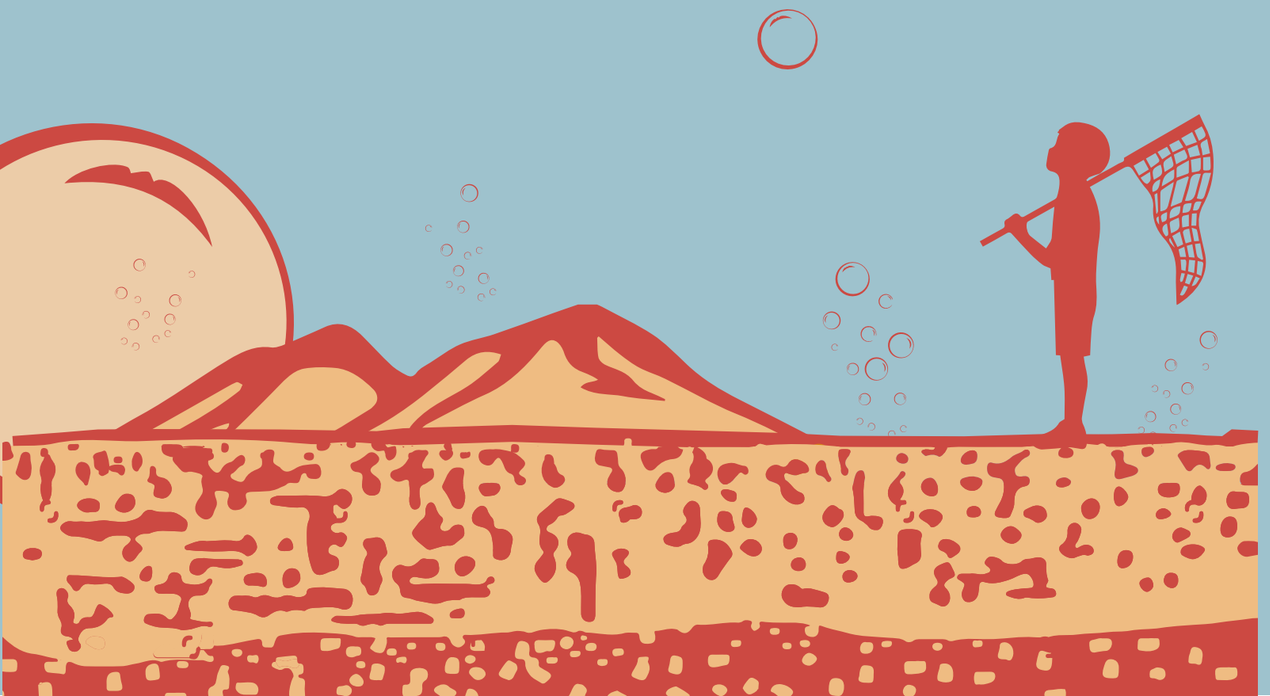

Carme Estruch Puig

Flujos de CO_2 en ecosistemas mediterráneos: Influencia de factores bióticos y abióticos

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FLUJOS DE CO_2 EN ECOSISTEMAS MEDITERRÁNEOS: INFLUENCIA DE FACTORES BIÓTICOS Y ABIÓTICOS

Carme Estruch Puig

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Memoria presentada por Carme Estruch Puig para optar al Grado de Doctora por la Universidad de Valencia. Esta Tesis ha sido dirigida por el Dr. Francisco I. Pugnaire de Iraola, Profesor de Investigación del CSIC en la Estación Experimental de Zonas Áridas y codirigida por la Dra. Cristina Armas Kulik, Investigadora Ramón y Cajal en la EEZA-CSIC.

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– “Nunca se ha de pensar en toda la calle de una vez, ¿entiendes? Sólo hay que pensar en el paso siguiente, en la inspiración siguiente, en la siguiente barrida. Nunca nada más que en el siguiente”.

-Michael Ende

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Índice

Summary

In this Thesis we address the effect of the main biotic and abiotic factors on CO_2 fluxes in drylands. With this purpose, we assessed the effect of plant communities and soil properties on CO_2 fluxes, and their relationship with soil humidity and temperature. From these CO_2 fluxes we focused our analyses in soil respiration, and in several cases we also analyzed atmosphere CO_2 fixation by plants (photosynthesis) Specifically, our objectives were to analyze 1) the effect of plant communities in soil respiration and their relationship with seasonal changes in temperature and humidity, 2) soil respiration responses to sudden changes in soil moisture in a range of temperatures typical of arid zones, and relate them to the composition of the microbial community involved, 3) the contribution to soil respiration of the three main soil biota groups involved in soil respiration (roots, mycorrhiza and bulk soil), and 4) the links between plant community fluxes and soil respiration in a chronosequence of abandoned land to determine ecosystem capacity to act as a carbon source or carbon sink.

In the first chapter we addressed soil respiration under different plant species canopies and bare soil. The different plant species influence CO_2 emissions through specific effects on soil humidity, temperature, C allocation, organic matter or microbial communities. We determined CO_2 emissions in soils beneath different plant species and bare soils their response to temperature in a semiarid environment over a 20-mo manipulative experiment. We altered soil temperature under the canopy of four plant species differing in functional type and activity, and in bare soil, and measured monthly fluxes to establish seasonal patterns of CO_2 release. We found an exponential relationship between soil respiration and soil temperature that shifted from negative in the dry and warm season to positive in the relatively wet and cool season. A model that include soil temperature, soil water content and plant species identity effects on CO_2

emissions explained 74% of the seasonal variation in soil respiration rate in this system, species identity playing a significant and probably the strongest effect on soil respiration rate. Our data showed the importance that plant species composition plays on annual CO_2 emissions at the community level.

In the second chapter we addressed the soil respiration response to water pulses in a range of high temperatures (30ºC-60ºC) that are typical in arid and semiarid environments. We determined optimal soil respiration temperature after rapid rewetting with different water pulses, and assessed soil microbial communities (SMC) in three different soils from contrasted semiarid environments. We used short incubation times with four temperatures and three watering regimes and determined the structure of soil microbial communities (SMC). Soil respiration responses to water supply depended on temperature and soil origin. Optimum temperatures in sandy soils (desert and alpine) were well above 50ºC while in clay soils were lower. Soils showed marked differences in SMC, and differed with depth. Our data show that soil respiration pulses depended on temperature if not completely dry, and that optimum temperatures were well above the general assumption of 35ºC in these semiarid environments. Our results also evidenced the dependence of soil respiration responses on soil depth, which showed higher respiration pulses in the upper soil layers.

In the thirds chapter we addressed the contribution of the three main soil community groups (roots, mycorrhiza and the bulk soil community) in the total soil respiration. In order to determine the sensitivity of these components to environmental drivers we set up an experiment to address the effect of plant community composition, soil age and warming on soil respiration rate during the 2014-2015 winter. We tested differences among microbial, fungal and root respiration using an exclusion technique to assess the effect of plant community (open grasslands vs oak woodland) in two field sites differing in geologic soil age (92 and 137 kyr). We also used open top chambers

(OTC) to simulate global change effects on grasslands. Our results showed that arbuscular mycorrhizal fungi were the main drivers of differences recorded between soils of different age, and that those differences were linked to nutrient availability. Bulk soil respiration was more sensitive to temporal variation than mycorrhizal or root respiration. Soil age affected CO_2 flux from grasslands but not under oak canopies, likely due to differences in SOM content which moderate CO_2 fluxes. Overall our study shows that the ability of grasslands to mitigate CO_2 emissions depends on interactions between vegetation and their rhizosphere and soil microbial communities.

In the fourth chapter we addressed changes in the carbon balance along a chronosequence from abandoned farmlands, in a semiarid environment. We addressed changes in C balance along a chronosequence of land abandonment in a semiarid environment and assessed the consequences of secondary succession on C sequestration capacity at community scale. We used a closed-chamber method to estimate the contribution of whole-plants and bare soil to whole-ecosystem C exchange. Plant community composition and cover strongly affected C balance. Overall, whole-ecosystem C exchange shifted from C source to C sink with succession. However, only after 63 years of agriculture abandonment the system did recover its natural C sequestration capacity. Thus, the capacity of semiarid ecosystems to recover native plant communities after anthropogenic disturbance may contribute to decrease C emissions in the long term.

This information is important to understand the role of drylands in the global carbon cycle. Moreover, brings information relevant in understanding the function of different ecosystems components and their implication in the net carbon balance. The information generated by this thesis is useful to improve predictions about carbon cycling in terrestrial ecosystems limited by water.

Resumen

En esta Tesis pretendemos entender el papel que tienen los principales factores bióticos y abióticos en el balance de CO_2 entre el suelo, vegetación y la atmósfera en ecosistemas limitados por el agua. Para ello hemos estudiado el papel de la vegetación y el suelo en las emisiones de CO_2 del ecosistema, y su relación con la temperatura y humedad. Hemos tenido en cuenta tanto las emisiones del suelo (respiración), como los flujos de CO_2 de la vegetación (fotosíntesis y respiración de las plantas). En concreto, los objetivos han sido analizar 1) el efecto de la vegetación sobre la respiración del suelo y su relación con los cambios estacionales de temperatura y humedad; 2) la respuesta a cambios bruscos en la humedad de suelos de distinta procedencia en un rango de temperaturas elevadas típicas de las zonas áridas, junto con el estudio de la comunidad bacteriana de dichos suelos; 3) la contribución a la respiración del suelo de los tres grupos principales de organismos involucrados en la respiración del suelo (raíces, micorrizas y comunidad de microorganismos de vida libre del suelo); y 4) los vínculos entre el intercambio de CO_2 que se produce entre la comunidad de plantas y la atmósfera (fotosíntesis y respiración) y la respiración del suelo, que determinan la capacidad del ecosistema de actuar como fuente o sumidero de C a lo largo de la sucesión secundaria.

En el primer capítulo medimos las emisiones de CO_2 en suelos bajo diferentes especies de plantas y en claros para seguir su respuesta temporal a la temperatura y la humedad en un ambiente semiárido. Para esto alteramos la temperatura del suelo bajo la copa de cuatro especies de plantas (dos gramíneas y dos arbustos) y en suelo desnudo, y tomamos medidas mensuales de respiración del suelo. Con esto establecimos patrones de respiración estacional, y vimos la relación entre respiración y temperatura del suelo; la relación de la respiración del suelo con la temperatura pasó de ser negativa en la

estación seca a positiva en la temporada húmeda. Ayudándonos de un modelo empírico que incluía la temperatura y humedad del suelo y la identidad de cada especie, explicamos el 74% de la variación estacional de la tasa de respiración. Nuestros datos muestran la importancia que tiene la identidad de las diferentes especies vegetales en las emisiones anuales de CO_2 del suelo en los sistemas semiáridos.

En el segundo capítulo evaluamos las respuestas en la respiración del suelo a pulsos de agua en un rango de temperaturas elevado (30-60ºC) que se alcanza habitualmente en los suelos de los sistemas áridos y semiáridos. Para ello sometimos distintos suelos a tres niveles de riego, simulando pulsos de agua (suelo seco, baja intensidad de lluvia, alta intensidad de lluvia); los incubamos durante una hora y analizamos su respuesta inmediata. Para el experimento utilizamos suelos procedentes de tres ambientes limitados por el agua (suelos arcillosos y suelos arenosos de ambientes semiáridos a baja altitud, y suelos arenosos de ambientes secos alpinos), y a dos profundidades (0-5 y 10-30 cm), en los que analizamos la estructura de las comunidades microbianas del suelo. Comprobamos que la respuesta de la respiración del suelo a los pulsos de agua dependía de la temperatura y del origen del suelo, y establecimos temperaturas óptimas alrededor de los 50ºC en suelos arenosos (semiárido y seco alpino) mientras que en suelos arcillosos no se alcanzó un óptimo. Los suelos mostraron diferencias marcadas en la estructura de las comunidades microbianas según su procedencia y profundidad. Nuestros resultados mostraron que la respuesta del suelo a los pulsos de agua depende de la temperatura, y que se alcanzan niveles óptimos en un rango de temperaturas mucho más elevadas de las descritas hasta ahora.

En el tercer capítulo estudiamos la contribución de diferentes comunidades de organismos del suelo (raíces, micorrizas, organismos de vida libre) a la respiración del suelo y su respuesta a factores ambientales como la vegetación, la edad del suelo o la

manipulación del ambiente. Para separarlos, instalamos mallas de nylon de diferente tamaño de poro para impedir el crecimiento de los distintos componentes bióticos. Comparamos comunidades de plantas anuales frente a robledales en suelos que diferían en edad geológica (92.000 años vs 137.000 años de antigüedad). Además, utilizamos cámaras abiertas de efecto invernadero (en inglés OTC) con la intención de simular un aumento de la temperatura del aire. Nuestros resultados mostraron que la vegetación ejercía un papel muy importante en la respiración de los distintos grupos del suelo de forma que en los robledales la respiración no variaba entre suelos de distintas edades, mientras que en los pastizales sí que observábamos diferencias entre edades de geológicas. La presencia de micorrizas arbusculares era el principal factor para explicar las diferencias observadas en tasas de respiración en suelos de distintas edades en los pastizales, y esas diferencias se relacionaban con la disponibilidad de nutrientes (N y C). La respiración de la comunidad de vida libre del suelo aumentó debido a los cambios ambientales producidos por las OTC, mientras que eso no ocurrió con la comunidad de raíces y micorrizas. En general, nuestro estudio demuestra que en pastizales, la comunidad del suelo puede reducir las emisiones de CO_2 en función de los factores que influyen en la abundancia y actividad de micorrizas arbusculares, como la edad del suelo o la vegetación.

En el cuarto capítulo hemos abordado los cambios en los flujos de carbono que se producen entre una comunidad arbustiva y la atmósfera a lo largo de una cronosecuencia de abandono de campos en un ambiente semiárido. En este capítulo evaluamos la capacidad de distintos estadios sucesionales de secuestrar carbono de la atmósfera. Utilizamos un sistema de cámara cerrada para estimar la contribución de la parte aérea de la vegetación y la respiración del suelo en el intercambio de CO_2 entre el sistema y la atmósfera. Comprobamos que el porcentaje de la cantidad de cubierta

vegetal tiene un efecto determinante en las tasas de intercambio de CO_2 y en las diferencias encontradas en las comunidades de distintos estadios sucesionales. Así, las comunidades pasaron de ser emisor de CO_2 a la atmósfera a ser sumideros a medida que la sucesión avanzaba. Sin embargo, sólo se recuperó la capacidad neta de secuestro de carbono después de 63 años de abandono, momento a partir del cual el ecosistema actuó como sumidero. La recuperación de las comunidades vegetales incrementa la captación de carbono en estos sistemas.

Esta información es relevante para comprender el papel de los ambientes áridos y semiáridos en el secuestro de carbono de la atmósfera. Además, aporta información sobre el funcionamiento de los distintos componentes del ecosistema y su papel en el intercambio de CO_2 suelo-atmósfera y ecosistema-atmósfera. Estos componentes normalmente se estudian como un conjunto a modo de caja cerrada, por lo que la información generada por esta Tesis es de utilidad para mejorar las predicciones sobre el ciclo del carbono en ecosistemas limitados por el agua.

Introducción general

Uno de los principales retos científicos de la actualidad es entender cómo la acumulación de CO_2 en la atmósfera afectará al funcionamiento de los ecosistemas. Esto es debido a que el dióxido de carbono absorbe la radiación infrarroja, lo que conlleva el calentamiento global de la atmósfera. La capacidad de mitigar las emisiones de CO_2 a la atmosfera está muy relacionada con la cubierta vegetal, que lo puede captar de la atmósfera. A nivel global ha habido un aumento de la biomasa vegetal en los ecosistemas naturales en los últimos años, en parte como respuesta al aumento de CO_2 atmosférico (Zhu *et al.*, 2016). Se estima que los ecosistemas terrestres son capaces de fijar en torno a un 25% de las emisiones de CO_2 de origen antropogénico (Le Quéré *et al.*, 2015), un almacenaje que se distribuye entre la cubierta vegetal y en el suelo.

El 41% de los ecosistemas terrestres está limitado por el agua (UN, 2011), y comprenden las regiones áridas, semiáridas y subhúmedas secas (Abraham de Vázquez, 2008), que albergan el 38% la población mundial (Reynolds *et al.*, 2007). Debido a su gran extensión y a pesar de poseer una escasa cubierta vegetal, estas zonas tienen un papel fundamental en el secuestro de carbono (Ahlström *et al.*, 2015).

Los modelos climáticos apuntan a un aumento de la temperatura media anual y a un cambio en los patrones de precipitación en muchos sistemas (IPCC, 2014), lo que puede modificar el balance de carbono a nivel global. En ambientes limitados por el agua la interacción entre temperatura y humedad controla las emisiones de CO_2 del suelo (Reichstein *et al.*, 2003; Rey *et al.*, 2011) y está fuertemente relacionado con la actividad de la vegetación (Rey *et al.*, 2002; Maestre & Cortina, 2003; Reichstein *et al.*, 2003).

En las largas estaciones secas características de los ecosistemas áridos y semiáridos, los eventos de lluvia dan lugar a grandes emisiones de CO_2 (Fierer &

Schimel, 2003; Chatterjee & Jenerette, 2011a) que suponen una fracción importante de la respiración anual (Kim *et al.*, 2012; Ataka *et al.*, 2014; Fan *et al.*, 2015), e incluso pequeños episodios de lluvia durante épocas secas pueden desencadenar respuestas mayores que grandes eventos de lluvia durante periodos húmedos (Rey *et al.*, 2011). La temperatura a la que se producen estos eventos de lluvia puede afectar la magnitud de las emisiones de CO_2, ya que la temperatura afecta a la disponibilidad de sustrato (Conant *et al.*, 2011; Sierra, 2012), la actividad enzimática (Davidson *et al.*, 2006b; Davidson *et al.*, 2012) y el metabolismo microbiano (Davidson *et al.*, 2006b). En un escenario de cambio global en el que esperamos un aumento de las temperaturas medias anuales y cambios en los patrones de precipitación, que serán cada vez más erráticos (IPCC, 2014), es importante entender estos mecanismos, porque hay pocos estudios que midan el efecto de la temperatura más allá de los 35ºC (Lloyd & Taylor, 1994; Richardson *et al.*, 2012).

La respiración del suelo se debe a la actividad de sus tres componentes bióticos: raíces, micorrizas y la comunidad microbiana (hongos saprófitos y bacterias de vida libre). Determinar cómo la respiración del suelo responde a los factores que constituyen el cambio global (incremento de CO_2, incremento de temperatura, mayor disponibilidad de nutrientes, cambios de usos del suelo, contaminación, etc.) es complicado debido a la multitud de factores implicados y a la divergencia en respuestas que pueden tener los componentes bióticos del suelo. Por ejemplo, la comunidad de vida libre es más sensible a cambios de temperatura y humedad (Hartley *et al.*, 2007; Heinemeyer *et al.*, 2007), mientras que raíces y micorrizas pueden ser menos sensibles a la variabilidad ambiental (Zhu, 2003; Heinonsalo *et al.*, 2010; Hu *et al.*, 2013), por lo que su respuesta está más ligada al metabolismo de la cubierta vegetal (como fotosíntesis o fenología) que a cambios ambientales (Hartley *et al.*, 2007; Churchland *et al.*, 2013).

En general, las raíces y la comunidad microbiana del suelo representan (cada una) un 40-60% de la respiración total, mientras que la de las micorrizas representa hasta un 25%, aunque esto varía dependiendo del ecosistema, e incluso puede variar temporalmente dentro de un mismo sistema (Heinemeyer *et al.*, 2007; Heinonsalo *et al.*, 2010; Zhang, 2013; Barba *et al.*, 2016). El régimen climático puede favorecer que algunos grupos del suelo tengan una mayor representación en detrimento de otros; por ejemplo, aunque el micelio extra radical de las micorrizas puede sobrevivir los rigores del verano (Brito *et al.*, 2011), sabemos que la sequía reduce su abundancia en el suelo (Herzog *et al.*, 2013). También sabemos que en suelos ricos en nutrientes la comunidad de bacterias es más abundante (Moore *et al.*, 2010; Gerz *et al.*, 2016), o que los distintos grupos funcionales de plantas determinan el desarrollo de comunidades en la rizosfera (Bunn *et al.*, 2015). Por tanto, es de particular interés estudiar por separado la contribución de los distintos componentes bióticos del suelo y su relación con la vegetación, el tipo de suelo, la temperatura y la humedad.

Los principales intercambios de carbono entre el ecosistema terrestre y la atmósfera se producen a través de la vegetación y el suelo. En condiciones estables, el intercambio neto de carbono entre el ecosistema y la atmósfera (NEE, acrónimo del término en inglés *Net Ecosystem Exchange*) es el resultado del balance entre la productividad primaria neta (NPP o *Net Primary Productivity*) y la respiración del ecosistema (ER o *Ecosystem Respiration*). La vegetación es el principal responsable de la NPP mediante la fijación de CO_2 atmosférico. A su vez, la respiración del suelo supone tres cuartas partes de la respiración del ecosistema (Law *et al.*, 2001).

La estructura de la comunidad vegetal afecta a la capacidad del ecosistema para capturar CO_2 de la atmósfera (Sundquist *et al.*, 2008; Ostle *et al.*, 2009). La fijación de carbono atmosférico a través de la fotosíntesis genera material vegetal que se acumula

tanto en la parte aérea de las plantas como en el suelo a través de la hojarasca, las raíces o la emisión de exudados radicales (Bais *et al.*, 2006). Tanto la hojarasca como la materia orgánica del suelo y los exudados son consumidos por la comunidad heterótrofa del suelo y parte de estos compuestos son de nuevo liberados a la atmósfera.

Además, la composición y tipo de materia vegetal que llega al suelo (exudados, raíces u hojarasca) viene determinada por las estrategias de crecimiento de las plantas, que producen compuestos más o menos fáciles de descomponer (García Palacios *et al.*, 2013; Reich, 2014; Hasibeder *et al.*, 2015). De esta forma, la vegetación influye en el ciclo del carbono tanto a través de sus efectos directos (fotosíntesis, respiración) como indirectos, afectando a la actividad de los organismos del suelo (e.g., Tedeschi *et al.*, 2006; Tang *et al.*, 2009; Luan *et al.*, 2011; Novara *et al.*, 2014) a través de la hojarasca (Zhang *et al.*, 2013) que influye en la tasa de descomposición de la materia orgánica (Holden *et al.*, 2013; Osono *et al.*, 2013). Para mejorar los modelos de flujo de CO_2 a nivel de ecosistema es necesario relacionar el intercambio de CO_2 entre el suelo y la atmósfera con el balance de carbono que se produce en la cubierta vegetal (a través de la fotosíntesis y respiración de las plantas).

Objetivos

Con el fin de comprender los procesos que regulan el balance de carbono en los ambientes limitados por el agua, los objetivos que se abordan en cada uno de los capítulos de esta tesis son:

1. Estudiar el efecto que ejerce la vegetación sobre la variación temporal de la respiración del suelo, en función de la temperatura y humedad, y posterior modelización del patrón anual de respiración del suelo en ambientes semiáridos (Capítulo I).
2. Explorar la respuesta de la respiración del suelo, en muestras procedentes de distintos ambientes limitados por el agua y de dos profundidades distintas, a los pulsos de agua, simulando episodios de lluvia que se producen en estos sistemas cuando las temperaturas del suelo superan los 30ºC. Vincular estas respuestas con la estructura de las comunidades bacterianas (Capítulo II).
3. Estudiar la importancia relativa de los tres grupos principales de organismos del suelo (raíces, micorrizas y comunidad libre del suelo) en la respiración del suelo, y cómo varía la contribución de cada componente en función de factores como tipo de vegetación o edad del suelo (Capítulo III).
4. Explorar cómo la sucesión secundaria afecta la capacidad del ecosistema de actuar como fuente o sumidero de C en función del balance entre flujos de carbono en la vegetación (fotosíntesis y respiración) y la respiración del suelo (Capítulo IV).

Métodos generales

Medidas con IRGA

Los analizadores de gas por infrarrojo (IRGA) miden la concentración de CO_2 en un volumen determinado de aire. Esta detección se fundamenta en el hecho de que los gases como el CO_2 absorben fotones en el rango del infrarrojo. Mediante la cuantificación de la absorción de luz en el espectro infrarrojo se puede determinar la concentración de CO_2.

En nuestras medidas hemos utilizado sistemas de cámara cerrada, que miden la tasa de respiración/fotosíntesis midiendo el cambio en la concentración de CO_2 dentro de la misma cámara (Figura 1).

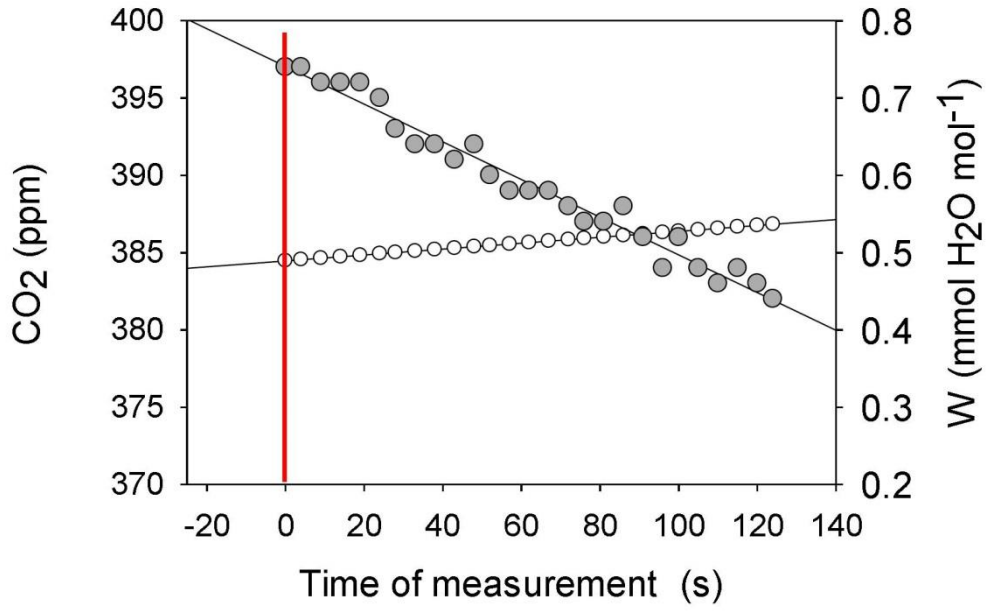

Figura 1. Ejemplo de una medida de fotosíntesis, los puntos grises corresponden a la concentración de CO_2 y los puntos blancos a la de vapor de agua.

Del valor obtenido de la pendiente de la concentración de CO_2 (ppm/unidad de tiempo) se calcula la concentración de moles de CO_2 que hay en nuestra muestra a partir

de la obtención de los moles de aire que hay en nuestra cámara de medida, que se obtiene de la ley de los gases nobles, usando la ecuación:

$$P \times v = n \times R \times T \quad (1)$$

Donde P es la presión atmosférica (kPa), V es el volumen de aire, R es la constante universal de los gases (8,31 × 10^{-3} L kPa mol-1), T es la temperatura (K) y n es la concentración de moles de aire.

Cámaras de medida

En nuestros experimentos hemos utilizado tres cámaras distintas (Figura 2), dos para medir respiración del suelo y una adaptada para medir el intercambio de gases en plantas.

Figura 2. Cámaras de medida utilizadas en nuestros experimentos

Cámaras para medir respiración del suelo

Utilizamos dos cámaras de medida, una asociada a un IRGA Li-Cor 6400 (Lincoln, Nebraska, USA) y una SRC-1 acoplada a un IRGA EGM4 (PP Systems, Amesbury, MA, USA).

Cámara para medir intercambio de gases en plantas

Utilizamos una cámara fabricada en policarbonato transparente (96 x 94 x 80 cm), que reduce la entrada de luz PAR en un 10%. La cámara lleva dos ventiladores instalados en

el interior (12 V, 0.14A) para mezclar el aire. El techo de la cámara no está fijo, para permitir la ventilación antes del inicio de las medidas. La base de la cámara está recubierta de espuma y cubierta por una falda de un material plástico que se fija al suelo con una cadena metálica en cada medida para reforzar el sellado de la cámara. La cámara se conecta a un IRGA EGM4 (PP Systems, Amesbury, MA, USA). Para medidas en oscuridad, se cubre con un material plástico opaco que anula la entrada de la luz.

Áreas de estudio

Los experimentos que conforman esta Tesis se han desarrollado en cinco áreas de estudio pertenecientes a sistemas mediterráneos secos o semiáridos, con un periodo de sequía que va principalmente de mayo a septiembre.

Área de estudio 1 (**Capítulos I y II**), se localiza en la cara norte de Sierra Alhamilla, Almería (37º 01'N, 02º 25'O, ~350 m altitud), es de clima mediterráneo semiárido con una precipitación media anual (PMA) de 230 mm y una temperatura media anual (TMA) de 17.9ºC. La vegetación está compuesta por una comunidad arbustiva. El suelo tiene un alto contenido de arcillas y está clasificado como Orthic solonchak (Martínez-García *et al.*, 2011). El suelo desnudo se caracteriza por una alta presencia de costra biológica. Las especies dominantes son las gramíneas *Stipa tenacissima* L. y *Lygeum spartum* (L.) Kunth, y los arbustos *Salsola genistoides* Juss. Ex Poir. y *Hammada articulata* (Moq) O Bolós & Vigo, ambos de la familia Chenopodiaceae.

Área de estudio 2 (**Capítulo II**), se localiza en la Rambla del Saltador, en la zona sur de la Sierra de Los Filabres, Almería (37º 08'N 2º 22'O, 630 m altitud). Presenta un clima semiárido (PMA 235 mm, TMA 17.9ºC) y un suelo aluvial arenoso, pobre en

nutrientes, clasificado como Regosol Eútrico. La especie dominante de la zona es el arbusto *Retama spherocarpa* L.

Área de estudio 3 (**Capítulo II**), se localiza en la cara norte de Sierra Nevada, Granada (37° 05'N, 03° 23'O, 2700 m altitud). El clima es mediterráneo seco (PMA 690 mm, TMA 3.9ºC). El suelo es más bien arenoso, similar al área de estudio 2, clasificado como Leptosol Dístrico y la vegetación es la típica de un sistema alpino con arbustos de porte almohadillado (Mas información en Schöb *et al.*, 2013).

Área de estudio 4 (**Capítulo III**), se localiza en Santa Cruz, California (37º 00' N, 122º 11' O, ~100 m altitud). El clima es mediterráneo (PMA 771 mm, TMA 13.9ºC). Los suelos están clasificados como Loamy Molisol (Aniku & Singer, 1990) y provienen de sedimentos marinos, contienen feldespatos y cuarzo procedentes de una loma granítica. Las dos parcelas de estudio pertenecen a una secuencia tectónica, siendo formaciones con 92.000 y 137.000 años de antigüedad (Mas información en Moore *et al.*, 2010). La vegetación típica es una pradera de herbáceas anuales dominada por especies invasoras provenientes del continente europeo y con agrupaciones de robledales.

Área de estudio 5 (**Capítulo IV**), se localiza en la planicie de Los Llanos de Rueda, en la cuenca de Tabernas, Almería (37º 05'N, 2º 22'O, ~500m altitud) con clima semiárido (PMA 235mm, TMA ~18ºC). En esta zona se identificó una cronosecuencia (~100 años) de suelos abandonados provenientes de la agricultura, que va desde parcelas roturadas en los últimos 5 años hasta campos abandonados hace más de 80 años y una comunidad arbustiva con esparto típica de la zona. El suelo está clasificado como Orthic solonchak. Las especies arbustivas dominantes en cada uno de los tiempos de abandono en estas parcelas son *Thymelaea hirtusa* (L.) Endl. (~15 años de abandono), *Artemisia barrelieri* (Besser) Soják (~60 años de abandono), *Hammada*

articulata (Moq.) O. Bolos & Vigo y *Salsola oppositifolia* Desf. (~65 años de abandono), *Stipa tenacissima* L. y *Helianthemum almeriense* Pau (>84 años de abandono y comunidad nativa) (Mas información en Lozano *et al.*, 2014).

Diseño Experimental

Para poder responder a los diferentes objetivos planteados en esta Tesis se abordaron varias metodologías que se exponen a continuación. Para un mejor seguimiento, estas metodologías serán introducidas con más detalle en los diferentes capítulos.

Tareas de campo

Para varios de los objetivos abordados se realizaron manipulaciones experimentales en campo.

Manipulación de la temperatura

Con el fin de estudiar los efectos a largo plazo de la temperatura sobre la respiración del suelo, en las áreas de estudio **1** y **4** se introdujeron elementos para modificar la temperatura del suelo (**1**) y el aire (**4**).

En marzo de 2012 aplicamos dos tratamientos de temperatura (incremento y disminución) y un control (sin tratamiento) a individuos de cuatro especies seleccionadas repartidos al azar y en suelo desnudo. Los tratamientos consistieron en manipular el color de la superficie del suelo mediante la aplicación de una capa de arena de cuarzo: blanca para disminuir la temperatura y negra para aumentar la temperatura. Con este procedimiento se consiguió una diferencia de 1.9ºC entre tratamientos.

En noviembre de 2013 establecimos 5 cámaras abiertas para incrementar la temperatura en dos parcelas del **área de estudio 4**. Aunque no se observaron diferencias significativas entre tratamientos en cuanto a la temperatura del aire, sí observamos ciertos efectos que se explicarán con más detalle en el **capítulo III**.

Manipulación de la comunidad del suelo

Con el fin de separar la contribución a la respiración total del suelo que realizan los tres principales grupos de organismos del suelo (raíces de las plantas, micorrizas, comunidad heterotrófica del suelo), en el **área de estudio 4** utilizamos mallas de exclusión (Heinemeyer *et al.*, 2007) para crear barreras físicas que impidan la entrada de los diferentes organismos en la porción de suelo que queremos estudiar. Utilizamos anillos de PVC de 25 cm de profundidad, con tres perforaciones de 5 cm de diámetro a 7 cm del borde superior del anillo, y los cubrimos con malla de nilón de 41 μm de luz en los tratamientos para impedir la entrada de las raíces, o de 1 μm para impedir la entrada de raíces y micorrizas. Utilizamos como control un tercer anillo (7 cm de longitud) que permitía el crecimiento de toda la comunidad del suelo. Los anillos se colocaron de forma que quedaran 2 cm de PVC por encima de la superficie del suelo para poder instalar la cámara de medida.

Para el resto de los experimentos en los que se utilizaron anillos de respiración utilizamos collares de PVC de 5 cm de profundidad, para afectar lo mínimo posible a la comunidad del suelo original. Todos los anillos de respiración del suelo utilizados en esta Tesis tenían 10.3 cm de diámetro.

Efecto de la vegetación

En las diferentes áreas de estudio abordamos el efecto que tiene la vegetación sobre la respiración del suelo (**1** y **4**) y el balance de C en la comunidad (**2**).

