Universidad Rey Juan Carlos

TESIS DOCTORAL

Impactos del cambio global en el intercambio de gases suelo-atmósfera y propiedades microbianas en ecosistemas áridos a distintas escalas espaciales

Autora:

Angela Lafuente García-Ubero

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Programa de Doctorado en Conservación de Recursos Naturales
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Resumen general

Antecedentes: En zonas áridas de todo el mundo, los primeros centímetros de la superficie del suelo que se encuentra entre las manchas dispersas de vegetación perennes están dominados por la costra biológica. Se trata de comunidades compuestas por cianobacterias, algas, hongos, bacterias, líquenes y musgos que juegan un papel fundamental regulando la humectación y secado del suelo tras la precipitación, los ciclos biogeoquímicos del carbono y el nitrógeno y la composición de las comunidades microbianas del suelo. Como consecuencia del cambio climático se prevé un aumento de la temperatura y cambios en el régimen de precipitaciones en zonas áridas de todo el planeta. Sin embargo, se desconoce el efecto que estos cambios puedan tener en la hidrología de los suelos cubiertos con costra biológica. Asimismo, se desconoce si la costra biológica puede modular los flujos de gases de efecto invernadero y en las principales comunidades microbianas asociadas con los flujos de N_2O y CH_4 en respuesta al cambio climático. Además de los cambios en temperatura y precipitación consecuencia del cambio climático, el aumento de la deposición de nitrógeno atmosférico ocasionado por las actividades antrópicas puede también desempeñar un papel clave en el intercambio de gases de efecto invernadero entre el suelo y la atmósfera en zonas áridas.

Objetivos y Métodos: El objetivo de esta Tesis Doctoral es comprender cómo el cambio global y la costra biológica afectan a la dinámica de humectación y secado del suelo y al intercambio suelo-atmósfera de gases traza (N_2O, CH_4 y CO_2) así como a las comunidades microbianas asociadas a dicho intercambio.

En el primer capítulo se analizó ocho años de registros continuos de datos meteorológicos y de humedad del suelo en un experimento de simulación del cambio climático (control, aumento de 2.5 °C de temperatura, reducción del 33% de la precipitación y combinación de ambos), con el objetivo de evaluar si

el cambio climático afecta al comportamiento hidrológico de suelos cubiertos con costra biológica.

En el segundo capítulo se estudió cómo, tras ocho años de simulación del cambio climático en el mismo experimento utilizado en el capítulo 1, áreas con un alto y bajo desarrollo de costra biológica afectan a los flujos de N_2O y CH_4, así como a la abundancia de genes funcionales relacionados con bacterias desnitrificantes y metanotrofas.

En el tercer capítulo se evaluó como la simulación de cuatro niveles de deposición de N atmosférico afectan a los flujos de N_2O, CH_4 y CO_2 en un experimento a largo plazo situado en el centro de la Península Ibérica.

En el cuarto capítulo se utilizaron datos de un muestreo global estandarizado y modelos de ecuaciones estructurales para evaluar los efectos directos e indirectos de variables climáticas (aridez, estacionalidad de las precipitaciones y temperatura media anual) y del suelo (carbono orgánico, pH y textura) sobre la abundancia total, la riqueza y la estructura de comunidades de microorganismos relacionados con los flujos de N_2O y CH_4 (portadores de los genes *nosZ* y *pmoA*, respectivamente).

Resultados: La costra biológica moduló la humectación y secado del suelo tras los eventos de precipitación. Tanto la cantidad de lluvia como un buen desarrollo de la costra biológica aumentaron las ganancias de agua del suelo después de dichos eventos, mientras que la humedad inicial del suelo, la intensidad de la lluvia durante el evento y el calentamiento experimental redujeron las ganancias de agua del suelo. La humedad inicial, la temperatura máxima y la cobertura de costra biológica, mediante su efecto en la evapotranspiración potencial y en el oscurecimiento del suelo aumentaron la tasa de secado del suelo. Sin embargo, observamos diferencias importantes entre los dos primeros años del experimento y cinco años después de su

montaje. Estos efectos se debieron principalmente a las importantes reducciones en la cobertura y la diversidad de la costra biológica observadas en el tratamiento de calentamiento.

La costra biológica moduló también el efecto del cambio climático en los flujos de gases traza, reduciendo las emisiones de N_2O y aumentando la fijación de CH_4. En suelos donde el grado de desarrollo de la costra biológica es bajo, la exclusión de las precipitaciones y su combinación con el calentamiento aumentaron las emisiones de N_2O. Por el contrario, un buen desarrollo de la cobertura de costra biológica provocó una fuerte reducción de los flujos de N_2O bajo estos tratamientos (~ 96% y ~ 197%, respectivamente). En suelos con bajo desarrollo de costra biológica, el calentamiento solo y en combinación con la exclusión de la lluvia redujeron el consumo de CH_4. Sin embargo, en suelos con un buen desarrollo de costra biológica, la exclusión de lluvia sola y en conjunto con el calentamiento, aumentaron la captación de CH_4. Además, la abundancia general de genes funcionales relacionados con bacterias desnitrificantes y metanotrofas fue mayor en zonas con alto que en las de bajo desarrollo de la costra biológica.

El aumento de la deposición de N condujo a un aumento constante de las emisiones de N_2O, probablemente debido a los aumentos en los procesos de nitrificación microbiana y / o desnitrificación del suelo. Sin embargo, solo los niveles intermedios de deposición de N redujeron la captación de CH_4 y tendieron a reducir las emisiones de CO_2, lo que sugiere la existencia de puntos de inflexión entre las condiciones saturadas y no saturadas de N.

A escala global, la abundancia y la riqueza de los microorganismos metanotrofos no se asociaron con el clima o las propiedades del suelo. Sin embargo, variables como la temperatura media anual, la estacionalidad de las lluvias, el C orgánico, el pH y el contenido de arena estuvieron altamente correlacionados con la composición de su comunidad. La aridez, el contenido en arena y el pH del suelo

se correlacionaron con la abundancia, la riqueza y la composición de la comunidad microbiana portadora del gen *nosZ*.

Conclusiones: El trabajo desarrollado en esta tesis pone de manifiesto la importancia de los estudios a largo plazo para comprender los efectos del cambio climático en la humectación y secado del suelo en ecosistemas semiáridos. El grado de desarrollo de la costra biológica afecta a la capacidad de los suelos de estos ambientes para intercambiar gases de efecto invernadero con la atmósfera. Los resultados obtenidos sugieren que comunidades de costra biológica bien desarrolladas podrían contrarrestar el impacto del calentamiento y la alteración en los patrones de lluvia en los flujos de gases de efecto invernadero del suelo, destacando su importancia y la necesidad de preservarlas para minimizar los impactos del cambio climático en los ecosistemas semiáridos. El aumento en la deposición de nitrógeno atmosférico a largo plazo aumenta las emisiones de N_2O y reduce las tasas de fijación de CH_4 (al menos niveles intermedios de deposición de N), contribuyendo al actual cambio climático. El aumento en la aridez consecuencia del cambio climático alterará las comunidades de microorganismos metanotrofos y portadores del gen *nosZ*. La esperada reducción de la cubierta vegetal como consecuencia del cambio climático y el aumento de la aridez global alterará directa e indirectamente las comunidades microbianas oxidadoras del metano, lo que podría afectar al intercambio neto de CH_4 con la atmósfera. Estos cambios en la aridez y el carbono orgánico del suelo también afectarán a las bacterias desnitrificantes portadoras del gen *nosZ* reduciendo la capacidad de los suelos áridos y semiáridos de llevar a cabo el paso final de la desnitrificación (la reducción de N_2O a N_2), favoreciendo la emisión de N_2O a la atmósfera. En conjunto, nuestros resultados nos ayudan a comprender mejor los factores ambientales que causan cambios en la abundancia, riqueza y estructura de los genes *nosZ* y *pmoA* en suelos áridos y semiáridos. Esta información puede utilizarse para refinar los

modelos utilizados para pronosticar cambios en los flujos de gases de efecto invernadero en el futuro.

Introducción general

Zonas áridas: características e importancia

Existen distintos criterios para definir a las zonas áridas. Uno de los más utilizados es el índice de aridez (IA = Precipitación/Evapotranspiración Potencial), propuesto por la UNEP (1992), y que se basa en el déficit de agua respecto a la demanda hídrica de un determinado lugar. Según este criterio, una zona es considerada árida cuando su IA es menor de 0.65. Las zonas áridas a su vez pueden subdividirse en hiperáridas (IA < 0.05); áridas (0.05 < IA < 0.2), semiáridas (0.2< IA < 0.5); y seco-subhúmedas (0.5 < IA < 0.65) (UNEP, 1992; Figura 1). Climáticamente, las zonas áridas tienen regímenes de lluvia caracterizados por largos períodos de sequías interrumpidos por eventos de precipitación relativamente esporádicos (D'Odorico & Bhattachan, 2012). Esta característica podría intensificarse debido al actual cambio climático. Así, los modelos más recientes sugieren que el régimen de lluvias en muchas zonas áridas del mundo se caracterizará por un número menor de eventos de lluvia más concentrados en el tiempo (Weltzin *et al.*, 2003; Loik *et al.*, 2004; Trenberth, 2011; D'Odorico & Bhattachan, 2012; Singh & Kumar, 2015). Además, el cambio climático está ya aumentando la variabilidad de las precipitaciones en muchas zonas áridas del mundo (Easterling, 2000; Weltzin *et al.*, 2003; Hughes & Diaz, 2008; Huang *et al.*, 2016a; Tietjen *et al.*, 2017), que de por sí es muy elevada (D'Odorico & Bhattachan, 2012; Singh & Kumar, 2015).

Encontramos zonas áridas en todos los continentes (Figura 1), y es el bioma dominante en algunos de ellos, como en Oceanía, África y Asia, donde ocupa el 85%, 75% y 51% de su superficie, respectivamente (Prăvălie, 2016). Las zonas áridas cubren entre el 40 y el 45% del total de la superficie terrestre del planeta (Prăvălie, 2016; Huang *et al.*, 2017), y en ellas se asienta más del 30% de la población humana (Reynolds *et al.*, 2007; Davies *et al.*, 2012). Además, el 50% del ganado y el 40% de la agricultura que alimenta a la población mundial se desarrolla en estas zonas (UNEP, 1992; Thornton, 2002; Safriel & Adeel, 2005). Pese a la limitación hídrica de estos sistemas y la baja fertilidad de sus suelos

(Schimel, 2010, 2018; Wang *et al.*, 2012; Delgado-Baquerizo *et al.*, 2013b; Jiao *et al.*, 2016), algunas zonas áridas presentan una alta diversidad y numerosas especies endémicas que se han adaptado a las duras condiciones de estos sistemas (Davies *et al.*, 2012; Ward, 2016).

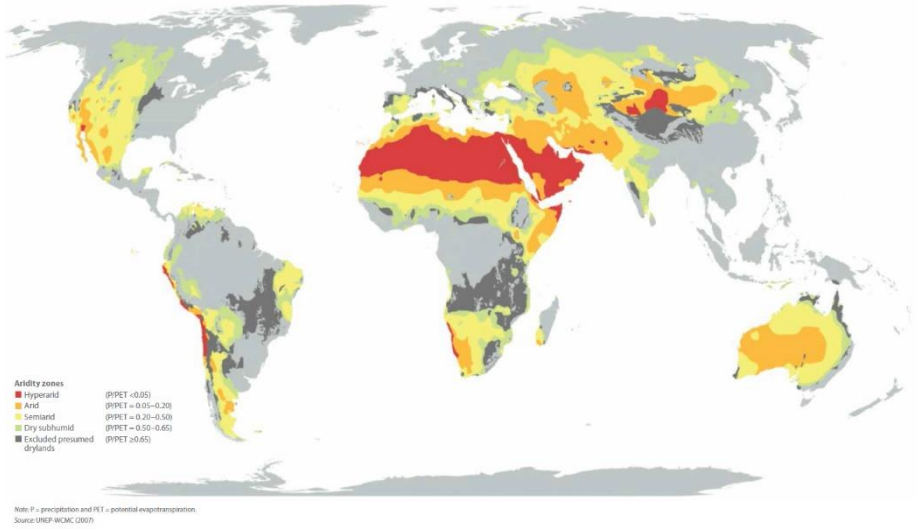

FIGURA 1 Mapa mundial de la zonas áridas y subtipos. Fuente UNEP-WCMC (2007).

Las zonas áridas constituyen sistemas muy frágiles cuya preservación es clave para la subsistencia de los casi 2.5 billones de seres humanos que viven en estas regiones (Reynolds *et al.*, 2007; United Nations, 2017) tanto por la gran cantidad de recursos que proporcionan (casi todas las reservas de petróleo del mundo y numerosos metales como el cobre, la plata o el oro), como por su importancia para mantener la biodiversidad en el planeta y para paliar los efectos del cambio climático (Davies *et al.*, 2012; OPEC, 2017). Aproximadamente el 40% de la producción primaria neta ocurre en regiones áridas (Wang *et al.*, 2012), por lo que su potencial para secuestrar carbono a escala global convierte estas regiones en sitios clave (Lal, 2004; Poulter *et al.*, 2014; Huang *et al.*, 2017).

En los ecosistemas áridos, la actividad de los organismos, los ciclos de

nutrientes, la respiración del suelos o la productividad vegetal están fuertemente controlados por la disponibilidad de agua (Huxman *et al.*, 2004; Yan *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015; García-Palacios *et al.*, 2018). Estudios recientes muestran como, a escala global, conforme aumenta la aridez, se produce una reducción del carbono orgánico y el nitrógeno total del suelo (Delgado-Baquerizo *et al.*, 2013b; Jiao *et al.*, 2016). Los pequeños pulsos de lluvia característicos de las zonas áridas (Austin *et al.*, 2004; Huxman *et al.*, 2004; Reynolds *et al.*, 2004) pueden aumentar la mineralización de la materia orgánica al activar a los microorganismos del suelo lo que daría lugar a un aumento del N inorgánico del suelo disponible para la vegetación (Wang *et al.*, 2014). Sin embargo, es posible que estos pequeños pulsos no sean suficientes para activar a la vegetación que sigue limitada por la disponibilidad de agua, de forma que este N inorgánico es consumido por los microorganismos en los procesos de nitrificación y desnitrificación (Vitousek, 2004; Schlesinger & Bernhardt, 2013).

Gases de efecto invernadero en zonas áridas: relevancia de sus flujos y su sensibilidad a la deposición de N y al cambio climático

La concentración atmosférica de los gases de efecto invernadero (GEI) ha aumentado en las últimas décadas contribuyendo al cambio climático (Pachauri & Meyer, 2014). En 2015 se superó por primera vez desde que tenemos registro las 400 ppm de dióxido de carbono (CO_2) en la atmósfera, pero desde 1800 la concentración atmosférica de CO_2, metano (CH_4) y óxido nitroso (N_2O) ha aumentado un 145%, 249% y 122% respectivamente (Meinshausen *et al.*, 2017). Si bien los flujos y la dinámica del CO_2 se han estudiado ampliamente en los últimos años (Pendall *et al.*, 2013; Vicca *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015, 2018; Lo Cascio *et al.*, 2017), otros gases con un importante efecto invernadero, como el N_2O o el CH_4, cuyos potenciales de calentamiento son 298 y 25 veces, respectivamente, mayores que el del CO_2 (Nakicenovic & Swart, 2000), han recibido menos atención (Flechard *et al.*, 2007; Soussana *et al.*, 2007;

Snyder *et al.*, 2009; Ussiri *et al.*, 2009; Dijkstra *et al.*, 2013; Tate, 2015).

