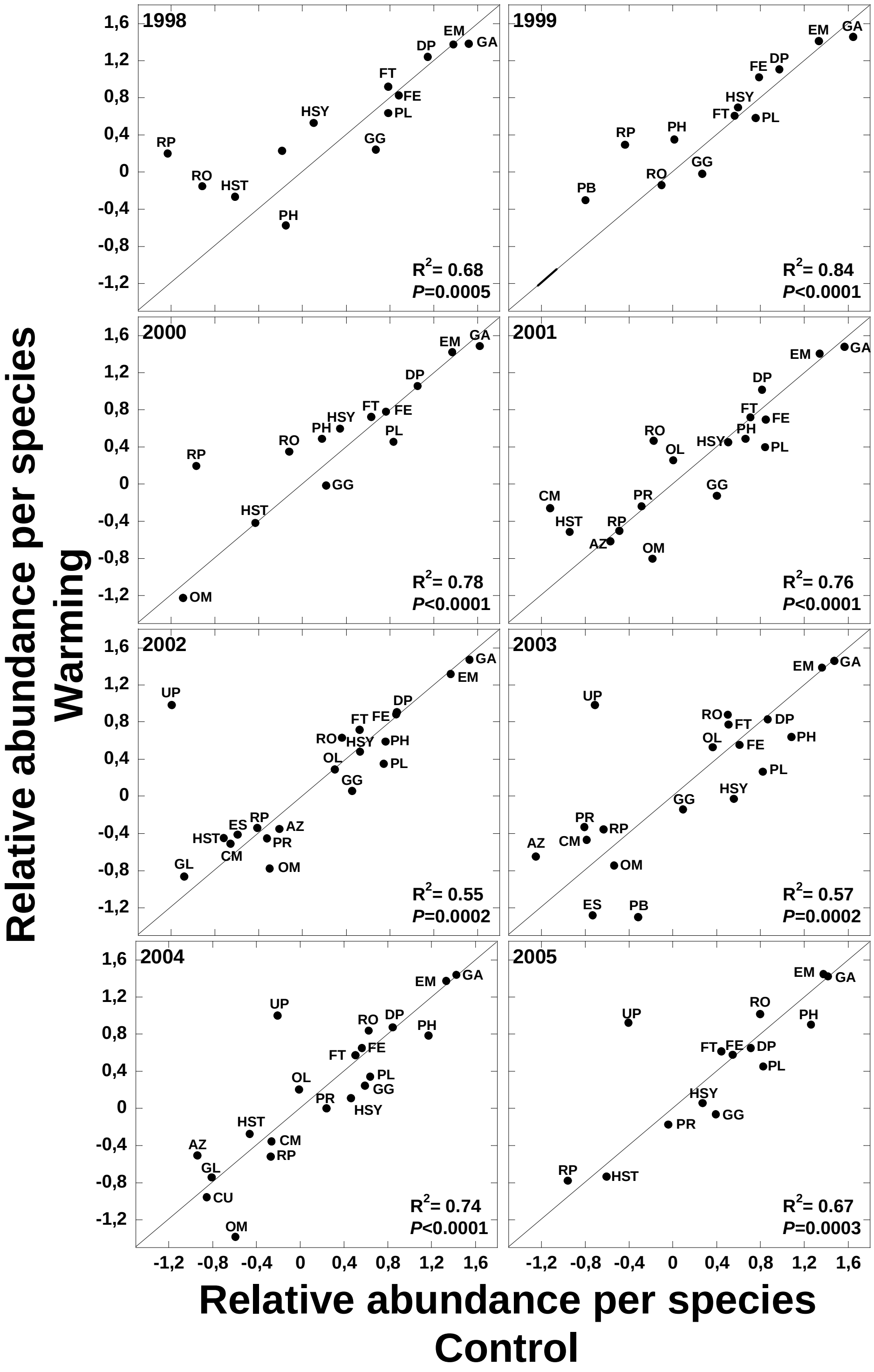

Figure 10. Annual relationship between the relative abundances (in logarithms) of the different species in warming and control plots for the studied eight-year period (1998-2005). Each point represents the data for each species present in both warming and control treatments. For the acronyms of the species present in treated and control plots see Fig 9.