Para ello, en el **área de estudio 1** seleccionamos un total de 36 individuos de cada una de las cuatro especies (*Stipa tenacissima*, *Lygeum spartum*, *Salsola genistoides* y *Hammada articulata*) y 36 puntos de suelo desnudo en un diseño factorial no pareado. Estos individuos y las áreas de suelo desnudo fueron seleccionados al azar en un área de 1 ha. Los collares de PVC se instalaron siempre en la cara Este de los arbustos para que las medidas fueran más comparables entre ellas.

Para estudiar el efecto de temperatura sobre la respiración en respuesta a los pulsos de agua, recogimos suelos procedentes de claros de las **áreas de estudio 1, 2 y 3**. En cada sitio el suelo se recogió en cinco puntos distintos, a dos niveles de profundidad (0-5 cm y 10-30 cm) y dentro de un área de 20 × 20 m. Los suelos a las dos profundidades se recogieron de puntos diferentes para evitar correlación espacial. El suelo se tamizó en una malla de 2 mm para eliminar piedras y raíces, y se homogeneizó. Así obtuvimos una muestra compuesta de cada lugar y de cada profundidad sobre la que aplicar las incubaciones que se llevaron a cabo en el laboratorio. Se siguieron protocolos estándar de esterilidad para tomar las submuestras destinadas a análisis moleculares.

Para analizar la interacción entre la edad del suelo y la vegetación sobre la respiración de los distintos componentes bióticos del suelo (raíces, micorrizas, comunidad libre del suelo), en el **área de estudio 4** manipulamos la comunidad del suelo bajo los dos tipos de vegetación (anuales y robledales) y en las dos parcelas geológicas (92.000 y 137.000 años de antigüedad) con las mallas de exclusión descritas anteriormente.

Para evaluar el efecto que tiene la composición de la comunidad vegetal sobre los flujos de CO_2 a lo largo de la sucesión secundaria, seleccionamos 3 parcelas de 30 m^2 en cada etapa sucesional del **área de estudio 5**, ampliamente descritas por Lozano *et al.* (2014). Dentro de cada etapa sucesional escogimos cinco individuos de las especies con mayor cobertura y cinco puntos de suelo desnudo, repartidos al azar, para medir la respiración del suelo.

Variación estacional de la respiración

Todas las medidas de respiración del suelo y flujos de CO_2 en planta tomadas en el campo fueron repetidas en el tiempo para captar la variación estacional de los intercambios de gases. Las medidas de respiración del suelo e intercambio de gases de

la vegetación se realizaron en las horas de máxima actividad (especificadas en cada capítulo), para determinar la franja horaria de medida nos basamos en estudios anteriores para las medidas de la vegetación y en datos de observaciones empíricas para la respiración del suelo, intentando reducir al máximo la variación diurna.

En el **área de estudio 1** hicimos un seguimiento de la respiración del suelo durante 20 meses (**Capítulo III**). En el **área de estudio 4** tomamos medidas periódicas de la respiración del suelo (con periodos de medida de entre dos y cuatro semanas durante la época húmeda) durante un año y medio. En el **área de estudio 5** realizamos tres campañas de medidas de intercambio de CO_2 en el sistema planta-suelo (incluyendo tanto respiración del suelo y planta como fotosíntesis) a lo largo de una estación de crecimiento.

Medidas micro meteorológicas

Instalamos sondas de tipo ECH_2O (Decagon Devices, Pullman, WA, USA) en las **áreas de estudio 1, 2 y 3** para monitorizar la temperatura y la humedad a 5 cm de profundidad del suelo; y sondas HOBO (Onset Computer, Bourme, MA, USA) en el **área de estudio 4** para monitorizar la temperatura y la humedad a 5 cm de profundidad del suelo y la temperatura del aire a 5 cm de altura.

Tareas de laboratorio

Incubaciones

Los suelos utilizados para evaluar el efecto de la temperatura sobre los pulsos de respiración procedían de las **áreas de estudio 1, 3 y 4**. Recogimos suelo de distintas áreas a dos profundidades (0-5 cm y 10-30 cm). Los suelos se recogieron de distintos puntos y se tamizaron con una malla de 2 mm; con ellos creamos una muestra compuesta para cada profundidad y localización. Los suelos se mantuvieron a

temperatura ambiente (25ºC) y se dejaron secar al aire por un período de 24 horas; todas las incubaciones se realizaron durante la semana siguiente a la recogida de suelos y se estableció una secuencia aleatoria de incubaciones para evitar un sesgo en los resultados.

Cada submuestra de suelo se usó solo una vez, y se utilizaron seis submuestras para cada combinación de temperatura, humedad, profundidad y procedencia. Para ello se emplazaban 30 g de suelo en un tarro sellado de 600 ml. Los suelos se sometieron a cuatro temperaturas (30ºC, 40ºC, 50ºC, 60ºC) y 3 niveles de humedad (suelo seco al aire con una humedad <5% de la capacidad de campo, 10-11% de la capacidad de campo, 50-57% de la capacidad de campo). Las incubaciones fueron de una hora. Después de ese tiempo se extrajeron 20 ml de aire con una jeringuilla y 10 ml de ese aire se hacían pasar por un IRGA EGM-4 (PPSystems) programado para medidas en modo estático. En cada grupo de incubación se controlaba la concentración de CO_2 del aire de partida midiendo jarras vacías y selladas, incubadas junto con el resto (n=3).

Análisis de la comunidad bacteriana

Se analizó la comunidad bacteriana de los suelos procedentes de las **áreas de estudio 1, 3 y 4** mediante la secuenciación de librerías de amplicones del gen marcador bacteriano rRNA 16S mediante la tecnología Illumina MiSeq. Para la extracción de ADN de las muestras de suelo se empleó el kit PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA) siguiendo las instrucciones del fabricante. Posteriormente se midió la concentración del ADN extraído mediante fluorimetría (Quant-iT™ PicoGreen® dsDNA Assay Kit by Thermo Fisher Scientific, Walthman MA, USA 02451) y esta medida se usó como un estimador de la biomasa microbiana (Marstorp *et al.*, 2000; Taylor *et al.*, 2002; Wagg *et al.*, 2014). La amplificación del gen rRNA 16S y secuenciación de amplicones se llevó a cabo en la empresa LifeSequencing

S.L. (Valencia, España). Para las PCR se emplearon los primers Illumina-341-F (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') e Illumina-805-R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') que flanquean las regiones V3-V4 del gen. (Klindworth *et al.*, 2013). Los productos de PCR fueron purificados y se aplicó el protocolo de Illumina MiSeq para la preparación de las librerías (http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). La librería fue secuenciada mediante el kit MiSeq reagent Kit v3 de la plataforma Illumina, que permite secuenciar cada fragmento desde los dos extremos, generando datos de alta calidad (secuencias "pair-end"), con un tamaño promedio esperado de 300 pares de bases por secuencia. Los análisis bioinformáticos se realizaron mediante la plataforma QIIME 1.9.1 (http://qiime.sourceforge.net/). En el **capítulo I** se encuentra una descripción más detallada del proceso y posterior análisis bioinformáticos.

Análisis de Carbono orgánico, Carbono y Nitrógeno total

En el **área de estudio 1** analizamos el carbono orgánico que contenían los suelos en muestras tomadas un año después de haber aplicado los tratamientos de manipulación de temperatura y durante la época de crecimiento y actividad de las plantas (marzo). Las muestras se analizaron por colorimetría, utilizando el método de oxidación húmeda (Nelson & Sommers, 1996). También analizamos suelo procedente de las **áreas de estudio 1, 2, 3 y 4** para determinar el C y N total por combustión seca. El suelo procedente de las **áreas 1, 2 y 3** se analizó usando un analizador LECO Truspec C/N analyser (St. Joseph, MI, USA); para el **área de estudio 4** los análisis se hicieron por

combustión seca con un analizador elemental Carlo Erba NA1500 (Thermo Scientific Inc., Waltman, MA, USA).

Cuantificación de raíces y micorrizas

En el **área de estudio 5** analizamos muestras para determinar el contenido de raíces en cada uno de los tratamientos. La extracción se realizó de forma manual; las raíces se pesaron después de secar en estufa (70ºC) durante una semana. Determinamos el grado de colonización por micorrizas siguiendo el protocolo de (Emam, 2016) y se cuantificaron por el método de intersección (McGonigle *et al.*, 1990), mediante el conteo de 100 intersecciones por muestra en un microscopio óptico a 400x de magnificación. Cuantificamos la longitud de las hifas que contenía el suelo siguiendo el protocolo descrito por Treseder *et al.* (2007) usando 5 g de suelo.

Ecuaciones utilizadas

Respiración del suelo

La respiración del suelo (SR) se puede representar como una función de la temperatura y la humedad, como se muestra en la siguiente ecuación (Rey *et al.*, 2011):

$$SR = F(T) \times F(W) \quad (2)$$

Donde SR corresponde a la emisión de CO_2 del suelo (µmol m^{-2} s^{-1}), F (T) es la fracción de SR en función de la temperatura, y F (W) es la fracción de SR en función de la humedad.

F (T) se puede determinar mediante una curva exponencial que coincide con la ecuación de Arrhenius:

$$F(T) = \alpha\, e^{\beta T} \quad (3)$$

Donde α y β son constantes que se obtienen de la pendiente de la curva exponencial y T es la temperatura (ºC) del suelo.

Del cálculo de la constante de β se extrae la relación:

$$Q10 = e^{10\,\beta} \quad (4)$$

donde Q10 es una medida relativa para estimar el cambio en la respiración debido a un aumento de 10 ºC de temperatura (Davidson *et al.*, 2006a).

En el **capítulo I** de esta Tesis hemos establecido la relación con la humedad siguiendo una relación lineal:

$$F(W) = \gamma\, w \quad (5)$$

Donde γ es una constante y w es el contenido de agua del suelo en ($m^3\ m^{-3}$).

Para establecer la respuesta relativa a los pulsos de agua en base a la temperatura, hemos utilizado el índice $SR_{(I)}$ basado en el índice RII (Armas *et al.*, 2004)

$$SR_{(I)} = SR_{(Wet)} - SR_{(Dry)} / SR_{(Wet)} + SR_{(Dry)} \quad (6)$$

siendo $SR_{(Dry)}$ la respiración del suelo seco y $SR_{(Wet)}$ la respiración del suelo después de recibir un pulso de agua. $SR_{(I)}$ contiene unidades relativas y estándares que pueden variar entre 1 y -1.

Flujos de CO_2 de la vegetación

El intercambio neto de cada especie de planta (PNE) depende de la respiración (PR) y la fotosíntesis (PH).

$$PNE = PH - PR \quad (7)$$

De esta forma, de nuestras medidas con cámara (**capítulo IV**), obtuvimos PH (µmol $m^{-2}\ s^{-1}$) por substracción a los valores medidos con una cámara transparente PNE (µmol $m^{-2}\ s^{-1}$) y los valores obtenidos con una cámara oscura PR (µmol $m^{-2}s^{-1}$). Seguimos el consenso de que valores positivos indican emisión de CO_2 a la atmosfera, y valores negativos indican captación de carbono.

Flujos de CO_2 del ecosistema

Los flujos de CO_2 del ecosistema se establecen entre la respiración del suelo y la vegetación teniendo en cuenta el área ocupada (cobertura de la planta = cov_p, cobertura del suelo desnudo = cov_s, en m^2) por cada elemento en el sistema de acuerdo a las siguientes ecuaciones:

$$GR = \sum_{i=1}^{N} \left(PR_i \times \frac{Cov_{Pi}}{100} \right) + \left(SR \times \frac{Cov_S}{100} \right) \quad (8)$$

$$GPP = \sum_{i=1}^{N} \left(PH_i \times \frac{Cov_{Pi}}{100} \right) \quad (9)$$

$$NEE = \sum_{i=1}^{N} \left(PNE_i \times \frac{Cov_{Pi}}{100} \right) + \left(SR \times \frac{Cov_S}{100} \right) \quad (10)$$

GR es la respiración bruta del ecosistema (µmol m^{-2} s^{-1}), GPP es la producción primaria bruta del ecosistema (µmol m^{-2} s^{-1}), y NEE es el intercambio neto de CO_2 del ecosistema (µmol m^{-2} s^{-1}) y es una medida de la capacidad de secuestro de C del ecosistema. Los datos de cobertura vegetal y suelo desnudo para nuestros sitios de estudio se extrajeron de Lozano *et al.* (2014).

Análisis estadísticos

Utilizamos distintos análisis estadísticos para comprobar los objetivos planteados; aquí exponemos por capítulos los análisis más relevantes, aunque en cada capítulo se detalla cada uno de ellos junto con otros análisis complementarios.

Utilizamos **modelos lineales generales** (**GLM)** para explorar los efectos de la vegetación, tipo de suelo, temperatura y periodo de medida sobre nuestras variables respuesta (respiración del suelo, flujos en las plantas, flujos a nivel de comunidad).

En el **capítulo I** utilizamos GLM repetidos en el tiempo, con especie (*Stipa tenacissima*, *Lygeum spartum*, *Salsola genistoides*, *Hammada articulata* y suelo desnudo) y tratamiento de temperatura (incremento, disminución) como factores fijos

para determinar su efecto sobre la variable respuesta respiración del suelo. En el **capítulo II** utilizamos GLM con lugar de origen del suelo (área de estudio 1, 3 o 4), profundidad (0-5 cm y 10-30 cm de profundidad), humedad (suelo seco al aire <5% capacidad de campo, 10-11% capacidad de campo, 50-57% capacidad de campo) y temperatura (30ºC, 40ºC, 50ºC, 60ºC) como factores fijos, y respiración como variable respuesta. En el **capítulo III** utilizamos GLM repetidos en el tiempo con tipo de vegetación (anuales, robledales), edad del suelo (92.000, 137.000 años) y comunidad del suelo (raíces, micorrizas, comunidad libre del suelo) como factores fijos sobre la variable respuesta respiración del suelo. En un segundo análisis utilizamos GLM con medidas repetidas en el tiempo para testar diferencias entre tratamientos (OTC, control) en este test incluimos edad del suelo (92.000, 137.000 años), tratamiento (OTC, control) y comunidad del suelo (raíces, micorrizas, comunidad libre del suelo) como factores, y la variable respuesta fue la respiración del suelo. En el **capítulo IV** utilizamos varios GLM repetidos en el tiempo con especie vegetal (6 niveles) como efecto fijo y PAR y temperatura del aire como covariables, para testar el efecto sobre tres variables respuesta a nivel de sistema planta-suelo (PNE, PH y PR). También utilizamos GLM repetidos en el tiempo con comunidad vegetal-etapa sucesional (5 niveles) como factor fijo para analizar las variables respuesta comunitarias SR, GR, GPP, NEE.

Para todos ellos se comprobó la normalidad (distribución normal de los residuos) y la homogeneidad de las varianzas, y se controló la correlación (falta de independencia) y/o variabilidad espacial y temporal en los modelos que así lo requerían. Las medias resultantes de los análisis se compararon mediante el test post-hoc LSD de Fisher. La significación de los modelos y test se estableció a un $P<0.05$.

En el **capítulo I** comprobamos mediante regresiones la relación entre temperatura y respiración del suelo, y entre humedad y respiración para cada especie de planta y en

suelo desnudo**.** En el **capítulo IV** usamos regresiones para estudiar la relación entre variables como el C, N y C/N con la respiración del suelo y la cantidad de hifas, y también para estudiar la relación entre biomasa de raíces y respiración del suelo.

Todos los análisis se realizaron usando las funciones glm y glmer en R (R-Core Team, 2015) mediante la interfaz InfoStat (Di Rienzo *et al.*, 2015). El resto de análisis estadísticos se realizaron con el paquete estadístico InfoStat.

En el **Capítulo II,** para el análisis de las comunidades microbianas se aplicaron técnicas de estadística multivariante. Para ello, tratamos los datos de composición taxonómica obtenidos mediante análisis bioinformáticos con el paquete PRIMER7 (Playmouth, UK) generando análisis de clusters y heatmaps. Los datos a nivel de unidades taxonómicas operativas (OTUs) se llevaron a cabo exportando la matriz de OTUs y los datos de betadiversidad obtenidos con Qiime (índice Unifrac ponderado) a PRIMER7 para la generación de Análisis de Coordenadas Principales (PCoA) y análisis de similitudes (ANOSIM).

Chapter I:

Species identity affects soil respiration responses to moisture and temperature in a semiarid scrubland: implications to carbon fluxes

Carme Estruch, Petr Macek, Cristina Armas, Nuria Pistón, Francisco I. Pugnaire.

Abstract

Soil CO_2 fluxes account for ca. 25% of the annual terrestrial ecosystems emissions and are highly sensitive to temperature and moisture, being influenced by plant community composition. The different plant species influence CO_2 emissions through specific effects on soil humidity, temperature, C allocation, organic matter or microbial communities. We determined CO_2 emissions in soils beneath different plant species and bare soils their response to temperature in a semiarid environment over a 20-mo manipulative experiment. We altered soil temperature under the canopy of four plant species differing in functional type and activity, and in bare soil, and measured monthly fluxes to establish seasonal patterns of CO_2 release. We found an exponential relationship between soil respiration and soil temperature that shifted from negative in the dry and warm season to positive in the relatively wet and cool season. A model that include soil temperature, soil water content and plant species identity effects on CO_2 emissions explained 74% of the seasonal variation in soil respiration rate in this system, species identity playing a significant and probably the strongest effect on soil respiration rate. Our data showed the importance that plant species composition plays on annual CO_2 emissions at the community level.

Introduction

Soil respiration is the second largest CO_2 flux to the atmosphere and represents three quarters of ecosystem respiration (Law *et al.*, 2001). In drylands, soils can store up to 260 Petagrams of soil organic carbon (SOC) (Serrano-Ortiz *et al.*, 2012) making them critical actors in the global CO_2 budget. Future scenarios for drylands predict an increase in temperature and changes in precipitation patterns, which will become more unpredictable (IPCC, 2014). Such changes may enhance water scarcity and restrain respiration responses to temperature (Bahn *et al.*, 2010; Rey *et al.*, 2011) increasing the complexity of modeling C fluxes in these systems.

Plants in semiarid environments create "islands of fertility" that modify both above- and below-ground environmental conditions (e.g., Cerdà, 1998; Pugnaire *et al.*, 2004) and determine the structure of understory plant and soil communities (Martínez-García *et al.*, 2011; Hortal *et al.*, 2015; Aguilera *et al.*, 2016) which ultimately modulate soil respiration (Metcalfe *et al.*, 2011). Differences in plant species identity can change the amount and kind of C compounds released into the soil affecting, for instance, SOC. Thus, while some species provide easily decomposable C compounds, others produce recalcitrant materials, slowing organic matter turnover and contributing to decrease soil respiration rates (García Palacios *et al.*, 2013; Hasibeder *et al.*, 2015). But while the effects of root biomass on soil respiration are clear (e.g., Hopkins *et al.*, 2013), overall soil respiration seems to be regulated by plant species identity as much as by root biomass *per se* (Johnson *et al.*, 2008). This issue is related to plant strategies; for example, Curiel Yuste *et al.* (2004) found that differences between evergreen and deciduous species in mixed forests affected soil responses to temperature, which differed among seasons and were caused by changes in plant metabolism. Plant activity also affects soil respiration and, for instance, C allocation to roots decreases during

drought periods (Padilla *et al.*, 2015) lowering soil respiration rates (Hasibeder *et al.*, 2015). Several reports have emphasized the importance of spatial heterogeneity in patchy environments, reporting differences in soil respiration due to vegetation (Maestre & Cortina, 2003; Castillo-Monroy *et al.*, 2011; Oyonarte *et al.*, 2012). Barba *et al.* (2013) even reported variations in soil respiration rates caused by proximity to different plant species, but there is still little information on the role of species identity in soil respiration responses. There is, however, evidence showing how the combined effects of plant characteristics and temperature can affect soil CO_2 fluxes (Bahn *et al.*, 2010; Chen *et al.*, 2014b).

Overall, these reports evidence the need of better approaches to fine-tune soil respiration estimates as a function of the plant species present in patchy environments. Only by understanding plant species role in soil respiration processes we would be able to improve predictions for future scenarios of global change.

Here we report an experiment where we manipulated soil temperature in bare soil and under four plant species and recorded soil respiration rate over 20 months. We aimed to examine how soil temperature manipulations may affect soil respiration rates and to determine how soil respiration under different plant species would be affected by the interaction with soil temperature under varying conditions of soil water content. We hypothesized that 1) The response of soil respiration to temperature and humidity is determined by the plant species; and 2) the temporal variation in CO_2 emissions is determined by the interaction between soil temperature and humidity and how the different plant species react to this interaction. Since all our selected species are perennial, we further expected that 3) understory soil activity will respond swiftly to temperature and humidity for most of the season but, during periods of plant dormancy, soil respiration associated to these species will decrease. We used this information to

build a model to assess the importance of seasonal soil CO_2 fluxes in arid environments taking in account the different strategies of plant species against seasonal variation in humidity (wet-dry periods) and the interaction with temperature variation along the year.

Methods

Study area

The study area was in the northern foothills of the Sierra Alhamilla range, Almería, Spain (37º01' N, 002º25' W, ~350 m elevation); the climate is Mediterranean semiarid (230 mm of mean annual precipitation) with a dry period between June and September and a mean annual temperature of 17.9 ºC. Soils are orthic solonchak with calcic regosol inclusions (Martínez-García *et al.*, 2011). The selected area is a native scrub community and we selected four dominant perennial species as target; two tussock grasses, *Stipa tenacissima* L. and *Lygeum spartum* (L.) Kunth, and two shrubs, *Salsola genistoides* Juss. ex Poir. and *Hammada articulata* (Moq) O Bolós & Vigo (both Chenopodiaceae). All species are hereafter referred to by genus.

Experimental design

On March 2012 we selected at random 36 individuals of each of the four dominant species and bare soil patches spread in an area of ~1 ha. Individuals were at least 1 m apart from each other. We inserted 5-cm deep PVC collars 10.3 cm in diameter in the East aspect of each individual. Each plot was randomly assigned to one of three treatments with twelve replicates each. In two treatments we manipulated albedo with a thin layer of colored sand (black sand Zolux© for temperature increase; white sand Zolux© for temperature decrease), and added nothing to the control group. The two treatments with colored sand changed soil texture in the same way but differed in their effect on soil temperature. The two colored sand applications were considered a

temperature treatment in statistical analyses. Thus, we performed two different comparisons, one to test the effect of different soil temperature on soil respiration, and in the other we compared the effects of both sand applications with control (no sand added) plots. We did the latter test because we altered soil surface texture in addition to temperature and we considered sand application a texture treatment.

We measured monthly soil respiration rates between May 2012 and November 2013 with a portable EGM-4 infrared gas analyzer connected to an SRC-1 chamber (PPSystems, Amesbury, MA, USA). Measurements were made along three consecutive days per month between 12:00 and 17:00 GMT. Within this time frame readings were steady as all measurements were carried out in sunny days with similar temperature; measurements order was randomized across treatments and replicates to avoid biases.

Field parameters

We monitored hourly soil temperature and volumetric soil water content (SWC) in bare soil for all three treatments between March 2012 and November 2013 using ECH_2O probes (Decagon Devices Pullman, WA, USA) installed at a depth of 5 cm. Additionally, soil temperature was recorded manually at each respiration measurement using a thermocouple.

We estimated the volume of selected plants at the beginning of the experiment by measuring height and canopy diameter. Soil organic carbon (SOC) was determined by the wet oxidation method (Nelson & Sommers, 1996) at the beginning of the growing season, one year after the treatment onset (March 2013).

Soil Respiration Modeling

To model respiration rate we established two periods based on mean air temperature and precipitation (Fig S1); 1) the **wet period** stretched from October to March, had mild

temperatures and included most rain periods; and 2) the **dry period**, from April to September, predominantly dry and warm.

We considered soil respiration (SR) as a function of soil temperature Rt and soil water content Rw (Rey *et al.*, 2011) using the equation:

$$SR = F(T) \times F(W) \quad (1)$$

We first analyzed the effects of soil temperature on soil respiration per plant species and bare soil for each period (wet and dry). For each treatment (increase, decrease, control) and species we calculated the mean soil respiration value (i.e. mean of 12 plants per species or bare soil) per day of measurements with the SRC-1 chamber. Thus we included in the model three values per day of measurement and plant species (or bare soil). We adjusted the relationship between temperature and soil respiration following the Arrhenius equation:

$$F(T) = \alpha\, e^{\beta T} \quad (2)$$

where Rt is soil respiration (µmol CO_2 m^{-2} s^{-1}) as a function of temperature; T is temperature (ºC); and α and β are fitted parameters from the exponential curve.

We calculated soil temperature sensitivity (Q10) using the equation:

$$Q10 = e^{\beta T} \quad (3)$$

To address the relationship between soil moisture and soil respiration for the whole period we used a different approximation. We continuously monitored SWC in bare soils (n=3) but not in other positions. In the model, we included mean daily values of this SWC monitored in bare soil as the independent variable and relate it to daily mean respiration rate in control treatment and across species, including the bare soil. We only used control plots to test soil respiration relationships with moisture because

we were unable to measure the effect of sand application on soil moisture. The best adjustment was a linear relationship described as:

$$F(W) = \gamma\, w \quad (4)$$

where Rw is soil respiration (μmol CO_2 m^{-2} s^{-1}) as a function of moisture; w is volumetric water content (m^3 m^{-3}) and γ is the fitted parameter from a linear ecuation.

For each species, we fitted the relationship between soil respiration, soil temperature and soil moisture from equation 1 in each of the two periods (dry and wet) following procedures from Rey *et al.* (2011), and determined the best model based in r^2 from a linear regression between observed and modeled values. We interpolated the data with the mean daily values of temperature and SWC obtained from the ECH_2O probes at the time of our measurements (12:00-17:00 GTM) to build a year model of soil C respired beneath each species and period.

Statistical analysis

Differences in soil temperature, soil water content, and soil respiration were analyzed using general linear models (GLM) in a complete factorial design that included repeated-measures analysis; we checked the assumption of normality and homoscedasticity of variance. We controlled the effect of temporal variability in the analysis, the repeating unit in time were the collars. Soil respiration was analyzed considering plant species and bare soil (5 levels) and temperature treatments (2 levels) as fixed-factors. To test solely the effect of manipulating soil with sand (i.e., excluding the temperature effect), we performed a second analysis to compare sand addition one treatment level that includes data from soils were black and white sand was added with controls using soil temperature as covariate. Treatment means were compared using the LSD Fisher post-hoc test ($p < 0.05$).

Values from the exponential curve (equation 1) were transformed to natural log. Linear regressions were then compared using species as a dummy category to determine differences in soil respiration beneath the canopy of different species in response to soil temperature. The same analysis was used to compare the response of plant species to soil water content. GLMs were conducted using glm and glmer functions in R (R-Core Team, 2015) using the implemented interface in InfoStat statistical package (Di Rienzo *et al.*, 2015). All other statistical analyzes were performed with the InfoStat statistical package.

Results

Soil respiration responses

Treatments affected soil temperature, showing a mean annual difference of 1.90ºC between the increased (23.31 ± 0.01ºC) and decreased (21.42 ± 0.01ºC) treatments. The different plant species were grouped into two canopy sizes, as *Hammada* and *Lygeum* were notably smaller than *Salsola* and *Stipa* (Fig. S2a). Canopy size had no effect on the amount of SOC but affected soil temperature; the two plant species with smaller canopies had higher mean soil temperatures than the bigger ones, and even higher than bare soil (Fig. S2).

Plant species differed in soil respiration over time (Table 1, Fig. 1a) and, overall, soil respiration was higher in the wet period. Soil respiration rates were not related to plant functional type, since the shrub *Hammada* (Chenopodiaceae) and the tussock grass *Lygeum* (Poaceae) showed the highest rates. In fact, soil respiration rates were related to canopy size and were high in soils under plants with smaller canopies (Fig. S2a) like *Hammada* (0.64 ± 0.02 µmol CO_2 m^{-2} s^{-1}) and *Lygeum* (0.60 ± 0.02 µmol CO_2 m^{-2} s^{-1}), followed by soils under *Salsola* (0.53 ± 0.01 µmol CO_2 m^{-2} s^{-1}) and *Stipa* (0.48 ± 0.01 µmol CO_2 m^{-2} s^{-1}), all significantly above bare soil respiration rates (0.42 ± 0.01

µmol CO_2 m^{-2} s^{-1}) (Fig S3). Soil respiration under *Hammada* was highest all along the measuring period, while soil respiration under *Lygeum* was high only during the wet period, remaining very low during the dry period. Soils under *Stipa* and *Salsola* had overall low respiration rates, the former showing very low rates in the dry period — even lower than bare soil— or under *Salsola*. Bare soil remained lowest for most of the year, except in the dry period where for some days it was higher than both tussock grass species and *Salsola*. There were no differences in soil respiration between temperature treatments and this lack of differences did not change with time (Table 1, Fig. 1b). Independently from temperature treatment, the addition of sand increased soil respiration during the rainy periods (Fig. 1c).

	df	**F**	**P**
GLM_1			
Species	4	3.83	**0.004**
Temperature treatment	1	0.03	0.861
Time	13	128.20	**<0.001**
Species:T_treat	4	0.24	0.913
Species:Time	52	2.64	**<0.001**
T_treat:Time	13	1.57	0.086
Species:T_treat:Time	52	0.82	0.816
GLM_2			
Species	4	29.71	**<0.001**
Sand Application	1	18.37	**<0.001**
Time	13	172.11	**<0.001**
Soil Temperature	1	6.51	**0.011**
Species: Sand Application	4	0.92	0.450
Species:Time	52	3.80	**<0.001**
Sand Application :Time	13	3.15	**0.001**
Species: Sand:Time	52	0.87	0.737

Table 1. Results from general linear models (GLM) with soil respiration rate as the dependent variable. In GLM_1 plant species, soil temperature treatments and time were fixed factors. In GLM_2 plant species, sand application and time were fixed factors, and soil temperature was included as a covariate.

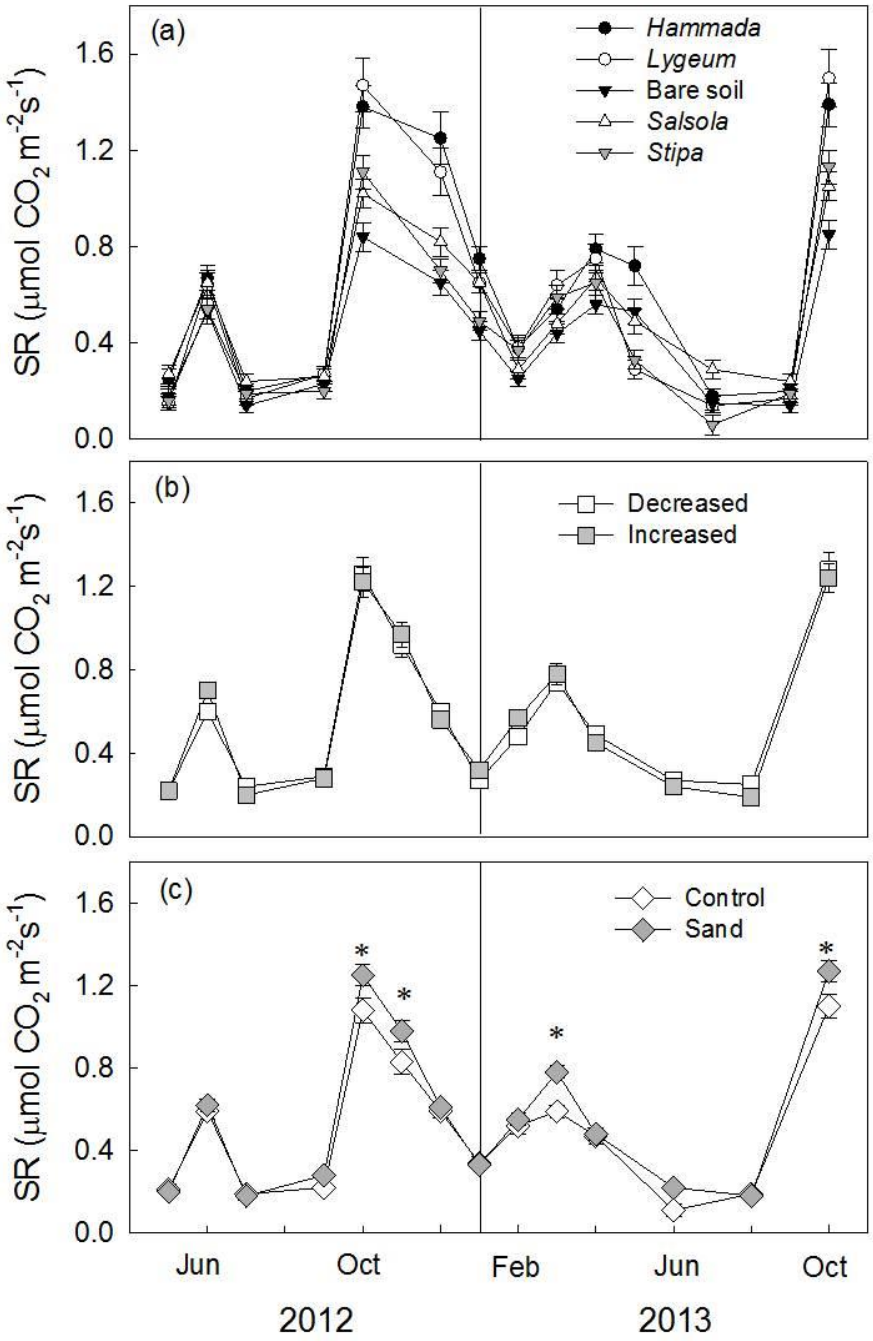

Figure 1. Soil respiration rate beneath the canopy of four plant species and bare soil along the 20 months of the experiment (a); between the two soil temperature treatments (b), and between control and sand addition treatments (i.e., the effect of applying coarse sand to the soil) (c). In the last panel, asterisk indicate significant differences in soil respiration ($p<0.05$) between control and addition treatment. Symbols and bars represent mean values ±1 SE.