Tradicionalmente se ha considerado que los flujos de GEI en las zonas áridas son bajos debido a la falta de agua y a la escasez de nutrientes que caracteriza estos ecosistemas y que limita la actividad microbiana (Dalal & Allen, 2008). Esto ha generado un sesgo en el que la gran mayoría del conocimiento sobre el intercambio de N_2O y CH_4 entre el suelo y la atmósfera, así como la ecología microbiana asociada, proviene de estudios realizados en ecosistemas más húmedos (Oertel *et al.*, 2016). Sin embargo, en la última década, varios estudios han reportado flujos elevados de N_2O en suelos áridos y semiáridos después de pulsos de lluvia (Barton *et al.*, 2008, 2013; Zaady *et al.*, 2013), y han descrito los suelos de las zonas áridas como un importante sumidero global de CH_4 atmosférico (Striegl *et al.*, 1992; Potter *et al.*, 1996; Liu *et al.*, 2016; Zhou *et al.*, 2019). Estos resultados sugieren que, dada la extensión de estos ecosistemas, los flujos de N_2O y CH_4 en zonas áridas pueden ser más relevantes a escala global de lo tradicionalmente reconocido (Martins *et al.*, 2015; Hu *et al.*, 2017).

El nitrógeno (N) es uno de los principales gases presentes en la atmósfera. Sin embargo, la mayor parte de este gas se encuentra en forma no reactiva (N_2) y, por lo tanto, no disponible para los seres vivos excepto para aquellos capaces de fijar el N_2 atmosférico (algunas cianobacterias, bacterias y arqueas; Madigan et al., 2008). Esto hace que el N sea, junto con el agua, uno de los principales factores limitantes para plantas y microorganismos en la mayoría de ecosistemas (Hooper & Johnson, 1999; Fenn *et al.*, 2003; Li *et al.*, 2009). Las emisiones antrópicas de formas de N reactivas (i.e. NH_3, NO_x, HNO_3) a la atmósfera proceden principalmente de la actividad industrial, los motores de combustión, la ganadería y la fertilización en la agricultura (Bouwman, 1998; Snyder *et al.*, 2009; Fowler *et al.*, 2013; Kim *et al.*, 2015; Van Lent *et al.*, 2015). El aumento en la deposición de N en zonas áridas como consecuencia de estas actividades antrópicas es una fuente importante de N y podría estar asociado a

un aumento de la fertilidad del suelo (Chapin *et al.*, 1986; Vitousek *et al.*, 1997; Bobbink *et al.*, 2010; Ochoa-Hueso *et al.*, 2011a). Sin embargo, por encima de la carga critica (el nivel máximo de deposición de N que puede soportar un sistema sin que este tenga efectos nocivos en el mismo (Nilsson & Grennfelt, 1988), y que en ecosistemas mediterráneos se ha estimado entre 3-17 kg N ha^{-1} a^{-1} (Fenn *et al.*, 2008, 2010), la deposición antropogénica de N puede causar desequilibrios en el ciclo N, eutrofización y acidificación del suelo (Bobbink *et al.*, 2010; Ochoa-Hueso *et al.*, 2013), influir en la descomposición de la materia orgánica y la actividad microbiana (Lo Cascio *et al.*, 2017), y también estimular el intercambio de gases de efecto invernadero entre el suelo y la atmósfera (Mosier *et al.*, 1991; Bowden *et al.*, 2000).

Los modelos climáticos más recientes prevén aumentos de temperatura de hasta 4 °C en algunas regiones debido al cambio climático (Pachauri & Meyer, 2014), así como cambios en el régimen de precipitaciones, con menos eventos pero más intensos (D'Odorico & Bhattachan, 2012), y un aumento generalizado de la aridez (Fu & Feng, 2014). En zonas áridas, la duración, frecuencia y severidad de las sequias ya ha aumentado en las últimas décadas y los modelos climáticos prevén que estas sigan aumentando en los próximos años (Pachauri & Meyer, 2014). El aumento de la aridez y las sequias afectan a las comunidades vegetales reduciendo la producción primaria, lo cual afecta negativamente al funcionamiento de los ecosistemas (Gaitán *et al.*, 2014; Sohoulande Djebou *et al.*, 2015). Por otro lado, las comunidades de hongos y bacterias del suelo juegan un papel muy importante en el funcionamiento del ecosistema ya que intervienen en la descomposición de la hojarasca, el reciclado de nutrientes y la producción primaria (Bardgett & Van Der Putten, 2014; Delgado-baquerizo *et al.*, 2016). En zonas áridas, estas comunidades microbianas se ven afectadas por cambios en temperatura, aridez y humedad como los que se prevé que ocurran como consecuencia del cambio climático. Así, Maestre et al. (2015a), observaron una clara reducción tanto en la diversidad como en la abundancia

de bacterias y hongos del suelo de zonas áridas al aumentar la aridez (resultados similars han sido encontratos también por Huang et al., 2018; Neilson et al., 2017; Nielsen & Ball, 2015). Estos cambios en las comunidades microbianas pueden afectar a los distintos procesos que dependen de ellas, como el intercambio de gases suelo-atmósfera. Así, experimentos de cambio climático han visto como la fijación de C en suelos áridos disminuye con el calentamiento, aumentando las emisiones de CO_2 del suelo (Shen *et al.*, 2009; Maestre *et al.*, 2013; Yan *et al.*, 2014; Darrouzet-Nardi *et al.*, 2015). También se han visto que las emisiones de N_2O aumentan con la humedad del suelo y como un aumento de la temperatura podría reducir la capacidad de las zonas áridas de actuar como sumideros de CH_4 y N_2O (Dijkstra *et al.*, 2013). Sin embargo, son todavía escasos los experimentos en los que se evalúe el efecto conjunto del calentamiento global y los cambios pronosticados en los patrones de lluvia sobre los flujos de N_2O y CH_4 en los suelos de zonas áridas (Darrouzet-Nardi *et al.*, 2015; Guan *et al.*, 2018).

La costra biológica del suelo y su importancia en los ecosistemas áridos

La costra biológica del suelo (Figura 2) es una comunidad formada por musgos, líquenes y microorganismos (cianobacterias, hongos, otras bacterias y arqueas) que viven en los primeros milímetros de la superficie del suelo (Weber *et al.*, 2016). Estas comunidades están especialmente bien desarrolladas en las zonas áridas de todo el mundo, donde la falta de agua limita la cobertura y el desarrollo de la vegetación vascular (Eldridge & Greene, 1994; Castillo-Monroy & Maestre, 2011; Maestre *et al.*, 2011; Weber *et al.*, 2016). Según el grupo de organismos dominante, la costra biológica puede ser de cianobacterias (como la que domina en muchas zonas de EE.UU; Belnap & Lange, 2002), algas verdes (presente sobre todo en zonas de dunas costeras; Schulz et al., 2016), musgos (dominantes en muchos ecosistemas áridos de China; Yang et al., 2019) o líquenes (como las que podemos encontrar en las zonas gipsícolas de España;

Escolar et al., 2012; Figura 3).

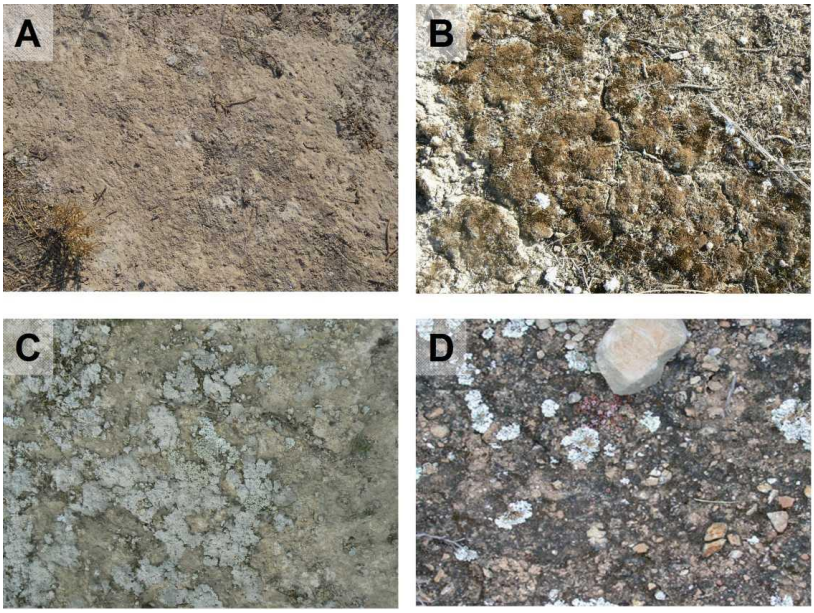

FIGURA 2 Tipos de costra biológica basados en el grupo morfológico dominante. A) Suelo desnudo (desprovisto de vegetación). B) Costra biológica dominada por musgos (Briófitos). C) Costra biológica dominada por líquenes y D) Costra biológica dominada por cianobacterias. Fuente: (Castillo-Monroy y Maestre, (2011).

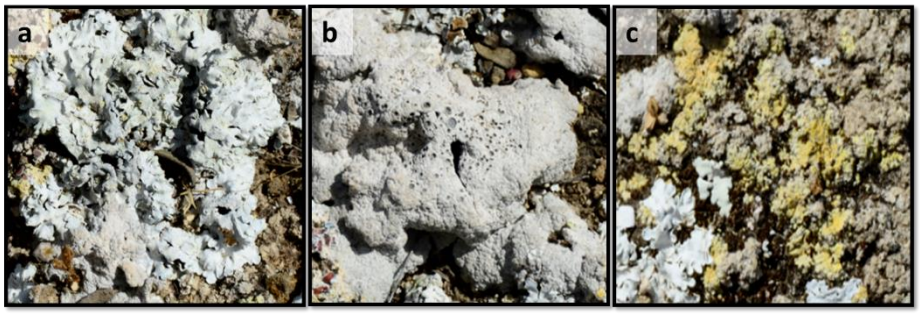

FIGURA 3 Detalle de algunas de las especies más comunes que forman parte de la costra biológica del suelo en ecosistemas gipsícolas españoles: *Squamarina lentigera* (a), *Diploschistes diacapsis* (b) y *Fulgensia subbracteata* (c).

En las zonas áridas la costra biológica es un componente biótico

predominante que regula múltiples procesos y propiedades bióticas y abióticas del suelo como la respiración (Maestre *et al.*, 2013), los flujos de CH_4 y N_2O (Aschenbach *et al.*, 2013; Zaady *et al.*, 2013; Porada *et al.*, 2017), el ciclo del N (Reed *et al.*, 2012), la porosidad (Felde *et al.*, 2014), la temperatura (Couradeau *et al.*, 2016) o la infiltración (Eldridge *et al.*, 2010; Lafuente *et al.*, 2018), por nombrar algunos. Además, y al igual que las plantas vasculares, las comunidades de costra biológica ofrecen en los suelos en los que se desarrollan un hábitat para las comunidades microbianas (Delgado-Baquerizo *et al.*, 2018a) y de fauna del suelo (Neher, 1999; Darby *et al.*, 2006, 2010; Darby & Neher, 2016), influyendo en su abundancia y actividad (Delgado-Baquerizo *et al.*, 2016a). El efecto de la costra biológica en la estructura y función del ecosistema depende en gran medida de su composición específica, diversidad y grado de desarrollo (Bowker *et al.*, 2011; Chamizo *et al.*, 2012a, 2013; Maestre *et al.*, 2012a; Whitney *et al.*, 2017).

El cambio climático está afectando la biota de los ecosistemas terrestres de múltiples maneras (Peñuelas *et al.*, 2013) y las comunidades de costra biológica no son una excepción. Diversos estudios realizados en distintas regiones del mundo muestran cómo el aumento de la temperatura y los cambios en los regímenes de precipitaciones, como los pronosticados para la segunda mitad de este siglo, afectan negativamente a la cobertura y a la actividad de los organismos que componen la costra biológica (Escolar *et al.*, 2012; Johnson *et al.*, 2012; Maphangwa *et al.*, 2012; Zelikova *et al.*, 2012; Maestre *et al.*, 2013, 2015b; Ferrenberg *et al.*, 2015; Ladrón de Guevara *et al.*, 2018). Ferrenberg et al. (2015) observaron que un aumento de 2 °C y 4 °C en la temperatura y un aumento en la frecuencia de los eventos de lluvia provocaron grandes alteraciones en la composición de las comunidades que forman las costras biológicas, favoreciendo el crecimiento de cianobacterias a expensas de líquenes y musgos, generando grandes alteraciones en los procesos y atributos ecosistémicos que dependen de estos organismos. Resultados similares se

observaron en la región mediterránea, donde un aumento de 2-3 °C en la temperatura, donde la cobertura de costra biológica dominada por líquenes disminuyó drásticamente y aumentó ligeramente la cobertura de musgos (Escolar *et al.*, 2012; Ladrón de Guevara *et al.*, 2014, 2018; Maestre *et al.*, 2015b).

Las comunidades que forman la costra biológica tienen una fuerte influencia en el ciclo hidrológico de las zonas áridas a través de cambios en la retención de agua (Cantón *et al.*, 2004), en la rugosidad del suelo (Eldridge & Rosentreter, 1999) o en la temperatura (Couradeau *et al.*, 2016), lo que a su vez determina la infiltración, la escorrentía y la evapotranspiración (Belnap, 2006; Chamizo *et al.*, 2016b) y la difusión de gases (Ball *et al.*, 1999; Smith *et al.*, 2018). Dada la gran importancia que estos organismos tienen regulando las ganancias y pérdidas de agua del suelo, los ciclos de nutrientes y los flujos de gases de efecto invernadero en zonas áridas, existe un interés creciente en la influencia que el cambio climático podría ejercer sobre estas comunidades y sobre los procesos ecosistémicos asociados a ellas (Grote *et al.*, 2010; Reed *et al.*, 2012, 2016; Maestre *et al.*, 2013; Ladrón de Guevara *et al.*, 2014; Delgado-Baquerizo *et al.*, 2015b; Escolar *et al.*, 2015). Además, diversos estudios han sugerido la necesidad de tener en cuenta a la costra biológica en los modelos biogeoquímicos y de realizar nuevos esfuerzos para entender y pronosticar su papel en futuras emisiones de gases de efecto invernadero desde los ecosistemas terrestres (Maestre *et al.*, 2013; Weber *et al.*, 2015). A pesar de ello, apenas existen hasta la fecha estudios que evalúen el efecto de la costra biológica como modulador de los procesos hidrológicos y los flujos de N_2O y CH_4 (potentes gases de efecto invernadero) en respuesta al cambio climático utilizando aproximaciones experimentales.