1998, Lloret et al. 2003). However, after several years, the abundance of shrubs began to decline while *Pinus halepensis* started to gain dominance as a consequence of its increasing plant size and the likely change in the partitioning of resources (Martínez-Sánchez et al. 2003). Probably, the reductions in the abundance of some small and medium size shrubs such as *Fumana ericoides* and *Dorycnium pentaphyllum* were also due to the shading in the subcanopy environment produced by the taller shrubs.

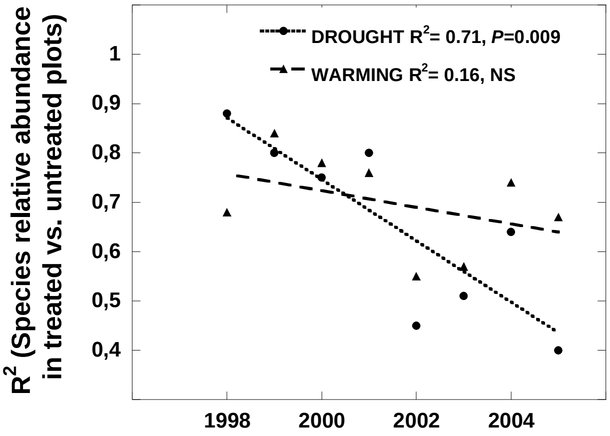

Figure 11. Reduction of the R^2 of the annual relationship between the relative abundances of the different species in treated and control plots for the studied eight-year period (1998-2005). Each dot represents the data for each year (see Fig 9, 10).

In drought and warming plots there were increases in the relative abundance of shrubs over herbs and trees, represented only by *Pinus halepensis*. Our results suggest that drier and warmer conditions in the Mediterranean area may enhance the competitive disadvantage of *Pinus halepensis* in favour of native shrubs and consequently, may slow down the post-fire transformation from a shrub to tree dominated vegetation. Other negative competitive effects of shrubs of the Cistaceae family on the establishment of *Pinus halepensis* have been described in burnt stands (Martínez-Sánchez et al. 2003). The negative effects of low water availability on *Pinus halepensis* could accumulate over different life phases. For example, it has been described that seedlings of *Pinus halepensis* are very susceptible to summer drought (Martínez-Sánchez et al. 2003, Daskalakou and Thanos 2004). Additionally, low water availability may affect the growth of pines (Borghetti et al. 1998), resulting in them reaching a size that allows them to compete successfully for water and nutrients with coexisting species later (Martínez-Sánchez et al. 2003). The increased shrub/pines ratio in the warming treatment may also be explained by a reduction in water availability in sensitive growing periods of the pines during the early and middle successional stages. Although *Pinus halepensis* has been widely used for restoration of burned areas, high fire recurrences could decrease its abundance (Lloret et al. 2003, Lloret and Vilà 2003) because it needs 7-10 years to produce enough viable seeds to ensure regeneration (Kutiel 1997, Ne'eman and Izhaki 1999, Daskalakou and Thanos 2004). Therefore, lower water availability in future may increase this pre-reproductive period if pine recruitment, growth and reproduction are affected.

There is evidence of a current increase in shrub abundance in different plant communities around the world as consequence of climate change and changes in land use and management (Brown and Archer 1999, Van Auken 2000, Sturm et al. 2001). Zavaleta and Kettley (2006) showed how shrub invasion in Californian grasslands increased the total ecosystem carbon storage, the level of which was still increasing 25 years after the shrub establishment. In our case, the biomass accumulation was not significantly different between treatments and controls, although differences may appear in the longer term (Peñuelas et al. *in press*, Prieto 2007).

We analyzed changes in the species composition of the community by comparing patterns of species abundance in control and treated plots and we found constant positive linear correlations between them. Thus, the least abundant species in control plots were also the least abundant in treated plots. Several studies have shown that, under drier and warmer climatic conditions, uncommon species are more likely to disappear from the community than abundant species due to their low population