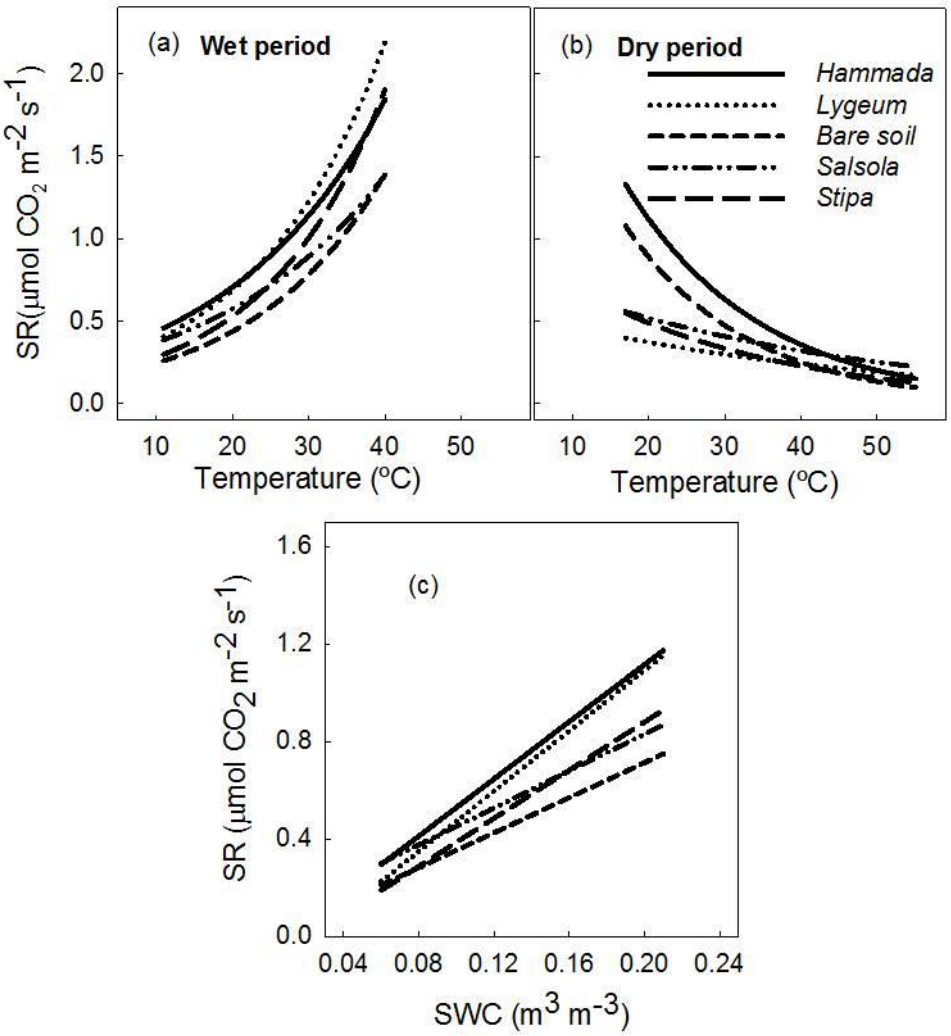

Figure 2. Soil respiration rate as a function of soil temperature (from Equation 1) modeled across different species and bare soil. We used daily mean values from data measured manually, for each treatment (increase, decrease, control) and species, including the bare soil of each sampling date. Results are shown for the wet (a) and dry periods (b). Relationship between maximum soil respiration rate and soil volumetric water content (modelled following Equation 3) (c), respiration data were obtained from the main value of each species and sampling date using only the control plots. Volumetric soil water content are the mean values from the ECH_2O proves (n=3) for every date.

Relationship between soil respiration, temperature and moisture

There was an exponential relationship between soil respiration and temperature, irrespective of the season. But whereas the relationship was positive during the wet period, it was negative during the dry period. Q_{10} values were below 2 during the wet period, showing a positive but low sensitivity to temperature, and below 1 during the

dry period, reflecting the negative relationship between temperature and soil respiration (Table 2, Figs. 2a and b).

The relationship between soil respiration and volumetric water content was positive and linear for all species and for bare soil all year round (Fig. 2c). The response to water content was different between bare soil and all the species but *Stipa*, and the only species with different slopes were *Stipa* and *Hammada* (Fig 2c, table 2).

During the wet period, *Hammada* and *Lygeum* responded to temperature in a similar way and different to bare soil (Table S1), while *Salsola* and *Stipa* behaved like bare soil (Fig. 2a, Table 2). During the dry period *Hammada* and *Salsola* responded similarly to temperature, and very differently from bare soil, while the two tussock grass species responded in the same way, similar to bare soil (Fig. 2b, Table 2).

	Especies	**α**	**β**	**Q10**	
Wet period	*Hammada*	0.27 ± 0.09	0.05 ± 0.01	1.62	a
	Lygeum	0.21 ± 0.06	0.06 ± 0.01	1.79	ac
	Bare soil	0.13 ± 0.03	0.06 ± 0.01	1.79	b
	Salsola	0.24 ± 0.06	0.04 ± 0.01	1.55	b
	Stipa	0.15 ± 0.03	0.06 ± 0.01	1.90	c
Dry period	*Hammada*	3.51 ± 0.69	-0.06 ± 0.01	0.57	a
	Lygeum	0.58 ± 0.18	-0.02 ± 0.01	0.80	b
	Bare soil	3.15 ± 0.95	-0.06 ± 0.01	0.53	a
	Salsola	0.84 ± 0.18	-0.02 ± 0.01	0.79	b
	Stipa	1.05 ± 0.33	-0.04 ± 0.01	0.68	b

Table 2. Mean (± 1 SE) values for α and β parameters in the exponential curves (Equation 1) and Q10 values (Equation 2) relating soil respiration and soil temperature in the understory of each species in two different periods, wet and dry. All α and β coefficient estimations had significant curve fits ($p<0.05$). Different letters in the last column indicate differences in soil respiration equations among species and bare soil.

Seasonal soil respiration patterns

We built a general model quantifying the annual rate of soil respiration under the different species and in bare soil (Fig. 3). In the wet period, temperature was the best

predictor of soil respiration; in the dry period, both temperature and humidity were required to accurately predict soil respiration. The model reflects how CO_2 emissions are concentrated in the wet season. It is also noticeable how rain events modify soil respiration during the dry season, although residues distribution showed that the model is less accurate at the extremes (e.g., rain pulses in dry periods), overestimating low values and underestimating high values (data not shown). However, the empirical model explained 74% of the variance in soil respiration along the year, and shows robustness in prediction. The model reflected differences in soil respiration under different plant species and their responses to rain events during the dry period. As expected, soil respiration under *Hammada* was predominant throughout the year. Annual C respired by soil biota (including plant roots and biological soil crusts) ranged from 378 g C m^{-2} yr^{-1} in bare soil to 675 g C m^{-2} yr^{-1} under *Hammada*, with soils under *Stipa* (431 g C m^{-2} yr^{-1}), *Lygeum* (456 g C m^{-2} yr^{-1}), and *Salsola* (544 g C m^{-2} yr^{-1}) in between. Each species contributed differently to total C emission depending on the season (Fig. 4).

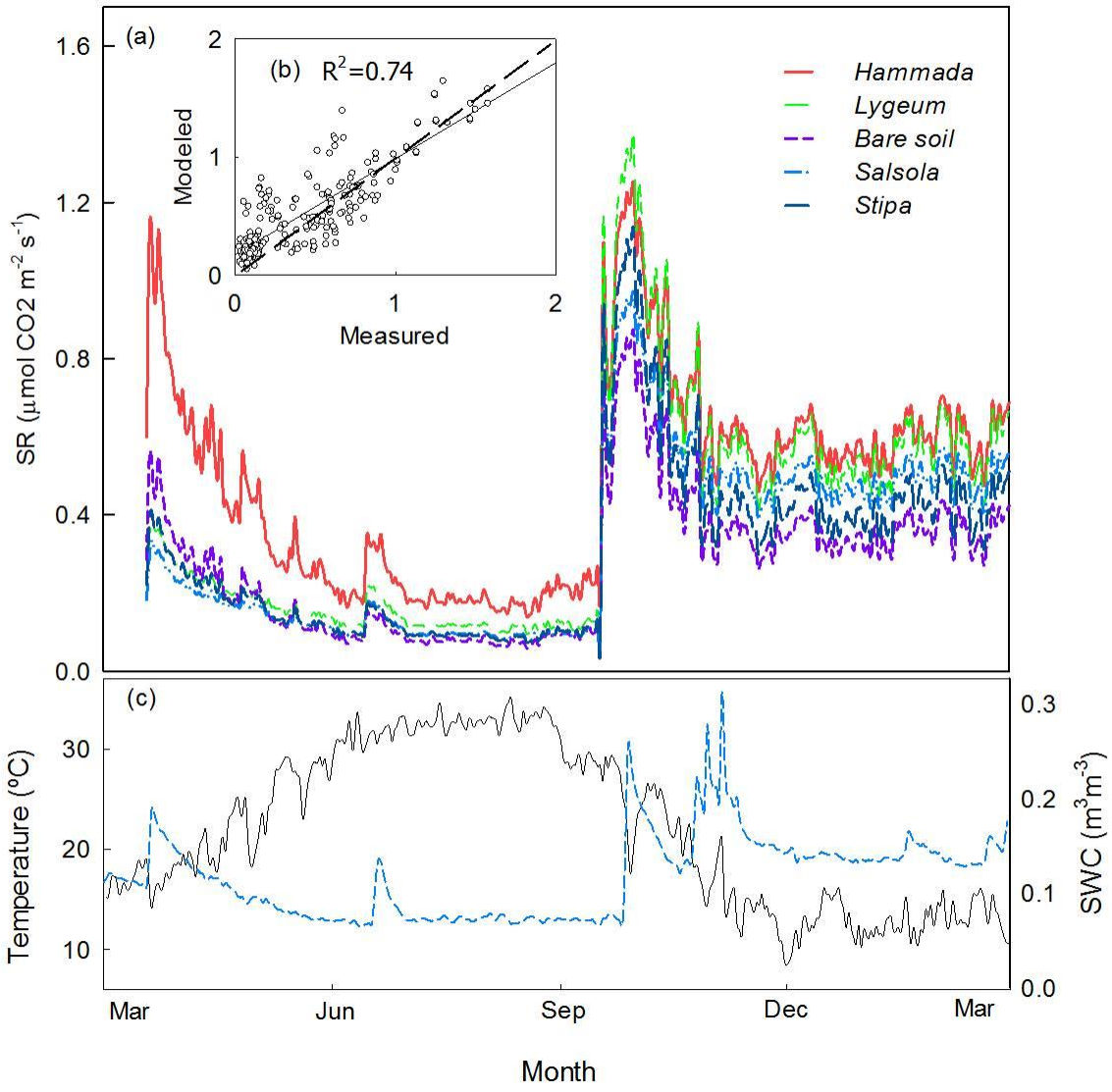

Figure 3. Modelled soil respiration rate between March 2012 and March 2013. Modelled values obtained from the interpolation of α, β and γ parameters (from Equations 1 and 2) with soil temperature and water content (a). Measured vs modeled values for soil respiration (b). Daily mean soil temperature (grey) and soil moisture (blue) at 5 cm depth (c).

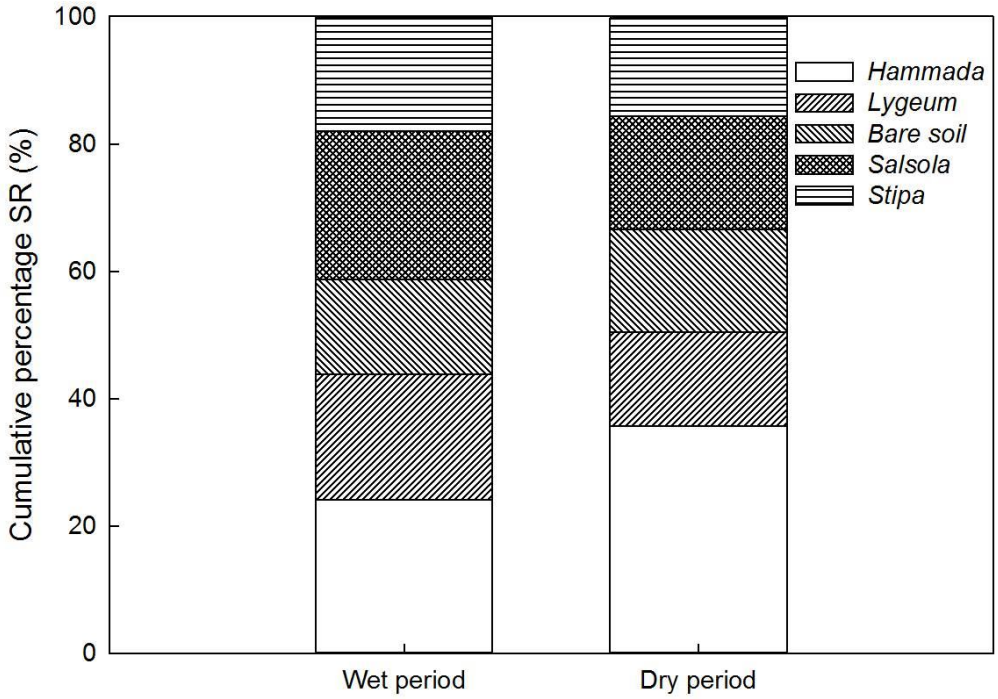

Figure 4. Cumulative contribution (percent) of each species to total soil respiration (SR) over the wet and dry periods. Values were computed from the empirical model.

Discussion

Our data show the importance of the relationships between temperature and water availability on CO_2 fluxes in water-limited environments. In a patchy environment, differences in plant identity contributed to differentiate responses of soil respiration to environmental variability, which determined differences in annual emissions at community level. Our data showed the importance of plant identity for carbon balance through specific species effects on seasonal soil respiration dynamics.

Temperature, plant identity and soil respiration

Long warming treatments did not affect soil respiration responses in our study, as reported in similar semiarid environments (de Dato *et al.*, 2010; Wagg *et al.*, 2014), although others reported decreases in soil CO_2 emission rates with extended warming and depending on water availability (Rey *et al.*, 2011; Chang *et al.*, 2014). Nonetheless,

soil respiration in our field site depended mostly on temperature; i.e., the link between mean daily temperature and soil respiration was positive under a wide range of soil water availability but was negative under water limitation. This response does not depend on temperature legacies, at least for the duration of our experiment, since the response to temperature did not change with the long term temperature treatments application. We only observed differences between the two periods, dry and wet.

Plant identity was the main factor affecting seasonal patterns of soil respiration in our field site, which was functional type or SOC. Species identity also determined differences in soil respiration in a study by Chang *et al.* (2014). Effects of plant species on soil respiration are driven by their effects on belowground soil communities (Martínez-García *et al.*, 2011; Hortal *et al.*, 2013; Hortal *et al.*, 2015; Aguilera *et al.*, 2016), root activity and density (Han *et al.*, 2014) and/or the combination of both, resulting in different respiration rates (Maestre & Cortina, 2003; Oyonarte *et al.*, 2012). However, the heterogeneity created by plants affects soil C fluxes, and small-scale variability could explain variability at the whole ecosystem scale (Barba *et al.*, 2013).

Seasonal soil respiration responses

The seasonal variation in soil respiration rates was dependent on water availability, as water limitation triggered negative responses to temperature. In dry periods soil under plant understories can be drier than soil in bare patches (Oyonarte *et al.*, 2012) and differences depend on the strategy of each species. Some species are able to maintain higher soil moisture under the canopy through hydraulic lift (Prieto *et al.*, 2012) while other enter in dormancy during stress periods (Norton *et al.*, 2016). For example, Oyonarte *et al.* (2012) found in a tussock grass community that C released to the atmosphere by bare soil during summer was higher than C released by soils under tussocks, which agree with our data from soils under Stipa, and at least for a short

period with the other tussock grass species Lygeum; meanwhile soils under *Salsola* occasionally emitted same C rates than bare soil on dry periods, and soils under Hammada always emitted more C than bare soil irrespective of the season.

Implications for CO_2 flux

Seasonal variations were satisfactorily predicted by our empirical model including soil temperature, moisture, and plant type. However, part of the variation remained unexplained by the model, and was most likely related with rain pulses in the dry period. Cueva *et al.* (2015) showed that sudden changes in moisture across multiple vegetation types ended in errors of soil CO_2 efflux estimations. Water pulses in relatively dry periods can trigger a swift liberation of labile compounds accumulated during periods of low activity, e.g., during drought (Richardson *et al.*, 2012; Meisner *et al.*, 2015; Liu *et al.*, 2016) which can be up to 40% of C released annually in drylands (López-Ballesteros *et al.*, 2016). Our data bring new information to reduce the error related to heterogeneity, although there is still uncertainty on the inclusion of rainfall pulses to improve predictions on CO_2 flux modeling.

The importance of accounting for spatial heterogeneity in C emission models has been widely discussed (Reichstein *et al.*, 2003; Rey *et al.*, 2011; Oyonarte *et al.*, 2012; Cueva *et al.*, 2015), particularly in arid environments where the patchy distribution of plant species with contrasting strategies contribute to increase system heterogeneity. Plant community composition is important when analyzing ecosystem responses to environmental drivers, and errors in the estimation of CO_2 fluxes by previous models (Davidson *et al.*, 2006a; Cueva *et al.*, 2015) are partly attributable to plant patchiness. Our study also shows that water availability affects soil respiration responses to temperature in dry environments and that depending on whether the period was more or less humid, the response of soil respiration to temperature shifted from positive to

negative. Previous reports tended to underestimate fluxes at low soil moisture (Reichstein *et al.*, 2003) but how water is handled in a model determines the different responses observed in a system (Rey *et al.*, 2011).

Conclusion

Our data emphasize the importance of plant identity in the release of CO_2 from the soil to the atmosphere, and the complex interactions between soil humidity and temperature occurring in a semiarid plant community. They also show the seasonal control of soil respiration by temperature, which is modulated by plant species identity. The response to soil temperature and soil humidity was not uniform among plant patches, as respiration rates respond to a combination of drivers that need to be addressed to improve predictions in a scenario of climate change.

Appendix

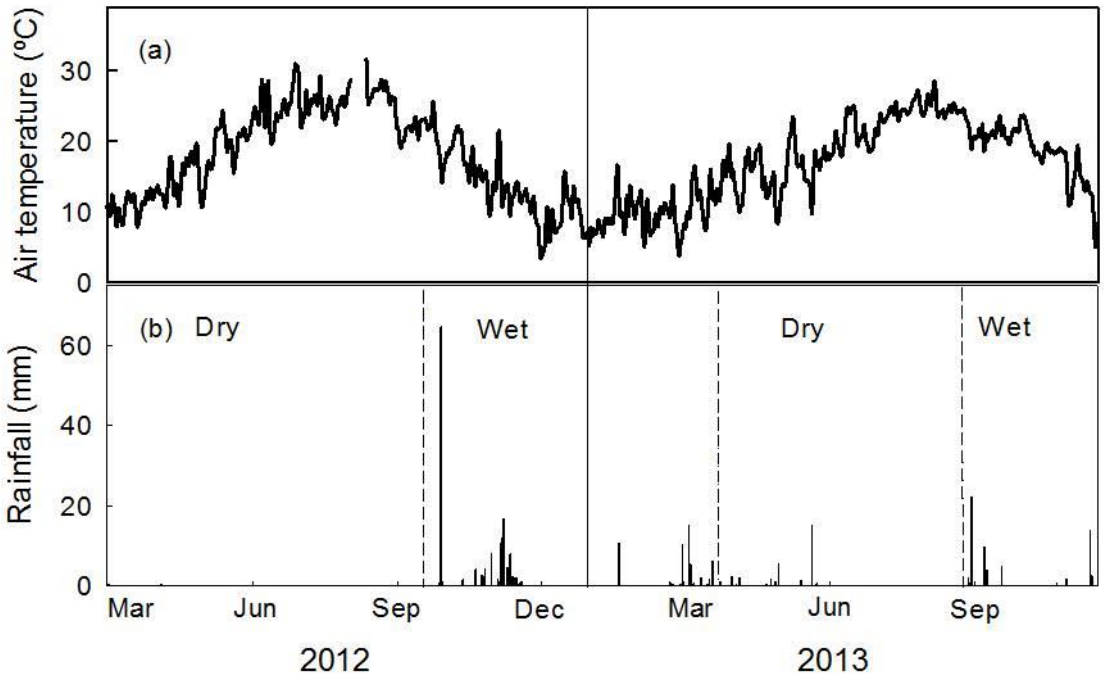

Figure S1. Daily mean air temperatures (b), and rainfall (b) in a nearby climatic station for the period of measurements. Perpendicular slashed lines show the different periods considered (dry or wet). Data were obtained from Tabernas meteorological station (Junta de Andalucía Regional Government,http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController?action=Init).

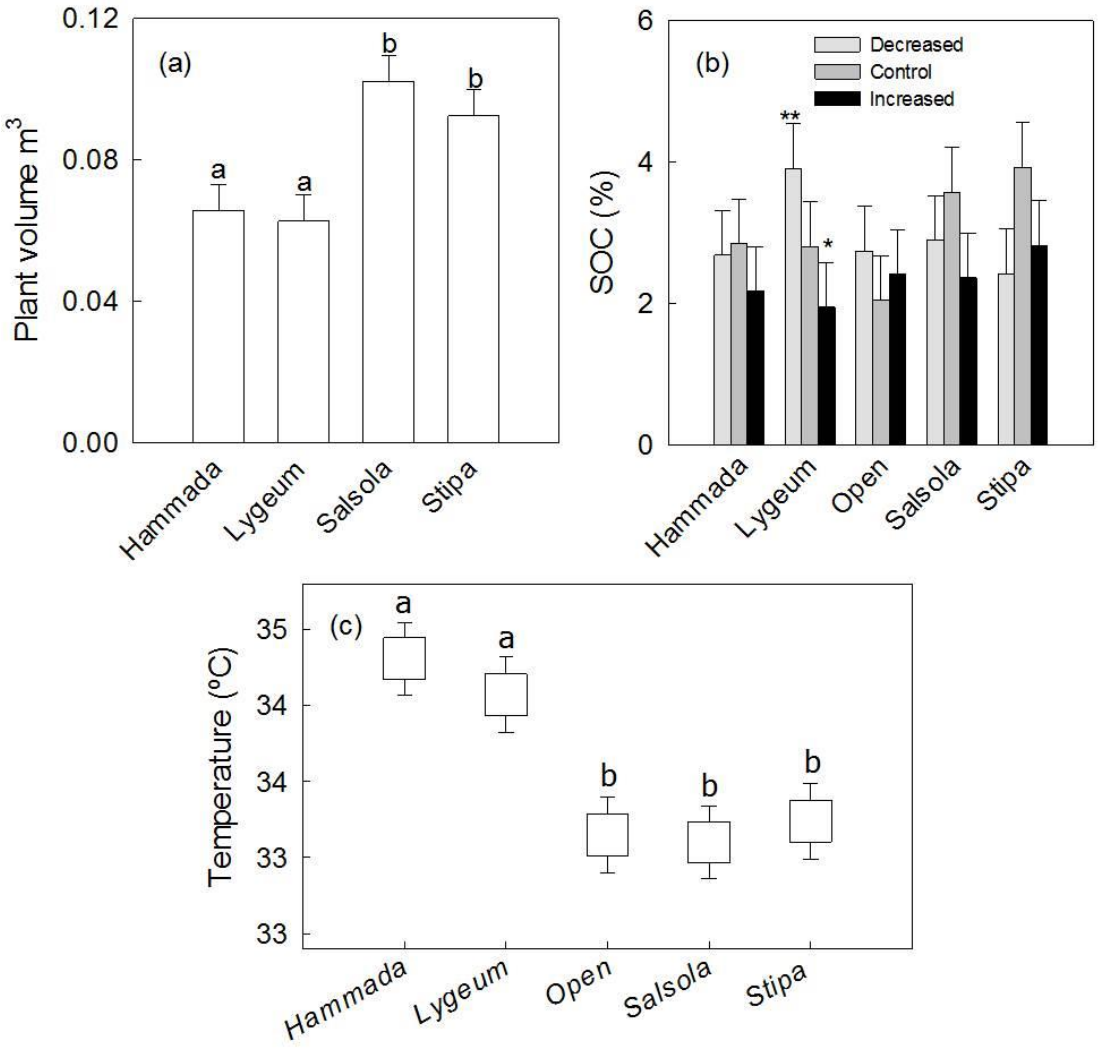

Figure S2. Species plant volume (m^3) (a); Soil Organic Carbon (SOC) content beneath the canopy of species and bare soil and for each temperature treatment (increase, decrease, control) (b) and; mean soil temperature for the whole period at 3.5 cm depth under different plant species and bare soil (c). Bars represent mean values ±1 SE, n= 36. Different letters indicate significant differences ($p<0.05$) after LSD Fisher post-hoc tests among species (a, c); asterisk indicates significant differences observed within a specie (b).

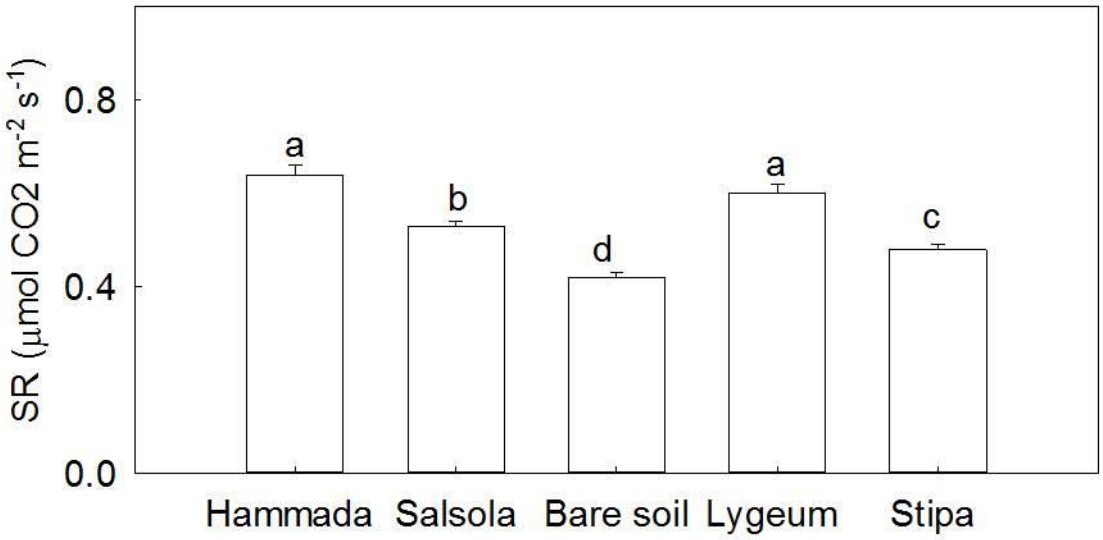

Figure S3. Mean (±1SE) soil respiration rate among plant species and bare soil for the entire experimental period. Different letters indicate significant differences ($p<0.05$) among species.

a) Ln soil R vs Temperature	Wet			
	Lygeum	**Bare soil**	**Salsola**	**Stipa**
Hammada	0.82	**<0.01**	**0.03**	0.08
Lygeum	1	**<0.01**	**0.01**	**0.04**
Bare soil		1	0.12	**0.05**
Salsola			1	0.70

b) Ln soil R vs Temperature	Dry			
	Lygeum	**Bare soil**	**Salsola**	**Stipa**
Hammada	**<0.01**	**<0.01**	0.65	**<0.01**
Lygeum	1	0.63	**0.01**	0.13
Bare soil		1	**<0.01**	0.30
Salsola			1	**<0.01**

c) Soil R vs Humidity				
	Lygeum	**Bare soil**	**Salsola**	**Stipa**
Hammada	0.23	**<0.01**	0.29	**<0.01**
Lygeum	1	**0.04**	0.90	0.11
Bare soil		1	**0.03**	0.65
Salsola			1	0.09

Table S1. Resulting P-Values from the comparison of linear regression functions across different species (each species including bare soil was considered a dummy variable in the regression analyses). Regressions analyzed soil respiration as a function of soil temperature along the wet cold period (a) and dry warm period (b), and analyzed soil respiration as a function of soil water content during the entire period (c). Bold numbers indicate differences between functions. Data in the regression analysis on soil respiration as a function of temperature was ln-transformed.

Chapter II:

Soil respiration responses to water pulses in 30-60 ^{o}C temperature range in dry environments

Carme Estruch, José A. Morillo, Francisco I. Pugnaire

Abstract

Understanding soil respiration pulses after rewetting and their interaction with temperature is critical in drylands, where environmental variables, soil microbial communities and soil properties mediate soil respiration pulses. We determined optimal soil respiration temperature after rapid rewetting with different water pulses, and assessed soil microbial communities (SMC) in three different soils from contrasted semiarid environments. We used short incubation times with four temperatures and three watering regimes and determined the structure of soil microbial communities (SMC). Soil respiration responses to water supply depended on temperature and soil origin. Optimum temperatures in sandy soils (desert and alpine) were well above 50ºC while in clay soils were lower. Soils showed marked differences in SMC, and differed with depth. Our data show that soil respiration pulses depended on temperature if not completely dry, and that optimum temperatures were well above the general assumption of 35ºC in these semiarid environments. Our results also evidenced the dependence of soil respiration responses on soil depth, which showed higher respiration pulses in the upper soil layers.

Introduction

Understanding drivers of soil respiration is critical to anticipate the impacts of global warming on carbon cycling. In drylands there are important respiration pulses after rainfall events (Fierer & Schimel, 2003; Chatterjee & Jenerette, 2011a) being a significant part of the annual CO_2 efflux from these soils (Kim *et al.*, 2012; Ataka *et al.*, 2014; Fan *et al.*, 2015). The accumulation of labile substrates during preceding drought periods can induce sudden fluxes of soil C just after rewetting (Birch, 1958; Borken & Matzner, 2009; Jenerette & Chatterjee, 2012; Richardson *et al.*, 2012; Meisner *et al.*, 2015; Liu *et al.*, 2016). Because knowledge about mechanisms is poor and its relationship with temperature remains unclear, the focus on short-term responses is important to understand this process.

Temperature is a critical driver of soil respiration in dry environments, where small increases in temperature enhance CO_2 release through its effects on substrate availability (Conant *et al.*, 2011; Sierra, 2012) and soil enzymatic activity (Davidson *et al.*, 2006a; Davidson *et al.*, 2012). There is an overall positive correlation between temperature and soil respiration (Bond Lamberty & Thomson, 2010) which varies with soil type (Chatterjee & Jenerette, 2011b; Richardson *et al.*, 2012; Hamdi *et al.*, 2013). Temperature also influences microbial metabolism (Davidson *et al.*, 2006a), which may be linked to short-term soil respiration responses. The optimum soil respiration temperature is an indicator of thermal adaptation of soil microbial communities. However, and despite the fact that temperatures at soil surface in drylands can easily reach 60^{o}C, most reports on optimal soil respiration temperature focused on temperate systems, where soil temperatures seldom go beyond 35ºC (Lloyd & Taylor, 1994; Fang & Moncrieff, 2001; Davidson *et al.*, 2006a; Richardson *et al.*, 2012; Guntiñas *et al.*, 2013). Richardson *et al.* (2012), however, reported optimum temperatures up to 45ºC in

a semiarid environment, and Hamdi *et al.* (2011) found significant increases in soil respiration between 40 and 50^{o}C in dryland soils, showing that soil metabolism may be adapted to the high temperatures frequent in such environments.

Soil properties such as texture can affect soil respiration responses as well, since labile C can be trapped into small pores and is made available only after water pulses, allowing different CO_2 release rates depending on soil texture (Van Gestel *et al.*, 1991; Cable *et al.*, 2008). In addition, differences along the soil profile can modulate soil respiration responses to water and temperature (Rey *et al.*, 2005; Shamir & Steinberger, 2007; Rey *et al.*, 2008). Since microbial communities differ with depth, different rates of activity could be expected between shallow and deep soil layers. Indeed, changes in soil microbial biomass (Miralles *et al.*, 2012; Bastida *et al.*, 2014), enzymatic activity (Yu & Steinberger, 2012), carbon mineralization (Rey *et al.*, 2005) and basal respiration (Yu & Steinberger, 2011) have been recorded at different depths in semiarid environments, even at the centimeter scale (Pasternak *et al.*, 2013), suggesting a stratification of soil microbial communities (SMC). Soil microbial communities in the soil surface are exposed to huge diel and seasonal fluctuations in temperature and humidity, unlike communities in deeper soil layers, more buffered against such changes.

We focused on factors affecting short-term soil respiration responses in order to clarify mechanisms controlling CO_2 release pulses by looking at temperature effects as a function of water availability in three contrasted environments. We tested how different soils respond to water pulses in the 30-60^{o}C range, more similar to the conditions found in our systems, and linked SMC to soil respiration rate.

For this purpose, we carried out an experiment based on short-term lab incubations of soils from three contrasting environments within the semiarid climate of SE Spain and analyzed the structure of soil bacterial communities. Specifically, we

tested 1) whether optimum temperatures of warm, semiarid soils were above 35^{o}C; 2) how temperature modulates soil respiration responses to water pulses; and, finally, 3) whether soil microbial communities differed among soils and depths.

Methods

Field sites

We sampled bare soil in three different field sites in southeast Spain (Table 1), two in hot environments and one in cold, high elevation mountains. The first site (Desert-clay) is in the northern foothills of the Sierra Alhamilla range, Tabernas basin (Almería province), on an orthic solonchak soil with calcic regosol inclusions (Martínez-García *et al.*, 2011); vegetation in this site is a scrub community composed by small shrubs such as *Hammada articulata* or *Salsola genistoides* and tussock grasses like *Lygeum spartum* and *Stipa tenacissima*. The second site was in the Rambla del Saltador valley (Desert-sand), in the southern aspect of the Sierra de Los Filabres range on a loamy sand soil (Prieto *et al.*, 2010) with a community dominated by *Retama spherocarpa* shrubs. The two sites differed in soil characteristics and elevation (350 versus 650 m) but shared similar climate; both are semiarid with similar precipitation rates (around 250 mm) and mean annual temperature (ca. 18ºC). The third site (Alpine-sand) is located in the northern aspect of the Sierra Nevada range (Granada, Spain) at 2700 m elevation. Climate is dry Mediterranean, with 690 mm mean annual precipitation and 3.9ºC mean annual temperature, dominated by an alpine cushion plant community. Soil in this site has similar characteristics as the Desert-sand location, a loamy sand soil (Schöb *et al.*, 2013) but differs in climate and elevation (Table 1).

Site Code	Location	Elevation (m)	Dominant vegetation	Soil type	MAT (ºC)	MAP (mm)
Desert-clay	37°01' N 02°25 'W	350	Desert Scrub *L. spartum S. tenacissima, H. articulata, S. genistoides*	Orthic solonchak with calcic regosol inclusions[1]	17.9 [1]	242 [1]
Desert-sand	37° 10′N 21° 22′W	650	Monospecific shrub *Retama Sphaerocarpa* [2]	Loamy sand, [2] Eutric Regosol	18.2 [2]	250 [2]
Alpine-sand	37°03' N 03°23' W	2700	Alpine cushion-plant community [3]	Loamy sand, [3] Leptosol	3.9 [3]	690 [3]

[1] (Martínez-García *et al.*, 2011)
[2] (Prieto *et al.*, 2010)
[3] (Schöb *et al.*, 2013)

Table 1. Field sites characteristics; location, soil type, mean annual temperature (MAT) and precipitation (MAP).

Soil sampling and incubation procedure

We collected soils in September 2013. In each field site we randomly sampled five points in gaps between shrubs at least 5 m apart from each other within a 20x20 m plot. We carefully extracted soil samples from two different depths, 0-5 cm and 10-30 cm, in different sites to avoid spatial correlation effects. We sieved and homogenized soil to 2 mm removing pebbles and roots. We obtained a composite sample for each field site and depth. Pooling soil samples from the same microhabitat reduces variability while allowing testing for differences between soil origins and could be considered as technical replicates (Rodríguez Echeverría *et al.*, 2013). Soils were brought to the lab, air-dried for 24 h and stored at room temperature (~25ºC) until the experiment started to avoid heat shock. Total N and C soil content were determined in three dry subsamples per site and depth using a LECO Truspec C/N analyser (St. Joseph, MI, USA). To

reduce changes in soil microbial communities with time, we did all incubations within one week of collection.