El papel de los microorganismos en los flujos de gases suelo-atmósfera

Las comunidades microbianas del suelo son los principales responsables bióticos del intercambio entre el suelo y la atmósfera de gases de efecto invernadero como el N_2O y CH_4 (Dalal & Allen, 2008). Por ejemplo, aproximadamente dos tercios del CH_4 que se encuentra en la atmósfera lo producen microorganismos (Le Mer & Roger, 2001), pero los suelos aeróbicos son también el único sumidero biológico para este CH_4 atmosférico (Dalal & Allen, 2008; Conrad, 2009). La nitrificación y la desnitrificación son los procesos responsables de más de dos tercios de las emisiones de N_2O del suelo a la atmósfera (Dalal & Allen, 2008; Butterbach-Bahl *et al.*, 2013). Los microorganismos nitrificantes transforman el NH_4^+ en NO_3^-, liberando N_2O a la atmósfera como un subproducto (Bremner, 1997). La desnitrificación (es decir, la reducción de NO_3 a NO, N_2O y finalmente a N_2) es un proceso de varios pasos llevado a cabo por diferentes microorganismos y sus enzimas, incluida la *óxido nitroso reductasa* (codificada por el gen *nosZ*), que cataliza el último paso de la desnitrificación (Bremner, 1997; Canfield *et al.*, 2010). En los suelos de zonas áridas, que están secos (es decir, en condiciones aeróbicas) la mayor parte del tiempo, la nitrificación se asume como el proceso dominante (Delgado-Baquerizo *et al.*, 2016b). Es por ello que el gen *nosZ*, presente en bacterias desnitrificantes, así como los factores que afectan su abundancia y actividad, han sido poco estudiados en zonas áridas, al asumirse que estas zonas la mayor parte del tiempo no reúnen las condiciones anaeróbicas necesarias para que la desnitrificación tenga lugar (Philippot *et al.*, 2007; Hallin *et al.*, 2017). Sin embargo, los agregados del suelo y los esporádicos pulsos de precipitación crean condiciones anaeróbicas puntuales favorables para la desnitrificación en estos suelos, por lo que la desnitrificación y los microorganismos portadores del gen *nosZ* pueden ser más relevantes de lo convencionalmente esperado en estos ecosistemas (Austin *et al.*, 2004; Ley *et al.*, 2018; Wang *et al.*, 2019).

Por otro lado, los microorganismos metanógenos producen CH_4 en

condiciones anaeróbicas (por ejemplo, arrozales, sedimentos lacustres o humedales) a través de un proceso llamado metanogénesis (Le Mer & Roger, 2001). La producción de CH_4 en las zonas áridas, caracterizadas por precipitaciones poco frecuentes y escasas, no se espera que sea elevada (Castaldi *et al.*, 2006). Sin embargo, estudios recientes han observado la presencia de microorganismos metanógenos en la costra biológica que podrían contribuir en mayor o menor medida a la producción de CH_4 en zonas áridas con un alto desarrollo de la costra biológica (Angel & Conrad, 2013). Por otro lado, los microorganismos metanotrofos poseen la enzima metano monooxigenasa (codificada por el gen *pmoA*), que oxida el CH_4 en condiciones aeróbicas, lo que reduce potencialmente las concentraciones de CH_4 en la atmósfera (McDonald & Murrell, 1997; Holmes *et al.*, 1999; Conrad, 2009). Debido a sus requisitos aeróbicos, estos microorganismos sí son reconocidos miembros de las comunidades microbianas en las zonas áridas (Angel & Conrad, 2009; Tate, 2015; Martins *et al.*, 2016).

Debido a la creciente evidencia de la relación entre abundancia de genes funcionales y funcionalidad, diversos estudios han relacionado la abundancia de los genes *nosZ* y *pmoA*, y los flujos de N_2O y CH_4 entre el suelo y la atmósfera (Nazaries *et al.*, 2013b; Powell *et al.*, 2015; Martins *et al.*, 2017). La mayoría de estos estudios se han centrado en ecosistemas concretos como bosques templado boreales (Martins *et al.*, 2017), bosques (Bengtson *et al.*, 2009; Nazaries *et al.*, 2013b), zonas pantanosas (Peltoniemi *et al.*, 2016) o suelos agrícolas (Lammel *et al.*, 2015). Sin embargo, pese a la importancia a escala global de los GEI, carecemos de estudios globales y en particular de zonas áridas, que se centren en la abundancia de estos genes funcionales y que relacionen genes con flujos de GEI.

Estructura y objetivos generales de la tesis

El objetivo general de esta tesis es evaluar cómo distintos aspectos del cambio ambiental global (cambio climático y deposición de nitrógeno) afectan a aspectos clave relacionados con la hidrología y el intercambio de gases de efecto invernadero entre el suelo y la atmósfera en ecosistemas áridos a distintas escalas espaciales (local y global). Para poder cumplir este objetivo la tesis se estructura en cuatro capítulos, que se describen a continuación:

En el **Capítulo 1** se estudia cómo la costra biológica del suelo afecta a la dinámica de humectación y desecación del suelo después de eventos de lluvia bajo distintos escenarios de cambio climático en un experimento a largo plazo ubicado en Aranjuez (Madrid). Con este capítulo se pretende evaluar la importancia de las costras biológicas en la hidrología del suelo y cómo su papel puede verse afectado por el cambio climático en el que estamos inmersos, aspectos clave a la hora de entender los impactos del cambio climático en la actividad de los microorganismos responsables de los flujos de gases de efecto invernadero en el suelo.

En el **Capítulo 2** se analizan los flujos de gases de efecto invernadero (N_2O y CH_4) y genes funcionales relacionados con los mismos (*pmoA* y *nosZ*), así como el efecto que la costra biológica tiene sobre estos flujos, bajo distintos escenarios de cambio climático en un experimento a largo plazo ubicado en Aranjuez (Madrid; Figura 4a).

En el **Capítulo 3** se exploran los efectos que el aumento de la deposición de nitrógeno atmosférico tiene sobre los flujos de gases de efecto invernadero (N_2O, CH_4 y CO_2) en un experimento a largo plazo ubicado en la reserva natural de El Regajal-Mar de Ontígola (Madrid; Figura 4b).

En el **Capítulo 4** se estudia la abundancia, riqueza y estructura de comunidades microbianas que portan genes relacionados con la desnitrificación y la oxidación del metano en zonas áridas de todo el planeta. En concreto, se analizó cómo variables climáticas y propiedades físicas del suelo determinan la abundancia,

riqueza y estructura de las comunidades microbianas portadoras de los genes funcionales *nosZ* y *pmoA*.

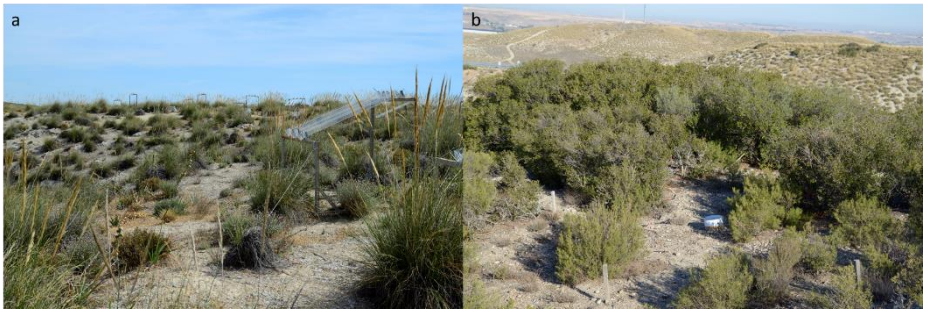

FIGURA 4 Estación experimental de Aranjuez (a) y reserva natural de El Regajal-Mar Ontígola (b).

Capítulo 1 / Chapter 1

Simulated climate change affects how biocrusts modulate water gains and desiccation dynamics after rainfall events

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Photo credits: Felipe Gutierrez

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Abstract

Soil surface communities dominated by mosses, lichens and cyanobacteria (biocrusts) are common between vegetation patches in drylands worldwide and are known to affect soil wetting and drying after rainfall events. While ongoing climate change is already warming and changing rainfall patterns of drylands in many regions, little is known on how these changes may affect the hydrological behaviour of biocrust-covered soils. We used 8 years of continuous soil moisture and rainfall data from a climate change experiment in central Spain to explore how biocrusts modify soil water gains and losses after rainfall events under simulated changes in temperature (2.5 °C warming) and rainfall (33% reduction). Both rainfall amount and biocrust cover increased soil water gains after rainfall events, whereas experimental warming, rainfall intensity and initial soil moisture decreased them. Initial moisture, maximum temperature and biocrust cover, by means of enhancing potential evapotranspiration or by soil darkening, increased the drying rates and enhanced the exponential behaviour of the drying events. Meanwhile, warming reduced their exponential behaviour. The effects of climate change treatments on soil water gains and losses changed through time, with important differences between the first two years of the experiment and five years after its setup. These effects were mainly driven by the important reductions in biocrust cover and diversity observed under warming. Our results highlight the importance of long-term studies to understand soil moisture responses to ongoing climate change in drylands.

Key words

biological soil crusts, drying, drylands, rainfall exclusion, soil moisture, warming, wetting.

Introduction

Arid, semi-arid and dry-subhumid ecosystems (drylands) have rainfall regimes characterized by long periods without rainfall, which also takes place as discrete pulses that largely control the activity of organisms and the rate of ecosystem processes such as nutrient cycling, soil respiration or plant productivity, to name a few (Austin et al., 2004; Huxman et al., 2004; Reynolds et al., 2004). Ongoing climate change is increasing the variability of rainfall in many drylands worldwide (D'Odorico & Bhattachan, 2012; Singh & Kumar, 2015), a trend that will likely continue and be enhanced in the future (Easterling, 2000; Hughes & Diaz, 2008; Weltzin et al., 2003). Current forecasts indicate that the rainfall regime of many drylands worldwide will be characterized by a lower number of more concentrated rainfall events (Dore, 2005). Therefore, understanding the factors affecting water gains and losses after rainfall events is crucial not only to understand how dryland ecosystems function, but also how they are responding to ongoing climate change (Collins *et al.*, 2014).

Biocrusts are communities formed by mosses, lichens and microorganisms (cyanobacteria, fungi, other bacteria and archaea) that live on the soil surface in drylands worldwide (Weber et al., 2016). These communities are a prevalent biotic component in these ecosystems, where they control the exchange of elements and energy between the atmosphere and the soil (Pointing & Belnap, 2012). Biocrusts largely affect the hydrological cycle of drylands by controlling soil properties such as soil water retention (Cantón et al., 2004), albedo (Rutherford *et al.*, 2017), surface roughness (Eldridge & Rosentreter, 1999) and temperature (Couradeau *et al.*, 2016), which affect infiltration and runoff generation and evaporation losses (Belnap, 2006; Berdugo et al., 2014; Chamizo et al., 2012). The hydrological impacts of biocrusts are, however, largely dependent on: i) their composition and degree of development (Chamizo et al., 2012, 2013) and ii) the amount, duration and intensity of rainfall events (Berdugo et al., 2014; Chamizo et al., 2016). However,

most of our knowledge on the hydrological impacts of biocrusts come from short-term studies (i.e., typical length below two years; Cantón et al., 2004; Chamizo et al., 2016; Zaady et al., 2014; but see Berdugo et al., 2014). Conducting studies over multiple years is of great importance to capture inter-annual rainfall variability, which is typically very high in drylands (D'Odorico & Bhattachan, 2012), and hence to better understand how biocrusts affect soil water gains (i.e difference between the initial and the maximum moisture achieved during the rainfall event) and losses (i.e the slope of the drying curve) after rainfall events of different amount, duration and intensity (Chamizo et al., 2016).

Climate change is impacting the biota of terrestrial ecosystems in multiple ways (Peñuelas *et al.*, 2013), and biocrust constituents are not an exception. Increases in temperature and changes in rainfall regimes such as those forecasted for the second half of this century have been found to negatively affect the performance and cover of lichen- and moss-dominated biocrusts in South Africa (Maphangwa et al., 2012), Spain (Escolar et al., 2012; Maestre et al., 2013, 2015) and the USA (Ferrenberg et al., 2015; Johnson et al., 2012; Zelikova et al., 2012). These changes are leading to shifts in the composition of biocrust communities, with reported increases in cyanobacteria at the expense of mosses and lichens (Ferrenberg *et al.*, 2015), which underlie the dramatic changes in nitrogen (Reed *et al.*, 2012; Delgado-Baquerizo *et al.*, 2014) and carbon (Escolar et at., 2015; Grote et al., 2010; Ladrón de Guevara et al., 2014; Maestre et al., 2013) cycling observed under simulated climate change in biocrust-dominated ecosystems. Despite the growing interest and literature on the impacts of climate change on both biocrusts and the ecosystem processes associated to them (Reed *et al.*, 2016), no study so far has evaluated how climate change-induced changes in biocrust communities, affect their role as modulators of water gains and losses after rainfall events. Given the important roles played by biocrusts as determinants of rainfall infiltration

(Berdugo et al., 2014; Chamizo et al., 2016), such studies are critical to better understand the hydrological impacts of ongoing climate change on dryland ecosystems worldwide.

Here, we evaluate how biocrusts modulate the effect of simulated climate change on soil water gains and losses after rainfall events in a semiarid ecosystem from central Spain. For doing so we use eight years of data from an ongoing manipulative experiment where soil moisture is being continuously measured in areas with different biocrust cover and under warming and rainfall exclusion treatments (Maestre *et al.*, 2013). This unique dataset allowed us to explore how: a) forecasted changes in rainfall and temperature may affect water gains and losses after rainfall events across multiple years with contrasting climatic conditions, and b) simulated climate change alter the role of biocrusts as modulators of these hydrological features. We hypothesize that climate change-induced effects on biocrust communities, being the most noticeable the reduction in the cover and photosynthetic activity of dominant lichen species - already observed during the first years of the experiment (Escolar et al., 2012; Maestre et al., 2013, Ladrón de Guevara et al., 2014), will influence their capacity to affect water gains and losses after rainfall events (Berdugo *et al.*, 2014).

Material and Methods

Site description

This study was conducted in the Aranjuez Experimental Station, located in central Spain (40º01'55.7''N-3º32'48.3''W; 590 m.a.s.l). Its climate is Mediterranean semi-arid, with average annual rainfall and temperature of 358 mm and 15 ºC, respectively (data available since 1977 from Aranjuez Meteorological Station, 40º04'N-3º32'W; 540 m.a.sl, and from an *in situ* meteorological station). The area typically registers a marked dry period from June to September with very few rains of little intensity (June, July, August and

September average 19, 9, 9 and 22 mm of precipitation respectively from 1981-2010; see also Figures 1 and S1 and Table 1 for a more detailed description of the climatic conditions throughout the study period). Soils are classified as Gypsiric Leptosols (IUSS Working Group WRB 2006), with pH, organic carbon and total nitrogen content values ranging between 7.2-7.7, 9-32 mg/g soil and 0.8-4 mg/g soil, respectively, depending on the microsite (open areas, vegetation and biocrust) considered (see Castillo-Monroy et al., 2010 for more details). The vegetation is dominated by *Macrochloa tenacissima* (L.) Kunth (18% of total cover), *Retama sphaerocarpa* (L) Boiss and *Helianthemun squamatum* Pers. (6% of total cover for both shrubs). The open areas between vascular plant patches are covered with a well-developed biocrust community that covers ~34% of the soil surface and is dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia* (Scop.) Timdal, and *Psora decipiens* (Hedw.) Hoffm. (see Maestre et al. 2013 for a full species checklist).