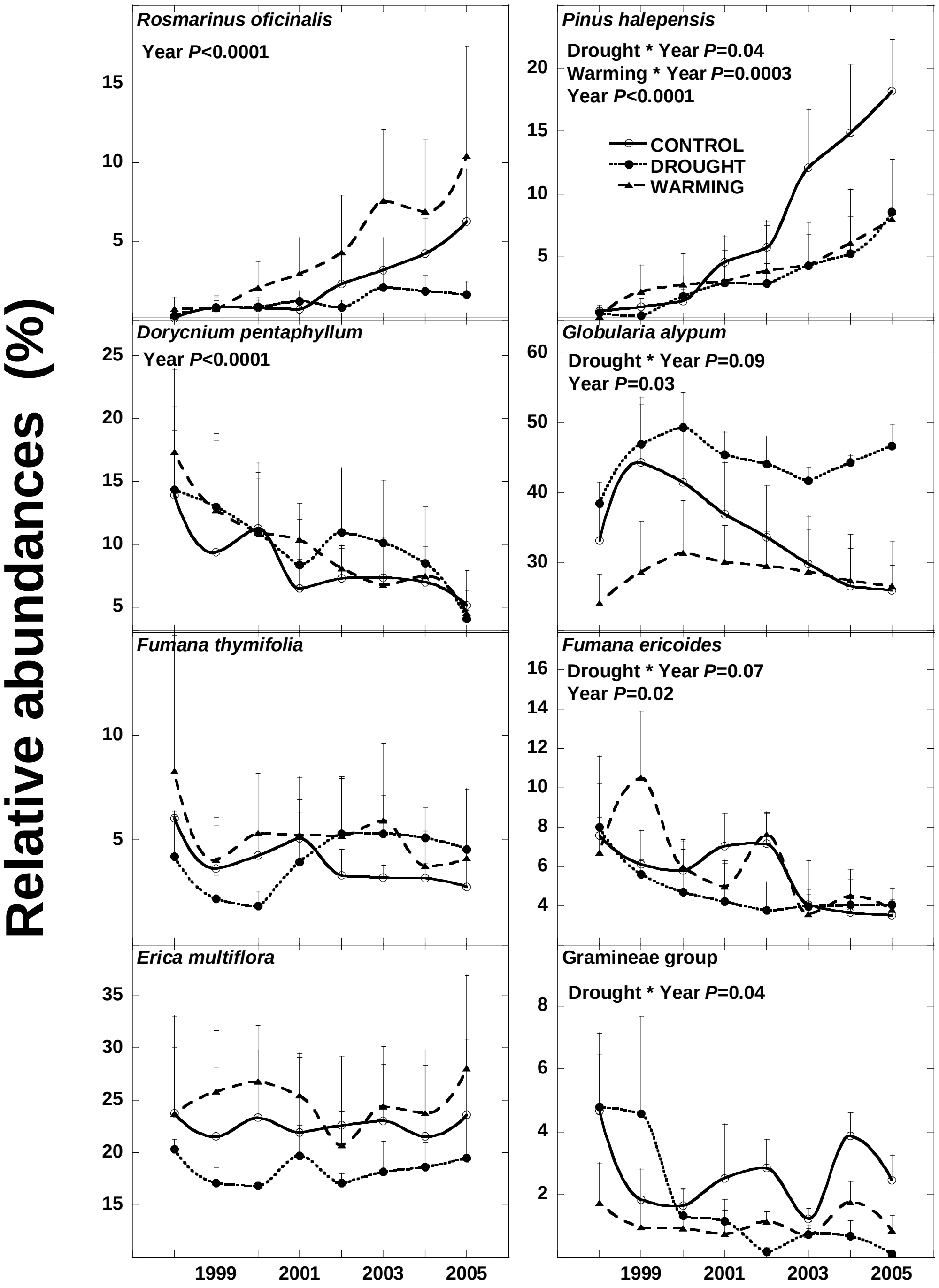

Figure 12. Patterns of the relative abundances of dominant and common species during the seven-year study period in control, warming and drought treatments. Whenever significant (RM-ANOVA), the effects of the studied factor or interaction are depicted in the corresponding panel. Bars indicate the standard errors of the mean (n=3 plot means).

densities (Tilman and El Haddi 1992, Symstad et al. 2003, Smith and Knapp 2003, Lloret et al. 2004). Lloret et al. (2004) described the same relationships for seedlings less than 1-year old in the treated and in control plots suggesting that species that produce fewer recruits are more likely to disappear in drier or warmer scenarios. However, they found that in the drought treatment there were more idiosyncratic changes in the drier years (species abundance in drought treatment was not as strongly correlated to the abundance in the control plots). Interestingly in our study, the correlation coefficients between species abundance in control and drought plots decreased with time of treatment (Figures 9 and 10). These trends support the hypothesis that the likelihood of being locally extinct is firmly related to species abundance at low to intermediate levels of drought, but it tends to become species-specific when conditions become more extreme.