We subjected soils to four different temperatures (30ºC, 40ºC, 50ºC and 60ºC) and 3 water treatments, air-dry, low water input (33 μl g^{-1}), and high water input (150 μl g^{-1}). For the orthic solonckhad soil (Desert-clay) low and high inputs equaled to 10% and 50% of soil water holding capacity (SWC) respectively; for the loamy sand soils (Desert-sand and alpine) low and high inputs equaled to 11% and 57% of SWC respectively. As we wanted to get short-term soil responses of the original soil microbial community, we exposed each soil sample only to one temperature and water treatment. We had 6 subsamples per microhabitat, temperature, and water treatment, totaling 72 samples (3 water levels x 4 temperatures x 6 replicates) per site and depth. Soil samples (30 g) were placed on 600 ml sealed jars 1 h before water addition, and incubation started 10 minutes afterward. Jars remained open until we placed them on the incubation chamber, which allowed handling 3 groups of 6 jars at a time. Short-term laboratory incubations (1 h) allowed us to focus on respiration pulses occurring just after rewetting (Fierer & Schimel, 2003), preventing the decline of substrate availability (Leifeld & von Lützow, 2014) and changes in the soil microbial community. After 1 h incubation, we extracted 20 ml of air from each jar with syringes and injected 10 ml of air in an IRGA EGM-4 (PPSystems) set in steady mode to obtain the CO_2 concentration in air (ppm). We did the incubations at different temperatures randomly to avoid confounding effects with pre-incubation period, and controlled background CO_2 concentration level by measuring sealed, empty jars before every incubation period (n = 3).

DNA extraction and sequencing

We extracted DNA from 0.25 g of homogenized soil from each sample using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) following manufacturer's directions. DNA quantification, 16S rRNA genes amplification, and carried out Illumina sequencing by LifeSequencing S.L. (Valencia, Spain). DNA quantification of extracts was made through a fluorimetric quantification method (Quant-iT™ PicoGreen® dsDNA Assay Kit by Thermo Fisher Scientific, Walthman MA, USA 02451), and used as an estimator of total microbial biomass in soils (Marstorp *et al.*, 2000; Taylor *et al.*, 2002; Wagg *et al.*, 2014). We amplified the V3-V4 regions of the bacterial 16S rRNA gene with the primers Illumina-341-F (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and Illumina-805-R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') that included Illumina adapter overhang nucleotide sequences (Klindworth *et al.*, 2013). We determined the DNA concentrations of each purified PCR product sample by the fluorimetric method. Amplicons were generated, cleaned, indexed and sequenced according to the Illumina MiSeq 16S Metagenomic Sequencing Library Preparation protocol (http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). The final library was paired-end sequenced at 2×300 bp using a MiSeq Reagent Kit v3 on the Illumina MiSeq platform.

Bioinformatic analysis

Illumina data were analyzed using the QIIME 1.9.1 pipeline (http://qiime.sourceforge.net/) (Caporaso *et al.*, 2010). As a first step, we removed the low quality tails of raw sequences, forward and reverse, and those sequences were

merged to obtain the complete sequence. Once trimmed and assigned to samples, sequences were processed using the QIIME´s UCLUST *de novo* method to cluster sequences in operational taxonomic units (OTUs) at the 97% identity level excluding singletons. We selected the most abundant sequence of each OTU as representative and subsequently aligned using PyNAST against the Greengenes core set (DeSantis *et al.*, 2006) version gg_13_5. Possible chimeric sequences were identified using the Uchime tool (Edgar *et al.*, 2011), generating a non-chimeric OTU list. We assigned taxonomic affiliations to OTUs using the Ribosomal Data Project (RDP) Classifier at a confidence threshold of 80% (Wang *et al.*, 2007), and removed sequences not assigned to the Bacteria domain from the dataset, alpha and beta-diversity analysis were performed with the QIIME script core_diversity_analysis.py with normalized data to obtain equal number of reads per sample (30220 reads). Sequences were deposited under NCBI Sequence Read Archive (SRA) with accession numbers SRX1825973–SRX1825990 (http://www.ncbi.nlm.nih.gov/sra/SRP076207).

Data treatment and statistical analysis

To calculate soil respiration we applied the ideal gas equation

$$P \times V = n \times R \times T \quad (1)$$

Where P is the observed air pressure (kPa), V is the volume of the jar extracting the volume occupied by the soil (by measuring the volume occupied by the soil once introduced into the jar), R is the universal gas constant ($8.31 \times *10^{-3}$ L kPa mol^{-1}), T is the observed temperature (K) and n is air concentration in moles, once we calculate the moles of air in the jar we obtained the moles of CO_2 in the jar from the concentration in ppm obtained from the IRGA. We evaluated the relationship between soil temperature and soil respiration using a linear model from which we empirically obtained the maximum respiration rates from each soil origin depending on water treatment. To

calculate the sensitivity in the response to water events among different temperatures and soils, we used an index $SR_{(I)}$ based on the RII index (Armas *et al.*, 2004)

$$SR_{(I)} = (SR_{(Wet)} - SR_{(Dry)}) / (SR_{(Wet)} + SR_{(Dry)}) \quad (2)$$

Were $SR_{(I)}$ is the relative response in soil respiration to water addition, $SR_{(Wet)}$ is the soil respiration rate at the respective water treatment and $SR_{(Dry)}$ is the respiration rate at the dry treatment.

We tested differences in soil respiration with general lineal models (GLM) using site origin, depth, moisture, and temperature as factors. We established optimum T from post hoc values from the model. One replicate consisted in the mean value of six subsamples to solve the potential problem of pseudo-replicated analysis. Prior to model, we tested whether the fourth interaction was significant and eligible to be excluded from the model. To test for differences in soil respiration we used the gls function of nlme package in R and performed post-hoc analyses using LSD tests (Pinheiro & Bates, 2000). Differences in total C, N, soil microbial DNA, Shannon index and total Observed OTUs (n=3) among sites and depths were tested with GLM using site origin and depth as factors, and means compared with LSD post-hoc analysis. All tests were done using R (R core team, 2013) through the infoStat statistical software (Di Rienzo *et al.*, 2015). For bacterial communities analysis, we imported tables of taxonomic composition obtained with Qiime in the software package PRIMER7 (PRIMER-E Ltd) for the generation of dual hierarchical cluster analysis and analysis of similarity (ANOSIM). The OTU-level analysis was performed exporting the weighted Unifrac dissimilarity matrix produced in Qiime to PRIMER7 to generate Principal Coordinate's analysis (PCoA) and ANOSIM analysis.

Results

Soil properties

There were differences among soils, with Desert-clay and Alpine-sand having the richest and poorest C and N content, respectively. In all cases, shallow soils had significantly more C than deeper soils. Nitrogen was similar in the Desert-clay and Desert-sand soils, with higher values on the shallow layer, while in the Alpine-sand soils the two layers did not differ (Table S1). In Desert-sand and Desert-clay we recorded temperature at 3 depths (5 cm, 10 cm and 30 cm deep; Fig. S1). Our records at 5 cm depth showed diel temperature amplitudes of 20ºC, and in half the recorded days maximum temperatures were above 40ºC, reaching maximum values of 49ºC in Desert-sand and 47ºC in Desert-clay during the warmer days of August. Soil surface in summer may be well above 60ºC (F.I. Pugnaire *per. obs.*). For Alpine-sand we recorded temperature 10 cm deep (Fig. S1); the maximum value recorded was 26ºC, while maximum values for Desert-sand and Desert-clay at 10 cm depth were 37 and 33ºC respectively.

Soil respiration

There was a significant site x SWC x depth interaction ($p=0.01$; Table S2, Fig. 1a), respiration rates being similar between depths on the dry treatment, while wet soils maintained higher respiration rates in the upper layers independently of the water treatment. The interaction between soil origin and temperature was independent of soil depth ($p<0.01$; Fig. 1b), showing that soil respiration in Desert-clay increased with temperature up to 60ºC, while for Desert-sand and Alpine-sand respiration did not show an increase between 50ºC and 60ºC. Post hoc analysis also showed that soil respiration rates were not significantly different between soils from different sites at 30 and 40ºC, while they were significant between 50ºC and 60ºC (Fig. 1b). Soil respiration responses to water addition were different between depths in alpine and desert climates, while

responses to temperature were different between clay and sand soils independently of soil layer.

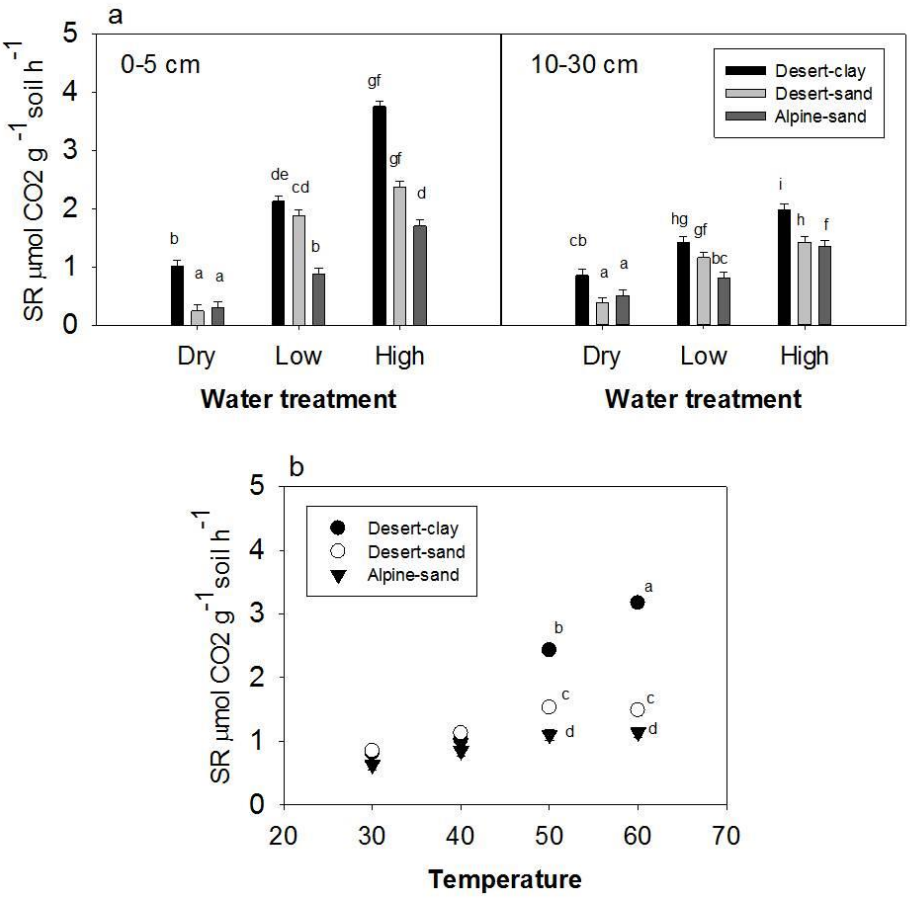

Figure 1. Relationship between soil respiration (SR) and water treatment in shallow and deep soil layers (GLM, $p<0.001$) (a); relationship between SR and temperature at three sites (GLM, $p<0.001$) (b). Low letters show significance level ($p<0.05$) among sites, depths or treatments.

Desert-clay soils presented a maximum sensitivity to water addition at 40ºC (Fig. 2a, b) while shallow sand-soils appeared in general more sensitive at 30ºC (Fig. 2c, e). Sandy soils had similar behavior among temperatures (Fig 2c, d, e, f). When we looked at absolute soil respiration values, Desert-clay increased with SWC (Fig. 3a, b) while Desert-sand responded strongly to low SWC but saturated soon, showing that the strong response capacity to water addition was not dependent of the amount of water received (Fig. 3c, d). In Alpine soils, respiration rate increased little with low water input an increased as SWC increased (Fig. 3e, f). There was a positive correlation between

maximum soil respiration and soil C in all three treatments (Fig S2 a), and with microbial biomass in the water addition treatments (Fig. S2b).

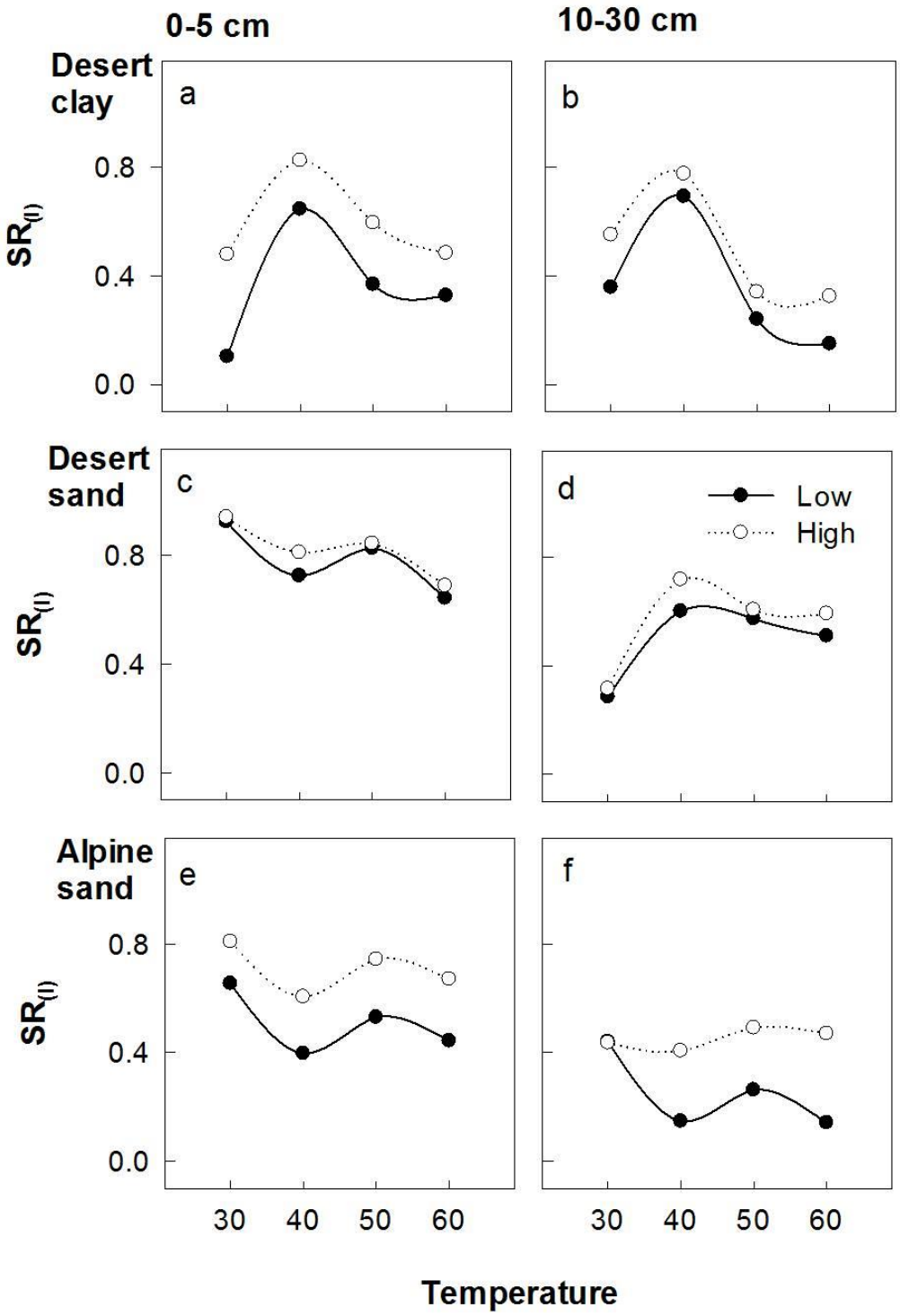

Figure. 2. Soil respiration sensitivity $SR_{(I)}$ to water pules calculated from RII index, depending on temperature, soil origin and depth. Y axis represent standard relative values between 1 and -1.

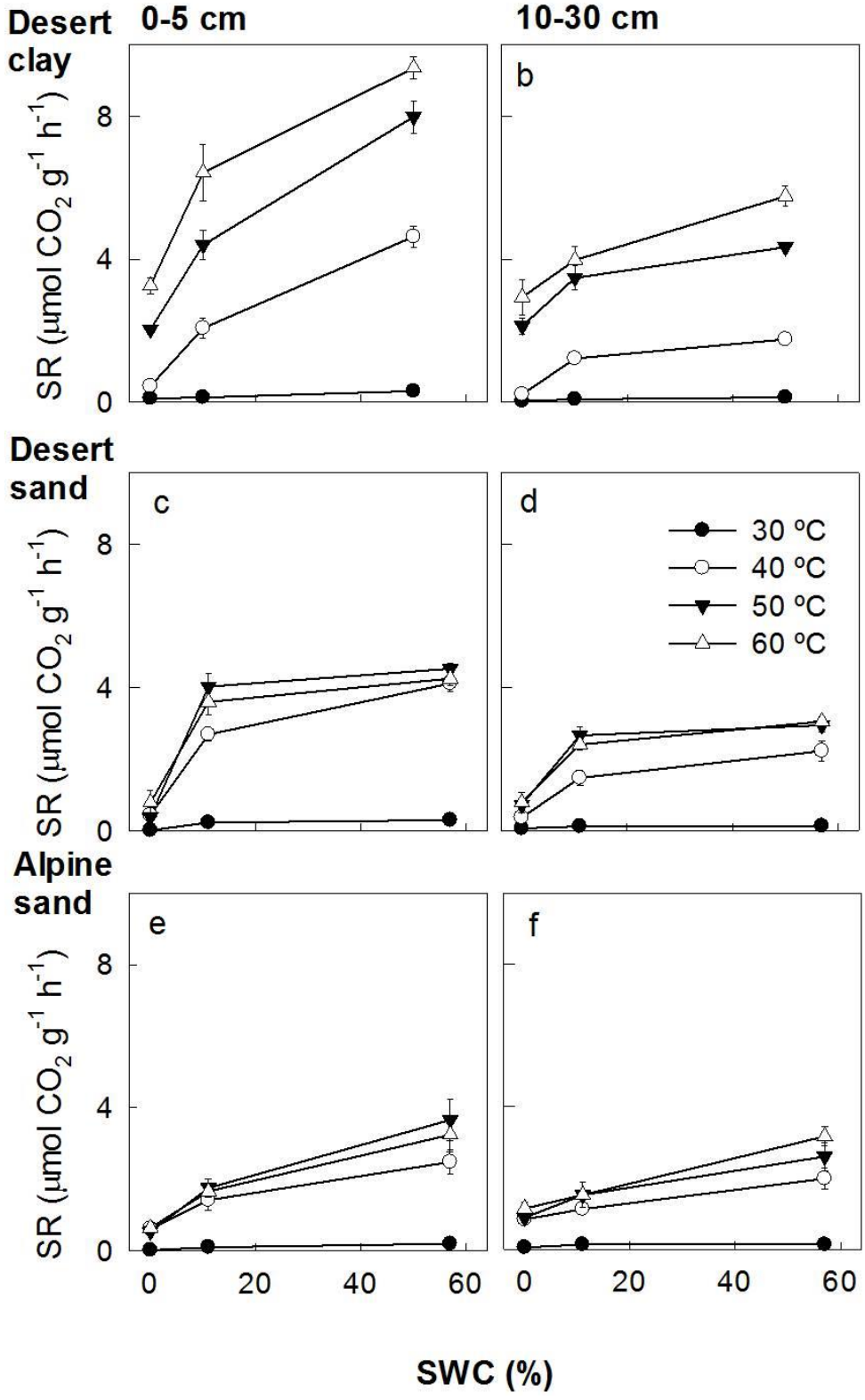

Figure 3. Soil respiration (SR) increase with soil water content (SWC) depending on temperature, soil origin, and depth. All points represent the mean ±1 SE.

Bacterial Communities

Microbial biomass was at least 10-fold higher in soils collected from the two deserts than in the alpine soil. Shallow Desert-clay layers showed the highest values of microbial DNA followed by shallow Desert-sand soils (Table 2). Microbial DNA was higher in shallow soils than in deeper soil layers in all three sites. In deep Alpine-sand soils, the extremely low concentrations of microbial DNA only allowed the quantification in one of the samples, as the extracts concentration were close to the detection limit of the DNA-quantification method (Table 2).

Site	**Depth** (cm)	**Total DNA** ($\mu g\ g^{-1}$soil)	**Shannon's index**	**OTUs number**
Alpine-sand	0-5	0.05 ± 0.01^{a}	9.55 ± 0.07^{b}	3613 ± 449^{b}
	10-30	$0.01 \pm$ n.d*	8.27 ± 0.07^{a}	1363 ± 449^{a}
Desert-sand	0-5	0.50 ± 0.11^{b}	10.69 ± 0.20^{d}	5932 ± 162^{d}
	10-30	0.09 ± 0.11^{cd}	10.65 ± 0.20^{d}	5618 ± 162^{d}
Desert-clay	0-5	0.83 ± 0.05^{d}	10.08 ± 0.10^{c}	4697 ± 113^{b}
	10-30	0.33 ± 0.05^{bc}	9.61 ± 0.10^{b}	3765 ± 113^{c}

Table 2. Soil bacterial communities characteristics. Values are mean ± 1 SE (n = 3) from the GLM analysis. Small letters indicate the significance level ($p<0.05$) prom the LSD post hoc analysis

After quality filtering, trimming and detection of PCR-chimeras, the molecular analysis of bacterial communities yielded 1,011,895 high-quality reads (428.04 ± 14.21 bp) which were clustered in 27,406 bacterial OTUs (excluding singletons). OTUs richness obtained by rarefaction curves (Fig. S3) showed higher bacterial diversity in shallow than in deep soil layers, and in Desert-sand than in the other two field sites, being in Alpine-sand much lower (Table S3). The Shannon-Weaver diversity index followed the same pattern, showing the bacterial community in Desert-sand samples to be the most diverse, followed by Desert-clay and Alpine-sand soils. In Alpine-sand and Desert-clay sites, microbial communities were more diverse in shallow than in deep soil layers, and there were not differences in Desert-sand soils (Table 2).

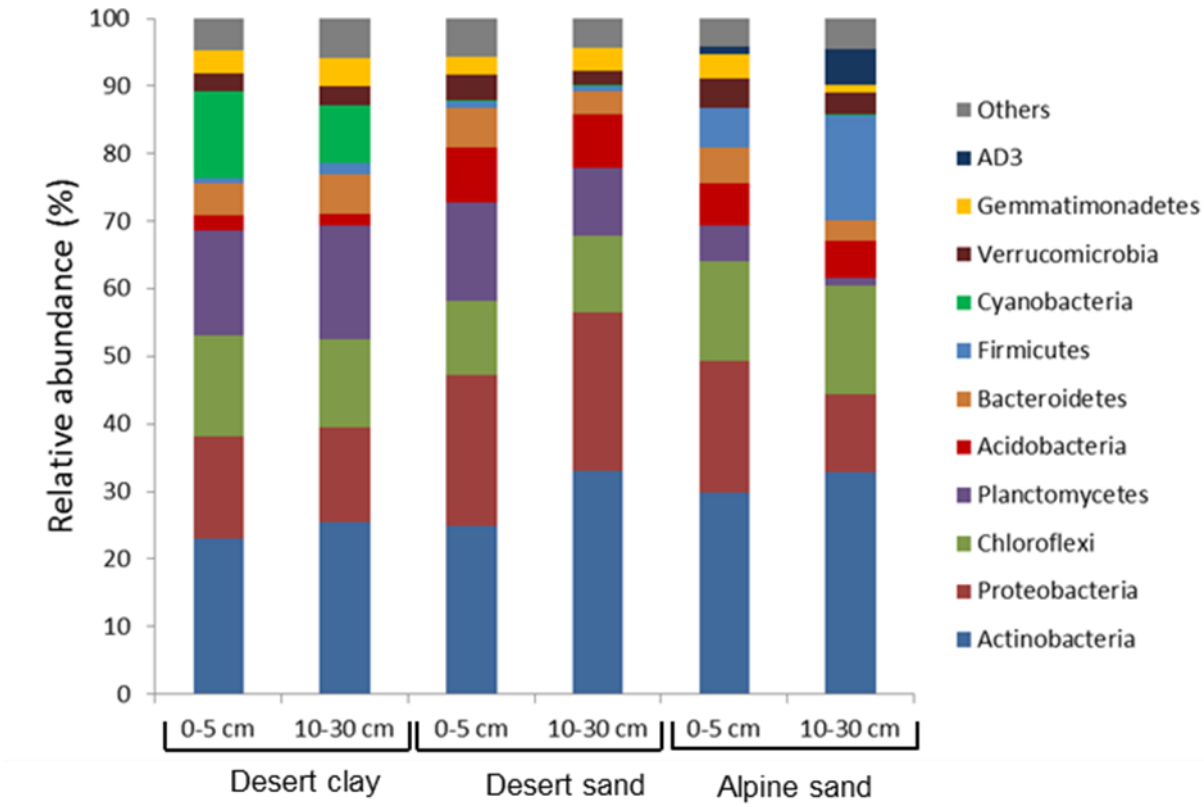

Figure 4. Relative abundance of phyla in soil bacterial communities at different depth in three semiarid locations (n=3). Less abundant phyla were grouped as "Others".

Actinobacteria, Proteobacteria and Chloroflexi were the most abundant and ubiquitous phyla (Fig. 4; Table S3). Within Proteobacteria most groups were Alphaproteobacteria (Table S4).The three more abundant phyla accounted for more than 50% of bacterial communities in all samples (in terms of relative read abundance), exceeding by far this percentage in Desert-sand and Alpine-sand soils. With the exception of Verrucomicrobia, Bacteroidetes and Gemmatimonadetes, which did not show marked association with a particular soil type or site, most bacterial phyla followed specific patterns. For example, Planctomycetes were very abundant in desert soils (up to 16% in clay soils) but much less frequent in cold alpine soils (<6%). By contrast, Acidobacteria were more abundant in clay than in sandy soils (Table S4). Other phyla showing specific patterns were Cyanobacteria, abundant in clay soils but

nearly absent in sandy soils; Firmicutes, represented at the class level by Bacilli in shallow soils and by Clostridia in deep alpine soils (Table S4); and AD3 bacteria in deep alpine soils but absent in desert samples.

Soil communities clustered together by site, with the exception of Alpine samples (Fig. 5). In fact, deep alpine samples were apart, likely reflecting extreme conditions. In Desert-sand soils, shallow and deep samples clustered separately, opposite to Desert-clay samples which showed no differentiation at the phyla level.

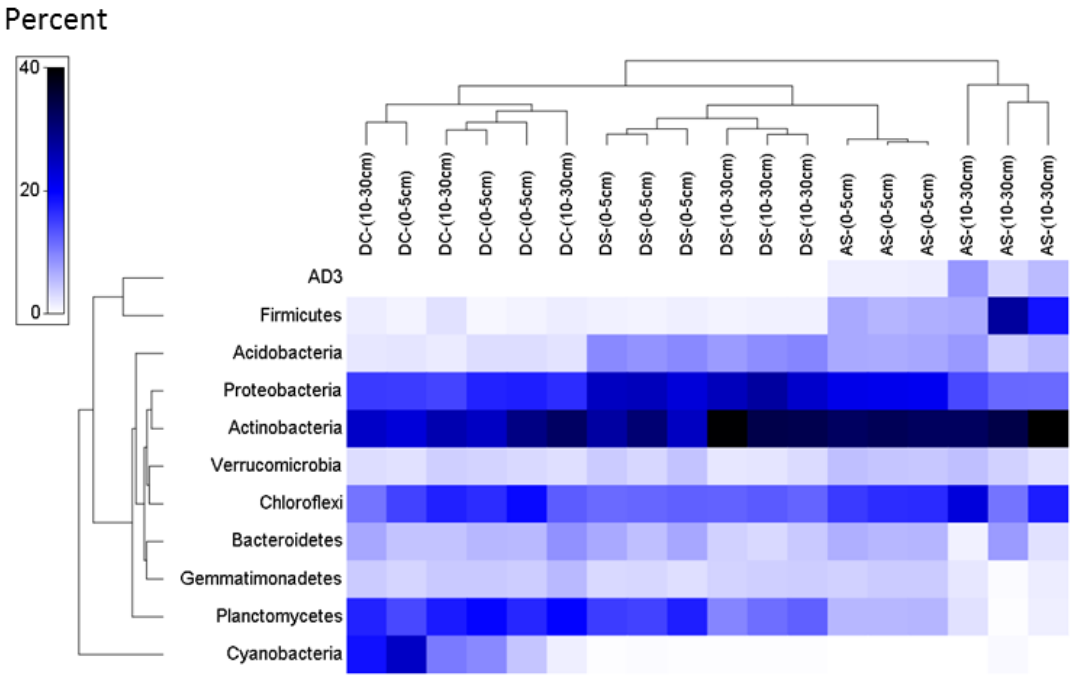

Figure 5. Dual hierarchical cluster analysis of soils based on Bray-Curtis dissimilarity matrix of bacterial communities at phyla level. The heatmap represents the relative abundance of predominant bacterial phyla. Legend: Alpine-sand (AS), Desert-clay (DC) and Desert-sand (DS). Sampling depth is indicated. Less abundant phyla were grouped as "Others".

Actinobacteria, the most abundant phyla in all soils, included extremophilic genus like *Geodermatophilus* and *Modestobacter* (Geodermatophilaceae), abundant in desert environments (Fig. S4). Other well-known extremophilic organisms like *Halomonas*

(Proteobacteria), *Deinoccocus* (Deinoccocus-Thermus) and *Geobacillus* (Firmicutes) were also present but with lower abundance (data not shown).

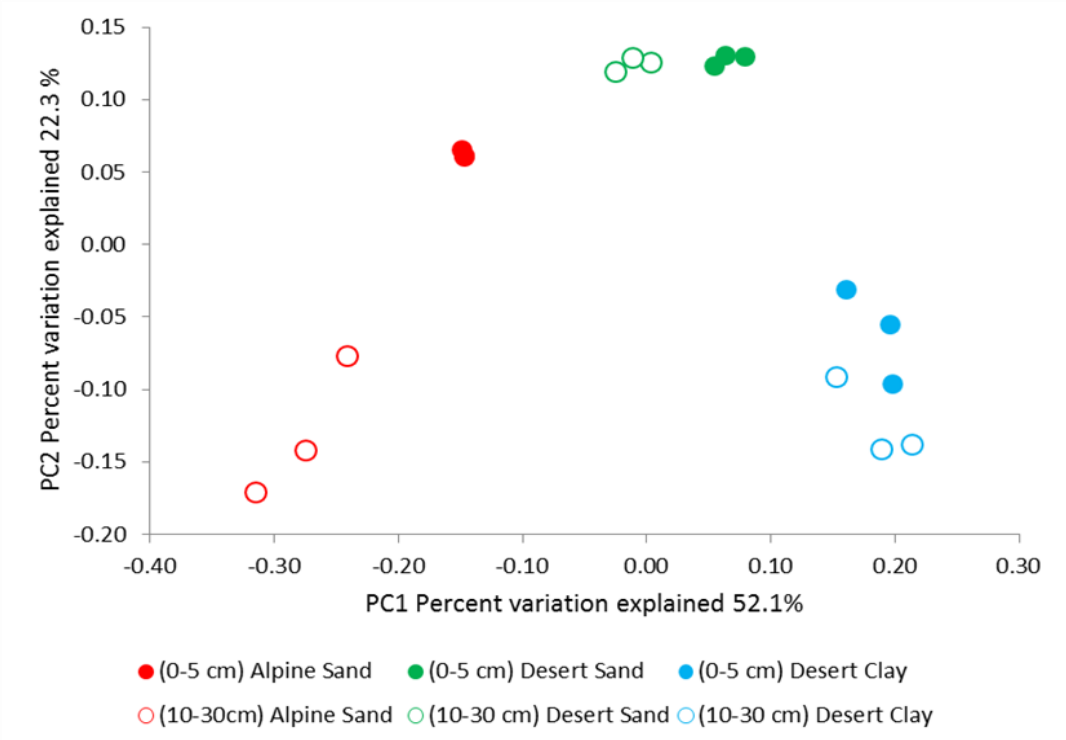

Figure 6. Principal coordinates analysis (PCoA) of weighted UniFrac distances among samples of different soils and depths.

A β-diversity analysis at OTU level using a phylogenetic distance metric (weighted Unifrac) showed patterns similar to the phyla, i.e., soils clustered by site, being spatial differentiation with depth more evident in Alpine-sand soils (Fig. 6). Results of a two-way crossed Analysis of Similarities test (ANOSIM) comparing weighted Unifrac distances confirmed the differences between microbial communities in the three environments ($R=1$, $p<0.01$), as well as the differences between shallow and deep soil layers ($R=0.87$, $p<0.01$).

Discussion

Our data show that, in semiarid environments, soil respiration responses to water pulses were determined by temperature and differed depending on soil origin and depth.

Microbial community size and structure varied in soils from different sites and depths showing a variety of phyla adapted to water stress, which should have an effect on the recorded soil respiration responses. We also evidenced that optimal temperature in short-term soil respiration responses were well above the suggested optimum of 35ºC and more in accordance to temperatures registered in these systems.

Water effects on soil respiration

The CO_2 released after soil rewetting can reach up to 70% of the annual soil respiration budget in arid environments (Ataka *et al.*, 2014). Our data proved that small water contributions may have important effects on CO_2 pulses and this effect depends on temperature, with differential responses with depth at the scale of centimeters. Microbial mineralization of C is likely responsible of such pulses (Fierer & Schimel, 2003; Kim *et al.*, 2012) since long, dry periods lead to the accumulation of labile substrates (Jenerette & Chatterjee, 2012) which can be released after rains. Differences in soil respiration were site-dependent, Desert-clay soils showing the largest CO_2 release rate. CO_2 fluxes were related to total soil C (Fig. S4) suggesting a link between labile C in the soil and drying and rewetting cycles (Birch, 1958; Fierer & Schimel, 2003; Meisner *et al.*, 2015). The release of labile substrate is higher in top soil layers, more exposed to wet-dry fluctuations, and is where otherwise OM accumulates.

The fact that sandy soils (desert and alpine) responded similarly to temperature but differed in their response to water availability suggests that processes controlling both responses are different. In fact, microbial communities seemed more responsible to water on desert soils, and recent studies reported changes in microbial communities within two hours after rewetting (Barnard *et al.*, 2015), linking peaks in soil respiration to changes in bacterial community functions. In addition, communities from desert environments are adapted to deal with drought and rewetting cycles, explaining the

responses to low water inputs in Desert-sand soils. Opposite, soils in wetter environments such as the alpine are expected to be less sensitivity to rewetting processes, which is what we found.

Soil respiration responses to temperature

CO_2 pulses after rewetting were temperature-dependent, with optimal temperature consistently above 35ºC on the sandy soils throughout the different sites and depths (Hamdi *et al.*, 2011; Richardson *et al.*, 2012). Our data did not support the hypothesis that warmer climates would have higher optimum temperatures, since the alpine site also had high optimum temperatures. But, while our range of temperatures did not allow us to determine optimal temperature in clay soils, sand soils stabilized at 50 ºC (Fig. 1). Alpine-sand and Desert-sand soils differed in location, climate and vegetation but shared the same texture, suggesting that soil texture is a better predictor of temperature optimum than climate (Van Gestel *et al.*, 1991; Cable *et al.*, 2008).