Experimental design and monitoring

We established a fully factorial experimental design with three factors, each with two levels: biocrust cover (poorly developed biocrust communities with cover <25% vs. well-developed biocrust communities with cover >50%), warming (WA, control vs. ~2.5ºC temperature increase), and rainfall exclusion (RE, control vs. ~33% reduction in total annual rainfall). Ten replicates per combination of treatments were established, resulting in a total of 80 experimental plots. To simulate warming, we used hexagonal open top chambers (OTCs) made of methacrylate and with a size of 40 cm x 50 cm x 32 cm. To intercept rainfall, we built a 1.2 m x 1.2 m x 1 m metallic frame supporting three V-shaped methacrylate gutters that cover ~37% of the surface. The methacrylate gutters had a 20º inclination and on the lower side was a PVC gutter connected by a hose to a tank to collect the excluded rainfall. The WA

and RE treatments were setup in July and November 2008, respectively (see Maestre et al. 2013 for additional details on the treatments and on their performance).

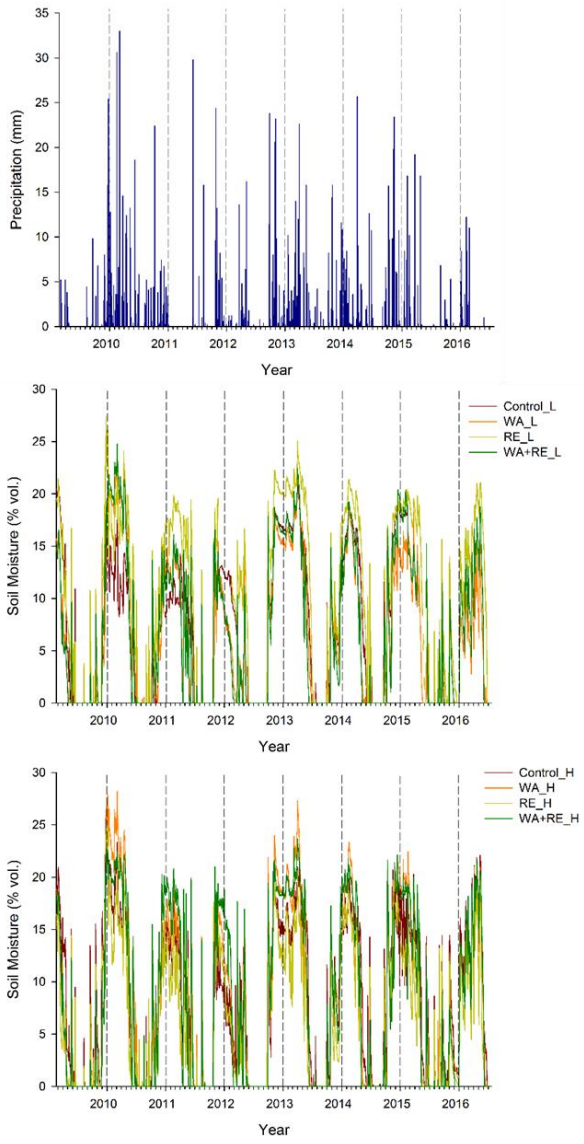

FIGURE 1 Rainfall events registered during the experiment (upper panel, blue bars) and soil moisture (0-5 cm depth) measured by automated sensors in low (L) and high (H) biocrust cover plots (middle and lower panels, respectively). WA = Warming and RE = Rainfall exclusion.

Air temperature/humidity, soil moisture, rainfall and biocrust cover have been monitored from 2009 to 2016. Soil moisture (0–5 cm depth) was continuously monitored every 2.5 hours in all treatments using a total of 23 replicated automated sensors, 2-4 replicates per treatment (EC-5 soil moisture sensors, Decagon Devices Inc., Pullman, WA, USA). Relative air humidity, air temperature and rainfall accumulated every 10 min was also monitored using an on-site meteorological station (OnsetCorp 2.8., MA, USA). Within each plot, the total cover of the visible biocrust components (lichens and mosses) were estimated at the beginning of the experiment and once a year hereafter using high-resolution photographs, as indicated in Maestre et al. (2013).

Identification and characterization of wetting and drying events

We identified wetting and drying events following the approach of Berdugo et al. (2014). We define a wetting event as any rain registered with the *in-situ* pluviometer. Rainfall events separated by at least 2.5 h without rainfall in between were considered as different wetting events. Drying events are defined as periods of ten consecutive days without any registered rain after a rainfall event. Per these criteria, we described 521 wetting events and 56 drying events from February 2009 to July 2016 (Figure 1).

We used in our analyses different covariates that are important to determine soil water gains and losses after rainfall events in drylands (Berdugo et al., 2014). Four covariates were used when analyzing wetting events: initial soil moisture, rainfall amount, rainfall intensity and the interaction between rainfall amount and intensity. Rainfall intensity was estimated as the maximum rainfall achieved in intervals of 10 minutes. We selected these covariates because they may have opposite effects on soil water gains, i.e. while rainfall amount increases soil water content, a higher intensity of the event may reduce it because it is directly related to runoff (Gardner et al., 1999). Initial soil moisture was estimated as the soil water content before the rainfall event. We

used two covariates when analyzing drying events: the maximum temperature reached during the drying event and soil moisture at the beginning of this event, estimated as the water content after the rainfall event.

We characterized water gains as the difference in soil volumetric water content (VWC) between initial moisture preceding a given rainfall event (i.e. minimum moisture) and the maximum moisture achieved during this event. Drying events were characterized by means of the slope and the shape of a drying curve, which describes how soil moisture decreases through time. The drying curves were obtained from the 56 drying events recorded. Each drying curve was fitted to a linear equation

$$bx + c \quad (1)$$

to obtain the slope (b) of the drying curve, and to a quadratic equation

$$ax^2 + bx + c \quad (2)$$

to obtain the shape (a) of the drying curve. In Equation 2, $a = 0$ indicates a linear decay, $a > 0$ an exponential decay and $a < 0$ an inversed exponential decay. To improve the fitting of the drying events, we multiplied a by R^2, the coefficient of determination from the quadratic regression, to obtain the shape parameter aR^2 (Berdugo *et al.*, 2014). The shape parameter transforms poorly fitted drying curves to an aR^2 value of 0, as these curves are considered artefacts of the statistical method and have no biological meaning.

Statistical analyses

We used generalized linear models to analyze the influence of the experimental treatments on the wetting and drying events. In the case of wetting events, we used the model

$$WG = Ppt*I10 + H0 + OTC*Cv + RS \quad (3)$$

TABLE 1 Description of the climatic conditions registered during the three periods identified in our study (1-2, 2-5 and 5-8 years after the setup of the experiment).

		Period		
		1-2	**2-5**	**5-8**
Mean Annual Rainfall (mm)		232.6	288.4	260.6
Mean Temperature (°C)		15.8	15.9	14
Mean I10 (mm/hr)		0.54	0.88	0.58
Maximum WG	**Control**	1.77 [0 - 9.32]	1.48 [0 - 19.31]	1.24 [0 - 19.55]
	WA	0.70 [0 - 6.55]	1.22 [0 - 26.94]	0.98 [0 - 21.08]
	RE	1.67 [0 - 9.52]	1.66 [0 - 22.61]	1.44 [0 - 21.00]
	WA+RE	0.61 [0 - 4.34]	1.32 [0 - 22.82]	1.03 [0 - 23.11]
Average water loss	**Control**	-0.51 [-1.19 - 0.18]	-0.53 [-1.66 - 0.77]	-0.41 [-1.45 - 0.58]
	WA	-0.48 [-1.21 - -0.01]	-0.38 [-1.56 - 0.21]	-0.40 [-1.50 - 0.48]
	RE	-0.71 [-1.24 - -0.04]	-0.49 [-1.72 - 0.16]	-0.54 [-1.79 - 0.09]
	WA+RE	-0.58 [-1.07 - -0.26]	-0.56 [-1.76 - 0.02]	-0.41 [-1.45 - 0.37]

Note. Water gains and losses are expressed as volumetric water content (%VWC) per day (data are means with range in brackets). RE = rainfall exclusion; WA = warming.

where WG is the maximum water gain at 0–5 cm depth, Ppt is the total amount of rainfall registered during the event, I10 is the rainfall intensity, H0 the soil moisture preceding the rain (hereafter initial soil moisture), OTC is the effect of the open top chambers, Cv is the cover of visible biocrust components (measured from the photograph taken closest to the date of the event) and RS is the effect of rainfall shelters. We introduced the interaction between WA and biocrust cover because we have previously reported negative effects of WA on the cover of biocrusts in our experiment (Escolar et al. 2012, Maestre et al. 2013). In the case of desiccation events, we built two models for the two parameters of the drying curve:

$$Slope = Tmax + H0 + OTC*Cv + RS \quad (4)$$

$$Shape = Tmax + H0 + OTC*Cv + RS \quad (5)$$

where Tmax is the maximum temperature achieved during the drying event, H0 is the initial soil moisture of this event, and the other terms are those used in

Equation 3.

Because biocrusts tend to colonize areas with initial low cover through time (Maestre *et al.*, 2015b), and to further investigate whether the effects of the treatments on the wetting and drying events changed through time, we repeated the analyses described above for three periods: 1-2 years, 2-5 years and 5-8 years after the setup of our experiment. We selected these periods because they showed contrasted patterns of biocrust cover under our climate change treatments (Figure 2). We performed all statistical analyses using the "lm" function of the R 3.3.2 statistical software (R Development Core Team, 2011). Data associated with this study are available in Figshare (Lafuente et al., 2018).

Results

Throughout the study period, mean annual temperature was 15.1 °C, mean annual rainfall was 276.5 mm and rainfall intensity (I10) ranged from 0.02 - 15.2 mm/hr (Table 1). Soil moisture content accurately matched rainfall events during this period (Figure 1). Soil water gains under both WA and the combination of WA and RE decreased when the effects of main covariates (i.e., initial soil moisture and biocrust cover) were controlled (Figure 3b). Initial soil moisture of dry soils (i.e., preceding rainfall events) was lower under climate change treatments, especially during the first and last stages of the experiment (Figure 3c). Meanwhile, initial soil moisture after rainfall events showed variations throughout the experiment. In the first stage of the experiment, wetted soils under the WA and WA + RE treatments showed a lower initial moisture (Figure 3d). In the second stage of the experiment, initial soil moisture of wetted soils was lower under the RE and WA treatments, but not under the combination of both (Figure 3d). However, towards the last stage of the experiment, RE, WA and the combination of both had a lower initial soil moisture after rainfall events (Figure 3d).

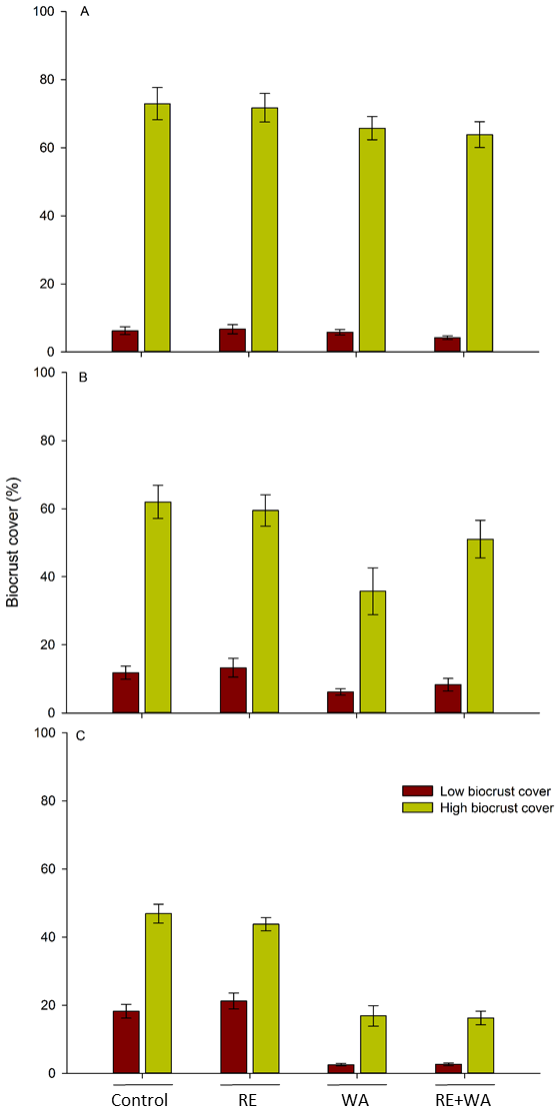

FIGURE 2 Cover of biocrusts in the different treatments throughout the duration of the experiment: 0-2 years (A), 2-5 years (B), and 5-8 years (C). WA = Warming and RE = Rainfall exclusion. Data are means ± SE (n = 10).

Climatic covariates (H0, Ppt, I10 and interaction PptxI10 and Tmax) were major drivers of both the maximum water gain during wetting events and the slope and shape of the drying curve (Table 2; Figure 4). During wetting events, rainfall amount increased water gain. However, rainfall intensity decreased soil

water gain during these events, even in the case of those with higher rainfall amounts (Table 2, Figure S.2). Initial soil moisture negatively influenced water gains during rainfall events. During the drying events, initial moisture and maximum temperature increased both drying rates and the exponential behavior of the drying curve (Table 2; Figure 4b, c).

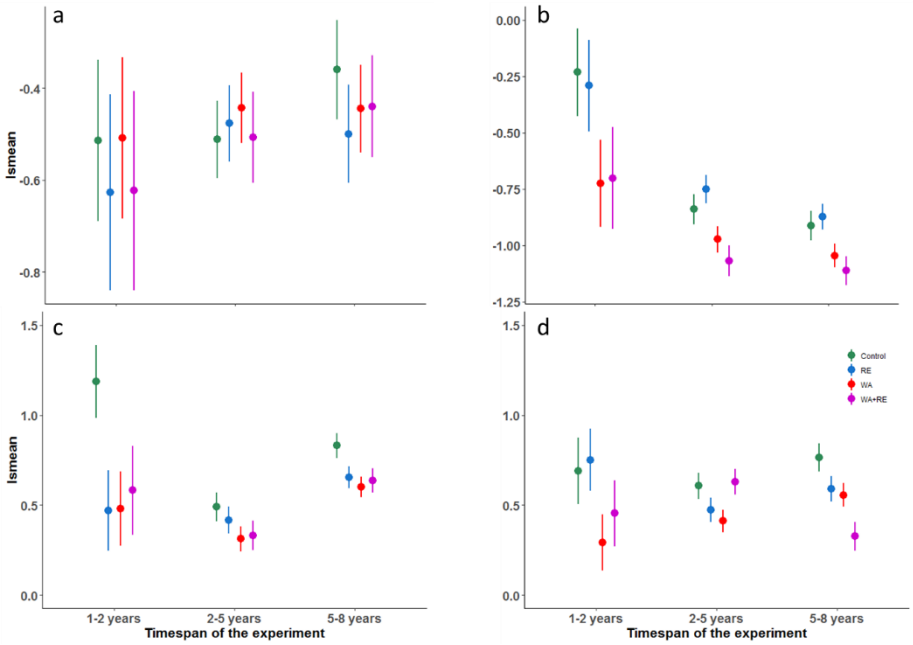

FIGURE 3 Least squared means (lsmean) for the slope of the desiccation curve (a), soil water gains (b), initial soil moisture prior to soil wetting (c) and initial soil moisture prior to desiccation (d) throughout the duration of the experiment. WA = Warming and RE = Rainfall exclusion. Data are lsmeans ± confidence intervals (n = 21).