In a scenario of non-random species loss, ecosystem responses should largely be influenced by persistent common or dominant species (Sternberg et al. 1999). We found that the treatment effects on the relative abundance of dominant species were species-specific according to attributes related to their ecology, phenology and water use or regeneration strategy. The direction and rate of change in their relative abundance will determine the changes at stand level because *Globularia alypum* and *Erica multiflora* were directly responsible for 75 % of the biomass accumulation throughout the study period (46 % and 29 % respectively) (Prieto 2007). Stand dynamics are also indirectly determined by these species because changes in the canopy may affect the use of the space by other species (Fowler 1986, Holmgren et al. 2000, Pugnaire et al. 2004, Tirado and Pugnaire 2004, Lloret et al. 2005).

In drier conditions, *Globularia alypum* tended to maintain a dominant position in relation to other species. However, their reduced shoot elongation of established plants in drought plots (Llorens et al. 2004, Peñuelas et al. *in press*) implies that drought treatment could have an effect on the plant architecture. This is probably related to the high mortality of branches observed in drought periods. These branches begin to dry from the apex to the base, followed by the appearance of new sprouts. Other authors have also shown the large potential of plant structure and canopy to acclimate when faced with environmental limitations (Niinemets and Valladares 2004, Sardans et al. 2006). The maintenance of dominance in drought conditions agrees with findings from previous ecophysiological experiments in the studied plots. They showed that *Globularia alypum* has a prodigal or non-conservative water use strategy, suggesting that this would be advantageous in the competition for water (Llorens et al. 2003).

The relative abundance of *Erica multiflora* in control plots did not vary throughout the succession occurring in the studied period, as shown by the almost constant values of 20-25 % measured in control plots. In warming plots *Erica multiflora* tends to increase, but this tendency is not statistically significant. Previous studies have shown that *Erica multiflora* is very sensitive to warmer temperatures. Accordingly in the warming treatment seedling density increased (Lloret et al. 2004), and shoot elongation in adults was significantly enhanced (30 %) (Llorens et al. 2004, Peñuelas et al., *in press*), probably due to an advanced growth phenology (Prieto 2007). These results suggest that in future warmer conditions *Erica multiflora* may maintain a dominant position in the latter stages of the succession. Additionally, Sardans et al. (2006) suggested that *Erica multiflora* may persist in increasingly disturbed Mediterranean ecosystems. However, a trend for it to decrease in abundance in response to higher fire recurrences, because of its life-cycle and regenerative traits was indicated by Lloret et al. (2003), so further research is needed to improve our understanding of the complex responses of this species to changing environmental conditions.

In summary, drier conditions in Mediterranean areas may seriously affect species richness and diversity recovery after fire due to lower levels of plant establishment and reduced growth rates. These effects may especially reduce the presence of species with current low abundances although species-specific responses may also occur, related to biological attributes. In our case, drier conditions in earlier successional stages may increase the competitive disadvantage of the obligate seeder tree *Pinus halepensis* in favour of native resprouter shrubs. Moreover, the water spender strategy of species such as *Globularia alypum* may allow it to take advantage of these new drier conditions. However, continued study in later successional stages is needed to monitor the changing species assemblies, their changing competitive relationships, and thus the effects at stand level that may need a longer period to show statistically significant changes.

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CONCLUSIONES

Las **conclusiones** de los cinco capítulos que conforman este trabajo de investigación ponen de manifiesto la **complejidad** de los efectos que pueden tener unas condiciones más cálidas y secas en la estructura, composición y funcionamiento de los matorrales en Europa y particularmente en el matorral mediterráneo. Tal y como se hipotetizó en la introducción general, en base a las premisas de la bibliografía consultada y de los resultados de los estudios previos realizados en las mismas parcelas de estudio, los efectos detectados variaron según el sitio (latitud, clima, litología,...), el estado sucesional de la comunidad, las especies, la variable o el parámetro monitorizados, la estación y año de medida e incluso las especificidades concretas, en lo que se refiere a temperatura y a precipitación, del periodo en que se realizó la medida.