Soil bacterial community and soil respiration

The correlation between maximum soil respiration and soil microbial DNA in water-added treatments shows the extent to which the microbial community can mediate respiration pulses. Bacterial communities were quite different in the different soils. Actinobacteria, one of the largest phyla, was predominant in all three soils. This group of gram-positive bacteria has a broad distribution and highly variable physiological and metabolic properties (Ventura *et al.*, 2007). Some Actinobacteria groups are metabolically active at extremely low water levels (Stevenson & Hallsworth, 2014) and dominate desert environments. It includes well-known thermotolerant and xerotolerant taxa often isolated in arid environments (Kurapova *et al.*, 2012; Mohammadipanah & Wink, 2016) which can reach abundances in the order of 72-88% in the hyperarid Atacama Desert (Crits-Christoph *et al.*, 2013). Genera like *Geodermatophilus* and

Modestobacter (Geodermatophilaceae) are thermophilic bacteria adapted to desiccation and high UV radiation, respectively (Chanal *et al.*, 2006; Harwani, 2013). Many of these groups dominated bacterial communities in our soils, with maximum abundance in Desert-sand soils. Other groups such as Acidobacteria, linked to short-term responses after desiccation periods (Barnard *et al.*, 2013, 2015), have been detected in our soils, mostly sandy. The presence of AD3 in Alpine-sand soils is not surprising since they have been reported as abundant in alpine and subalpine sub-surface soils (Costello *et al.*, 2009; Tas *et al.*, 2014; Ji *et al.*, 2016). Finally, the high presence of cyanobacteria in Desert-clay soils can be linked to their desiccation-resistance mechanism (Rajeev *et al.*, 2013) and high capacity to respond to rewetting. The fact that soil communities were all different evidence how environmental factors such as climate and soil type and depth shape soil microbial communities and how they mediate soil respiration responses to environmental factors.

Conclusion

Our data shown that soil respiration pulses after rewetting depended on temperature and soil origin, suggesting that microbial communities are responsible of differences in soil respiration rate among sites and depths, although more analysis are needed to clearly establish the link. Optimum temperatures in dry environments were in the range of 50ºC on sandy soils, much higher than expected. Our data support the idea that global temperature increases will boost soil respiration rates, releasing C stored in soils in arid environments.

Appendix

Site	**Depth** (cm)	**C** (g/100g soil)	**N** (g/100g soil)	**C/N ratio**
Desert-clay	**0-5**	2.64 ± 0.01a	0.13 ± 0.01ab	20.28
	10-30	2.33 ± 0.01b	0.07 ± 0.01c	33.68
Desert-sand	**0-5**	0.94 ± 0.03c	0.15 ± 0.04a	6.26
	10-30	0.69 ± 0.02f	0.08 ± 3.3E-03bc	8.62
Alpine-sand	**0-5**	0.85 ± 0.01d	0.05 ± 0.01c	17.14
	10-30	0.76 ± 0.02e	0.07 ± 0.01c	10.42

Table S1. Total C and N concentration (n=3) from the different soil origins and depths. Small letters indicate the significance level ($p<0.05$) among sites or depth.

Variable	DF	F Value	P Value
Site	2	147.79	**<0.01**
Depth	1	114.87	**<0.01**
SWC	2	391.23	**<0.01**
Tº	3	148.27	**<0.01**
Site: Depth	2	26.95	**<0.01**
Site: SWC	4	15.81	**<0.01**
Site: Tº	6	41.86	**<0.01**
Depth: SWC	2	47.08	**<0.01**
Depth: Tº	3	1.31	0.31
SWC:Tº	6	10.04	**<0.01**
Site: Depth:SWC	4	5.24	**0.01**
Site: Depth:Tº	6	1.49	0.26
Site: SWC:Tº	12	1.85	0.14
Depth: SWC:Tº	6	1.08	0.42

Table S2. Results from GLM comparing soil respiration values from the incubations among soil origin (Site) , Soil depth , soil water content (SWC) and Temperature, and their interaction.

Phyla	DC (0-5cm)		DC (10-30 cm)		DS (0-5 cm)		DS (10-30cm)		AS (0-5 cm)		AS (10-30 cm)	
	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
Actinobacteria	23.55	3.24	25.23	3.78	25.34	2.92	32.91	3.09	29.94	0.41	32.65	3.60
Proteobacteria	15.27	1.23	14.12	0.86	22.24	1.11	23.38	1.57	19.51	0.30	11.49	1.40
Chloroflexi	15.41	2.08	12.50	3.11	10.98	0.31	11.35	0.35	14.74	0.63	15.62	5.43
Planctomycetes	15.49	2.50	16.76	1.21	14.49	1.37	10.10	1.31	5.25	0.06	1.18	1.01
Acidobacteria	2.22	0.37	1.71	0.23	8.23	0.37	8.05	0.80	6.18	0.10	5.23	1.90
Bacteroidetes	4.84	0.43	6.13	1.76	5.66	0.94	3.30	0.57	5.42	0.31	3.47	3.28
Firmicutes	0.74	0.14	1.57	0.47	0.99	0.12	0.93	0.04	5.74	0.44	16.09	9.64
Cyanobacteria	11.67	9.51	9.22	7.97	0.20	0.04	0.14	0.02	0.06	0.02	0.17	0.26
Verrucomicrobia	2.67	0.50	2.73	0.58	3.61	0.76	2.03	0.43	4.26	0.27	3.34	1.28
Gemmatimonadetes	3.45	0.43	4.19	0.68	2.60	0.23	3.41	0.20	3.54	0.22	1.06	0.75
AD3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.21	0.07	5.11	2.17
Others	4.68	0.34	5.82	0.36	5.65	0.70	4.40	0.45	4.15	0.08	4.58	1.38

Table S3. Taxonomic composition of bacterial communities at phyla level in soil microcosms (n=3). Taxa are ordered in the table by average abundance in the data set. The abbreviations refer to the sampled soils: DC = Desert Clay, DS = Desert Sand and AS = Alpine Sand.SD indicates standard deviation.

Classes	DC (0-5cm)		DC (10-30 cm)		DS (0-5 cm)		DS (10-30cm)		AS (0-5 cm)		AS (10-30 cm)	
	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
p__Proteobacteria;c__Alphaproteobacteria	12.18	0.95	11.05	0.51	16.16	0.80	15.55	1.43	11.55	0.29	6.76	1.22
p__Actinobacteria;c__Actinobacteria	9.51	1.49	9.29	1.41	13.48	2.10	15.74	2.41	13.75	0.34	6.71	1.65
p__Actinobacteria;c__Thermoleophilia	4.63	0.61	4.04	0.74	5.19	0.61	8.32	0.70	10.21	0.14	15.62	2.29
p__Planctomycetes;c__Planctomycetia	6.55	0.44	9.55	1.17	6.61	0.74	5.86	0.82	2.85	0.13	0.96	0.76
p__Planctomycetes;c__Phycisphaerae	8.92	2.10	7.17	0.21	7.84	0.63	4.16	0.47	2.38	0.13	0.21	0.24
p__Chloroflexi;c__Chloroflexi	4.39	0.97	3.16	1.93	4.13	0.33	3.64	0.34	5.61	0.25	1.37	0.91
p__Actinobacteria;c__Acidimicrobiia	2.90	0.41	4.28	0.89	2.65	0.32	3.09	0.26	4.55	0.12	4.03	1.25
p__Firmicutes;c__Bacilli	0.74	0.14	1.53	0.48	0.97	0.13	0.88	0.09	5.64	0.41	10.24	4.07
p__Proteobacteria;c__Betaproteobacteria	0.92	0.15	0.88	0.12	2.44	0.12	3.72	1.51	6.03	0.25	1.74	0.77
p__Actinobacteria;c__MB-A2-108	2.11	0.43	1.40	0.62	1.82	0.07	3.68	0.18	0.72	0.04	5.08	0.55
p__Verrucomicrobia;c__[Spartobacteria]	2.13	0.31	1.78	0.81	2.50	0.52	1.00	0.14	3.90	0.28	2.67	1.42
p__Chloroflexi;c__Thermomicrobia	4.08	0.68	3.68	0.44	1.89	0.02	1.73	0.10	1.16	0.15	0.65	0.18
p__Acidobacteria;c__[Chloracidobacteria]	0.46	0.13	0.38	0.18	4.28	0.42	3.11	0.53	2.75	0.22	1.29	1.04
p__Chloroflexi;c__TK10	1.01	0.13	0.54	0.20	1.35	0.03	1.46	0.04	2.34	0.11	5.11	1.48
p__Bacteroidetes;c__[Saprospirae]	1.11	0.28	0.44	0.09	3.49	0.75	1.84	0.36	4.35	0.22	0.45	0.20
p__Proteobacteria;c__Deltaproteobacteria	1.66	0.04	1.15	0.11	2.48	0.14	2.56	0.34	1.74	0.17	1.59	0.41
p__Cyanobacteria;c__Oscillatoriophycideae	5.66	3.28	5.15	4.68	0.01	0.01	0.00	0.01	0.00	0.01	0.04	0.06
p__Chloroflexi;c__Anaerolineae	3.58	0.32	3.17	0.55	0.84	0.12	0.56	0.11	1.41	0.09	1.02	0.12
p__Actinobacteria;c__Rubrobacteria	3.32	0.44	1.61	0.29	2.15	0.05	1.99	0.23	0.70	0.05	0.65	0.25
p__Bacteroidetes;c__Cytophagia	3.05	0.09	2.48	0.75	1.81	0.31	1.29	0.29	0.89	0.08	0.05	0.06
p__Gemmatimonadetes;c__Gemmatimonadetes	1.19	0.28	0.73	0.29	0.91	0.03	1.42	0.05	3.24	0.22	0.66	0.39
p__Acidobacteria;c__Acidobacteria-6	0.86	0.22	0.73	0.29	1.58	0.14	2.42	0.05	1.07	0.07	1.29	0.24
p__Acidobacteria;c__Solibacteres	0.40	0.04	0.27	0.12	1.23	0.12	0.89	0.10	1.78	0.16	1.51	0.20
p__Actinobacteria;c__Nitriliruptoria	1.09	0.12	4.59	0.41	0.06	0.01	0.09	0.03	0.00	0.00	0.00	0.00
p__Firmicutes;c__Clostridia	0.00	0.01	0.04	0.02	0.01	0.01	0.05	0.05	0.10	0.05	5.59	5.85
p__Proteobacteria;c__Gammaproteobacteria	0.49	0.10	1.01	0.15	1.15	0.22	1.53	0.58	0.19	0.03	1.26	0.84
p__Chloroflexi;c__Ellin6529	0.72	0.14	0.32	0.10	0.92	0.13	1.55	0.15	0.66	0.07	1.41	0.53
p__Gemmatimonadetes;c__Gemm-3	1.72	0.10	1.72	0.03	1.03	0.12	0.88	0.15	0.06	0.01	0.01	0.01
p__AD3;c__ABS-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.06	4.10	1.28
p__Cyanobacteria;c__Synechococcophycideae	2.34	2.22	1.90	1.56	0.03	0.02	0.00	0.01	0.00	0.00	0.00	0.00
p__Chloroflexi;c__Gitt-GS-136	0.88	0.11	0.89	0.35	0.51	0.10	0.87	0.17	0.07	0.01	0.84	0.58
p__Cyanobacteria;c__Nostocophycideae	2.67	3.18	1.41	1.19	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Others	8.69	0.42	13.62	2.42	10.46	0.94	10.11	0.80	9.29	0.15	17.13	2.52

Table. S4 Taxonomic composition of bacterial communities at class level in soil microcosms (n=3). Taxa are ordered in the table by average abundance in the data set. The abbreviations refer to the sampled soils: DC = Desert Clay, DS = Desert Sand and AS = Alpine Sand. SD indicates standard deviation, p phylum and c class.

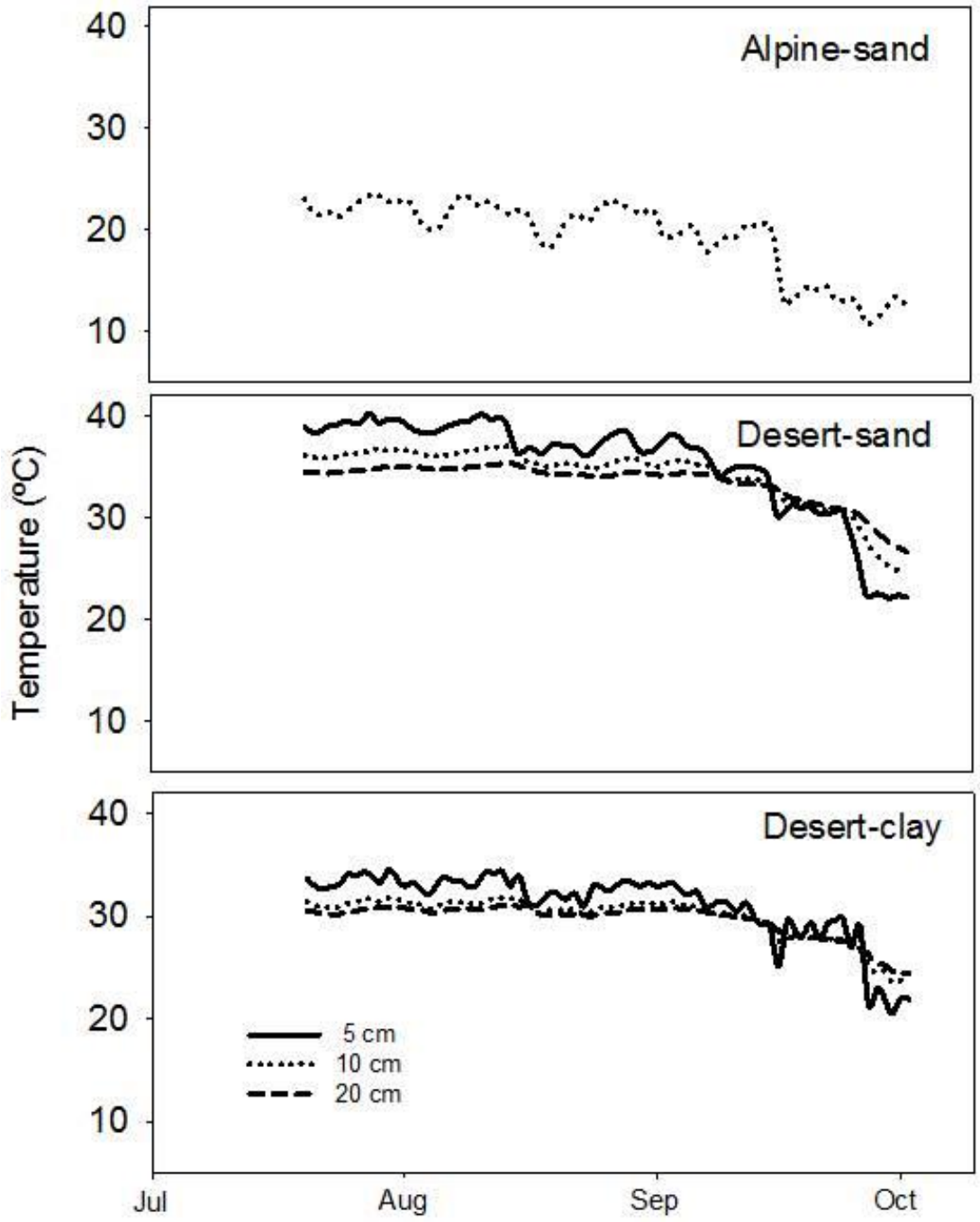

Figure S1. Soil temperature at 3 different depths (n=3), from ECH_2O probes. Values are shown as daily medium temperatures.

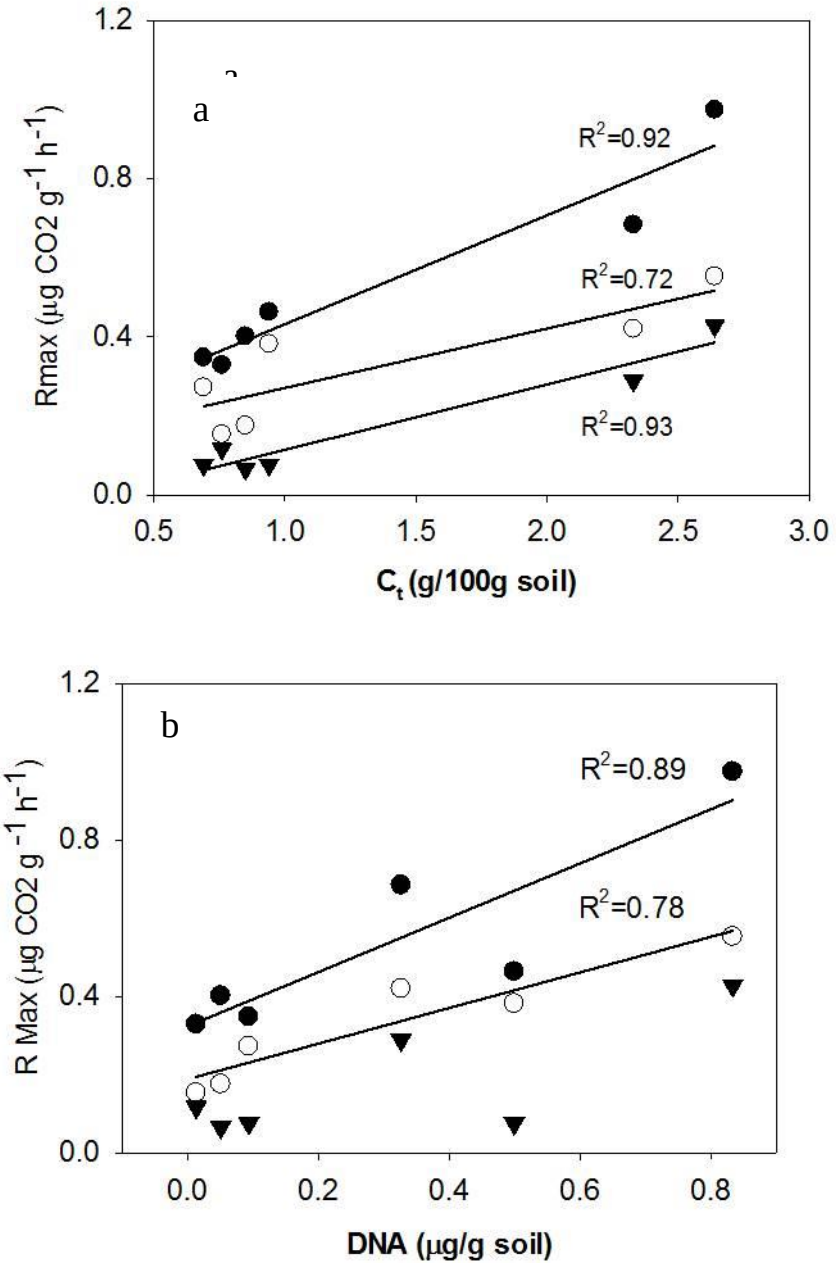

Figure. S2. Correlation between maximum soil respiration (R_{max}) and total carbon (C_t) in dry (solid triangles), low water (clear dots), and high water (solid dots) treatments. p values were 0.0024, 0.0312 and 0.001 respectively (GLM) (a). Linear regression between R_{max} and microbial DNA mass for dry (n. s., solid triangles), 10-11 % SWC (p = 0.0046, clear dots), and 50-57 SWC treatments (p = 0.0019, solid dots) (b).

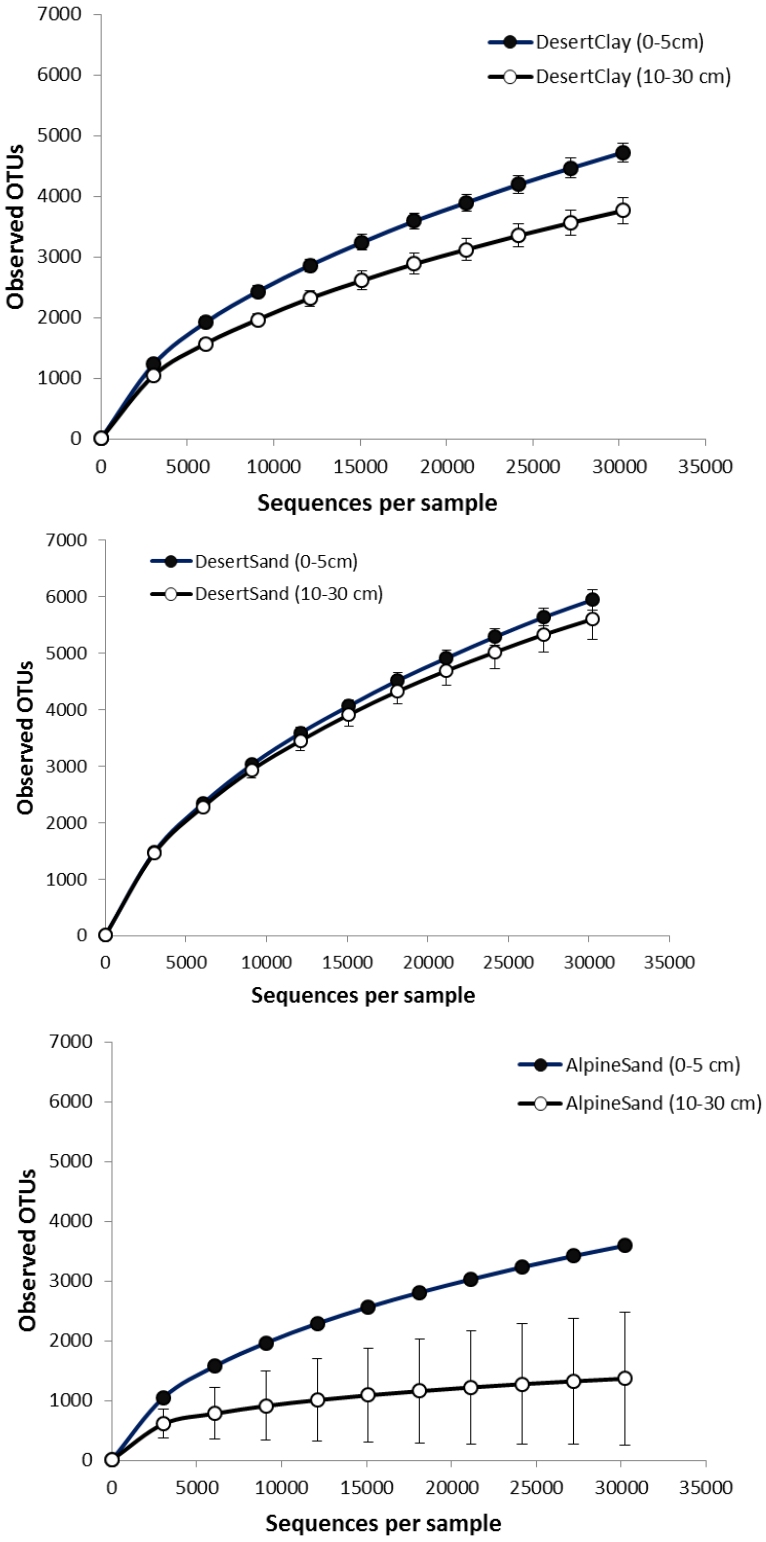

Figure S3. Rarefaction curves indicating the average observed number of operational taxonomic units for the different soil microcosms (n=3). Errors bars show ± standard deviation.

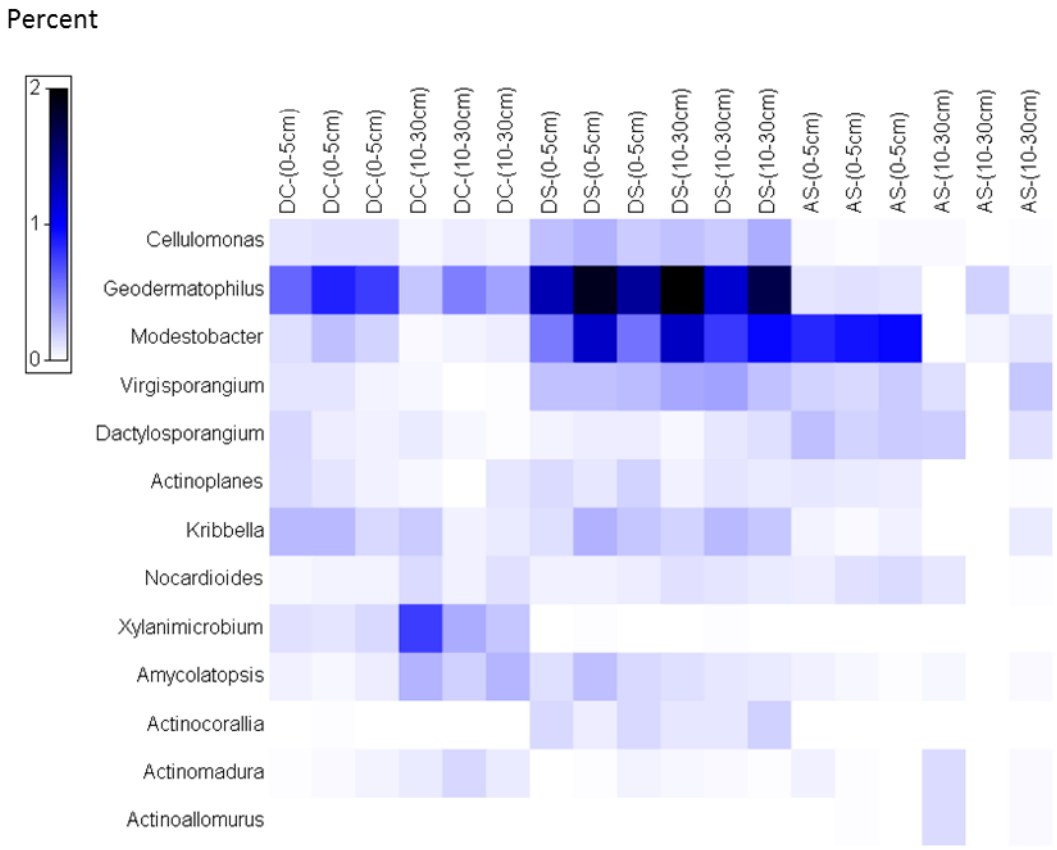

Figure S4. Heatmap representing the relative abundance of genera of the phylum Actinobacteria detected in the soil microcosms that has been previously associated with desert environments. Only genera with abundances >0.1% in at least one sample are represented. Legend: Alpine-Sand (AS), Desert-Clay (DC) and Desert-Sand (DS). Sampling depth is indicated.

CHO2

Chapter III:

Arbuscular mycorrhiza fungi mediate soil respiration response to environmental change in California grasslands

.

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Abstract

Soils of California grasslands store ca. 100 Tg of soil organic carbon (SOC) and almost 40% of those ecosystems are prone to land use changes. The fate of these carbon pools will largely depend on how the main responsibles of soil respiration -- i.e., roots, mycorrhiza, and 'bulk soil' communities-- respond to such changes. In order to determine the sensitivity of these components to environmental drivers we set up an experiment to address the effect of plant community composition, soil age and warming on soil respiration rate during the 2014-2015 winter. We tested differences among microbial, fungal and root respiration using an exclusion technique to assess the effect of plant community (open grasslands vs oak woodland) in two field sites differing in geologic soil age (92 and 137 kyr). We also used open top chambers (OTC) to simulate global change effects on grasslands. Our results showed that arbuscular mycorrhizal fungi were the main drivers of differences recorded between soils of different age, and that those differences were linked to nutrient availability. Bulk soil respiration was more sensitive to temporal variation than mycorrhizal or root respiration. Soil age affected CO_2 flux from grasslands but not under oak canopies, likely due to differences in SOM content which moderate CO_2 fluxes. Overall our study shows that the ability of grasslands to mitigate CO_2 emissions depends on interactions between vegetation and their rhizosphere and soil microbial communities.

Introduction

Globally, grasslands occupy 30% of emerged land and contain 10% of the organic carbon (SOC) stored in soils (Chaplot *et al.*, 2016) while providing multiple ecosystem services such as grazing, wildlife, and recreation. In California, grasslands store approximately 100 Tg of SOC and ca. 40% of these ecosystems are at risk of land use change (Byrd *et al.*, 2015). These systems are affected mostly by agricultural practices and widespread invasions (Mooney, 2007; IPCC, 2014) to the detriment of natural communities (Lenihan, 2003; Rao & Allen, 2010). Global change can have serious impacts on carbon stocks of these grasslands, as future scenarios for California forecast the extension of the warm and dry season (Byrd *et al.*, 2015; Pfeifer Meister *et al.*, 2016) which can reduce allocation of C compounds belowground (Fuchslueger *et al.*, 2016) contributing to decrease C stored in soil. In addition, warming enhances soil respiration (Reynolds *et al.*, 2015), and rainfall patterns can also have a strong effects on soil respiration, which peaks after rainfall following prolonged drought periods (Hoover *et al.*, 2016).

Plants have a regulatory effect on soil climate, moisture, and carbon and nutrient availability. This regulatory effect determines the composition and activity of soil microbial communities, including mycorrhizal associations. Californian grasslands are characterized by the presence of oak tree patches, that form "fertility islands" that accumulates high amounts of C and N and keep soils cooler (Waldrop & Firestone, 2004; Waldrop & Firestone, 2006). Soil nutrient status affects the composition of microbial communities (Thomson *et al.*, 2010; Uroz *et al.*, 2014) such that higher nutrient availability can decrease the fungi/bacteria ratio (Fierer *et al.*, 2009; De Vries *et al.*, 2012; Wan *et al.*, 2015), which has been observed to vary with soil age (Allison *et al.*, 2005; Moore *et al.*, 2010). Increasing soil moisture also increases the availability

of dissolved nutrients under plant canopies (Waldrop & Firestone, 2006) but, while Arbuscular mycorrhiza fungi (AM) dominate grasslands communities Ecto mycorrhizal fungi (EM) prevail under forested canopies, enhanced by nutrient availability.

Our understanding of how soil respiration responds to climate, as modulated by plant communities and soil age, is complicated by the divergent ways in which plants, mycorrhizae, and soil microorganisms may respond. Soil respiration can be separated into three main components; roots, the extra radical mycorrhizal fungi, and the non-mycorrhizal soil microbial community that we will herein call the 'bulk soil community'. These three groups may differ markedly in their response to climate warming and other factors, offering important insights into carbon partitioning under changing environmental conditions. For example, the bulk soil community is reported to be more sensitive to temperature (Hartley *et al.*, 2007; Heinemeyer *et al.*, 2007) while mycorrhizal fungi are capable to increase stabilization of C compounds derived from roots, turning respiration responses less sensitive to environmental variation (Zhu, 2003; Heinonsalo *et al.*, 2010; Hu *et al.*, 2013). Finally, fluctuations in rhizosphere (mainly roots and mycorrhizal fungi and bacteria associated) respiration responds more to aboveground processes (e.g., photosynthesis, plant phenology) than to temperature and humidity (Hartley *et al.*, 2007; Churchland *et al.*, 2013).

Soil respiration is roughly balanced between roots and soil microbial community but root respiration can range 30-70% depending on the ecosystem (e.g., Wang *et al.*, 2009; Heinemeyer *et al.*, 2012). Mycorrhizal respiration can represent up to 25 % of soil respiration (Heinemeyer *et al.*, 2007; Heinonsalo *et al.*, 2010; Zhang, 2013) while their categorization as part of the autotrophic (root) or heterotrophic respiration is controversial (Hopkins *et al.*, 2013). The importance of the different soil components on total soil respiration depends on factors that vary seasonally (Baldocchi *et al.*, 2006;

Heinemeyer *et al.*, 2007; Vallack *et al.*, 2012; Barba *et al.*, 2016). The climate regime may favor some groups in detriment to others; for example, although mycorrhizal extra radical mycelium can survive summer drought (Brito *et al.*, 2011), drought reduces their abundance in soils (Herzog *et al.*, 2013).

Here we addressed how root, mycorrhiza, and bulk soil respiration differ among vegetation types and soil ages, and how they respond to variations in temperature and moisture. On the Santa Cruz geological chronosequence (White *et al.*, 2008; Moore *et al.*, 2010; White *et al.*, 2012), we looked at the interaction between plant communities and soil age on SMC, to anticipate responses to global change. For this, we manipulated soil communities to partition their respiration contribution in the field.

Our hypotheses were that 1) Soil respiration in grassland communities is more sensitive than woodlands to external changes, such as temperature or soil nutrients availability, because their canopy is less effective in reducing environmental variation and have less SOM to buffer environmental fluctuations; 2) Old soils that are poor in nutrients emit less CO_2, and are more buffered against temporal fluctuation in temperature and moisture because they contain greater amounts of AM mycorrhizal fungi, that allocate C compounds derived from roots and produce recalcitrant compounds less available to be used quickly by soil community.

Methods

Field sites

Field sites were located in tectonically-uplifted marine terraces in the Santa Cruz chronosequence, near Wilder State Park, north of Santa Cruz. Soils were formed by marine sediment and derived from the Ben Lomond granite in the Santa Cruz Mountains. We selected two terraces widely described and classified in previous works (White *et al.*, 2008; Moore *et al.*, 2010; White *et al.*, 2012) differing in geological age.

The selected terraces were 92 kyr old (Young terrace) and 137 kyr old (Old terrace). Plant communities in the terraces were grasslands interspersed by oak trees. The climate is mediterranean with wet, cold winters and warm, dry summers. Mean annual temperature in Santa Cruz between 1948 and 2010 was 13.9ºC and mean annual precipitation was 770 mm (Western Regional Climate Center, http://www.wrcc.dri.edu/). California was under severe drought since 2011 to the experiment date, and Santa Cruz recorded extremely low precipitation (120 mm) in 2013.

Experimental design

In November 2013 we established five plots within each vegetation type (grassland and oak woodland) in both, the young and old terraces. We added a treatment to simulate the effects of global change on the grasslands consisting on open top chambers (OTC) to increase air temperature. In each plot we installed a soil partitioning experiment using the mesh exclusion technique described by Heinemeyer *et al.* (2007) to separate the 3 main components of the soil community; roots, mycorrhizal fungi, and bulk soil. Each block contained a PVC collar (7 cm) in the soil surface which acted as control. Two additional PVC collars (25 cm depth, perforated at 7 cm depth and covered by nylon mesh) were installed. One of the collars was covered with 41 μm mesh to avoid root growth inside and the other was covered by a 1 μm mesh to prevent root and mycorrhiza growth. In each plot we extracted and homogenized soil from 0 to 30 cm depth and filled the three collars with the same soil, letting them recover for one year.

Soil respiration and environmental measurements

We monitored soil respiration with an infrared gas analyzer (IRGA) EGM-4 (PPsystem,UK), from November 2013 to February 2014, to monitor the recovery of the soil after placing treatments (data not shown). From November 2014 to February 2015 we measured soil respiration periodically using a Licor 6400 adapted to a customized

soil respiration chamber, following manufacturer instructions. We recorded soil and air temperature, and volumetric water content hourly, from March to December 2014, on the different terraces, communities and treatments using a HOBO weather station (Onset Computer, Bourne, MA, USA).