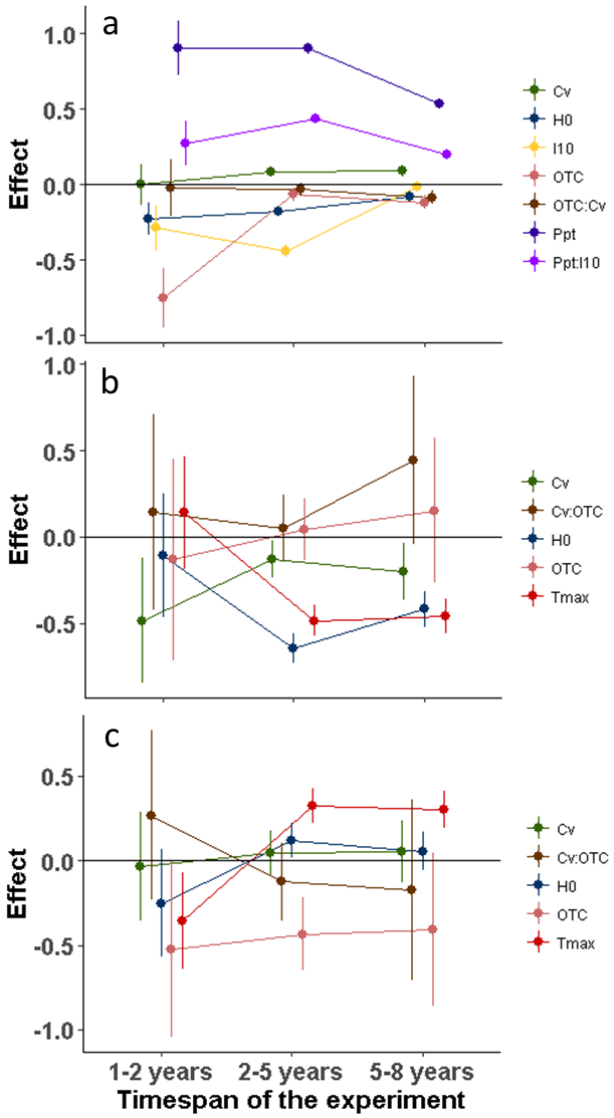

FIGURE 4 Standardized effect size of variables involved in predicting water gains (a), and the slope (b) and shape (c) of the desiccation curve after rainfall events throughout the duration of the experiment. Vertical lines represent 95% confidence intervals. Tmax = maximum temperature during desiccation event; H0 = initial soil moisture before the rainfall event; OTC = warming treatment; Cv = cover of biocrust; Ppt = summation of rainfall during the wetting event; I10 = rainfall intensity

Biocrust cover increased both water gains after rainfall events and the slope of the drying curve (Table 2). RE had a positive effect (0.04 as measured with standardized coefficients) only on water gains. WA decreased (-0.10 as measured with standardized coefficients) water gains, but also substantially reduced the exponential behavior of the drying curve (-0.35, as measured with standardized coefficients). These effects of WA were constant throughout the experiment but their magnitude decreased through time (Table 2; Figure 4). We also found an interaction between WA and biocrust cover when analyzing water gains, as the effect of biocrust cover on this variable was reduced (-0.06 as measured with standardized coefficients) under WA (Table 2).

TABLE 2 Estimates of standardized effects of covariates introduced when modelling water gains and losses (slope and shape of the drying curve) after rainfall events.

Predictor	Water Gain	Slope	Shape
H0	-0.12 ***	-0.51 ***	0.05
Tmax	-	-0.43 ***	0.27 ***
Ppt	0.61 ***	-	-
I10	-0.09 ***	-	-
Ppt × I10	-0.22 ***	-	-
OTC	-0.10 ***	-0.06	-0.35 ***
RS	0.04 *	-0.10	-0.02
Cv	0.08 ***	-0.19 ***	0.07
OTC × Cv	-0.06 ***	0.09	-0.06
Adjusted R^2	0.3945	0.2929	0.1060
F	810.7	55.05	16.47
d.f.	8/9934	6/777	6/777

Note. Cv = biocrusts cover; H0 = initial soil moisture before the considered rainfall event; I10 = rainfall intensity (mm/hr); OTC = warming treatment; Ppt = summation of rainfall during the wetting event; RS = rainfall shelter; Tmax = maximum temperature during desiccation event. df = degrees of freedom; F = Fisher statistic. ***p < .001. *p < .05.***

The effects of the different treatments on water gains and losses varied through time. For instance, the effects of WA on water gains were negative throughout the experiment and particularly important during the first years (Figure 4a). Biocrust cover negatively affected the slope of the drying curves throughout the experiment (Figure 4b, c). In addition, the effects of initial moisture and maximum temperature varied throughout the experiment (Figure 4b,c). The effects of both initial moisture and maximum temperature on the slope of the drying events shifted to negative after the first 2 years of the experiment. The effect of these covariates on the shape of the drying events was the opposite. The effect of WA on the response variables evaluated was similar throughout the experiment.

Discussion

Our analyses of an 8-year dataset showed variable effects of simulated climate change on soil water gains and losses after rainfall events in a biocrust-dominated semiarid ecosystem. A 2.5 °C WA reduced water gains after rainfall events, and affected the way the soil dried up after them by reducing both the slope and exponential behavior of the drying curve. An important result of our study is that the effects of treatments on soil water gains and losses changed through time, with important differences between the first 2 years of the experiment and after 5 years since its set-up. These effects were mainly driven by the important reductions in biocrust cover and diversity observed under WA (Escolar *et al.*, 2012), which were similar to those reported in other climate change experiments conducted with biocrusts (Ferrenberg *et al.*, 2015). Our findings thus emphasize the importance of conducting long-term experiments to accurately characterize potential responses to climate change in hydrological variables such as water gains and losses after rainfall events.

Drivers of soil water gains and losses after rainfall events

Soil water gains were dependent on both the amount and intensity of rainfall events. However, there was an interaction between both precipitation attributes. The greater the rainfall event, the larger the soil water gains. Nevertheless, this effect was modulated by rainfall intensity; despite its magnitude, if the intensity of a rainfall event was high, soil water gains turned to be lower than expected. Many studies have evaluated the effects of the amount and intensity of rainfall on infiltration and runoff (e.g., Cantón et al., 2004; Chamizo et al., 2012; Faist et al., 2017; Kidron & Yair, 1997). Our results are similar to those of Chamizo et al., (2012) and Kidron & Yair (1997), who observed that biocrust-dominated soils had the ability to reduce runoff when compared to bare ground soils under low intensity rainfall events. However, when the intensity of the rainfall event was high, biocrusts lost their capacity to reduce runoff. Faist et al., (2017), observed that under high intensity rainfalls the capacity to reduced runoff was dependent on the successional stage of the biocrusts, late successional biocrusts had the capacity to reduce runoff, whereas early successional biocrust lost this capacity. Moreover, the higher the initial soil moisture, the lower the water gains after rainfall events. Fine textured soils show numerous cracks when are dry, which can considerably increase water infiltration. However, these cracks seal when they are wetted, and consequently runoff is favored under these conditions (Chamizo et al., 2012). In addition, wetted soils present a higher total pore volume filled with water, which reduces the capacity of the soil to further gain water (Gardner *et al.*, 1999). As found by Berdugo et al. (2014), soil moisture after rainfall events, combined with the maximal temperature registered during the drying event, affected soil water losses through desiccation.

The effect of biocrusts on water gains and losses after rainfall events is complex but of paramount important since they cover large areas of the soil surface in drylands worldwide. A large body of literature has discussed how

biocrusts enhance or decrease infiltration and runoff (see Chamizo et al., 2016 for a recent review). Reductions in infiltration by biocrusts have been described when these organisms smooth the soil surface (Belnap et al., 2003; Eldridge et al., 2000) and under intense rainfalls due to the production of exopolysaccharides by biocrust-forming cyanobacteria (Warren, 2003). On the other hand, biocrusts increase soil stability (Eldridge & Leys, 2003), porosity (Felde et al., 2014) and surface roughness (Belnap, 2006). Other studies have highlighted how biocrust-dominated soils increase water gains and reduce runoff after rainfall events, maintaining higher moisture conditions in the soil surface (Cantón et al., 2004; Chamizo et al., 2012, 2013, 2016, 2016). Our results agree with these studies, as we found a significant positive effect of well-developed biocrusts on soil water gains after rainfall events (Table 2). This effect is likely to be driven by the increase in soil surface roughness by dominant lichens, which reduces runoff and increases infiltration, and by the positive effects of these organisms on soil pore formation, which positively affect infiltration (Bowker et al., 2013; Chamizo et al., 2012).

We found that well-developed biocrusts increased both the slope and exponential behavior of the drying events (Table 2). Soils with a well-developed biocrust community gained more water after rainfall events, but also had higher drying rates. These results mimic those from Berdugo et al. (2014), who used another mid-term dataset (6 years) from the same study area. Our results suggest that evaporation is higher in biocrust-dominated than in bare ground soils. We speculate that this could be consequence of the soil surface darkening by cyanobacteria (Rutherford *et al.*, 2017), which are abundant in our study area (Cano-Díaz et al., 2017), and by some lichen species (e.g. *Toninia sedifolia*), which would thus increase surface heating and enhance evaporation. The increase in soil surface roughness by biocrusts, which also increases the amount of soil surface that can be heated (Kidron & Tal, 2012), can also contribute to explain the results observed.

Soil water gains and losses under simulated climate change

The dynamics of soil wetting and drying after rainfall events was impacted by our climate change treatments, and by WA in particular. The increase in the mean annual temperature imposed by this treatment (~2.5 °C on average throughout the experiment) led to drier soils (average reduction in soil moisture ~1% throughout the experiment; Figure 3c and d). Similar responses have also been observed in experimental studies conducted in grasslands (Liancourt et al., 2012) and shrublands (León-Sánchez et al., 2016) from dryland areas. Our WA treatment decreased the observed water gains and reduced the exponential behavior of the desiccation curve, meaning that warmed plots gained less water and desiccated more gradually compared to other treatments (Figure 3b). These findings mimic those of Liancourt et al. (2012), who found that OTCs like those employed in our study significantly reduced soil moisture and decreased soil drying rates in a Mongolian grassland. These authors found that this reduction in the desiccation rate was a consequence of a reduction in wind speed due to the WA structures. However, we believe that the reduction in the desiccation rate observed in our case was due to a reduced water gain that led to a lower initial soil moisture (our OTCs were elevated 5 cm from the soil, hence allowed ventilation), and thus to a more progressive drying (reduction of the exponential behavior), instead of the abrupt drying observed in a more exponential curve.

Our RE treatment increased soil water gains after rainfall events. This result was unexpected because our shelters effectively excluded ~33% of incoming rainfall (Maestre et al. 2013). However, it can be partially explained by the fact that the initial soil moisture of these soils was lower under this treatment (Figure 3). Indeed, the water gains observed under this treatment did not differ from those found in the control plots when least squared means were controlled by the soil moisture at the beginning of each rainfall event (Figure 3b). We believe that the amount of rainfall may be playing a role on this effect of RE because soils are driven to saturation during the most intense events.

Indeed, under rainfall events below 2 mm, water gains under RE tended to be slightly lower than controls, whereas for larger rainfall events the exclusion of rainfall did not reduce soil water gains (Figure S2) due to soil saturation. Another possible explanation for these results is the fact that we only placed one sensor under each RE plot, which occupies an area of 2.64 m^2. As such, the soil moisture registered could have not provided an accurate measure of the soil surface moisture under this treatment. Therefore, the results of our study regarding the effect of rainfall shelters should be treated with caution and must be confirmed by additional studies.

Temporal trends of the hydrological impacts of climate change treatments

Initial soil moisture after rainfall events was lower under WA throughout the experiment. However, the effect of the combination of WA and RE on this variable differed depending on the period considered. In the first stage of the experiment, the combination of both treatments had no effect on initial soil moisture after rainfall events. After 2-5 years of simulated climate change, the combination of both treatments mitigated the initial soil moisture decrease caused by WA alone. Meanwhile, 5-8 years after the setup of the experiment the combination of WA and RE promoted important reductions in the moisture of wetted soils (Figure 3d). Because the effect of biocrusts cover has been controlled in our analyses, we hypothesize these changes are not directly driven by the loss of lichen cover but indirectly through changes in the soil properties promoted by the loss of lichen cover. These include an increase in hydrophobic compounds originated from the decomposition of lichens, a reduction in the quality of soil organic matter or shifts in the composition of the biocrust community (Delgado-Baquerizo *et al.*, 2015a; Asplund & Wardle, 2017).

The effects of initial soil moisture and maximum temperature on the slope and shape of the desiccation curve changed from positive to negative and from negative to positive, respectively, after 2 years of simulated climate change

(Figure 4b,c). In addition, 2 years after the set-up of the experiment, the cover of biocrusts started having a positive effect on water gains, whereas WA decreased its negative effect on this variable. These results highlighted the existence of feedbacks between soil moisture and biocrust cover, and suggest that the importance of these factors for water gains were altered once biocrusts cover decreased. D'Odorico et al., (2007) also showed a lack of linearity when evaluating the effects of vegetation on soil moisture in the Kalahari. After rainstorms, open areas located between vegetated patches were wetter but also dried faster than plant patches, which is in accordance to what we observed in our experiment.

During our experiment, WA decreased the cover of biocrusts by ~56 % (Figure 2). Just after 2 years of simulated climate change we already observed a decrease in the water gains under both WA and the combination of WA and RE (Figure 3b). However, the effects of our climate change treatments on initial soil moisture were not evident until 5 years after the setup of the experiment (Figure 3c, d). These results illustrate the hydrological consequences of reductions in biocrust cover due to simulated climate change. Moreover, some of these consequences took a larger lapse of time to become evident. Our findings further emphasize the importance and value of long-term studies, as some of the observed effects would have gone unnoticed if our study had had a shorter duration.

Concluding remarks

Our results indicate that ongoing climate change, and WA in particular, will affect the hydrological behavior of biocrust-dominated ecosystems both directly, by increasing evaporation, and indirectly, by reducing the cover of lichen-dominated biocrusts and by altering their ability to control water gains and losses after rainfall events. This study emphasizes how after 2 years of simulated WA, water gains decreased and desiccation occurred more gradually.

However, reductions in water gains under the combination of WA and RE were not noticeable until 5 years after the set-up of the experiment. Most of the hydrological studies focusing on biocrusts have a duration lower than 2 years (e.g Cantón et al., 2004; Chamizo et al., 2016; Zaady et al., 2014; but see Berdugo et al., 2014). To our knowledge, no previous study focusing on the hydrological impacts of climate change and biocrusts have used a temporal series as large as that employed here. The use of such a dataset provided insights on the hydrological effects of biocrusts under simulated climate change that would not have been evident if our experiment had lasted for a shorter period. These findings point to the importance of conducting mid- and long-term experiments when evaluating the hydrological responses of drylands to climate change, particularly when these are mediated by organisms that are highly sensitive to changes in climatic conditions, such as biocrusts. In addition, and by illustrating how biocrusts modulate the impacts of simulated climate change on soil water gains and losses after rainfall events, our study not only emphasizes the key role of biocrusts on these important hydrological attributes, but also contributes to advance our understanding of climate change impacts on the ecohydrology of drylands.