A continuación, presentamos los resultados más significativos obtenidos en cada uno de los capítulos así como las conclusiones que de cada uno de ellos deducimos.

Chapter 1-Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought. Reductions in primary productivity in the heat and drought year of 2003.

- Resultados más significativos del capítulo:

- En un sitio con clima Atlántico-húmedo como Gales, la **biomasa anual acumulada** estuvo relacionada positivamente con la **temperatura** del periodo de crecimiento. En cambio, en Garraf (Barcelona-España) con clima mediterráneo, la biomasa anual acumulada estuvo negativamente relacionada con la temperatura y positivamente relacionada con la **humedad del suelo**.
- Las mayores respuestas de la biomasa acumulada al tratamiento del **calentamiento** se encontraron en los sitios con menor **aridez**. Sin embargo, este tratamiento de calentamiento no afectó significativamente esta variable durante el periodo de estudio (a excepción de Hungría).
- Condiciones de temperatura extremas como las detectadas durante la **ola de calor** en Europa en el verano del 2003, resultaron en reducciones de la biomasa acumulada, aumentos de la producción de hojarasca y en una menor riqueza de especies en la mayoría de matorrales estudiados.
- En España, *Erica multiflora* creció más en las parcelas de calentamiento mientras que *Globularia alypum* creció menos en las de sequía. También observamos diferentes tendencias no significativas en las respuestas al calentamiento del **crecimiento** de las especies coexistentes en Gales, efectos de la sequía dependientes del año o no efectos.
- El **número de especies** por transecto en las parcelas de sequía en España fue significativamente menor que en las control. El resto de matorrales, en un estado más maduro, no presentó ningún cambio significativo.

- Conclusiones:

- Aunque las **respuestas al calentamiento de la biomasa anual acumulada fueron más positivas en aquellos países con menor aridez,** el hecho de que también encontremos especies mediterráneas, como *Erica multiflora,* que responden positivamente al calentamiento y el hecho de que fenómenos extremos como la ola de calor Europea en 2003 reduzcan la productividad primaria, **matizan** la hipótesis de que sea en los ecosistemas más fríos en los que el calentamiento global de lugar a una mayor **absorción de carbono**.
- La clara reducción en el número de especies por transecto en las parcelas de sequía en Garraf (Barcelona) sugiere que la riqueza de especies en aquellas comunidades en **proceso de recuperación** después de una perturbación, puede ser especialmente sensible al cambio climático respecto a otras comunidades en estadios más maduros.

Chapter 2- Effects of experimental warming and drought on photosynthesis, shoot elongation and biomass accumulation in the dominant species of a Mediterranean shrubland.

- Resultados más significativos del capítulo:

- Las tasas fotosintéticas y de conductancia estomática y la fluorescencia presentaron una **gran variabilidad** estacional y también entre las mismas estaciones de diferentes años. Tanto las temperaturas extremas (altas o bajas) como la baja disponibilidad de agua o la interacción de ambos factores dieron lugar a reducciones en el ratio Fv/Fm, fotosíntesis y conductancia estomática. El verano en 2003 y el invierno en 2005 fueron las estaciones en que la actividad de las plantas estuvo más limitada.

- Las **medidas instantáneas de fotosíntesis, conductancia estomática y fluorescencia** fueron sensibles al tratamiento de sequía sólo en ***Pinus halepensis***. El tratamiento de sequía tendió a reducir el ratio Fv/Fm durante la ola de calor en el verano del 2003 y además redujo globalmente la fotosíntesis y la conductancia estomática. Sin embargo, la sequía experimental no afectó significativamente ni la elongación de esta especie ni su biomasa acumulada.
- Las variables instantáneas de fotosíntesis y conductancia estomática para las **especies arbustivas** (*Erica multiflora* y *Globularia alypum*) fueron mucho menos sensibles a los tratamientos de calentamiento y sequía que el **crecimiento** medido como medida integrada de la elongación estacional. El calentamiento experimental produjo ramas más largas en *Erica multiflora* y la sequía experimental redujo la longitud de las ramas en *Globularia alypum.*
- La **biomasa acumulada** para estas especies no alcanzó variaciones significativas bajo los tratamientos a los que el crecimiento fue sensible. Sin embargo, s~~i~~*í* observamos tendencias como una mayor biomasa de *Erica multiflora* y *Globularia alypum* en las parcelas de calentamiento y sequía respectivamente.
- Ni el calentamiento ni la sequía experimental llegaron a afectar significativamente la biomasa acumulada aérea total en la **comunidad**.