Soil sampling

We collected samples from the first 10 cm of soil with a corer (6 cm diameter, 10 cm depth) at the beginning and at the end of the experiment. In the collected soil samples we determined root biomass, AM extra-radical hyphal length, AMF root infection, soil water content (SWC), and bulk soil C and N concentrations. At the beginning of the experiment we collected two cores near each plot more than 0.5 m apart. Due to methodological restrictions, at the end of the experiment we only collected one core from inside each collar, and a control sample from the intact soil. Cores were carefully extracted to preserve the structure and stored on plastic bags in a cooler until they reached the lab. The core extraction was performed under sterile conditions to first collect a subsample for molecular analysis (not included in this work). All cores were weighed and divided transversally in 4 subsamples, and each was weighed. Subsamples from the two cores extracted at the beginning of the experiment in each plot were combined.

Soil analysis

Soil water content was determined gravimetrically in one of the subsamples, after drying the soil at 70ºC for one week. Total N and C were measured by dry combustion in the presence of excess oxygen using a Carlo Erba NA1500 elemental analyzer (Thermo Scientific Inc.). All roots from a subsample were extracted manually and dried at 70ºC for a week. To determine AMF root colonization we followed the protocol described in Emam (2016) on roots randomly selected from a soil subsample. Ten-cm

roots segments were mounted on slides using polyvinyl lactoglycerol and AMF were quantified following the intersection method described by McGonigle *et al.* (1990) using 100 intersections per slide at 400× magnification. To quantify AM hyphal length in soil we followed the method described in Treseder *et al.* (2007), but instead of using 10g of soil we used 5g.

Statistical analysis

Differences in soil respiration between soil ages, plant community and soil components were tested with general linear models (GLM) repeated-measurements analysis, after checking for normal distribution and heteroscedasticity of variance. We controlled the effect of temporal variability within collars in the analysis. Plant community, warming treatment, soil community and terrace age were fixed factors. Treatment means were compared using the LSD Fisher post-hoc test ($p < 0.05$).

We compared differences in plant community and soil age in control plots to determine their effect on soil community respiration. We tested the effect of warming in the grassland only, and evaluated the link between soil respiration, root and hyphal length biomass and soil parameters using linear regression analysis. Analyses were conducted using glm and glmer functions in R (R-Core Team, 2015) using the interface implemented in InfoStat (Di Rienzo *et al.*, 2015). All other statistical analyzes were performed with the InfoStat statistical package.

Results

Soil and air diurnal temperatures fluctuated more in grasslands than under the oaks. The maximum air temperature measured in the grassland was 34.6 ± 0.4 ºC, whereas in the oak woodland was 21.0 ± 0.2 ºC At night, air temperatures under the oak canopy were slightly higher than in grasslands at around 12 ± 0.1 ºC while in grassland temperatures reached 8.9 ± 0.3 ºC (data shown as mean ± SE, Figure 1a). Soil temperature values

were higher on the grassland and had amplitude of ~5.2 ºC between day and night while soil temperature remained stable under the oak canopies (Fig. 1c).

The open top chambers (OTCs) intended to be a warming treatment did not increase air temperatures (Figure 1b) enough. However, OTCs produced a slightly higher air CO_2 concentration (~24ppm; $p<0.01$). The older terrace was warmer and wetter than the younger terrace, and also soil temperatures on grasslands showed stronger day-night fluctuations (Fig. 1d e). Soil moisture was higher in oak woodlands compared to open grasslands. Soil C and N concentrations and the C/N ratio were higher in the oak woodland (3.99 ± 0.18%, 0.29 ± 0.10%, and 13.80 ± 0.16 respectively) than in grasslands (2.10 ± 0.05%, 0.18 ± 0.10%, and 11.63 ± 0.07 respectively), with no significant differences between terraces regarding C and N (Table 1), although the C/N ratio was higher in the older (13.13 ± 0.14) than in the younger terrace (12.31 ± 0.10). The treatment collars did not significantly affect soil moisture content compared to undisturbed soil (Table 1).

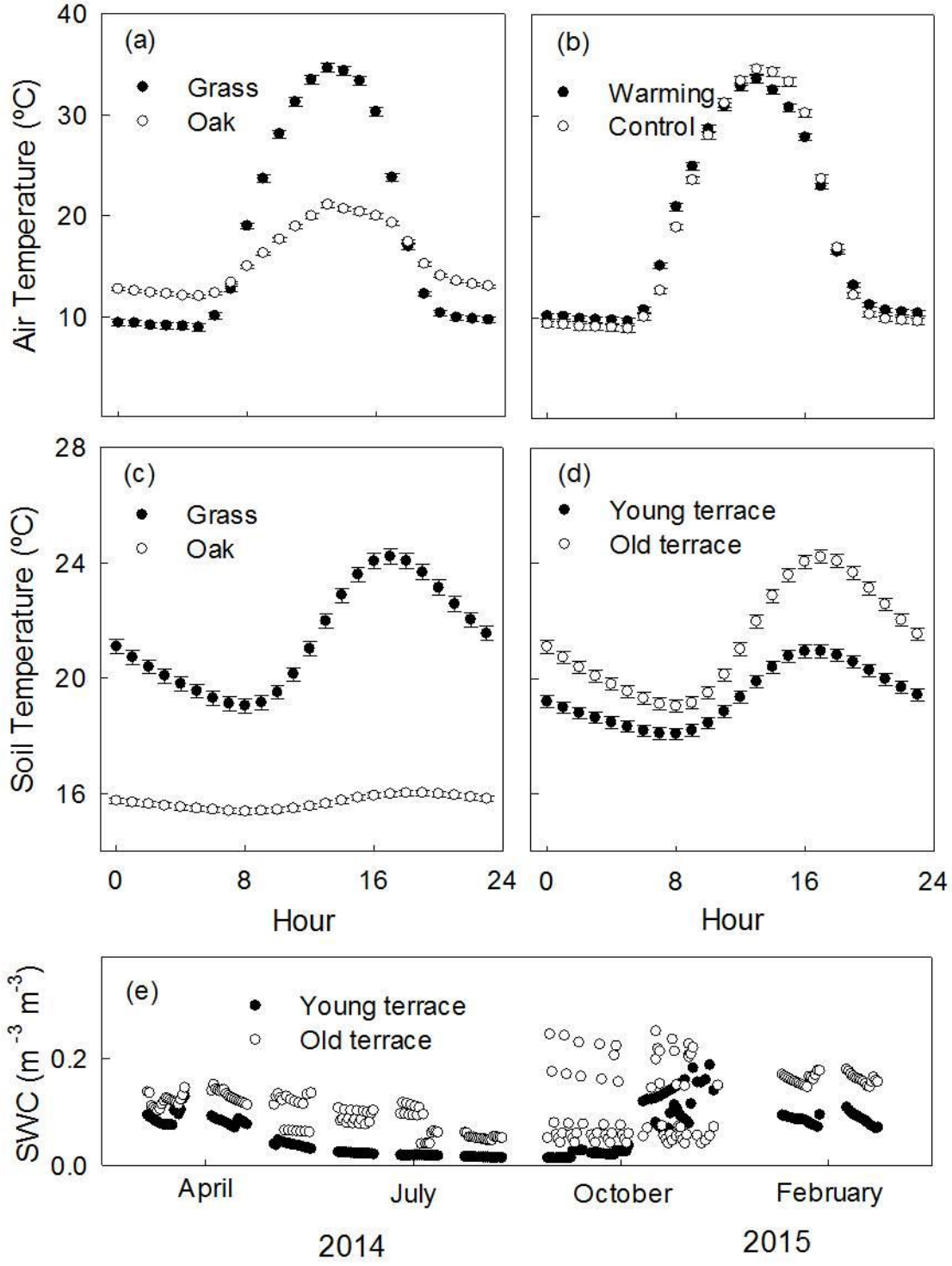

Figure 1. Average values for air temperature between vegetation (a) and open top chambers (OTC) (b); average soil temperature values between vegetation(c) and terraces(d), only in the grassland; Soil water content (SWC) between terraces(e).

Variable	**C**	**N**	**C/N**	**SWC** (control groups)	**Roots** (control groups)	**Roots** (only grasses)	**Hyphal lengths**	**AMF Root colonization**
Terrace	0.85	0.15	**<0.01**	0.60	0.20	**<0.01**	**<0.01**	0.24
Vegetation	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.92	-	**<0.01**	-
Treatment	-	-	-	-	-	0.36	-	0.62
Year	-	-	-	-	-	-	-	0.72
group	0.01	0.12	<0.01	0.60	**<0.01**	**<0.01**	**<0.01**	-
Terrace: vegetation	0.24	0.82	0.84	0.04	0.53	-	0.68	-
Terrace: treatment	-	-	-	-	-	0.31	-	0.44
Terrace :year	-	-	-	-	-	-	-	0.74
Terrace: group	0.44	0.34	0.84	0.69	0.60	**0.02**	0.50	-
Vegetation: group	0.81	0.80	0.97	-	0.68	-	**0.02**	-
Treatment: year	-	-	-	-	-	-	-	0.09
Treatment: group	-	-	-	-	-	0.18	-	-
Terrace:vegetation:group	0.27	0.15	0.56	0.72	0.62	-	0.88	-
Terrace:treatment:year	-	-	-	-	-	-	-	0.89
Terrace:treatment:group	-	-	-	-	-	0.33	-	-

Table 1. Summary of the p values obtained from different analysis GLM, to study the effect of different treatments on soil variables.

The mesh exclusion treatment prevented root growth inside collars. At the end of the experiment, control collars contained 76.11 ± 0.85 g dry root m^{-2} while in the exclusion plots it was less than 16 g dry root m^{-2} (Table S1). However, root abundance did not reach pre-treatment levels, as the amount of roots recorded in the exclusion treatment was much lower than in the undisturbed soil (between 144 ± 22 and 263 ± 98 g dry root m^{-2}).

Extra-radical hyphal length was significantly reduced in the bulk soil community treatment compared to the AMF & bulk soil community treatment (Table S1). We also found more hyphal length in the older than in the younger terrace (148.5 ± 10.9 m m^{-2} vs 107.7 ± 10.1 m m^{-2}, $p < 0.01$) and on grasses than under the oak canopies (155.8 ± 9.8 vs 100.4 ± 11.2 m m^{-2}, $p<0.01$). However, AM root colonization did not differ between treatments, terraces or years (Table 1).

We found a significant time x vegetation x terrace effect in grassland soil respiration. In the younger terrace, there was an increase in soil respiration in the oak woodland by mid-December, as soil moisture peaked after rainfall, which did not happen in the grassland. In the older terrace, respiration in both grassland and oak woodland peaked in wet winter months. Additionally, soil respiration was higher in the younger terrace compared to the older terrace (Figure 2, Table S2).

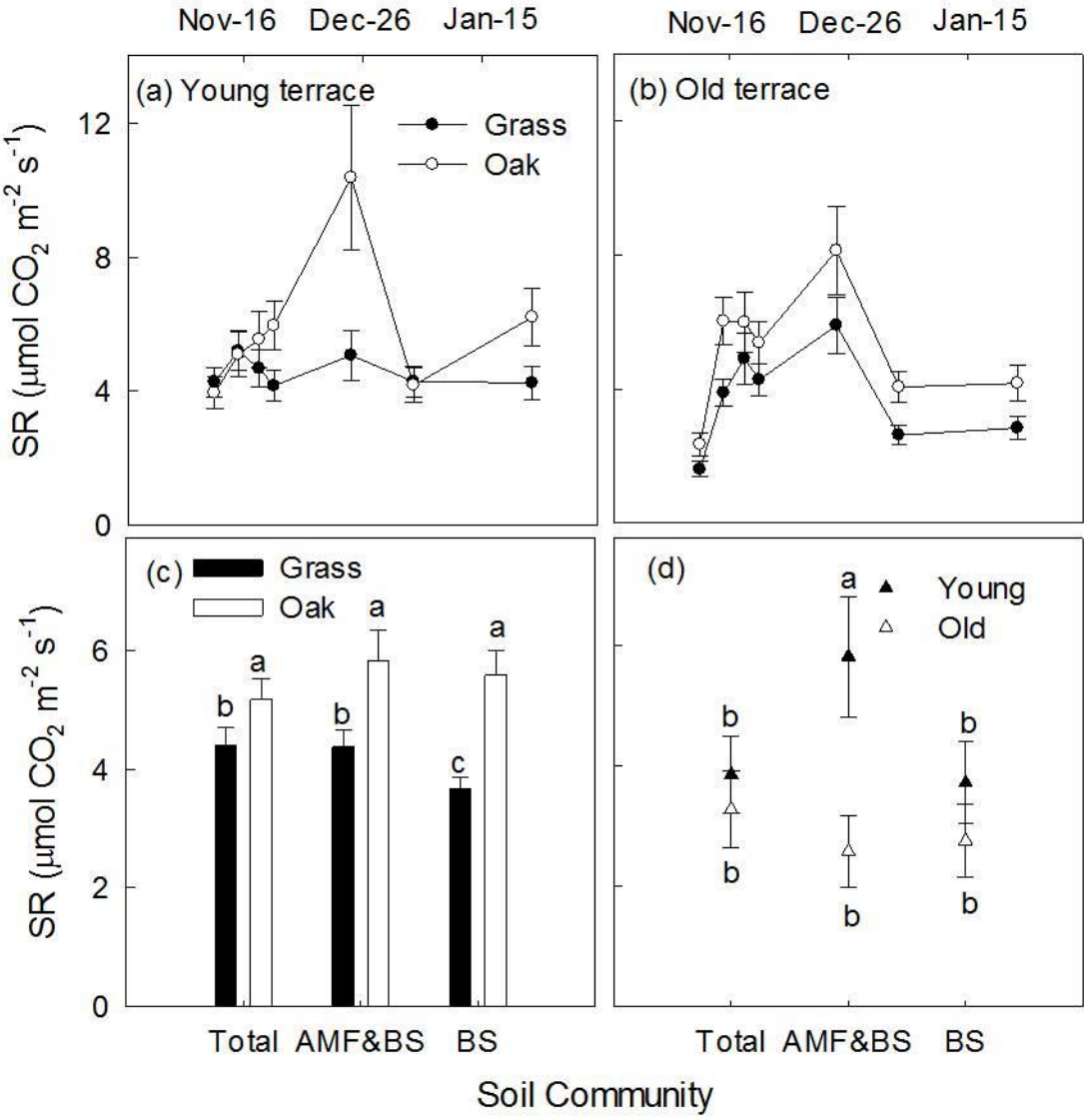

Figure 2. Differences in soil respiration (SR) between vegetation type at the two terraces(a, b), among vegetation type (c) and terrace age (d) depending on soil groups (Total = Total community, AM&BS= AM fungi and bulk soil community, BS= Bulk soil community). Data are mean values with ± 1 S.E

Respiration partitioning differed by plant community type. In the oak woodland there was no difference in respiration rate between exclusion treatments, suggesting that most respiration was from non-AM mycorrhizal soil heterotrophs. In the grassland, there was a significant decline between the bulk soil community treatment and the AM mycorrhizal treatment of about 20% of soil respiration, indicating the importance of AM mycorrhiza on the total soil respiration on grasslands. Roots and AM hyphal

respiration contribution to total soil flux was lower than the bulk soil contribution and, despite the high abundance of roots in the no exclusion treatment, soil respiration did not increase in comparison with the root exclusion treatments (Fig. 2c). Terrace age also affected how flux was partitioned among different soil components (Fig. 2d, Table S2). If we look at the AMF & bulk soil community collar, much more C was respired in the younger terrace than in the older terrace; when we look at bulk soil collars and total community collars, there were no differences in soil respiration between terraces.

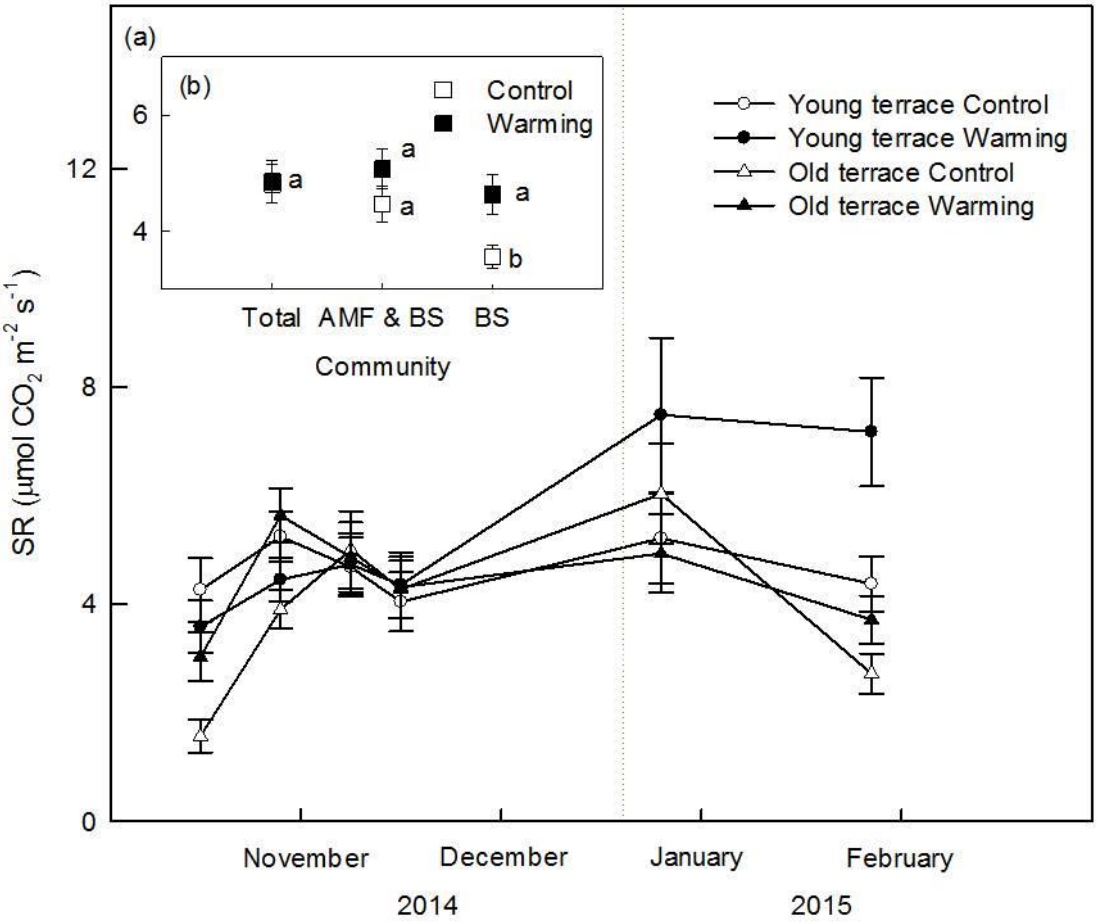

Figure 3. Soil respirations along time on the grassland plots on two terraces and under the two treatments (Control, warming) (a), and differences between treatments (b) on the different soil communities. (Total community, AM fungi and bulk soil community, Bulk soil community). Data are mean values with ± 1 S.E. different letters mean significant differences from the post hoc analysis. Dotted line indicates a strong rainy event.

The open top chambers (OTCs) increased soil respiration to a greater extent in the younger than in the older terrace ($p<0.01$, Table S2). Differences between the OTC treatment and control were primarily due to higher respiration by the bulk soil community, the total community and root exclusion treatments did not show differences between OTC treatments and (Fig. 3).

Across all terraces and treatments, soil respiration was positively correlated with total C, N and C/N ratio (Fig. 4 a,b,c). However, dispersion was still high indicating that other factors affected respiration. The C/N ratio had little influence on the response ($R^2= 0.13$). Extra-radical hyphal length was also correlated with N and C, and the relation was opposite to soil respiration values, while soil respiration increased with nutrient availability, the extra radical hypha length decreased (Fig. 4d,e); C/N ratio did only affect hyphal length in the bulk soil community (root and hyphal exclusion treatment), where there was a decrease in hyphal length biomass with an increase in the C/N ratio (Fig. 4f). There was also a slight but significant increase in soil respiration with root biomass (Fig. 5).

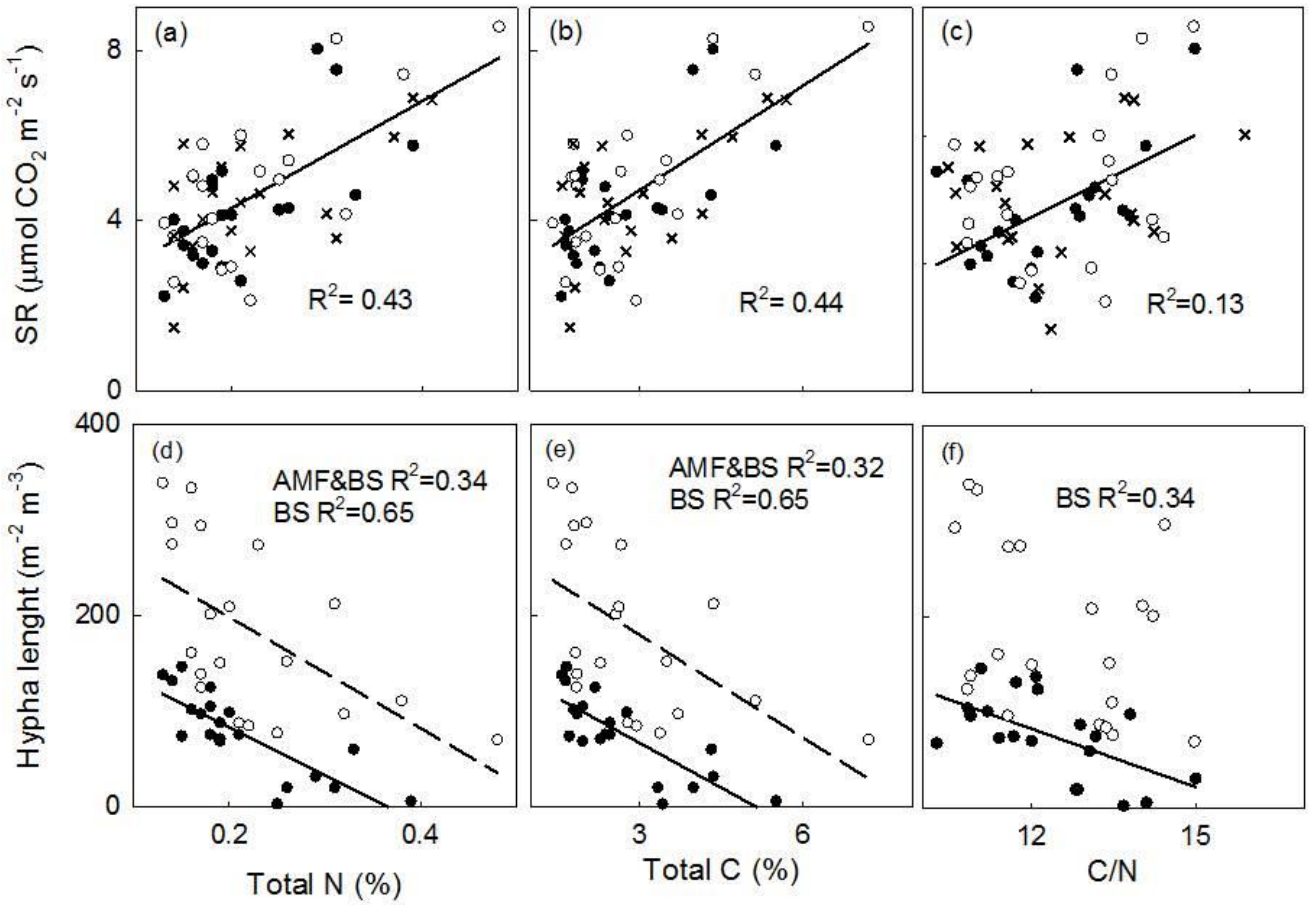

Figure 4. results from linear regression showing the relationship between medium soil respiration and total N (a), total C (b) and C/N ratio (c). Results from linear regression showing the relationship between hypha length and total N (d), total C (e) and C/N ratio (f). Soil respiration is the average values for the whole period, all R2 shown were significate ($p \leq 0.01$) (Total soil community: bold x; AM fungi and bulk soil community: clear circles; bulk soil: bold circles).

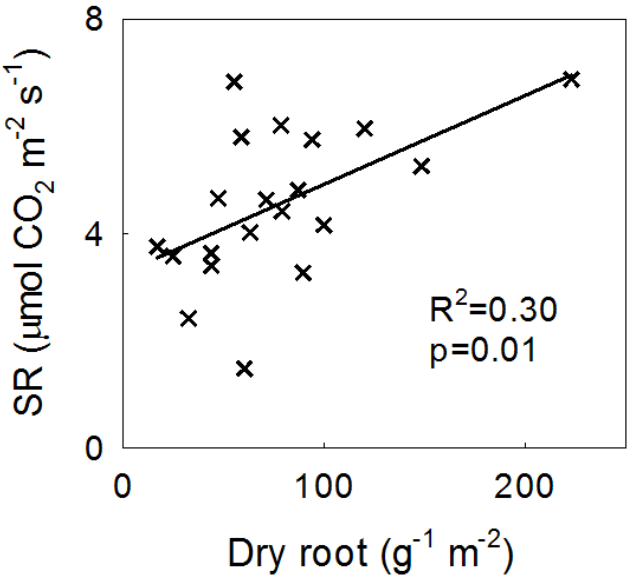

Figure 5. results from linear regression showing the relationship between root biomass and soil respiration on the total soil community in control plots. Soil respiration is the average values for the whole period.

Discussion

Our results show how AMF modulates the soil capacity to emit CO_2 in grasslands. The development of AMF is determined by factors such as plant community composition and soil properties such as nutrient availability (Allison *et al.*, 2005; De Vries *et al.*, 2012; Bunn *et al.*, 2015). Overall, plant community type had a stronger effect on AMF abundance than soil variables. The soil disturbance produced in our treatments did not preclude comparisons between different plant communities, soil type and environment, since all treatments started from the same starting point.

Plant community and soil age effects on soil community and soil respiration

In agreement with our first hypothesis, soil respiration was affected by plant community composition and, while oak patches behaved similarly in both soil ages, there were differences due to soil age in the grassland soil community that affected soil respiration. While the plant community did not affect the development of surface roots, AMF

abundance was higher in grasslands than under the oak canopies, as documented in previous studies (Treseder & Cross, 2006). In woodlads, greater accumulation of SOM reduces AMF abundance, because AMF are not capable to release N compounds from SOM, and it produces a disadvantage with other microbial groups such as ectomycorrhizal fungi (Averill *et al.*, 2014; Soudzilovskaia *et al.*, 2015).

Soil age effects depend on nutrient availability. Our study suggests that soils poor in nutrients have lower soil respiration rates possibly due to changes in AMF abundance. While AMF abundance was strongly affected by the presence of oak trees, in grasslands, hyphal lengths increased in the older terraces which could be related to reductions in soil nutrients as C and N content (Moore *et al.*, 2010; Terrer *et al.*, 2016). The negative relationship with N is in agreement with the mutualism-parasitism continuum concept (Johnson & Graham, 2013), which predicts that in nutrient-poor environments plants benefit more from symbiosis than in nutrient–rich environments (Gerz *et al.*, 2016).

Higher abundance of AMF in nutrient poor soils could increase C sequestration capacity of these soils. One of the ways in which the AMF act is by incorporate C derived from root exudates into hyphal tissues (Heinonsalo *et al.*, 2010; Clemmensen *et al.*, 2013) some of the hyphal components (chitin and glomalin) are highly recalcitrant and remain unaltered for long periods of time, being an important sink for terrestrial C (Treseder & Allen, 2000). Our results agree with the hypothesis that AMF increases C storage in soils (Heinonsalo *et al.*, 2010; Carrillo *et al.*, 2016), and disagree with recent hypothesis that also predict that AMF may increase CO_2 release by facilitating the access of saprophytes to SOM (Cheng *et al.*, 2012).

Soil community effects on total and seasonal response in soil respiration

Our second hypothesis was only partially supported because, although older soils with more hyphal length emitted less CO_2, with 16% lower soil respiration in the older terrace compared to the young terrace, older soil were not more buffered against environmental fluctuations. Many nutrient limited and low pH soils have been shown to have greater dominance of AMF fungi (Gerz *et al.*, 2016). If older, more weathered, and more nutrient limited soils develop more abundant hyphal networks, this could affect how C is cycled in soils by reducing rates of soil respiration, with implications for carbon sequestration in those ecosystems.

We established that AMF respiration was 20% of total respiration, similar to other studies on grasslands (Heinemeyer *et al.*, 2012). Since we used a shallow collar for the non-exclusion treatment, and root growth did not recover to pre-treatment levels, we were unable to accurately quantify undisturbed rates of root respiration. Although our data cannot be taken as a quantitative estimation of soil respiration in our ecosystem, since they were recovering from experimental disturbance, they can be used to compare relative differences among soil ages and plant communities.

The correlation between root biomass and soil respiration reflected low contribution of root respiration to total soil respiration. However, roots had great importance as regulators of ecosystem response because, in treatments with roots, the response to environmental changes such as soil age, warming, or plant communities, was buffered in comparison to treatments containing only mycorrhiza and bulk soil (Figs. 2b,c and 3b). This buffering effect could be related with root effects on soil water content; high abundance of roots reduce soil moisture (Luan *et al.*, 2011) and soil metabolic processes depend largely on soil moisture, especially in dry CA soils. Thus, soil moisture enhances soil respiration by increasing substrate supply as moisture

increases substrate diffusion (Davidson *et al.*, 2006a). Roots can also affect the C cycle through exudates, producing root exudates may 'prime' the microbial community to decompose SOM (Fuchslueger *et al.*, 2016), but exudates themselves are important components of SOM. Thus roots greatly control the C cycle.

Factors affecting soil community, and implications for global change scenarios

Our OTCs simulated a scenario with slightly higher CO_2 but did not increased air temperature most likely because their warming effect was compromised by the tall vegetation surrounding the chambers. They may have, in addition, affected factors that we failed to measure but led to differences in soil respiration. We can only guess that they modified the bulk soil community. In the presence of rhizosphere, OTCs had not effect on soil respiration rates, supporting the hypothesis that mycorrhiza increase C storage in soils (Heinonsalo *et al.*, 2010; Carrillo *et al.*, 2016) buffering the effects of environmental modification. OTCs effects on soil respiration was mostly observed in soils with low AM ratio (Moore *et al.*, 2010), further supporting our conclusion that mycorrhizas buffers the response in soil respiration against environmental changes.

Future global change scenarios will modify soil C dynamics by altering hyphal development through lower water availability, higher temperature or nutrients. In fact, some reports showed a decline in mycorrhizal fungi with drought (Brito *et al.*, 2011; Herzog *et al.*, 2013). The capacity of grassland communities to sequester carbon depends on several factors that need be considered in order to understand soil dynamics in those ecosystems. Soil nutrient availability will have a high impact in the development of AMF communities since we observed a negative effect between soil nutrients and soil hyphal lengths (Gerz *et al.*, 2016) Conservation and management

programs that take into account the impact of soil traits and plant communities on AMF development will contribute to mediate potential C losses from soils.

Conclusion

Changes in plant communities and soil traits (depending on soil age) affect soil community structure and both the partitioning of soil respiration and the response of the different components to changes in moisture and temperature. Our results show that in CA grasslands the AMF matrix is affected by vegetation type and soil age, which in turn mediates carbon fluxes from soil. Soil microbial communities in CA grasslands may mitigate CO_2 emissions depending upon factors that influence the abundance and activity of AMF fungi in soil.

Appendix

		Young terrace		Old terrace	
		Grass	Oak	Grass	Oak
C	Initial	2.27 ± 0.21^{de}	3.45 ± 0.33^{abc}	2.27 ± 0.21^{de}	3.95 ± 0.27^{ab}
	Total	1.90 ± 0.14^{e}	3.82 ± 0.55^{abc}	1.88 ± 0.13^{e}	3.91 ± 0.60^{abc}
	AMF & BS	1.81 ± 0.12^{de}	4.46 ± 1.01^{ab}	2.00 ± 0.15^{e}	3.17 ± 0.30^{bc}
	Bulk soil	1.85 ± 0.13e	3.69 ± 0.49^{abc}	1.90 ± 0.14^{e}	3.26 ± 0.33^{abc}
	Control	2.37 ± 0.14^{d}	5.15 ± 0.87^{ab}	3.01 ± 0.22^{c}	4.76 ± 0.89^{a}
N	Initial	0.19 ± 0.01^{efg}	0.24 ± 0.02^{bcd}	0.18 ± 0.01^{fg}	0.27 ± 0.02^{abc}
	Total	0.17 ± 0.01^{fg}	0.30 ± 0.04^{abc}	0.16 ± 0.01^{g}	0.28 ± 0.03^{abc}
	AMF & BS	0.17 ± 0.01^{fg}	0.33 ± 0.05^{ab}	0.17 ± 0.01^{fg}	0.24 ± 0.02^{cde}
	Bulk soil	0.17 ± 0.01^{fg}	0.28 ± 0.04^{abc}	0.16 ± 0.01^{g}	0.23 ± 0.02^{bcd}
	Control	0.20 ± 0.01^{def}	0.38 ± 0.06^{a}	0.25 ± 0.02^{bc}	0.31 ± 0.05^{abc}
C/N	Initial	11.71 ± 0.32^{fg}	14.21 ± 0.32^{abc}	12.53 ± 0.19^{def}	14.46 ± 0.26^{ab}
	Total	10.82 ± 0.17^{h}	12.92 ± 0.32^{cde}	12.92 ± 0.32^{fg}	14.21 ± 0.63^{abc}
	AMF & BS	10.93 ± 0.19^{h}	13.34 ± 0.40^{bcd}	12.13 ± 0.32^{fg}	13.64 ± 0.47^{abc}
	Bulk soil	10.91 ± 0.18^{h}	13.03 ± 0.034^{cde}	11.73 ± 0.36^{fg}	13.64 ± 0.47^{abc}
	Control	11.69 ± 0.30^{g}	13.46 ± 0.46^{abc}	12.17 ± 0.31^{efg}	15.5 ± 0.97^{a}
Root bio.	Initial	-	-	-	-
	Total	83.99 ± 18.31^{ab}	70.41 ± 12.30^{ab}	54.71 ± 11.46^{b}	97.71 ± 40.37^{ab}
	AMF & BS	15.39 ± 4.79^{c}	5.32 ± 3.25^{c}	14.42 ± 6.58^{c}	8.76 ± 4.36^{c}
	Bulk soil	15.50 ± 6.07^{c}	5.16 ± 2.48^{c}	6.84 ± 54.26^{c}	13.43 ± 7.67^{c}
	Control	263.04 ± 98.55^{a}	252 ± 83.75^{a}	144.25 ± 22.39^{a}	160 ± 28.70^{a}
AM length	Initial	89.27 ± 23.52^{cde}	99.19 ± 23.52^{cde}	147.81 ± 23.52^{bcd}	148.39 ± 23.52^{bcd}
	Total	-	-	-	-
	AMF & BS	210.29 ± 23.52^{ab}	103.60 ± 23.52^{cde}	266.72 ± 23.52^{a}	156.79 ± 23.52^{bc}
	Bulk soil	89.27 ± 23.52^{de}	46.56 ± 23.52^{e}	123.34 ± 23.52^{cd}	48.26 ± 23.52^{e}
	Control	-	-	296.75 ± 59.20^{a}	143.67 ± 24.84^{bcd}
SWC	Initial	13.85 ± 0.45^{b}	13.26 ± 0.61^{b}	15.78 ± 0.43^{b}	14.58 ± 0.61^{b}
	Total	15.63 ± 1.58^{b}	15.56 ± 1.41^{b}	14.13 ± 1.29^{b}	17.04 ± 1.41^{b}
	AMF & BS	16.61 ± 1.58^{b}	16.27 ± 1.41^{b}	15.30 ± 1.29^{b}	17.93 ± 1.41^{b}
	Bulk soil	16.21 ± 1.58^{b}	16.55 ± 1.41^{b}	15.50 ± 1.29^{b}	16.42 ± 1.41^{b}
	Control	15.66 ± 1.19^{b}	17.76 ± 1.41^{b}	14.84 ± 1.12^{b}	21.96 ± 1.41^{a}

Table S1. Soil properties of the different plant communities and terraces, including Total C (%) and N (%), C/N rate, dry root biomass (Root bio.; g^{-1} m^{-2}) AM extra radical hyphal length (m m^{-2} soil) and soil water content (SWC; %). The second column includes soil community treatments: Total= No exclusion, AMF & BS = root exclusion, Bulk soil = Root and hyphae exclusion, Control= no manipulation, end of experiment. Values are means ± SE. Values within a variable with different letters denote significant differences across vegetation types, soil ages and soil community treatments.