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Supplementary material

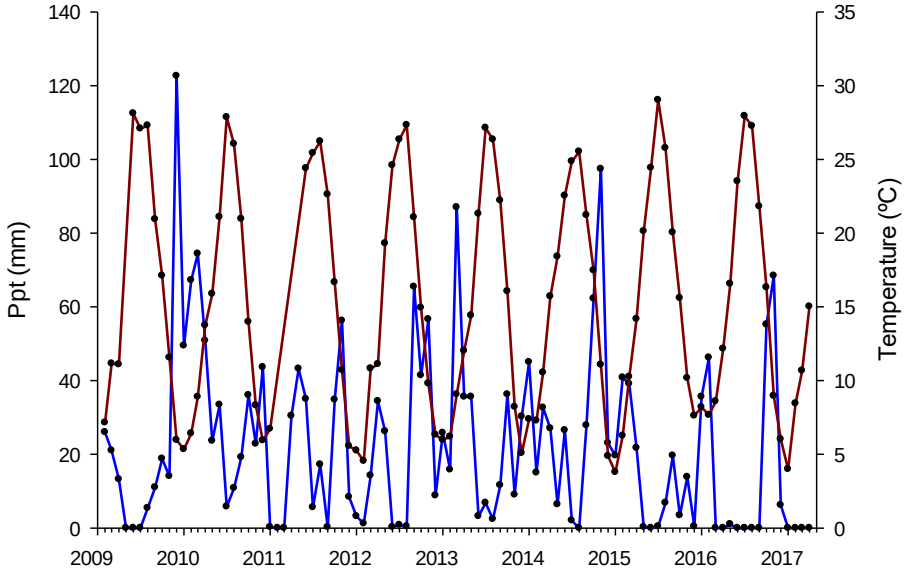

FIGURE S1 Monthly precipitation (mm) and mean monthly temperature (ºC) registered during the experiment (blue and red lines respectively).

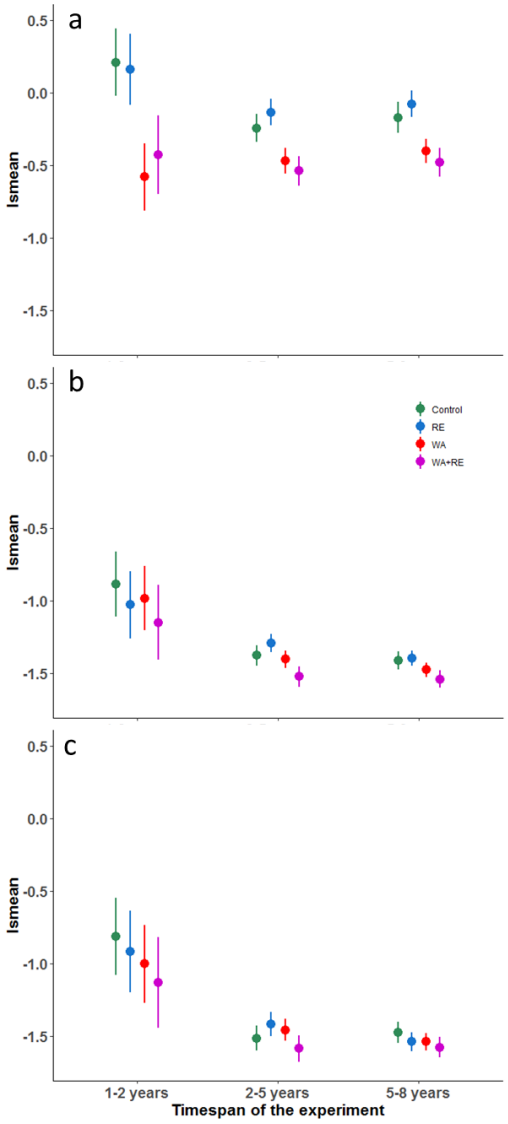

FIGURE S2 Least squared means (lsmeans) for different rainfall amounts (> 2 mm (a), < 2 mm (b) and < 0.5 mm (c)), registered throughout the duration of the experiment. WA = Warming and RE = Rainfall exclusion. Data are lsmeans ± confidence intervals (n = 21).

Capítulo 2/ Chapter 2

Biocrusts modulate the response of nitrous oxide and methane fluxes to climate change in a Mediterranean dryland

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Photo credits: Beatriz Gozalo (left) and Angela Lafuente (right)

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Abstract

Little is known about the role of biocrusts in regulating the responses of N_2O and CH_4 fluxes, and associated functional microbial communities, to climate change in drylands. Here, we aim to fill this gap of knowledge using an 8-year field experiment in central Spain where temperature and rainfall are being manipulated (~2 °C warming, 33% rainfall reduction and their combination) in areas with and without well-developed biocrust communities. Overall, areas with well-developed initial biocrusts showed reduced N_2O emissions, enhanced CH_4 uptake and higher abundances of functional genes linked to N_2O and CH_4 fluxes. Moreover, biocrusts modulated the responses of gases fluxes to climate change. For instance, in initial low-crusted soils, rainfall exclusion and its combination with warming increased N_2O emissions, whereas, under well-developed initial biocrust cover and for the same treatments, we found a sharp reduction in N_2O fluxes (~96% and ~197%, respectively). Warming and warming + rainfall exclusion treatments reduced CH_4 consumption in areas with low initial biocrust cover. However, well-developed initial biocrusts enhanced CH_4 uptake under rainfall exclusion and warming + rainfall exclusion treatments. Taken together, our results indicate that well-developed initial biocrust communities could counteract the impact of warming and altered rainfall patterns on the fluxes of N_2O and CH_4 from the soil, highlighting their importance and the need to preserve them to minimize climate change impacts on dryland ecosystems.

Keywords

Biocrust, *denitrifiers,* dryland, methane, *methanotrophs,* nitrous oxide.

Introduction

Most efforts to understand the main drivers of soil greenhouse gas (GHG) fluxes under global change scenarios have focused on carbon dioxide (CO_2) (Pachauri & Meyer, 2014). Relatively less is known about other greenhouse gases such as nitrous oxide (N_2O), and methane (CH_4), which have stronger greenhouse effects and can significantly affect feedback responses to climate change (Nakicenovic & Swart, 2000; Le Mer & Roger, 2001; Soussana *et al.*, 2007; Oertel *et al.*, 2016). This is especially true for dryland ecosystems (arid, semi-arid and dry-subhumid ecosystems), which cover 45% of the land surface (Prăvălie, 2016), and sustain over 40% of human population (Reynolds et al., 2007). The N_2O and CH_4 fluxes from dryland soils have been traditionally considered to be of little importance due to their typically low water and nutrient contents, which limit biological activity (Dalal & Allen, 2008). However, over the last two decades, multiple studies have reported elevated N_2O fluxes in arid and semiarid soils after rainfall pulses (Barton et al., 2008; Barton, Gleeson, Maccarone, Zúñiga, & Murphy, 2013; Zaady, Groffman, Standing, & Shachak, 2013), and early studies described dryland soils as a potential relevant global sink of atmospheric CH_4 (Potter, Davidson, & Verchot, 1996). The importance of drylands for understanding the global carbon cycle is well recognized (Poulter et al., 2014), and there is a growing number of studies exploring how ongoing climate change may affect key variables such as soil respiration (Darrouzet-Nardi, Reed, Grote, & Belnap, 2015; García-Palacios et al., 2018; Yan, Chen, Xia, & Luo, 2014). Yet how global warming and forecasted changes in rainfall patterns will affect N_2O and CH_4 fluxes in dryland soils remains poorly studied (Darrouzet-Nardi et al., 2015; Guan, Li, Zhang, & Li, 2018). Understanding the impacts of climate change on N_2O and CH_4 fluxes in dryland soils is thus of paramount importance to determine the response of drylands to projected changes in temperature and precipitation, and to improve prediction of biogeochemical models. Furthermore, the relevance of drylands for understanding net global GHG fluxes

will increase in the future, as their global extent will likely increase by 11-23% by the end of this century due to increases in aridity linked to ongoing climate change (Huang, Yu, Guan, Wang, & Guo, 2016).

The fluxes of N_2O and CH_4 from soils are largely carried out by highly specialized microbial communities. For instance, N_2O produced in both nitrification and denitrification processes (Bremner, 1997; Canfield, Glazer, & Falkowski, 2010; Firestone & Davidson, 1989) is reduced to N_2 by the *nosZ* carrying denitrifiers (Bremner, 1997; Canfield et al., 2010). In dryland surface soils, which are dry and hence under aerobic conditions most of the time, nitrification might be the dominant process (Delgado-Baquerizo *et al.*, 2016b) and, consequently, the *nosZ* gene (carried by denitrifying bacteria) and the factors affecting its abundance and activity have been poorly studied (Philippot *et al.*, 2007; Hallin *et al.*, 2017). However, aggregates and precipitation pulses create anaerobic conditions favourable for denitrification in dryland soils, which could represent a temporary sink for atmospheric N_2O, the substrate used by *nosZ* denitrifiers (Austin *et al.*, 2004; Ley *et al.*, 2018; Wang *et al.*, 2019). Likewise, under aerobic conditions, CH_4 oxidising bacteria (i.e. methanotrophs) use the CH_4 monooxygenase (encoded by the *pmoA* gene) to oxidise CH_4 (produced in anaerobic conditions in depth soils), constituting the only biological sink for atmospheric CH_4 (Conrad, 2009; Dalal & Allen, 2008). Previous experiments and observational studies (Nazaries *et al.*, 2013b; Powell *et al.*, 2015) have shown strong relationships between the abundance of *nosZ* and *pmoA* genes and GHG fluxes, and consequently functional genes have been used to predict these fluxes (Nazaries *et al.*, 2013b; Powell *et al.*, 2015). Unfortunately, most of our knowledge on *nosZ* and *pmoA* genes comes from mesic ecosystems (Martins et al., 2017; Nazaries et al., 2013; Powell et al., 2015; but see Martins, Nazaries, Macdonald, Anderson, & Singh, 2015), and we lack studies evaluating the changes in their abundance under global change scenarios in drylands.

The exchange of GHG between the soil and the atmosphere is also known to be highly sensitive to the activity of organisms living on the soil uppermost levels (Dalal & Allen, 2008). Biocrusts, soil surface communities composed by lichens, mosses, liverworts, fungi, algae, cyanobacteria, bacteria, and archaea, are a key biological component of dryland ecosystems worldwide (Weber, Büdel, & Belnap, 2016). Biocrusts regulate multiple soil biotic and abiotic properties and processes, including soil respiration, CH_4 uptake and N fixation and cycling (Reed *et al.*, 2012; Aschenbach *et al.*, 2013; Maestre *et al.*, 2013; Zaady *et al.*, 2013), porosity (Felde, Peth, Uteau-Puschmann, Drahorad & Felix-Henningsen, 2014) and infiltration (Eldridge et al., 2010; Lafuente, Berdugo, Ladrón de Guevara, Gozalo, & Maestre, 2018), to name a few. Moreover, biocrusts are home to particular soil microbial communities (Steven *et al.*, 2013; Delgado-Baquerizo *et al.*, 2018a). Given the importance of biocrusts in the GHG fluxes in drylands, repeated calls have been made to incorporate them into biogeochemical models aiming to forecast their future fluxes (Maestre et al., 2013; Weber et al., 2015). However, and to the best of our knowledge, no previous study has experimentally evaluated how biocrusts affect soil N_2O and CH_4 fluxes in response to forecasted changes in climate. Such studies are needed not only to advance our understanding of climate change impacts on drylands, where biocrusts are a prevalent biotic feature, but also to provide relevant data to refine simulation models employed to predict future N_2O and CH_4 fluxes across dryland biomes.

Herein, we used an 8-year warming and rainfall manipulation experiment located in central Spain (Maestre et al., 2013) to investigate: (i) the effects of simulated climate change (~2 ºC warming and ~33% rainfall reduction) on soil N_2O and CH_4 fluxes and the abundance of *nosZ* and *pmoA* functional genes; (ii) whether these effects are modulated by the presence and degree of development of biocrusts; and (iii) whether N_2O and CH_4 fluxes are related to the abundance of *nosZ* and *pmoA* functional genes, respectively.

Materials and methods

Study site

This experiment was conducted in the Aranjuez Experimental Station (central Spain; 40º01'55.7''N-3º32'48.3''W; 590 m.a.s.l). Its climate is Mediterranean semi-arid, with average annual temperature and rainfall of 15°C and 358 mm, respectively (data available since 1977 from the Aranjuez Meteorological Station, 40º04'N - 3º32'W; 540 m.a.sl). Soils are gypsum- derived (Gypsiric Leptosols, WRB 2006). Organic carbon (C), total nitrogen (N), and pH vary among the considered microsites (i.e. low and high biocrust cover) between 1.8-5.0%, 0.14-0.44%, and 6.6-7.2, respectively. Vegetation is dominated by *Macrochloa tenacissima* (L.) Kunth (18% of total cover), *Retama sphaerocarpa* (L) Boiss and *Helianthemun squamatum* Pers. (6% of total cover for both shrubs). Open areas between vascular plants are partially covered with a well-developed biocrust community dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia* (Scop.) Timdal, and *Psora decipiens* (Hedw.) Hoffm, which covers ~34 % of the soil surface (see Maestre et al. 2013 for a full species checklist).

Experimental design

A detailed description of the experimental design can be found in Escolar, Martinez, Bowker & Maestre, 2012. Briefly, we established a fully factorial experimental design with three factors, each with two levels: warming (control vs. ~2 ºC soil temperature increase), rainfall exclusion (control vs. 33% rainfall reduction) and initial biocrust cover (<25% [low] vs. >50% [high]). To simulate warming, we used 40 × 50 × 32 cm hexagonal open top chambers (OTCs) made of methacrylate, which were elevated 5 cm from the surface to avoid overheating (Fig. S1a). To intercept rainfall, we built a 1.2 × 1.2 × 1 m metallic

frame supporting three V-shaped methacrylate gutters that cover ~37% of the surface (Fig. S1b). Warming and rainfall exclusion treatments were setup in July and November 2008, respectively (see Maestre et al. 2013 for additional details). Ten replicates per combination of treatment were established, resulting in 80 experimental plots. In each plot, a polyvinyl chloride (PVC) ring (diameter = 20 cm, height = 7 cm) was inserted 5 cm into the ground at the start of the experiment for measuring GHG fluxes. Our warming treatments (warming and its combination with rainfall exclusion), significantly increased air temperatures by ~0.9°C and 1.1°C, respectively and soil temperatures by ~1.6°C and 2.3°C, respectively, compared to control plots (average of the 2008-2017 period). Our rainfall exclusion (RE) shelters excluded on average ~33% of the incoming rainfall (2008-2013 period).

Biocrust cover (i.e. lichens and mosses) was estimated annually using high resolution pictures since the setup of the experiment in 2008 as detailed in Maestre et al. (2013). Values obtained using these pictures are highly correlated with those obtained with *in situ* surveys (Ladrón de Guevara et al., 2018). Soil moisture in the top 5 cm was monitored every 2.5 h in all treatments in a subset of the plots (EC-5 soil moisture sensors, Decagon Devices Inc., Pullman, WA, USA). Air temperature and relative humidity were also monitored every 10 min using automated sensors (HOBO® U23 Pro v.2 Temp/ RH, Onset Corporation, Bourne, MA, USA).