- Conclusiones:

- Según nuestros resultados, no podemos inferir los efectos que tendrían unas condiciones más cálidas y secas en la acumulación de biomasa de especies mediterráneas a partir de los efectos de los tratamientos en las **variables ecofisiológicas instantáneas**, muy dependientes de las especificidades ambientales del periodo de muestreo.
- Los efectos de los tratamientos en la **elongación** de ramas fueron más consistentes para las especies dominantes arbustivas. Bajo unas condiciones más cálidas *Erica multiflora* podría crecer más lo que también se reflejó, aunque sólo como tendencia, en una mayor biomasa acumulada. Sin embargo, para especies como *Globularia alypum*, con un uso de agua no conservador y una gran capacidad rebrotadora, el efecto negativo de la sequía sobre la elongación de las ramas principales no impidió la tendencia positiva observada en la biomasa acumulada de esta especie en este tratamiento.
- Las **diferentes respuestas** de la biomasa acumulada por *Erica multiflora* y *Globularia alypum* en las parcelas de sequía (negativo y positivo respectivamente) pueden anular la aparición de una respuesta clara a nivel de biomasa total. Pero además, la falta de efectos de los tratamientos en la acumulación de biomasa a nivel de cubierta puede reflejar la necesidad un **periodo** de estudio más largo para la detección de respuestas secundarias de la vegetación (por ejemplo efectos en los nuevos individuos reclutados) o la necesidad de un mayor número de **réplicas** experimentales para aumentar la potencia del test.

Chapter 3- Acceleration of the onset of shrubland species spring growth in response to an experimental warming along a north-south gradient in Europe

- Resultados más significativos del capítulo:

- La fenología del crecimiento de *Vaccinium myrtillus* y *Empetrum nigrum* en Gales, *Deschampsia flexuosa* en Dinamarca, *Calluna vulgaris* en Holanda, *Populus alba* en Hungría y *Erica multiflora* en España fue sensible al calentamiento experimental en algunos años.
- Teniendo en cuenta todas las series de datos, un 68 % de las respuestas fueron avances en el día del crecimiento (el 23% fueron significativos) mientras sólo un 27 % fueron retrasos (el 9 % significativos).

- Conclusiones:

- Este estudio muestra los grandes efectos que puede tener el aumento de temperaturas en la fenología del crecimiento de primavera de las especies dominantes de diferentes matorrales europeos.
- El gradiente geográfico que dibuja los matorrales estudiados no determinó las especies sensibles al calentamiento ni la intensidad de la respuesta. Es decir, la fenología del crecimiento de primavera de las especies mediterráneas puede ser tan sensible al calentamiento como la de especies de distribución más septentrional.
- Nuestros resultados manifiestan la dificultad de hacer predicciones generales sobre los efectos del calentamiento global debido a las respuestas específicas y a la influencia de la interacción de las diferentes variables climáticas de un lugar. Consideramos que una

continuación de este estudio sería aconsejable para mejorar nuestra capacidad de prever si un avance en el periodo de crecimiento resulta en una mayor captación de carbono.

4- Precipitation-dependent autumn flowering of Globularia alypum and Erica multiflora, two co-occurring species in a Mediterranean shrubland, and its response to experimental drought and warming

- Resultados más significativos del capítulo:

- La floración de otoño-invierno de *Erica multiflora* y *Globularia alypum* mostró **variabilidad interanual** en las fechas de comienzo y pico de floración. Además la floración de *G. alypum* presentó dos patrones según el año (unimodal/bimodal) y los picos de floración máxima tuvieron diferente intensidad.
- La distribución de lluvias en primavera-verano y el **agua acumulada** en los meses de verano previos a la floración explicó en parte esta variación. Asimismo, encontramos relación entre la fecha del último **episodio de lluvia** de mediana o fuerte intensidad (mayor de 10 litros) justo antes del pico de floración y la fecha en la que éste se produjo.
- Los efectos del calentamiento y sequía experimentales en ***Globularia alypum*** dependieron del año aunque fue en el 2001 (año seco con distribución de lluvias muy irregular) cuando la floración de esta especies estuvo más afectada por la sequía experimental. Los tratamientos no afectaron la floración de ***Erica multiflora*** en ninguno de los cuatro años de estudio.