	Variable	DF	F-value	p-value
GLM 1	(Intercept)	1	1124.25	**<0.01**
	DOY	6	13.77	**<0.01**
	terrace	1	7.58	**0.01**
	Vegetation	1	23.13	**<0.01**
	group	2	0.85	0.43
	DOY:terrace	6	2.84	**0.01**
	DOY:Vegetation	6	1.69	0.12
	DOY:group	12	1.64	0.08
	terrace:Vegetation	1	0.03	0.87
	terrace:group	2	1.75	0.18
	Vegetation:group	2	1.64	0.20
	DOY:terrace:Vegetation	6	1.22	0.30
	DOY:terrace:group	12	0.5	0.92
	DOY:Vegetation:group	12	0.66	0.79
	terrace:Vegetation:group	2	1.22	0.30
	DOY:terrace:Vegetation:group	12	0.23	0.99
GLM 2	(Intercept)	1	12.87	**<0.01**
	DOY	5	7.57	**<0.01**
	terrace	1	9.56	**<0.01**
	treatment	1	4.54	**0.03**
	group	2	3.76	**0.02**
	air.temp	1	0.36	0.55
	DOY:terrace	5	3.87	**<0.01**
	DOY:treatment	5	1.27	0.27
	DOY:group	10	2.09	**0.02**
	terrace:treatment	1	0.10	0.74
	terrace:group	2	0.56	0.57
	treatment:group	2	1.35	0.26
	DOY:terrace:treatment	5	3.39	**<0.01**
	DOY:terrace:group	10	0.60	0.81
	DOY:treatment:group	10	0.62	0.79
	terrace:treatment:group	2	0.60	0.20
	DOY:terrace:treatment:group	10	1.02	0.42

Table S2. Results from GLM 1 comparing terrace vegetation type, DOY and group on the control plots, and GLM 2 comparing terrace, global change treatment, DOY and group on the control plots only on the grasslands.

Chapter IV:

Carbon sequestration capacity and secondary succession in a dry environment

Carme Estruch, Yudi M. Lozano, Cristina Armas, Francisco I. Pugnaire

Abstract

Human activities often alter natural plant communities which, after disturbance, undergo a process of secondary succession with important changes in C dynamics. In arid environments this process is very slow, and its links with C cycling are little known. We addressed changes in C balance along a chronosequence of land abandonment in a semiarid environment and assessed the consequences of secondary succession on C sequestration capacity at community scale. We used a closed-chamber method to estimate the contribution of whole-plants and bare soil to whole-ecosystem C exchange. Plant community composition and cover strongly affected C balance. Overall, whole-ecosystem C exchange shifted from C source to C sink with succession. However, only after 63 years of agriculture abandonment the system did recover its natural C sequestration capacity. Thus, the capacity of semiarid ecosystems to recover native plant communities after anthropogenic disturbance may contribute to decrease C emissions in the long term.

Introduction

Drylands comprise ca. 41% of terrestrial ecosystems (UN, 2011) and despite a scant plant cover they are critical carbon sinks at the global scale (Ahlström *et al.*, 2015) even though they lost part of their stored C pools as a consequence of land use changes (Plaza-Bonilla *et al.*, 2015). Recent reports suggest, however, that land abandonment and new dryland management practices would allow regeneration of native plant communities and contribute to increase stored C (Álvaro-Fuentes & Paustian, 2011; Plaza-Bonilla *et al.*, 2015).

Plant community structure has a strong influence on ecosystem processes that remove CO_2 from the atmosphere (Sundquist *et al.*, 2008) mainly through processes affecting soil respiration rates (Tedeschi *et al.*, 2006; Tang *et al.*, 2009; Luan *et al.*, 2011) such as litter production (Zhang *et al.*, 2013) and decomposition (Holden *et al.*, 2013; Osono *et al.*, 2013). The combination of soil respiration and plant metabolism drives net ecosystem exchange (NEE) (McMillan *et al.*, 2008; Béziat *et al.*, 2009; Ferlan *et al.*, 2011), an indicator of ecosystem capacity to remove CO_2 from the atmosphere. Changes in plant community structure, including cover and species richness, are evident during secondary succession (Chapin *et al.*, 2008) and reports from temperate and boreal systems have evidenced that successional changes affect C balance, as late-successional stages usually show higher C sequestration capacity than early stages (Noormets *et al.*, 2007; Goulden *et al.*, 2011; Wang & Epstein, 2013). However, changes in species composition and cover in semiarid systems during secondary succession are little known (Bonet, 2004; Bonet & Pausas, 2004; Lozano *et al.*, 2014), and very few reports address their contribution to C fluxes (Delgado-Balbuena *et al.*, 2013; Lozano *et al.*, 2014). In semiarid grasslands increases in water

availability drive NEE shifts from source to sink (Serrano Ortiz *et al.*, 2014) and soil respiration prevails while water limits photosynthesis (López-Ballesteros *et al.*, 2016).

Here we assessed ecosystem C sequestration capacity along a secondary succession chronosequence spanning ~100-years in an arid environment in SE Spain. Our aim was to test how plant community recovery influenced NEE since secondary succession changes plant cover, plant functional groups, plant species composition and soil characteristics (Lozano *et al.*, 2014; Gabarrón-Galeote *et al.*, 2015). We focused on the role of different plant strategies on the C cycle and expected in late successional stages both higher gross primary productivity (GPP) linked to increases in plant cover, and higher soil respiration rates linked to soil organic matter accruement. Ultimately, these changes would lead to an increase in NEE, providing a switch in C fluxes from source to sink between early- and late-successional stages. We expected these changes to be slow in semiarid systems due to overall low metabolic rates.

Methods

Field site

Our field site was in the Llanos de Rueda floodplains (37.05^{o}N, 2.22^{o}W, 503 m elevation) in the Tabernas basin (Almeria, Spain). The climate is semiarid with a mean annual precipitation of 235 mm, mild winters (mean minimum temperature 4.1^{o}C) and hot summers (mean maximum 34 ºC; Lázaro *et al.*, 2001). Soils are orthic solonchak with inclusions of calcic regosol, characterized by low organic matter content, low water holding capacity, moderate alkalinity (8.5 in pH), and low electrical conductivity. Our chronosequence of abandoned arable fields included five successional stages according to time of abandonment: from bare fields (1-4 years after abandonment) to the native semiarid community (>84 years; see more details in Lozano *et al.*, 2014). The mature community is a sparse scrubland with interspersed grasses, and the dominant

species change with successional stage from *Thymelaea hirsuta* (L.) Endl. (12-15 years old plots), to *Artemisia barrelieri* (Besser) Soják (56-60 years), *Hammada articula*ta (Moq.) O. Bolos & Vigo and *Salsola oppositifolia* Desf. (63-65 years) and, finally, *Stipa tenacissima* L. and *Helianthemun almeriense* Pau (>84 years, native communities); these species are hereafter referred to by genus only (Table 1).

Experimental design

We selected three 30 m^2 plots per successional stage and, within each, we randomly selected 5 individuals of the dominant species and a nearby bare spot to measure soil CO_2 fluxes. For statistical analyses we pooled all 5 plant individuals per species and gap measurements per plot (n=3). Plant species were measured at the successional stage where they were most abundant, typically 1-2 species per successional stage (Table 1), as they would represent optimum conditions for that stage. Measurements were used to calculate CO_2 fluxes at the community level, and were carried out along the 2014 growing season; in February, April and May at random across plants and gaps, and under full sun irradiance between 09:00-16:00 GMT in days with clear sky.

	Years after land abandonment				
	3	**12**	**56**	**63**	**Native (>84)**
Artemisia barrelieri	0.02±0.02	2.35±0.89	**17.96±3.28**	4.07±1.30	1.33±0.84
Hammada articulata		0.02±0.02	4.91±1.28	**10.09±1.70**	
Helianthemun almeriense			0.54±0.35	2.26±1.38	**17.38±1.16**
Salsola oppositifolia	0.02±0.01		0.57±0.42	**10.42±2.86**	0.71±0.38
Stipa tenacissima				0.03±0.03	**42.38±3.33**
Thymelaea hirsuta		**3.83±1.47**	0.30±0.30	0.45±0.23	
Total Cover (%)	10 %	78 %	94 %	83 %	78 %

Table 1. Percent cover of each of the dominant plant species along the different successional stages. Data are means ±1 SE, bold letters indicates the plant species that were measured in the corresponding successional stage. Data extracted from (Lozano *et al.*, 2014).

Closed chamber system for whole-plant gas exchange measurements

We built a large polycarbonate chamber attached to an aluminum frame (96 cm x 94 cm x 80 cm) with two small fans (12 W, 0.14 A) to thoroughly mix air inside. The chamber top was not fixed to allow ventilation prior to each measurement. Chamber isolation was secured with foam along the seams and a plastic flap at the base in each side covered with a heavy metal chain. Pérez-Priego *et al.* (2015) estimated that leakage from large chambers is negligible in short-term measurements (less than 3 min). The chamber was connected to an infrared gas analyzer (EGM4, PP Systems, Amesbury, MA, USA), and measurements started 10 seconds after the chamber was closed followed by a 15-second period of flux stabilization, recording data for 120 seconds. Air temperature and relative humidity were monitored inside the chamber with iButton sensors (Sunnyvale, CA, USA) 80 cm above the soil surface. PAR radiation was measured outside the chamber with a digital radiation meter PCE-174 (GHM, Erolzheim, Germany). PAR inside the chamber was reduced by 10% (data not shown).

We adjusted data to a linear fitting, more appropriate for short-term measurements (Koskinen *et al.*, 2014). We measured soil respiration from bare soil using an EGM4 infrared gas analyzer attached to a SRC-1 chamber following manufacturer (PP Systems, Amesbury, MA, USA) directions.

Plant CO_2 flux calculation

Considering one individual plant and its surrounding soil as a unit, we measured net CO_2 flux under light conditions to determine plant net exchange rate (PNE) and then covered the chamber with black, opaque fabric to estimate plant respiration (PR); plant photosynthesis (PH) was determined by subtraction from Equation 1. We show C emission as positive values and C fixation as negative.

$$PNE = PH\text{-}PR \quad (1)$$

CO_2 flux was obtained using the linear change in CO_2 concentration over time, controlling for chamber volume, air temperature, and water dilution effects (Pérez-Priego *et al.*, 2015).

We related C fluxes to the area occupied by the plant and considered the plant canopy plus the soil underneath as our measurement unit. When the plant canopy did not occupy the entire chamber area we extracted bare soil respiration from the area not covered by the plant from total fluxes using the following equation:

$$TE = F \times \frac{Ap}{100} + SR \times \frac{As}{100} \quad (2)$$

Where TE is total CO_2 flux recorded in the chamber; F is the fraction of CO_2 flux related to the plant that equals PNE when the measurement is done under light

conditions and PR under dark conditions (PNE and PR in Eq. 1), Ap is the projected canopy area, SR is the bare soil respiration rate, and As is the chamber area occupied by bare soil.

Community fluxes

We estimated the effect of plant composition and plant cover on CO_2 exchange by using the relative cover of every plant species and bare soil in each community (Table 1); we used mean values to assess fluxes of the different plant species combined with relative plant cover in each plot (30 m^2, n=3) using equations:

$$GR = \sum_{i=1}^{N} \left(PR_i \times \frac{Cov_P}{100} \right) + \left(SR \times \frac{Cov_S}{100} \right) \quad (3)$$

$$GPP = \sum_{i=1}^{N} \left(PH_i \times \frac{Cov_P}{100} \right) \quad (4)$$

$$NEE = \sum_{i=1}^{N} \left(PNE_i \times \frac{Cov_P}{100} \right) + \left(SR \times \frac{Cov_S}{100} \right) \quad (5)$$

Where GR is ecosystem gross respiration, GPP is ecosystem gross primary production, NEE is net ecosystem exchange (which indicates the C sequestration capacity), and *Cov* is the area occupied by the different plant species (p) and bare soil (s).

Statistical analysis

We used general linear models (GLM) with repeated-measures in a factorial design to test for differences in PNE, PH, and PR among the 6 selected plant species, which was the fixed factor, while PAR and air temperature were included as covariates. We also used GLM with repeated-measures to test for differences in soil respiration, NEE, GPP, and GR across plant successional stages (5 levels), which was the fixed factor. Means were compared using the LSD Fisher post-hoc test ($p < 0.05$) after checking the

assumptions of normal distribution and homoscedasticity of variances. Analyses were carried out using the glm and glmer functions in R (R-Core Team, 2015) using the interface implemented in InfoStat statistical package (Di Rienzo *et al.*, 2015).

Results

Soil and plant fluxes

Bare soil respiration (SR) significantly increased with successional time, reaching the highest values 63 years after agriculture abandonment, and decreased afterwards. SR peaked in April and the lowest values were obtained in May; only in the earliest successional stage (5 years after abandonment) SR did not change along the growing season (Fig. 1).

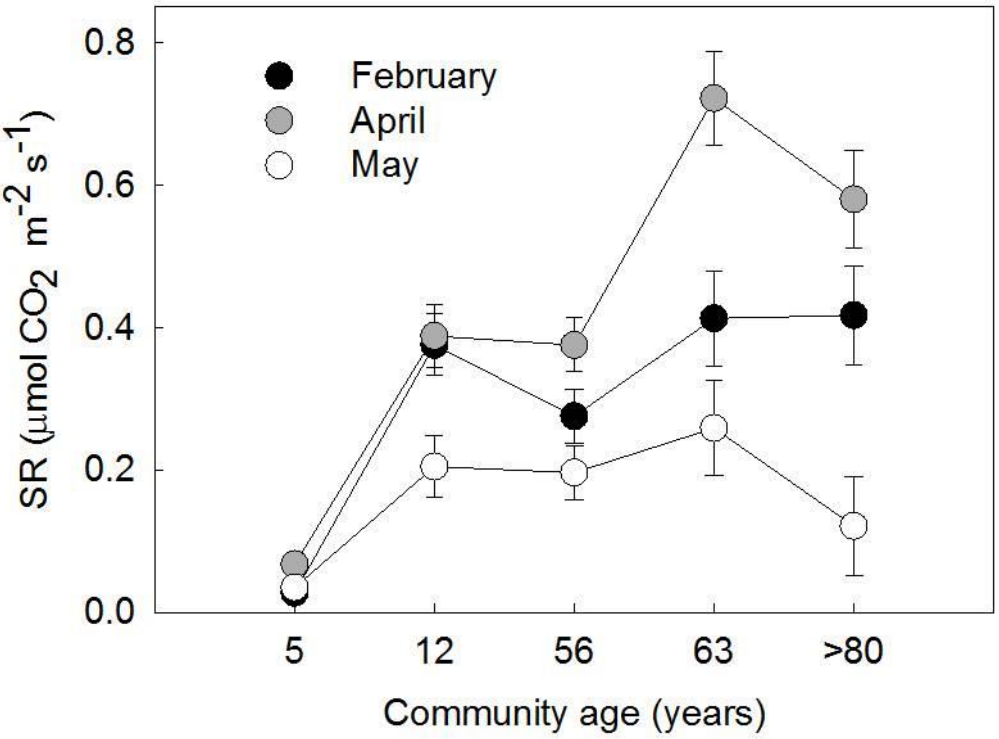

Figure 1. Changes in bare soil respiration (SR) at different successional stages. Symbols and bars are means ± 1 SE. Different letters indicate significant differences ($p < 0.05$) across months and successional stages after Fisher's LSD test; n= 3.

Carbon fluxes were affected by the interaction between plant species and time (Table 2). Overall, plant respiration (PR) remained steady along the growing season in each plant species; *Thymelaea* showed the highest PR rates of all species and for each measurement period (4.23 ± 0.87, 5.26 ± 0.87 and 3.81 ± 0.86 μmol CO_2 m^{-2} s^{-1} in February, April, and May, respectively) while *Salsola* peaked in April (4.52 ± 0.42 μmol CO_2 m^{-2} s^{-1}) with similar respiration rate as *Thymelaea* (Fig. 2a). Both *Thymelaea* and *Salsola* reached the highest PH rates across species in February and April (-8.01±1.15 and -8.38±1.23 and -10.33 ± 1.05 μmol CO_2 m^{-2} s^{-1} in February, and -11.10 ± 1.84 μmol CO_2 m^{-2} s^{-1} in April) while *Artemisia* showed the lowest rates across species and was later in the season (-1.4 ± 1.12 μmol CO_2 m^{-2} s^{-1}, May; Fig. 2b). All plant species were acting as C sinks (Fig. 2c) except *Artemisia* in May (PNE = 1.08 ± 0.45 μmol CO_2 m^{-2} s^{-1}), the only species recorded as C emitter. *Thymelaea* and *Salsola* showed the highest C sequestration rates (PNE) in February (-4.10 ± 0.72 and -6.07 ± 1.30 μmol CO_2 m^{-2} s^{-1}, respectively) and April (-4.64 ± 0.72 and -5.95 ± 1.30 μmol CO_2 m^{-2} s^{-1}, Fig. 2c). C sequestration rates in *Hammada*, *Heliantemum* and *Stipa* plants did not significantly change along the growing season (Fig. 2c).

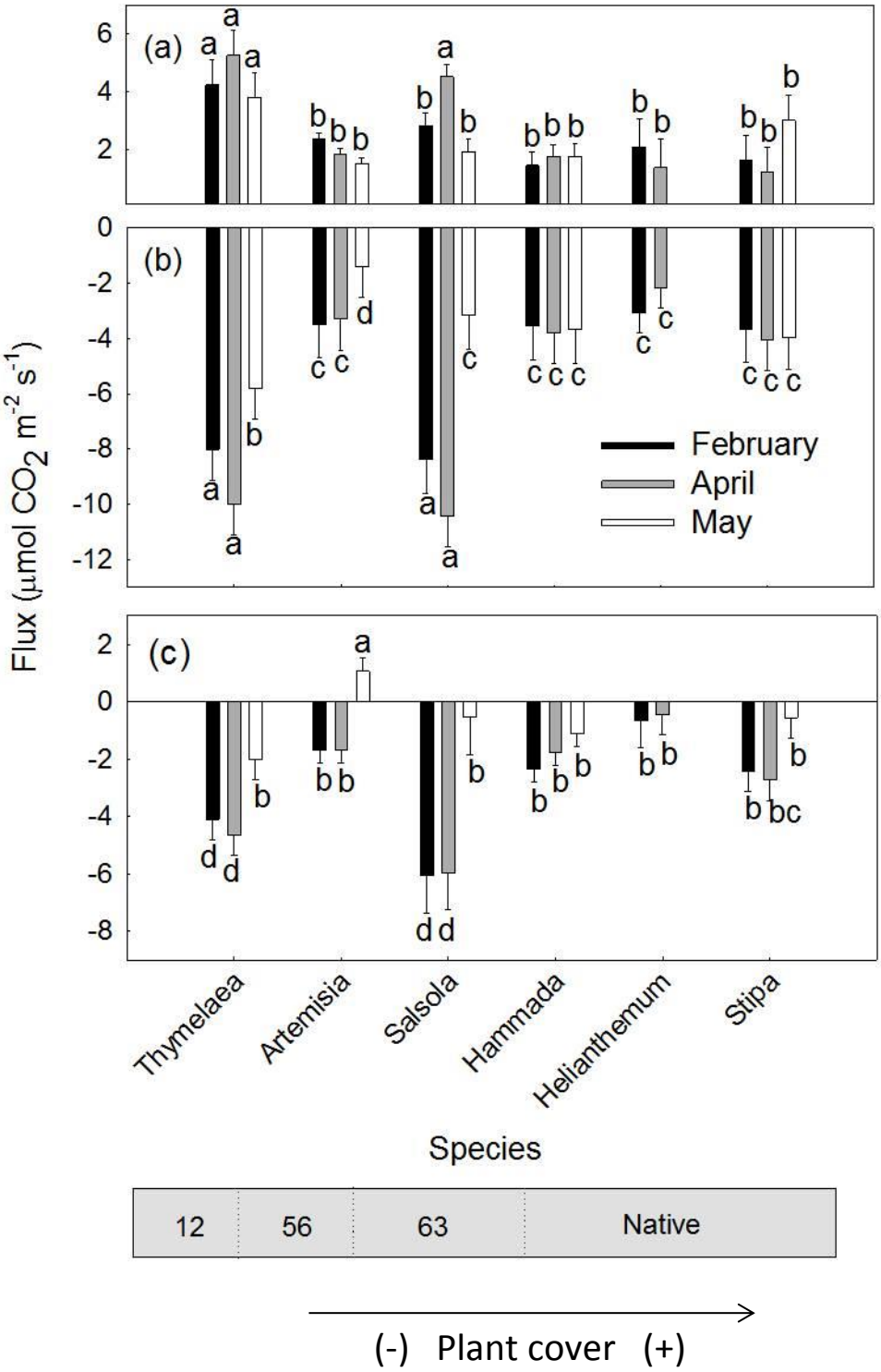

Figure 2. CO_2 fluxes in six different plant species at three measurement times: Plant Respiration (PR, a), Photosynthesis (PH, b), and Plant Net Exchange (PNE, c). Data are means ± 1 SE. Different letters show significant differences across months and species ($p<0.05$). The grey box below the figure indicates the successional stage at which the different plant species were measured. The arrow indicates the direction in which vegetation cover increases with time.

		df	F-value	p-value
Plant species				
PNE	Species	5	6.32	**<0.01**
	Month	2	21.09	**<0.01**
	PAR	1	4.02	0.06
	Temperature	1	1.16	0.29
	Species: Month	10	3.08	**0.01**
PH	Species	5	10.33	**<0.01**
	Month	2	11.12	**<0.01**
	PAR	1	2.69	0.59
	Temperature	1	0.30	0.77
	Species: Month	10	0.09	**0.02**
PR	Species	5	7.58	**<0.01**
	Month	2	1.30	0.28
	PAR	1	2.70	0.11
	Temperature	1	0.95	0.33
	Species: Month	10	3.88	**<0.01**
Plant community				
NEE	Successional stage (stage)	4	12.5	**<0.0001**
	Month	2	2.53	0.0968
	Stage: Month	8	2.09	0.0687
GPP	Stage	4	44.03	**<0.0001**
	Month	2	5.07	**0.0146**
	Stage: Month	8	2.26	0.0716
GR	Stage	4	23.8	**<0.0001**
	Month	2	1.08	0.354
	Stage: Month	8	1.37	0.2477

Table 2. Statistical results of the general linear models considering C fluxes of different plant species and communities as the dependent variables. Month and species (for plant species fluxes) and community age (for community carbon fluxes) and their interaction were included as fixed factors, and temperature and PAR as covariates (for plant species fluxes only).PNE = Plant net exchange; PH = Plant photosynthesis, PR = Plant respiration, NEE = Net ecosystem exchange, GPP = Gross primary production, GR= Gross respiration; n=3. Significant results are shown in bold ($p<0.05$).

Community-level balance

We recorded a significant effect of successional stage on community CO_2 flux - irrespective of the measuring season. GR and GPP increased monotonically with time after abandonment (Fig. 3) which translated into higher CO_2 uptake from the atmosphere (expressed as negative NEE values) and thus higher C sequestration as plant succession progressed. The 5 and 12-year stages acted as C emitters (0.27 ± 0.03 and

0.15 ± 0.05 μmol CO_2 $m^{-2}s^{-1}$, respectively) while NEE tended to zero 56 years after abandonment (0.10 ± 0.13 μmol CO_2 $m^{-2}s^{-1}$). Communities 63 years after abandonment and native communities acted as strong C sinks (-0.47 ± 0.12 and -0.85 ± 33 μmol CO_2 $m^{-2}s^{-1}$).

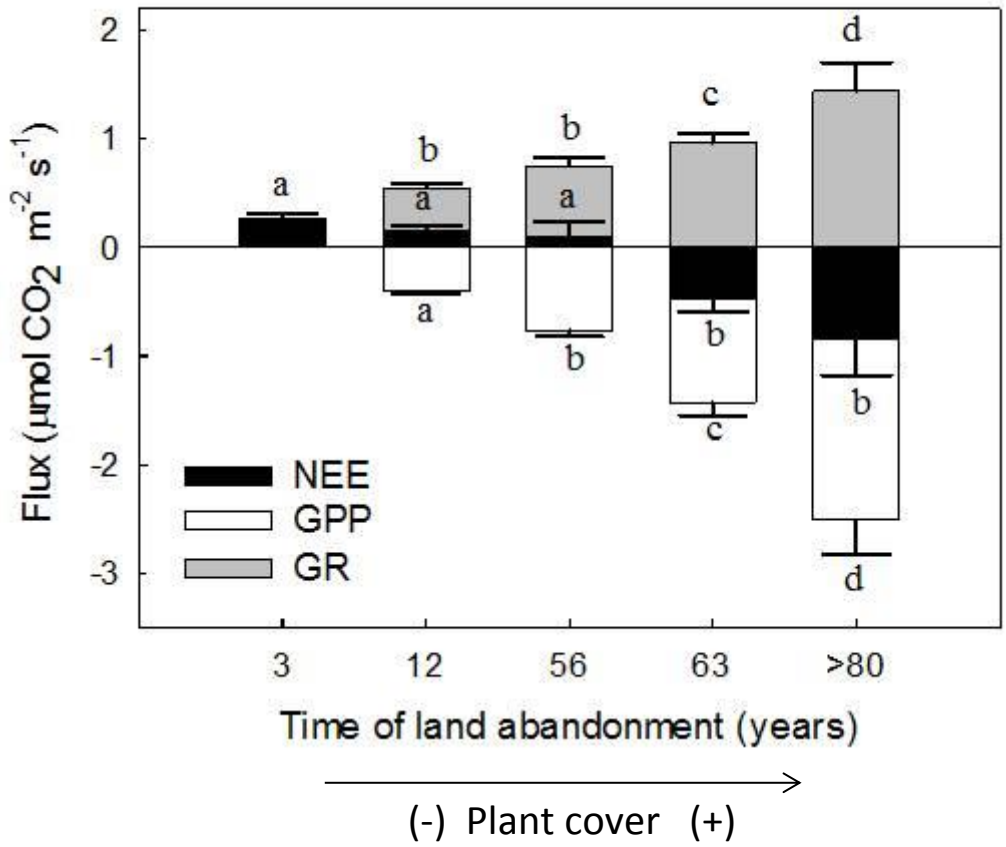

Figure 3. Integrated plant community C fluxes across successional stages for the whole period. The integration included the CO_2 fluxes data of each species and plant cover at every successional stage. Symbols are means ± 1 SE. Different letters within successional stages indicate significant differences ($p < 0.05$) among months after Fisher's LSD test; n= 3. The arrow indicates the direction in which vegetation cover increases with time.

Discussion

Our data show that C sequestration capacity by the plant community increased with successional time in a dry environment. This increase correlated with changes in plant community and in plant cover over time. Our data highlight how different plant species

influence CO_2 fluxes at community level. Agriculture drastically reduced the ability of semiarid systems to act as C sink, an ability regained through secondary succession after several decades of land abandonment.

Soil respiration and secondary succession

The increase of soil respiration rate over time was linked to the buildup of soil organic C and the increase of microbial biomass after land abandonment (Table S1; Lozano *et al.*, 2014). Plants can modify belowground C fluxes depending on factors such as allocation to roots, the presence of mycorrhizas, or the effects of soil exudates on microbial communities that affect soil respiration (De Deyn *et al.*, 2008; García Orenes *et al.*, 2010; Metcalfe *et al.*, 2011; Rey *et al.*, 2011). Plant community changes also affect soil respiration through changes in abiotic factors such as soil temperature and humidity, albedo and evapotranspiration rates (Chapin, 2003). All of them may have contributed to changes in soil respiration rate in our field plots after abandonment.

Plant species and C fluxes

Our data showed how different plant species influence CO_2 fluxes depending on the metabolism they have. For instance, fast-growing species with high C fixation rates vs low-growing species or species with different photosynthetic pathways affect C assimilation rates in different ways (Reich *et al.*, 2003). We recorded high rates of PH, PR and PNE in *Salsola* (the only C4 species in our set) and *Thymealea* (the earliest colonizer, and a fast growing species) which translated into higher CO_2 assimilation rates. Apart from these two species, we recorded no differences in other species nor between woody species and tussock grasses. High growth rates do not necessarily mean an increase in C sequestration capacity, as they tend to accumulate more labile C in their tissues (Aerts & Chapin, 1999) which is easily released into the atmosphere by mineralization (Zhang *et al.*, 2013). Supporting this line of evidence, Ward *et al.* (2009)

reported that removal of slow-growing species resulted in an increase of CO_2 fluxes from soil. Previous work also showed differences in NEE among plant functional types. For instance, Ferlan *et al.* (2011) assigned differences in NEE along secondary succession to changes in plant functional types such as grassland *vs* woody species, and recorded a shift from source to sink, as we also see in our chronosequence, with succession.

Secondary succession and ecosystem C sequestration capacity

Agriculture switched the system from sink to C emitter (Béziat *et al.*, 2009; Chen *et al.*, 2014a) but succession restored the pattern. The increase in NEE with plant cover supports previous reports which found a relationship between NEE and primary productivity, like Clark *et al.* (2004) in a temperate forest chronosequence and McMillan *et al.* (2008) in a boreal forest. C fixation was low in early successional stages and countered by soil C emissions in our system. In later successional stages, however, CO_2 fixed by a larger plant community was bigger than mineralized C, turning the system into a sink. The increase in C sequestration is related with increased GPP in semiarid ecosystems (Serrano Ortiz *et al.*, 2014). Changes in plant cover, aboveground biomass and soil organic matter combined to changes in C budgets in the different plant communities (Delgado-Balbuena *et al.*, 2013; Novara *et al.*, 2013; Novara *et al.*, 2014) explain the correlation that we found between successional age and C sequestration capacity. NEE along the chronosequence was steady along the growing season, suggesting that the decrease in PNE recorded in May (Fig. 2) was paralleled by a decrease in soil CO_2 emissions (Fig. 1), reflecting the fact the final C sequestration is the combination between C fixation by plants and the parallel release by soil respiration. Mean values of soil respiration and NEE where in the range of other reported for similar environments (Domingo *et al.*, 2011; Rey *et al.*, 2012).

The recovery of plant communities after disturbance needs long periods of time in dry environments because of limited resource supply. In a study carried out in a desert , Abella (2010) established the minimum period for ecosystem function recovery in 41 years. In our environment, a plant cover similar to the native community was not reached even after 63 years of land abandonment (Lozano *et al.*, 2014). Although the system was by then acting as a C sink, this period is longer that the 35 years reported, for instance, by Rosenzweig *et al.* (2016) for soil nutrients in a temperate grassland or the 50 years reported by Samaritani *et al.* (2011) in a temperate bog stressing the long time lapse that semiarid environments need to recover their carbon sequestration capacity after a major disturbance.

Conclusion

Our data show that secondary succession affects C sequestration capacity in a semiarid ecosystem. Changes in plant cover related to secondary succession impacted C fluxes, decreasing NEE with succession and turning this semiarid system into a C sink. Dry ecosystems need long periods of time to recover and reach pre-disturbance carbon sequestration capacity. By extension and functionality, dry ecosystems are important players in the mitigation of adverse global change effects on C sequestration but human activity may compromise their capacity to act as a C sinks.

Appendix

Stage (years)	**Fungi** (nmol g^{-1})	**Bacteria** (nmol g^{-1})	**Total Biomass** (nmol g^{-1})	**Basal Respiration** (mg CO_2-C Kg^{-1} day^{-1})	**pH**	**N** (g/Kg)	**C** (g/Kg)	**SOC** (g/Kg)	**C/N**
3	0.33	1.93	8.21	4.2	8.43	0.65	16.39	4.69	26.34
12	0.12	1.06	7.21	5.73	8.65	0.4	19.44	4.93	52.21
56	0.62	2.11	10.77	9.08	8.43	0.7	21.75	7.55	41.07
63	0.66	2.58	12.53	9.18	8.73	0.55	18.54	5.5	62.81
Native	0.79	3.73	16.65	8.37	8.32	1.14	21.48	9.55	19.29

Table S1. Soil pH, nitrogen (N), total carbon (C), organic carbon (SOC), C/N ratio, basal respiration, microbial biomass, and frequency of fungi and bacteria along the chronosequence (From Lozano *et al.*, 2014) .

General Conclusions

1. The seasonal control of soil respiration by temperature in a semiarid environment is modulated by water availability in the soil which switches the seasonal relationship between soil respiration and temperature from positive in humid periods to negative in dry periods.
2. In a semiarid shrub community the response of soil respiration to temperature and humidity is not uniform across plant species, as respiration rate reacts to a combination of drivers including plant identity. The inclusion of plant species identity in the temporal modeling of soil respiration greatly improves the proportion of variance explained by the model.
3. Soil respiration pulses after rewetting depend on temperature and soil origin, suggesting that microbial communities are responsible for the differences found in soil respiration rate among sites and depths.
4. Optimum temperatures in dry environments were in the range of 50ºC on sandy soils, much higher than expected. Our data support the idea that global temperature increases will boost soil respiration rates in these environments.
5. Soil community structure affects soil respiration responses to environmental changes. The bulk soil community is more sensitive to changes in temperature and humidity than the rhizosphere (roots and AMF fungi).
6. The AMF abundance is affected by vegetation type and soil age, which in turn mediates soil carbon fluxes in grasslands. Soil microbial communities in grasslands may mitigate CO_2 emissions depending upon factors that influence the abundance and activity of AMF in soil.
7. Secondary succession affects C sequestration capacity in a semiarid shrubland, turning the system into a C sink mainly due to changes in plant cover. Dry ecosystems need long periods of time to recover their pre-disturbance carbon sequestration capacity.