Greenhouse gas exchange measurements

We estimated soil-atmosphere N_2O and CH_4 fluxes in seven replicates per combination of treatments using the static chamber method (Bowden, Melillo, Steudler & Aber, 1991). From March 2015 to May 2016, 14 sampling campaigns were carried out, approximately once a month. Immediately before each measurement, a 20 cm diameter and 9 cm high PVC chamber was placed on top of each of the 56 permanent rings and sealed with a rubber band. Each chamber

had a sampling port in the top centre of the chamber that allowed air sampling and was covered with reflective material to thermally isolate it during the measurement. Gas samples were collected at 0, 30 and 60 min after chamber closure using a needle attached to a polypropylene syringe, transferred to 22 ml pre-vacuumed vials and kept at room temperature until analysis. We estimated N_2O and CH_4 concentrations in the gas samples using a HP-6890 gas chromatograph (GC), equipped with a headspace autoanalyser (HT3) (Agilent Technologies, Barcelona, Spain), a ^{63}Ni electron capture detector (for N_2O), and a flame-ionization detector fitted with a methaniser (for CH_4 detection). The carrier gas used was helium.

Soil sampling and analyses

Soil samples (0-2 cm) were collected five times during the study period (June, September and November 2015 and February and April 2016) from five replicates per combination of treatment. Each soil sampling always matched one of the gas measurement campaigns. Visible biocrusts were removed when present and then soils were stored in -20 ºC for DNA extractions. Total genomic DNA was extracted from 0.6 g of frozen soil using the PowerSoil DNA Isolation kit (MOBIO Laboratories, Inc. USA) according to manufacturer's protocol but with a slight modification during the cell lysis step (we used a tissue homogenizer [Precellys 24- dual. Bertin technologies, France] at a speed of 4500 rpm for 45 s, twice). DNA extractions yields ranged from 0.1 to 132.6 ng/µl, with an average of 6 ± 15 ng/µl (mean ± standard deviation). The abundances of two functional genes, *nosZ* and *pmoA*, were determined using nosZ2f/nosZ2r (Henry, Bru, Stres, Hallet & Philippot, 2006) and pmo189f/pmo650r (Bourne, McDonald & Murrell, 2001) primers, respectively. All primers were purchased from Integrated DNA Technologies, Australia. Each sample was quantified in duplicate, in a total volume of 10 µl using a BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). The reaction mixture

contained 1 µl of DNA template (2 ng/µl), 5 µl of SensiFast Sybr No-Rox Mix (2x) (Bioline, Australia), 0.3 µl of each primer (0.4 mM) and 0.4 µl of BSA (0.4 mg/ml). Thermal cycling conditions and primer sequences can be found in Table S1. The *nosZ* gene was cloned with pGEM-T Easy Vector kit according to manufacturer's instructions (Promega, Madison, USA) and transformed into *Escherichia coli* strain JM109 to perform calibration curves. The *pmoA* gene calibration curves were made form genomic DNA (*Methylosinus trichosporium*). Melt curve analyses were performed in each assay to verify the specificity of the amplicon products. Gene copy number per g dry soil normalised to extraction yield were calculated for both genes.

Statistical analyses

We estimated N_2O and CH_4 fluxes as described in Durán, Rodríguez, Morse & Groffman, (2013), and are reported as changes in milligrams or micrograms (for N_2O) per square metre per day [$\Delta(mg\ m^{-2}\ d^{-1})$]. In more than 90% of the cases, the increases in N_2O and CH_4 fluxes were linear (R^2 >0.7). Non-linear rates were discarded, and imputation of missing rates (per treatment) was performed using the missForest algorithm in the R package missForest (Stekhoven, 2013), which iteratively fills missing values in all columns of a data frame based on predictions from random forest models. For the iteration, we included the averaged soil moisture, air temperature and air humidity matching the treatment, date and time of the sampling. We estimated the 2.3% and 6.8% of the N_2O and CH_4 rates analysed in this study, respectively.

We first tested the effects of warming, rainfall exclusion and initial biocrust cover on N_2O and CH_4 fluxes and soil microbial gene abundance (*nosZ* and *pmoA* functional genes) with a repeated measures general linear mix effects model. We included the percentage of change in the biocrust cover over time as a covariate in the models to control for the observed changes in biocrust cover since the setup of the plots in 2008 (described in detail in Ladrón de

Guevara et al., 2018). As multiple interactions between initial biocrust cover and the climate change treatments were found (Table S2), we tested the effect of warming and rainfall exclusion (alone and combined) separately for low and high initial biocrust cover plots using the same model. These analyses were carried out using the function *lmer* in the R package *lmer4* (Bates 2015. Differences of least squares means for the factors of the mixed effects model were calculated using the function *difflsmeans* in the R package *lmerTest* (Kuznetsova, 2017). We compared differences in gas fluxes and gene abundances between the two levels of initial biocrust cover (low and high), within the treatment, with the student's t-test. Methane fluxes and soil microbial gene abundances were log transformed prior to analyses to improve normality. All statistical analyses were performed using R statistical software 3.4.0 (R Core Team, 2017). Data are available on Figshare ((Lafuente et al., 2019a).

Results

Effects of simulated climate change on N2O and CH4 fluxes

Nitrous oxide fluxes were very low in all cases, ranging from -10 to 20 μg m^{-2} d^{-1} (Figs 1a,b, and 2a,b). Moreover, we detected a high variability in the responses of N_2O fluxes with time. Even so, we also found clearly different N_2O flux patterns for low and high initial biocrust cover communities (Fig. 2 a,b). On average N_2O fluxes were lower in high than in low initial biocrust cover plots, although due to the high variability these differences were not significant (p=0.14; Fig. S2a). Biocrusts, however, regulated the responses of N_2O emissions to climate change. In the low initial biocrust cover plots, climate change treatments reduced N_2O fluxes (vs. control) in March, April and early July and increased them in late July and September (Fig. 1a, Table S3). On the other hand, in the high initial biocrust cover plots, although the variability was high -particularly during spring (Fig. 1b)-, we observed sharp reductions in overall N_2O

fluxes in the rainfall exclusion and warming + rainfall exclusion treatments (vs. the control; ~96% and ~197%, respectively; Fig. 2b, $p < 0.05$, Table S3).

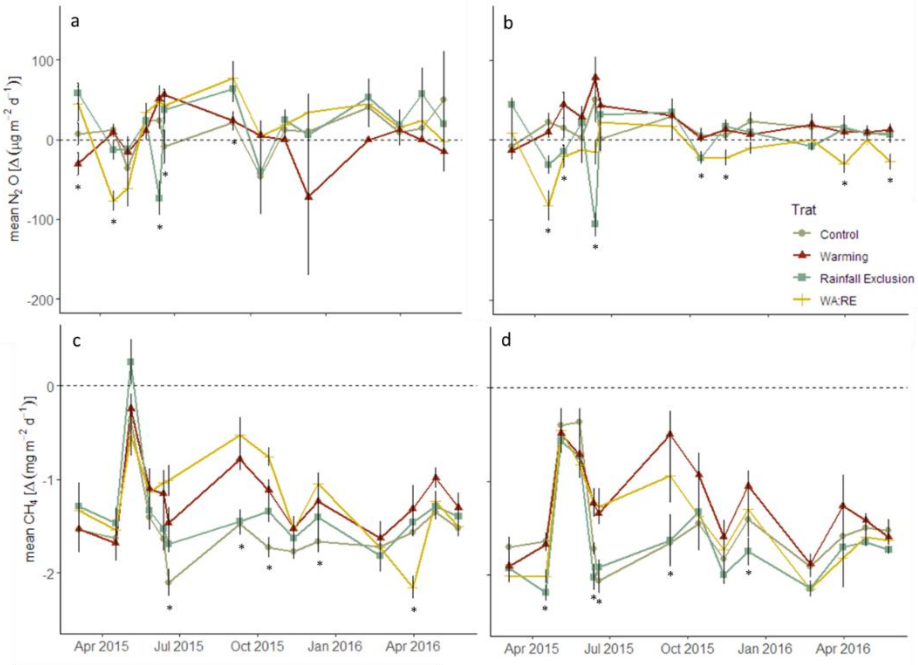

FIGURE 1 Temporal variation of N_2O (a,b) and CH_4 (c,d) fluxes in areas with low (left) and high (right) initial biocrust cover across the different climate change treatments evaluated throughout the study period. Data are means ± s.e. (n = 7). Asterisks in each panel show significant differences from the t-test ($p<0.05$). WA:RE = warming and rainfall exclusion combined.

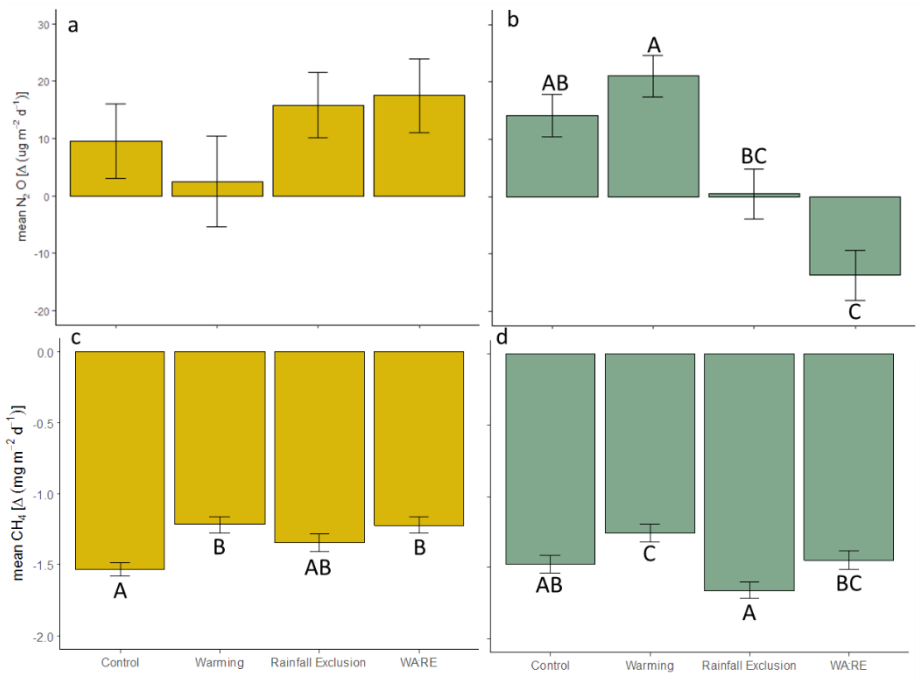

FIGURE 2 Average N_2O (a, b) and CH_4 (c,d) fluxes estimated in areas with low (left), and high (right) initial biocrust cover across the climate change treatments evaluated throughout the study period. Different letters indicate significant differences in pairwise comparisons between treatments by differences of least square means (P<0.05). Data represent means ± s.e. (n= 7). WA:RE = warming and rainfall exclusion combined.

Methane fluxes were also low and negative (i.e. CH_4 uptake) in most cases, ranging from -1.66 to -1.22 mg m^{-2} d^{-1} (Figs 1c,d and 2c,d). The CH_4 uptake was significantly higher in high than in low initial biocrust cover plots (p<0.01; Fig. S2b). The response of CH_4 fluxes to warming and rainfall exclusion was very variable throughout the study period, albeit important differences between low and high initial biocrust cover plots were observed (Fig. 2c,d). Biocrust modulated the responses of CH_4 fluxes to climate change. All treatments tended to decrease CH_4 uptake in the low initial biocrust cover plots, although the differences with control plots were only significant for the warming and warming + rainfall exclusion treatments (Table S3, Fig. 2c). Under high initial biocrust cover, only warming reduced CH_4 uptake (Table S3, Fig. 2d). In addition,

we observed a positive and significant (albeit weak) relationship between the across-plots changes in biocrust cover since the setup of the experiment and CH_4 uptake rates (R^2 = 0.02 and 0.02, $p < 0.05$, respectively; Fig. S3). Thus, those plots that had their biocrust cover reduced since the beginning of the experiment registered a lower CH_4 uptake.

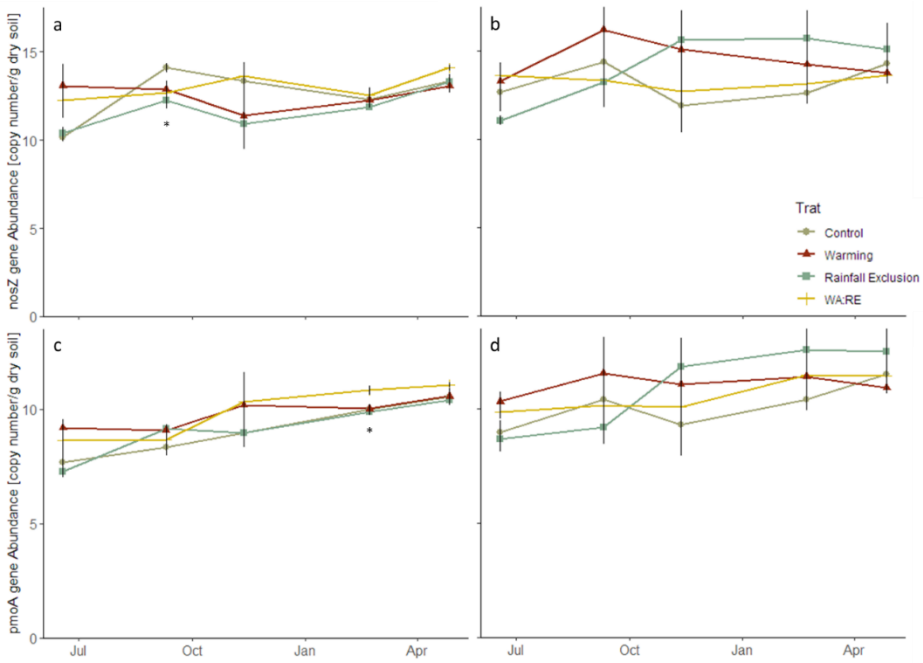

FIGURE 3 Temporal variation in the abundance of *nosZ* (a,b) and *pmoA* (c,d) genes in areas with low (left) and high (right) initial biocrust cover across the different climate change treatments evaluated throughout the study period. Data are log transformed means ± s.e. (n = 5). Asterisks in each panel show significant differences from the t-test (p<0.05). WA:RE = warming and rainfall exclusion combined.

Effects of climate manipulation on abundances of nosZ and pmoA genes

Both *nosZ* and *pmoA* genes were more abundant in high than in low initial biocrust cover plots ($p<0.05$; Fig. S2c,d). As found with N_2O and CH_4 fluxes, we observed a marked variability in *nosZ,* and *pmoA* gene abundance throughout the experiment (Fig. 3), as well as important differences in their responses to warming and rainfall exclusion treatments depending on initial biocrust cover (Figs 3 and 4).

Abundances of the *nosZ* gene ranged from $2.4x10^5$ to $7.8x10^7$ copy number g dry $soil^{-1}$ (Table S4, Fig. 4a, b). Warming, rainfall exclusion, and their combination significantly reduced the abundance of the *nosZ* gene (vs. the control) in the September sampling. In the low initial biocrust cover plots, its overall abundance was significantly higher at the warming + rainfall exclusion treatment than at the rainfall exclusion treatment (Table S4, Fig. 4a). We did not find any significant relationship between N_2O fluxes and *nosZ* gene abundance ($R^2 = 0.00$; $p=0.91$ and $R^2 = 0.02$; $p=0.07$ at low and high initial biocrust cover, respectively).

On average, the *pmoA* gene abundance ranged from $1.6x10^4$ to $8.3x10^5$ copy number g dry $soil^{-1}$ (Fig. 4c,d). The combination of warming and rainfall exclusion led to a significant overall increase in the *pmoA* gene abundance (~10%), but only under low initial biocrust cover (Table S4). A significant positive relation between CH_4 fluxes and the abundance of the *pmoA* gene was observed in the warming + rainfall exclusion plots, but only in the low initial biocrust cover plots ($R^2 = 0.13$; $p=0.04$).