- Conclusiones:

- La **floración en otoño-invierno** de *Erica multiflora* y *Globularia alypum* depende en gran medida de la lluvia acumulada y su distribución en primavera-verano.
- Esta relación entre floración y disponibilidad de agua y la gran plasticidad que muestra la floración de estas especies sugiere que florecer en otoño-invierno es una **estrategia** frente a cambios de temperatura y lluvia en el área Mediterránea. Sin embargo, cambios más importantes en la distribución de lluvias en primavera-verano pueden afectar a la floración de *Globularia alypum*.

5- Experimental Drought and Warming decrease diversity and slow down post-fire succession in a Mediterranean shrubland.

- Resultados más significativos del capítulo:

- La **riqueza** de especies vegetales y el **Índice de Shannon** estuvieron positivamente correlacionados con la lluvia acumulada durante la estación de crecimiento, y la sequía experimental afectó negativamente a ambas variables.
- También encontramos relaciones lineales positivas entre la **abundancia relativa** de las diferentes especies en las parcelas de calentamiento o sequía y su abundancia en las parcelas control. Sin embargo, estas relaciones en el tratamiento de sequía fueron debilitándose con el tiempo.
- En las parcelas control, la abundancia relativa de las especies arbustivas disminuyó con los años en pos de un aumento en la abundancia relativa de *Pinus halepensis*. Sin

embargo, en las parcelas de calentamiento y sequía los **arbustos** se mantuvieron en una alta proporción y la abundancia del **pino** no creció tanto como lo hizo en las parcelas control. Particularmente, *Globularia alypum* tendió a estar más representada en las parcelas de sequía.

- Conclusiones:

- Unas condiciones más secas en el área Mediterránea pueden afectar seriamente la **recuperación** de la composición y estructura de la comunidad después de una perturbación y estos efectos pueden estar relacionados con un menor establecimiento de plántulas y menores tasas de crecimiento.
- Las **especies** que actualmente son **poco frecuentes**, lo seguirán siendo bajo unas condiciones más cálidas y secas, aunque con el tiempo estas especies pueden presentar respuestas más idiosincrásicas (especialmente frente a las sequías).
- Las abundancias relativas de las **especies dominantes** y **comunes** presentaron respuestas específicas a los tratamientos relacionadas con características de la especie. Los tratamientos de calentamiento y sequía redujeron la habilidad competitiva de *Pinus halepensis* (germinador obligado) frente a los arbustos rebrotadores. La estrategia en el uso del agua pudo ayudar a *Globularia alypum* a mantener una posición dominante en las parcelas de sequía.
- Las respuestas de las diferentes especies, ya sean dependientes de su abundancia o de algún carácter específico que posean, pueden dar lugar a cambios en las **relaciones competitivas** y por lo tanto a cambios en la estructura y composición de los matorrales bajo un clima cambiante. En Garraf, la sequía y el calentamiento ralentizó

el proceso de transformación de una comunidad dominada por arbustos a una comunidad dominada por pinos.

En **resumen** podemos concluir que:

- Nuestros resultados confirman pero **matizan** la hipótesis de que en ecosistemas templados, y particularmente en los más fríos y húmedos, el calentamiento global de lugar a un mayor aumento en la **absorción de carbono**. Periodos como los vividos en el verano del **2003** (ola de calor europea) o en el invierno del **2005** (el más frío y seco del periodo de estudio en Garraf) pueden afectar negativamente la actividad de las plantas.
- Los resultados nos muestran como la sensibilidad de una comunidad o especie frente al cambio climático puede variar según la **variable** estudiada, el **año**, la **estación** o el **momento concreto** en el que éste tiene lugar.
- Diferentes **respuestas específicas** de especies co-dominantes en una comunidad, como las mostradas por *Erica multiflora*, *Globularia alypum* y *Pinus halepensis* en Garraf, sugieren que la dirección de los cambios ambientales puede dar lugar a cambios en sus relaciones competitivas, en sus abundancias y por lo tanto en los caracteres de la comunidad (estructura, composición, funcionamiento) a medio y largo plazo.