Referencias

Abella SR (2010) Disturbance and plant succession in the Mojave and Sonoran deserts of the American Southwest. Int J Environ Res Public Health, **7**, 1248-1284.

Abraham De Vázquez EM (2008) Tierras secas, desertificación y recursos hídricos. Ecosistemas, **17**, 1-4.

Aerts R, Chapin FS (1999) The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Advances in Ecological Research, **30**, 1-67.

Aguilera L, Armas C, Cea A, Gutiérrez J, Meserve P, Kelt D (2016) Rainfall, microhabitat, and small mammals influence the abundance and distribution of soil microorganisms in a Chilean semi-arid shrubland. Journal of Arid Environments, **126**, 37-46.

Ahlström A, Raupach MR, Schurgers G, Smith B, Arneth A, Jung M, Reichstein M, Canadell JG *et al.* (2015) The dominant role of semi-arid ecosystems in the trend and variability of the land CO2 sink. Science, **348**, 895-899.

Álvaro-Fuentes J, Paustian K (2011) Potential soil carbon sequestration in a semiarid Mediterranean agroecosystem under climate change: Quantifying management and climate effects. Plant and Soil, **338**, 261-272.

Allison VJ, Miller RM, Jastrow JD, Matamala R, Zak DR (2005) Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Science Society of America Journal, **69**, 1412-1421.

Aniku JRF, Singer MJ (1990) Pedogenic iron oxide trends in a marine terrace chronosequence. Soil Science Society of America Journal, **54**, 147-152.

Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: A new comparative index. Ecology, **85**, 2682-2686.

Ataka M, Kominami Y, Yoshimura K, Miyama T, Jomura M, Tani M (2014) In situ CO2 efflux from leaf litter layer showed large temporal variation induced by rapid wetting and drying cycle. PLoS ONE, **9**, e108404.

Averill C, Turner B, Finzi A (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature, **505**, 543-545.

Bahn M, Reichstein M, Davidson EA, Grünzweig J, Jung M, Carbone MS, Epron D, Misson L *et al.* (2010) Soil respiration at mean annual temperature predicts annual total across vegetation types and biomes. Biogeosciences, **7**, 2147-2157.

Bais H, Weir T, Perry L, Gilroy S, Vivanco J (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, **57**, 233-266.

Baldocchi D, Tang J, Xu L (2006) How switches and lags in biophysical regulators affect spatial-temporal variation of soil respiration in an oak-grass savanna. Journal of geophysical research, **111**, G02008.

Barba J, Curiel Yuste J, Martínez-Vilalta J, Lloret F (2013) Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. Forest Ecology and Management, **306**, 79-87.

Barba J, Curiel Yuste J, Poyatos R, Janssens IA, Lloret F (2016) Strong resilience of soil respiration components to drought-induced die-off resulting in forest secondary succession. Oecologia, **182**, 1-15.

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME Journal, **7**, 2229-2241.

Barnard RL, Osborne CA, Firestone MK (2015) Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. ISME Journal, **9**, 946-957.

Bastida F, Jehmlich N, Ondoño S, Von Bergen M, García C, Moreno JL (2014) Characterization of the microbial community in biological soil crusts dominated by Fulgensia desertorum (Tomin) Poelt and Squamarina cartilaginea (With.) P. James and in the underlying soil. Soil Biology and Biochemistry, **76**, 70-79.

Béziat P, Ceschia E, Dedieu G (2009) Carbon balance of a three crop succession over two cropland sites in South West France. Agricultural and Forest Meteorology, **149**, 1628-1645.

Birch HF (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil, **10**, 9-31.

Bond Lamberty B, Thomson A (2010) Temperature-associated increases in the global soil respiration record. Nature, **464**, 579-582.

Bonet A (2004) Secondary succession of semi-arid Mediterranean old-fields in south-eastern Spain: Insights for conservation and restoration of degraded lands. Journal of Arid Environments, **56**, 213-233.

Bonet A, Pausas J (2004) Species richness and cover along a 60-year chronosequence in old-fields of southeastern Spain. Plant Ecology, **174**, 257-270.

Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology, **15**, 808-824.

Brito I, De Carvalho M, Goss MJ (2011) Summer survival of arbuscular mycorrhiza extraradical mycelium and the potential for its management through tillage options in Mediterranean cropping systems. Soil Use and Management, **27**, 350-356.

Bunn RA, Ramsey PW, Lekberg Y (2015) Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. Journal of Ecology, **103**, 1547-1556.

Byrd KB, Flint LE, Alvarez P, Casey CF, Sleeter BM, Soulard CE, Flint AL, Sohl TL (2015) Integrated climate and land use change scenarios for California rangeland ecosystem services: wildlife habitat, soil carbon, and water supply. Landscape ecology, **30**, 729-750.

Cable JM, Ogle K, Williams DG, Weltzin JF, Huxman TE (2008) Soil texture drives responses of soil respiration to precipitation pulses in the sonoran desert: Implications for climate change. Ecosystems, **11**, 961-979.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pẽa AG *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods, **7**, 335-336.

Carrillo Y, Dijkstra FA, Lecain D, Pendall E (2016) Mediation of soil C decomposition by arbuscular mycorrizhal fungi in grass rhizospheres under elevated CO2. Biogeochemistry, **127**, 45-55.

Castillo-Monroy AP, Maestre FT, Rey A, Soliveres S, García-Palacios P (2011) Biological Soil Crust Microsites Are the Main Contributor to Soil Respiration in a Semiarid Ecosystem. Ecosystems, **14**, 835-847.

Cerdà A (1998) Soil aggregate stability under different Mediterranean vegetation types. Catena, **32**, 73-86.

Clark KL, Gholz HL, Castro MS (2004) Carbon dynamics along a chronosequence of slash pine plantations in North Florida. Ecological Applications, **14**, 1154-1171.

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD *et al.* (2013) Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. Science, **339**, 1615-1618.

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD *et al.* (2011) Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology, **17**, 3392-3404.

Costello EK, Halloy SRP, Reed SC, Sowell P, Schmidt SK (2009) Fumarole-supported islands of biodiversity within a hyperarid, high-elevation landscape on socompa volcano, puna de atacama, andes. Applied and Environmental Microbiology, **75**, 735-747.

Crits-Christoph A, Robinson CK, Barnum T, Fricke WF, Davila AF, Jedynak B, Mckay CP, Diruggiero J (2013) Colonization patterns of soil microbial communities in the Atacama Desert. Microbiome, **1**, 28.

Cueva A, Bahn M, Litvak M, Pumpanen J, Vargas R (2015) A multisite analysis of temporal random errors in soil CO_2 efflux. Journal of Geophysical Research G: Biogeosciences, **120**, 737-751.

Curiel Yuste J, Janssens IA, Carrara A, Ceulemans R (2004) Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity. Global Change Biology, **10**, 161-169.

Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin T (2006) The desert of Tataouine: An extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environmental Microbiology, **8**, 514-525.

Chang CT, Sabaté S, Sperlich D, Poblador S, Sabater F, Gracia C (2014) Does soil moisture overrule temperature dependence of soil respiration in Mediterranean riparian forests? Biogeosciences, **11**, 6173-6185.

Chapin FS (2003) Effects of plant traits on ecosystem and regional processes: A conceptual framework for predicting the consequences of global change. Annals of Botany, **91**, 455-463.

Chapin FS, Randerson JT, Mcguire AD, Foley JA, Field CB (2008) Changing feedbacks in the climate-biosphere system. Frontiers in Ecology and the Environment, **6**, 313-320.

Chaplot V, Dlamini P, Chivenge P (2016) Potential of grassland rehabilitation through high density-short duration grazing to sequester atmospheric carbon. Geoderma, **271**, 10-17.

Chatterjee A, Jenerette GD (2011a) Changes in soil respiration Q10 during drying-rewetting along a semi-arid elevation gradient. Geoderma, **163**, 171-177.

Chatterjee A, Jenerette GD (2011b) Spatial variability of soil metabolic rate along a dryland elevation gradient. Landscape ecology, **26**, 1111-1123.

Chen H, Fan M, Kuzyakov Y, Billen N, Stahr K (2014a) Comparison of net ecosystem CO2 exchange in cropland and grassland with an automated closed chamber system. Nutrient Cycling in Agroecosystems, **98**, 113-124.

Chen S, Zou J, Hu Z, Chen H, Lu Y (2014b) Global annual soil respiration in relation to climate, soil properties and vegetation characteristics: Summary of available data. Agricultural and Forest Meteorology, **198**, 335-346.

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO2. Science, **337**, 1084-1087.

Churchland C, Grayston SJ, Bengtson P (2013) Spatial variability of soil fungal and bacterial abundance: Consequences for carbon turnover along a transition from a forested to clear-cut site. Soil Biology and Biochemistry, **63**, 5-13.

Davidson EA, Janssens IA, Lou Y (2006a) On the variability of respiration in terrestrial ecosystems: Moving beyond Q10. Global Change Biology, **12**, 154-164.

Davidson EA, Samanta S, Caramori SS, Savage K (2012) The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biology, **18**, 371-384.

Davidson EA, Savage KE, Trumbore SE, Borken W (2006b) Vertical partitioning of CO2 production within a temperate forest soil. Global Change Biology, **12**, 944-956.

De Dato GD, De Angelis P, Sirca C, Beier C (2010) Impact of drought and increasing temperatures on soil CO2 emissions in a Mediterranean shrubland (gariga). Plant and Soil, **327**, 153-166.

De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters, **11**, 516-531.

De Vries FT, Manning P, Tallowin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H *et al.* (2012) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters, **15**, 1230-1239.

Delgado-Balbuena J, Arredondo JT, Loescher HW, Huber-Sannwald E, Chavez-Aguilar G, Luna-Luna M, Barretero-Hernandez R (2013) Differences in plant cover and species composition of semiarid grassland communities of central Mexico and its effects on net ecosystem exchange. Biogeosciences, **10**, 4673-4690.

Desantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol, **72**, 5069-5072.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2015) InfoStat, Versión 2015.

Domingo F, Serrano-Ortiz P, Were A, Villagarcía L, García M, Ramírez DA, Kowalski AS, Moro MJ *et al.* (2011) Carbon and water exchange in semiarid ecosystems in SE Spain. Journal of Arid Environments, **75**, 1271-1281.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, **27**, 2194-2200.

Emam T (2016) Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. Restoration Ecology, **24**, 35-44.

Fan Z, Neff JC, Hanan NP (2015) Modeling pulsed soil respiration in an African savanna ecosystem. Agricultural and Forest Meteorology, **200**, 282-292.

Fang C, Moncrieff JB (2001) The dependence of soil CO2 efflux on temperature. Soil Biology and Biochemistry, **33**, 155-165.

Ferlan M, Alberti G, Eler K, Batič F, Peressotti A, Miglietta F, Zaldei A, Simončič P, Vodnik D (2011) Comparing carbon fluxes between different stages of secondary succession of a karst grassland. Agriculture, Ecosystems and Environment, **140**, 199-207.

Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal, **67**, 798-805.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. Ecology Letters, **12**, 1238-1249.

Fuchslueger L, Bahn M, Hasibeder R, Kienzl S, Fritz K, Schmitt M, Watzka M, Richter A (2016) Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. Journal of Ecology, **104**, 1455-1465.

Gabarrón-Galeote MA, Trigalet S, Wesemael BV (2015) Soil organic carbon evolution after land abandonment along a precipitation gradient in southern Spain. Agriculture, Ecosystems & Environment, **199**, 114-123.

García Orenes F, Guerrero C, Roldán A, Mataix Solera J, Cerdà A, Campoy M, Zornoza R, Bárcenas G, Caravaca F (2010) Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. Soil & tillage research, **109**, 110-115.

García Palacios P, Maestre F, Kattge J, Wall D, García Palacios P, Klironomos J (2013) Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecology Letters, **16**, 1045-1053.

Gerz M, Bueno CG, Zobel M, Moora M (2016) Plant community mycorrhization in temperate forests and grasslands: Relations with edaphic properties and plant diversity. Journal of Vegetation Science, **27**, 89-99.

Goulden ML, Mcmillan AMS, Winston GC, Rocha AV, Manies KL, Harden JW, Bond-Lamberty BP (2011) Patterns of NPP, GPP, respiration, and NEP during boreal forest succession. Global Change Biology, **17**, 855-871.

Guntiñas ME, Gil-Sotres F, Leirós MC, Trasar-Cepeda C (2013) Sensitivity of soil respiration to moisture and temperature. Journal of Soil Science and Plant Nutrition, **13**, 445-461.

Hamdi S, Chevallier T, Ben Aïssa N, Ben Hammouda M, Gallali T, Chotte JL, Bernoux M (2011) Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. Soil Biology and Biochemistry, **43**, 1752-1758.

Hamdi S, Moyano F, Sall S, Bernoux M, Chevallier T (2013) Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. Soil Biology and Biochemistry, **58**, 115-126.

Han G, Xing Q, Luo Y, Rafique R, Yu J, Mikle N (2014) Vegetation types alter soil respiration and its temperature sensitivity at the field scale in an estuary wetland. PLoS ONE, **9**, e91182.

Hartley IP, Heinemeyer A, Evans SP, Ineson P (2007) The effect of soil warming on bulk soil vs. rhizosphere respiration. Global Change Biology, **13**, 2654-2667.

Harwani D (2013) Biodiversity of rare thermophilic actinomycetes in the great Indian Thar desert: an overview. Indo American Journal of Pharmaceutical Research, **3**, 934-939.

Hasibeder R, Fuchslueger L, Richter A, Bahn M (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phytologist, **205**, 1117-1127.

Heinemeyer A, Hartley IP, Evans SP, Carreira De La Fuente JA, Ineson P (2007) Forest soil CO2 flux: Uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology, **13**, 1786-1797.

Heinemeyer A, Tortorella D, Petrovičová B, Gelsomino A (2012) Partitioning of soil CO 2 flux components in a temperate grassland ecosystem. European Journal of Soil Science, **63**, 249-260.

Heinonsalo J, Pumpanen J, Rasilo T, Hurme KR, Ilvesniemi H (2010) Carbon partitioning in ectomycorrhizal Scots pine seedlings. Soil Biology and Biochemistry, **42**, 1614-1623.

Herzog C, Peter M, Pritsch K, Günthardt-Goerg MS, Egli S (2013) Drought and air warming affects abundance and exoenzyme profiles of Cenococcum geophilum associated with Quercus robur, Q. petraea and Q. pubescens. Plant Biology, **15**, 230-237.

Holden SR, Gutierrez A, Treseder KK (2013) Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. Ecosystems, **16**, 34-46.

Hoover D, Knapp A, Smith M (2016) The immediate and prolonged effects of climate extremes on soil respiration in a mesic grassland. Journal of Geophysical Research G: Biogeosciences, **121**, 1034-1044.

Hopkins F, Gonzalez Meler M, Flower C, Lynch D, Czimczik C, Tang J, Subke J-A (2013) Ecosystem-level controls on root-rhizosphere respiration. New Phytologist, **199**, 339-351.

Hortal S, Bastida F, Armas C, Lozano YM, Moreno JL, García C, Pugnaire FI (2013) Soil microbial community under a nurse-plant species changes in composition, biomass and activity as the nurse grows. Soil Biology and Biochemistry, **64**, 139-146.

Hortal S, Bastida F, Moreno JL, Armas C, García C, Pugnaire FI (2015) Benefactor and allelopathic shrub species have different effects on the soil microbial community along an environmental severity gradient. Soil Biology and Biochemistry, **88**, 48-57.

Hu Y, Rillig MC, Xiang D, Hao Z, Chen B (2013) Changes of AM Fungal Abundance along Environmental Gradients in the Arid and Semi-Arid Grasslands of Northern China. PLoS ONE, **8**, e57593.

Ipcc (2014) Summary for policymakers. In: *Climate Change 2014: Synthesis Report.* Cambridge University Press.

Jenerette GD, Chatterjee A (2012) Soil metabolic pulses: Water, substrate, and biological regulation. Ecology, **93**, 959-966.

Ji M, Van Dorst J, Bissett A, Brown MV, Palmer AS, Snape I, Siciliano SD, Ferrari BC (2016) Microbial diversity at Mitchell Peninsula, Eastern Antarctica: a potential biodiversity "hotspot". Polar Biology, **39**, 237-249.

Johnson D, Phoenix GK, Grime JP (2008) Plant community composition, not diversity, regulates soil respiration in grasslands. Biology Letters, **4**, 345-348.

Johnson N, Graham J (2013) The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant and Soil, **363**, 411-419.

Kim DG, Vargas R, Bond-Lamberty B, Turetsky MR (2012) Effects of soil rewetting and thawing on soil gas fluxes: A review of current literature and suggestions for future research. Biogeosciences, **9**, 2459-2483.

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, **41**, e1.

Koskinen M, Minkkinen K, Ojanen P, Kämäräinen M, Laurila T, Lohila A (2014) Measurements of CO2 exchange with an automated chamber system throughout the year: Challenges in measuring night-time respiration on porous peat soil. Biogeosciences, **11**, 347-363.

Kurapova AI, Zenova GM, Sudnitsyn II, Kizilova AK, Manucharova NA, Norovsuren Z, Zvyagintsev DG (2012) Thermotolerant and thermophilic actinomycetes from soils of Mongolia desert steppe zone. Microbiology, **81**, 98-108.

Law BE, Kelliher FM, Baldocchi DD, Anthoni PM, Irvine J, Moore D, Van Tuyl S (2001) Spatial and temporal variation in respiration in a young ponderosa pine forest during a summer drought. Agricultural and Forest Meteorology, **110**, 27-43.

Lázaro R, Rodrigo FS, Gutiérrez L, Domingo F, Puigdefábregas J (2001) Analysis of a 30-year rainfall record (1967-1997) in semi-arid SE spain for implications on vegetation. Journal of Arid Environments, **48**, 373-395.

Le Quéré C, Moriarty R, Andrew RM, Peters GP, Ciais P, Friedlingstein P, Jones SD, Sitch S *et al.* (2015) Global carbon budget 2014. Earth System Science Data, **7**, 47-85.

Leifeld J, Von Lützow M (2014) Chemical and microbial activation energies of soil organic matter decomposition. Biology and Fertility of Soils, **50**, 147-153.

Lenihan JM (2003) Climate change effects on vegetation distribution, carbon, and fire in California. Ecological Applications, **13**, 1667-1681.

Liu L, Wang X, Lajeunesse M, Miao G, Piao S, Wan S, Wu Y, Wang Z *et al.* (2016) A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. Global Change Biology, **22**, 1394-1405.

López-Ballesteros A, Serrano-Ortiz P, Sánchez-Cañete EP, Oyonarte C, Kowalski AS, Pérez-Priego Ó, Domingo F (2016) Enhancement of the net CO2 release of a semiarid grassland in SE Spain by rain pulses. Journal of Geophysical Research G: Biogeosciences, **121**, 52-66.

Lozano YM, Hortal S, Armas C, Pugnaire FI (2014) Interactions among soil, plants, and microorganisms drive secondary succession in dry environments. Soil Biology and Biochemistry, **78**, 298-306.

Luan J, Liu S, Wang J, Zhu X, Shi Z (2011) Rhizospheric and heterotrophic respiration of a warm-temperate oak chronosequence in China. Soil Biology and Biochemistry, **43**, 503-512.

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. Functional Ecology, **8**, 315-323.

Maestre FT, Cortina J (2003) Small-scale spatial variation in soil CO2 efflux in a Mediterranean semiarid steppe. Applied soil ecology, **23**, 199-209.

Marstorp H, Guan X, Gong P (2000) Relationship between dsDNA, chloroform labile C and ergosterol in soils of different organic matter contents and pH. Soil Biology and Biochemistry, **32**, 879-882.

Martínez-García LB, Armas C, Miranda JDD, Padilla FM, Pugnaire FI (2011) Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. Soil Biology and Biochemistry, **43**, 682-689.

Mcgonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist, **115**, 495-501.

Mcmillan AMS, Winston GC, Goulden ML (2008) Age-dependent response of boreal forest to temperature and rainfall variability. Global Change Biology, **14**, 1904-1916.

Meisner A, Rousk J, Bååth E (2015) Prolonged drought changes the bacterial growth response to rewetting. Soil Biology and Biochemistry, **88**, 314-322.

Metcalfe DB, Fisher RA, Wardle DA (2011) Plant communities as drivers of soil respiration: Pathways, mechanisms, and significance for global change. Biogeosciences, **8**, 2047-2061.

Miralles I, Domingo F, García-Campos E, Trasar-Cepeda C, Leirós MC, Gil-Sotres F (2012) Biological and microbial activity in biological soil crusts from the Tabernas desert, a sub-arid zone in SE Spain. Soil Biology and Biochemistry, **55**, 113-121.

Mohammadipanah F, Wink J (2016) Actinobacteria from arid and desert habitats: Diversity and biological activity. Frontiers in Microbiology, **6**, 1541.

Mooney H (2007) Past, present and future of a rapid-responding ecosystem: California grasslands. Trends in Ecology and Evolution, **23**, 296-297.

Moore J, Macalady JL, Schulz MS, White AF, Brantley SL (2010) Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. Soil Biology and Biochemistry, **42**, 21-31.

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. Black, C.A., ed. . Chemical methods. Madison, WI, USA. In: *Methods of soil analysis.* pp 961-1010.

Noormets A, Chen J, Crow TR (2007) Age-dependent changes in ecosystem carbon fluxes in managed forests in northern Wisconsin, USA. Ecosystems, **10**, 187-203.

Norton MR, Malinowski DP, Volaire F (2016) Plant drought survival under climate change and strategies to improve perennial grasses. A review. Agronomy for Sustainable Development, **36**, 29.

Novara A, Gristina L, La Mantia T, Rühl J (2013) Carbon dynamics of soil organic matter in bulk soil and aggregate fraction during secondary succession in a Mediterranean environment. Geoderma, **193-194**, 213-221.

Novara A, La Mantia T, Rühl J, Badalucco L, Kuzyakov Y, Gristina L, Laudicina V (2014) Dynamics of soil organic carbon pools after agricultural abandonment. Geoderma, **235-236**, 191-198.

Osono T, Azuma JI, Hirose D (2013) Plant species effect on the decomposition and chemical changes of leaf litter in grassland and pine and oak forest soils. Plant and Soil, **375**, 411-421.

Ostle N, Smith P, Fisher R, Ian Woodward F, Fisher J, Smith J, Galbraith D, Levy P *et al.* (2009) Integrating plant-soil interactions into global carbon cycle models. Journal of Ecology, **97**, 851-863.

Oyonarte C, Rey A, Raimundo J, Miralles I, Escribano P (2012) The use of soil respiration as an ecological indicator in arid ecosystems of the SE of Spain: Spatial variability and controlling factors. Ecological Indicators, **14**, 40-49.

Padilla FM, Miranda JD, Armas C, Pugnaire FI (2015) Effects of changes in rainfall amount and pattern on root dynamics in an arid shrubland. Journal of Arid Environments, **114**, 49-53.

Pasternak Z, Al-Ashhab A, Gatica J, Gafny R, Avraham S, Minz D, Gillor O, Jurkevitch E (2013) Spatial and Temporal Biogeography of Soil Microbial Communities in Arid and Semiarid Regions. PLoS ONE, **8**, e69705.

Pérez-Priego O, López-Ballesteros A, Sánchez-Cañete EP, Serrano-Ortiz P, Kutzbach L, Domingo F, Eugster W, Kowalski AS (2015) Analysing uncertainties in the calculation of fluxes using whole-plant chambers: random and systematic errors. Plant and Soil, **393**, 229-244.

Pfeifer Meister L, Bridgham S, Reynolds L, Goklany M, Wilson H, Little C, Ferguson A, Johnson B (2016) Climate change alters plant biogeography in Mediterranean prairies along the West Coast, USA. Global Change Biology, **22**, 845-855.

Pinheiro JC, Bates DM (2000) "Mixed-Effects Models in S and S-PLUS". Springer, 361.

Plaza-Bonilla D, Arrúe JL, Cantero-Martínez C, Fanlo R, Iglesias A, Álvaro-Fuentes J (2015) Carbon management in dryland agricultural systems. A review. Agronomy for Sustainable Development, **35**, 1319-1334.

Prieto I, Armas C, Pugnaire FI (2012) Water release through plant roots: New insights into its consequences at the plant and ecosystem level. New Phytologist, **193**, 830-841.

Prieto I, Kikvidze Z, Pugnaire FI (2010) Hydraulic lift: Soil processes and transpiration in the Mediterranean leguminous shrub Retama sphaerocarpa (L.) Boiss. Plant and Soil, **329**, 447-456.

Pugnaire FI, Armas C, Valladares F (2004) Soil as a mediator in plant-plant interactions in a semi-arid community. Journal of Vegetation Science, **15**, 85-92.

R-Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rajeev L, Da Rocha UN, Klitgord N, Luning EG, Fortney J, Axen SD, Shih PM, Bouskill NJ *et al.* (2013) Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. ISME Journal, **7**, 2178-2191.

Rao L, Allen E (2010) Combined effects of precipitation and nitrogen deposition on native and invasive winter annual production in California deserts. Oecologia, **162**, 1035-1046.

Reich P (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. Journal of Ecology, **102**, 275-301.

Reich PB, Buschena C, Tjoelker MG, Wrage K, Knops J, Tilman D, Machado JL (2003) Variation in Growth Rate and Ecophysiology among 34 Grassland and Savanna Species under Contrasting N Supply: A Test of Functional Group Differences. The New Phytologist, **157**, 617-631.

Reichstein M, Rey A, Freibauer A, Tenhunen J, Valentini R, Banza J, Casals P, Cheng Y *et al.* (2003) Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices. Global Biogeochemical Cycles, **17**, 15-11.

Rey A, Belelli Marchesini L, Were A, Serrano Ortiz P, Etiope G, Papale D, Domingo F, Pegoraro E (2012) Wind as a main driver of the net ecosystem carbon balance of a semiarid Mediterranean steppe in the South East of Spain. Global Change Biology, **18**, 539-554.

Rey A, Pegoraro E, Jarvis PG (2008) Carbon mineralization rates at different soil depths across a network of European forest sites (FORCAST). European Journal of Soil Science, **59**, 1049-1062.

Rey A, Pegoraro E, Oyonarte C, Were A, Escribano P, Raimundo J (2011) Impact of land degradation on soil respiration in a steppe (Stipa tenacissima L.) semi-arid ecosystem in the SE of Spain. Soil Biology and Biochemistry, **43**, 393-403.

Rey A, Pegoraro E, Tedeschi V, De Parri I, Jarvis PG, Valentini R (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology, **8**, 851-866.

Rey A, Petsikos C, Jarvis PG, Grace J (2005) Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. European Journal of Soil Science, **56**, 589-599.

Reynolds JF, Smith DMS, Lambin EF, Turner BL, Mortimore M, Batterbury SPJ, Downing TE, Dowlatabadi H *et al.* (2007) Global Desertification: Building a Science for Dryland Development. Science, **316**, 847-851.

Reynolds LL, Johnson BR, Pfeifer-Meister L, Bridgham SD (2015) Soil respiration response to climate change in Pacific Northwest prairies is mediated by a regional Mediterranean climate gradient. Global Change Biology, **21**, 487-500.

Richardson J, Chatterjee A, Darrel Jenerette G (2012) Optimum temperatures for soil respiration along a semi-arid elevation gradient in southern California. Soil Biology and Biochemistry, **46**, 89-95.

Rodríguez Echeverría S, Armas C, Pistón N, Hortal S, Pugnaire F, De Deyn G (2013) A role for below-ground biota in plant-plant facilitation. Journal of Ecology, **101**, 1420-1428.

Rosenzweig S, Carson M, Baer S, Blair J (2016) Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Applied soil ecology, **100**, 186-194.

Samaritani E, Siegenthaler A, Yli-Petäys M, Buttler A, Christin P-A, Mitchell EaD (2011) Seasonal Net Ecosystem Carbon Exchange of a Regenerating Cutaway Bog: How Long Does it Take to Restore the C-Sequestration Function? Restoration Ecology, **19**, 480-489.

Schöb C, Armas C, Guler M, Prieto I, Pugnaire FI (2013) Variability in functional traits mediates plant interactions along stress gradients. Journal of Ecology, **101**, 753-762.

Serrano-Ortiz P, Sánchez-Cañete EP, Oyonarte C (2012) The carbon cycle in drylands. In: *Recarbonization of the Biosphere: Ecosystems and the Global Carbon Cycle.* pp 347-368.

Serrano Ortiz P, Oyonarte C, Pérez Priego O, Reverter B, Sánchez Cañete E, Were A, Uclés O, Morillas L, Domingo F (2014) Ecological functioning in grass–shrub Mediterranean ecosystems measured by eddy covariance. Oecologia, **175**, 1005-1017.

Shamir I, Steinberger Y (2007) Vertical distribution and activity of soil microbial population in a sandy desert ecosystem. Microbial Ecology, **53**, 340-347.

Sierra CA (2012) Temperature sensitivity of organic matter decomposition in the Arrhenius equation: Some theoretical considerations. Biogeochemistry, **108**, 1-15.

Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, Van Bodegom PM, Cornwell WK, Moens EJ, Treseder KK, Tibbett M *et al.* (2015) Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. Global Ecology and Biogeography, **24**, 371-382.

Stevenson A, Hallsworth JE (2014) Water and temperature relations of soil Actinobacteria. Environmental Microbiology Reports, **6**, 744-755.

Sundquist ET, Burruss RC, Faulkner SP, Gleason RA, Harden JW, Kharaka YK, Tieszen LL, Waldrop MP (2008) Carbon Sequestration to Mitigate Climate Change:U.S. Geological Survey. pp 4p.

Tang J, Bolstad PV, Martin JG (2009) Soil carbon fluxes and stocks in a Great Lakes forest chronosequence. Global Change Biology, **15**, 145-155.

Tas N, Prestat E, Mcfarland JW, Wickland KP, Knight R, Berhe AA, Jorgenson T, Waldrop MP, Jansson JK (2014) Impact of fire on active layer and permafrost

microbial communities and metagenomes in an upland Alaskan boreal forest. ISME Journal, **8**, 1904-1919.
Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry, **34**, 387-401.
Tedeschi V, Rey A, Manca G, Valentini R, Jarvis PJ, Borghetti M (2006) Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Global Change Biology, **12**, 110-121.
Terrer C, Vicca S, Hungate BA, Phillips RP, Prentice IC (2016) Mycorrhizal association as a primary control of the CO2 fertilization effect. Science, **353**, 72-74.
Thomson B, Ostle N, Mcnamara N, Bailey M, Whiteley A, Griffiths R (2010) Vegetation Affects the Relative Abundances of Dominant Soil Bacterial Taxa and Soil Respiration Rates in an Upland Grassland Soil. Microbial Ecology, **59**, 335-343.
Treseder K, Cross A (2006) Global Distributions of Arbuscular Mycorrhizal Fungi. Ecosystems, **9**, 305-316.
Treseder K, Turner K, Mack M (2007) Mycorrhizal responses to nitrogen fertilization in boreal ecosystems: potential consequences for soil carbon storage. Global Change Biology, **13**, 78-88.
Treseder KK, Allen MF (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO2 and nitrogen deposition. New Phytologist, **147**, 189-200.
Un (2011) Global Drylands: A UN System-Wide Response. pp 123 United Nations Environment Management Group, United Nations.
Uroz S, Tech JJ, Sawaya NA, Frey-Klett P, Leveau JHJ (2014) Structure and function of bacterial communities in ageing soils: Insights from the Mendocino ecological staircase. Soil Biology and Biochemistry, **69**, 265-274.
Vallack HW, Leronni V, Metcalfe DB, Högberg P, Ineson P, Subke JA (2012) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. Plant and Soil, **352**, 405-417.
Van Gestel M, Ladd JN, Amato M (1991) Carbon and nitrogen mineralization from two soils of contrasting texture and microaggregate stability: Influence of sequential fumigation, drying and storage. Soil Biology and Biochemistry, **23**, 313-322.
Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, Van Sinderen D (2007) Genomics of Actinobacteria: Tracing the Evolutionary History of an Ancient Phylum. Microbiology and molecular biology reviews, **71**, 495-548.
Wagg C, Bender SF, Widmer F, Van Der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America, **111**, 5266-5270.
Waldrop M, Firestone M (2004) Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia, **138**, 275-284.
Waldrop MP, Firestone MK (2006) Seasonal Dynamics of Microbial Community Composition and Function in Oak Canopy and Open Grassland Soils. Microbial Ecology, **52**, 470-479.
Wan X, Huang Z, He Z, Yu Z, Wang M, Davis M, Yang Y (2015) Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. Plant and Soil, **387**, 103-116.

Wang J, Epstein HE (2013) Estimating carbon source-sink transition during secondary succession in a Virginia valley. Plant and Soil, **362**, 135-147.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, **73**, 5261-5267.

Wang W, Feng J, Oikawa T (2009) Contribution of Root and Microbial Respiration to Soil CO2 Efflux and Their Environmental Controls in a Humid Temperate Grassland of Japan. An International Journal Pedosphere, **19**, 31-39.

Ward SE, Bardget RD, Mcnamara NP, Ostle NJ (2009) Plant Functional Group Identity Influences Short-Term Peatland Ecosystem Carbon Flux: Evidence from a Plant Removal Experiment. Functional Ecology, **23**, 454-462.

White A, Schulz M, Vivit D, Blum A, Stonestrom D, Anderson S (2008) Chemical weathering of a marine terrace chronosequence, Santa Cruz, California I: Interpreting rates and controls based on soil concentration–depth profiles. Geochimica et Cosmochimica Acta, **72**, 36-68.

White AF, Schulz MS, Vivit DV, Bullen TD, Fitzpatrick J (2012) The impact of biotic/abiotic interfaces in mineral nutrient cycling: A study of soils of the Santa Cruz chronosequence, California. Geochimica et Cosmochimica Acta, **77**, 62-85.

Yu J, Steinberger Y (2011) Vertical Distribution of Microbial Community Functionality under the Canopies of Zygophyllum dumosum and Hammada scoparia in the Negev Desert, Israel. Microbial Ecology, **62**, 218-227.

Yu J, Steinberger Y (2012) Vertical distribution of soil microbial biomass and its association with shrubs from the Negev Desert. Journal of Arid Environments, **78**, 110-118.

Zhang K, Cheng X, Dang H, Ye C, Zhang Y, Zhang Q (2013) Linking litter production, quality and decomposition to vegetation succession following agricultural abandonment. Soil Biology and Biochemistry, **57**, 803-813.

Zhang Q (2013) Seasonal variations in soil respiration, heterotrophic respiration and autotrophic respiration of a wheat and maize rotation cropland in the North China Plain. Agricultural and Forest Meteorology, **180**, 34-43.

Zhu YG (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil–plant systems. Trends in plant science, **8**, 407-409.

Zhu Z, Piao S, Myneni RB, Huang M, Zeng Z, Canadell JG, Ciais P, Sitch S *et al.* (2016) Greening of the Earth and its drivers. Nature Climate Change, **6**, 791-795.