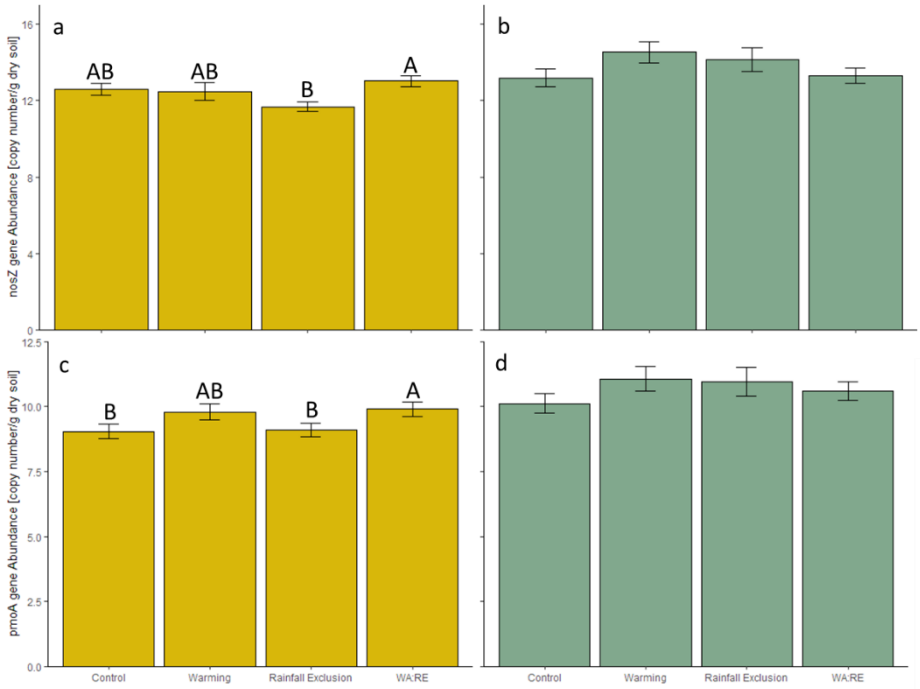

FIGURE 4 Log-transformed averaged abundances of *nosZ* (a,b) and *pmoA* genes (c,d) in areas with low (left), and high (right) initial biocrust cover across the climate change treatments evaluated throughout the study period. Different letters indicate significant differences in pairwise comparisons between treatments by differences of least square means (P<0.05). Data represent means ± s.e. (n= 5). WA:RE = warming and rainfall exclusion combined.

Discussion

Our study provides novel experimental evidence that biocrusts are key regulators of the responses of N_2O and CH_4 fluxes and associated functional genes to climate change drivers. On average, the presence of a well-developed biocrust community decreased N_2O emissions and increased CH_4 uptake. Biocrusts also regulated the temporal patterns of N_2O and CH_4 fluxes. For instance, and despite being highly variable in space and time, the combination of warming and rainfall exclusion led to a sharp reduction (197%) in average N_2O fluxes, but only in areas with high initial biocrust cover. The presence of a well-

developed initial biocrust community also mitigated the reductions in CH_4 uptakes observed for low initial biocrust under the combination of warming and rainfall exclusion. These results highlight the importance of considering biocrusts for better understanding ecosystem responses to climate change in drylands, and the need of considering them when forecasting future GHG fluxes from soils in these ecosystems, which are forecasted to cover more than 50% of the terrestrial surface by the end of this century (Huang *et al.*, 2016b).

Our results suggest that projected changes in temperature and precipitation will likely modify the capacity of dryland soils to exchange N_2O with the atmosphere. More importantly, these findings indicate that such responses depend on the degree of biocrust development. While in low initial biocrust cover areas rainfall exclusion (and its combination with warming) tended to increase N_2O emissions throughout the study period, in soils with a well-developed initial biocrust this treatment promoted a sharp decrease in the emissions of this GHG. Furthermore, the combination of warming and rainfall exclusion turned the soil under well-developed initial biocrusts into a net N_2O sink. These results highlight the ability of biocrusts to mitigate the effects of climate change on N_2O emissions, but also the importance of considering the interactions among different climate change drivers when evaluating potential future GHG emissions (Fig. 1b). Interestingly, despite in this field experiment the climate change treatments have reduced the original biocrust cover over the years (Ladrón de Guevara et al., 2018), we still found a sharp reduction in N_2O fluxes in the warming + rainfall exclusion treatment. Such a result suggests a strong legacy effect of the biocrust cover on soil functioning, similar to that reported in other mesic ecosystems with plants (Meisner, De Deyn, de Boer, & van der Putten, 2013), and further highlights the importance of these communities on driving the responses of drylands to global change drivers.

Climate change effects on N_2O fluxes are likely to be highly variable due to the key importance of climatic factors such as soil temperature and moisture

as major regulators of GHG emissions (Dijkstra et al., 2012; Zhou et al., 2016). Warming, and the associated increases in soil temperature, could enhance the metabolism of nitrifiers and denitrifiers, boosting N_2O emissions (Dalal, Wang, Robertson, & Parton, 2003). However, and particularly in drylands, climate change-driven reductions in soil moisture (either associated with warming or due to decreases in precipitation) can limit microbial metabolism and reduce atmospheric N_2O emissions (Chapuis-Lardy, Wrage, Metay, Chotte, & Bernoux, 2007). Overall, our rainfall exclusion and warming treatments promoted soil drying, as shown in Escolar et al. (2012) and Maestre et al. (2013). A detailed analysis of soil moisture changes after rainfall events in our experiment showed how biocrusts increased water gains after rainfall events but enhanced soil desiccation after rainfall pulses (Lafuente et al., 2018). Thus, the reductions in water availability due to our climate change treatments, particularly in plots with well-developed initial biocrusts, might explain the observed decreases in N_2O emissions in these plots. Alternatively, nutrient availability is also often highlighted as a key driver of dryland N_2O fluxes (Dalal & Allen, 2008; Dijkstra et al., 2013). A larger inorganic N availability under low initial biocrust might explain the highest N_2O emissions under global change scenarios observed in this treatment. Under aerobic conditions and high availability of N substrate (i.e. NH_4^+), nitrification is expected to dominate over denitrification (Dalal et al,, 2003; Weier, Doran, Power, & Walters, 1993), which have been reported to lead to an accumulation of inorganic N forms in global drylands (Delgado-Baquerizo et al., 2016). More importantly, we have found in our experiment that warming + rainfall exclusion treatments often lead to an accumulation of inorganic N under low, but not under high initial biocrust cover plots (Delgado-Baquerizo et al., 2014). Similarly, previous studies in our experimental site have found a higher potential nitrification rate and available NO_3^- in bare soil areas compared to areas with well-developed biocrusts (Castillo-Monroy, Maestre, Delgado-Baquerizo, & Gallardo, 2010), where DON is the dominant N form (Delgado-

Baquerizo, Castillo-Monroy, Maestre, & Gallardo, 2010). Thus, in drylands, a well-developed biocrust cover might be linked to a lower accumulation of inorganic N (Delgado-Baquerizo et al., 2014), therefore limiting the availability of substrate for the denitrification process, ultimately reducing N_2O fluxes to the atmosphere from incomplete denitrification leaks.

It is important to highlight the importance of the selected denitrification gene studied. Under aerobic conditions, nitrification produces N_2O as a by-product (Bremner, 1997; Canfield et al., 2010), a process that is expected to be important in drylands given their reported relatively high mineralization rates. However, denitrification is an anaerobic multistep process that also produces N_2O (Firestone & Davidson, 1989). Anaerobic soils are not dominant in drylands, but favourable conditions for denitrification can be created in soil aggregates or after precipitation pulses (Austin et al., 2004; Ley et al., 2018). The last step of the denitrification pathway consists on the conversion of N_2O into N_2, a step catalysed by the nitrous oxide reductase codified by functional gene *nosZ* (Philippot et al., 2007). Consequently, the *nosZ* gene has been used to estimate N_2O fluxes (Powell et al., 2015). Our climate change treatments had no detectable effects on this gene regardless the initial biocrust cover considered. However, the presence of a well-developed biocrust community increased the abundance of the *nosZ* gene (Fig. 4a, b) at the warming and rainfall exclusion treatments, which might also help explain, at least partially, the average lower rates of N_2O observed under well-developed initial biocrusts over the whole study.

Our climate change treatments consistently and relatively reduced CH_4 uptake, which supports other studies carried out in semiarid grasslands (Dijkstra et al., 2013). Methane oxidation requires gas diffusivity to provide atmospheric CH_4 to soil methanotrophs, a step catalysed by the CH_4 monooxygenase codified by the *pmoA* gene (Dalal & Allen, 2008). In more mesic ecosystems, decreased soil moisture would improve gas diffusivity, increase soil aeration and CH_4

oxidation. However, drylands are water limited ecosystems, so further reductions in soil moisture by our climate change treatments might have limited the activity of CH_4 oxidising bacteria (Galbally, Kirstine, Meyer, & Wang, 2008; Sullivan, Selmants, & Hart, 2013). Interestingly, we observed a positive correlation between changes in biocrust cover during the lifetime of our experiment and the CH_4 uptake. Put simply, the loss of biocrust cover through time observed in high initial biocrust cover plots (Ladrón de Guevara et al., 2018) has reduced CH_4 uptake (Fig. S3). Reductions in soil moisture promoted by the climate change treatments and enhanced soil desiccation rates after rainfall pulses by biocrusts (as discussed above) likely limited the metabolism of CH_4 oxidising bacteria, leading to a reduced CH_4 consumption. Increased temperature has been described previously to drive changes in the community composition of CH_4 oxidising bacteria (Mohanty, Bodelier, & Conrad, 2007) and these changes in community structure could explain the differences between treatments observed in CH_4 uptake (Nazaries et al., 2013).

The presence of a well-developed initial biocrust community increased overall *pmoA* gene abundance at the control and under rainfall exclusion. The well-known positive impacts of biocrusts on soil fertility (Weber et al., 2016) could underlie this increase in microbial abundance (Barger, Weber, Garcia-Pichel, Zaady, & Belnap, 2016; Maestre et al., 2011), which in turn might have contributed, at least partially, to increase the overall CH_4 uptake over the entire duration of this study (Le Mer & Roger, 2001). Moreover, CH_4 consumption in soils is associated to the more organic layers (Bull *et al.*, 2000). However, and in contrast with a previous study carried out in an Australian forest that found correlated gene abundances and GHG fluxes (Martins, Macdonald, Anderson, & Singh, 2016), we could not find a significant relationship between the overall abundance of the *pmoA* gene and overall CH_4 uptake. Methane uptake depends on the balance between gas diffusivity and metabolic stress (Luo, Kiese, Wolf, & Butterbach-Bahl, 2013). Thus, these results can be consequence of microbial

activity limitation due to water stress. Indeed, under low initial biocrust cover, we detected an increase in *pmoA* abundance at the warming + rainfall exclusion treatment (Fig. 4c). Despite such increase, this treatment did not show enhanced CH_4 uptake supporting that reductions in soil moisture could have limited microbial metabolism. Alternatively, we cannot discard that the interference of soil NH_4^+, which competes with CH_4 for the methane monooxigenase (King & Schnell, 1994), could be behind the lack of correlation between pmoA and CH_4 uptake observed.

Together, our findings highlight how biocrusts are essential regulators of soil-atmosphere N_2O and CH_4 fluxes and their responses to simulated climate change. They also show that functional microbial abundance (i.e. *nosZ* and *pmoA* carrying bacteria) can also be highly variable in time, providing evidence for seasonal patterns in these functionally important bacterial communities. Our results also illustrate how biocrusts affect temporal patterns in the fluxes of N_2O and CH_4 and associated functional genes from soils. On average, biocrusts legacy reduced the rate of N_2O emissions, increased the rate of CH_4 uptake and increased the abundance of both *nosZ* and *pmoA* genes. More importantly, they mitigated the reductions in CH_4 uptakes observed under the combination of warming and rainfall exclusion treatments. Our findings emphasize the importance of well-developed biocrust communities to mitigate the impacts of warming and altered rainfall patterns on the GHG fluxes from dryland soils, and thus the need to preserve them to minimize the negative consequences of ongoing climate change and to maintain ecosystem functioning in a warmer and drier world.

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Supplementary material

TABLE S1. Target gene, primer sequence and thermal conditions used in the quantitative pcr analyses.

Gene	Primer	Sequence (5' to 3')	Amplicon size (bp)	Reference	Thermal conditions
nosZ	nosZ2F	CGC RAC GGC AAS AAG GTS MSS GT	267	Henry et al., 2006	95 ºC - 300 s 95 ºC - 30 s 60 ºC - 30 s 72 ºC - 60 s 40 cycles
	nosZ2R	CAK RTG CAK SGC RTG GCA GAA			
pmoA	pmo189F	GGN GAC TGG GAC TTC TGG	500	Bourne et al. 2001	95 ºC - 180 s 95 ºC - 15 s 53 ºC - 15 s 72 ºC - 30 s 40 cycles
	pmo650R	ACG TCC TTA CCG AAG GT			

TABLE S2. Linear mix model of the effect of climate change treatments on N_2O and CH_4 fluxes (n = 7) and functional gene abundances (n=5). WA = warming, RE = rainfall exclusion and BSC = biocrust cover.

	N_2O		CH_4		nosZ		pmoA	
	F	*P*	F	*P*	F	*P*	F	*P*
warming	0.57	0.45	6.12	**0.02**	0.61	0.44	3.30	0.08
rainfall exclusion	1.59	0.21	0.47	0.50	0.10	0.76	0.00	0.99
BSC	1.53	0.22	9.41	**0.00**	9.56	**0.00**	12.87	**0.00**
WA x RE	0.49	0.49	1.33	0.25	1.81	0.19	0.03	0.85
WA x BSC	0.01	0.94	0.70	0.41	0.10	0.75	0.94	0.34
RE x BSC	12.50	**0.00**	7.99	**0.01**	0.00	0.95	0.07	0.79
WA x RE x BSC	1.92	0.17	1.33	0.25	6.20	**0.02**	1.23	0.28
change BSC	0.16	0.69	2.88	0.09	0.23	0.64	0.62	0.44

TABLE S3. Linear mix model of the effect of climate change treatments on N_2O and CH_4 fluxes under low and high biocrust cover, and pairwise comparisons between treatments by differences of least square means. WA = warming, RE = rainfall exclusion and BSC = biocrust cover.

		Low BSC		High BSC			Low BSC		High BSC	
		F	*P*	F	*P*		t	*P*	t	*P*
N_2O	WA	0.21	0.65	0.01	0.93	control - WA	0.60	0.56	-0.93	0.36
	RE	1.73	0.20	19.19	**0.00**	control - RE	-0.54	0.60	1.84	0.08
	WA x RE	0.15	0.70	3.99	0.06	control - WA x RE	-0.68	0.50	3.75	**0.00**
	change in BSC	0.12	0.73	0.12	0.74	WA - RE	1.13	0.27	-2.77	**0.01**
						WA - WA x RE	1.27	0.21	-4.68	**0.00**
						RE - WA x RE	-0.14	0.89	1.91	0.07
CH_4	WA	1.04	0.32	0.96	0.33	control - WA	-2.33	**0.03**	-2.11	**0.05**
	RE	1.90	0.18	8.24	0.01	control - RE	-1.40	0.17	1.96	0.06
	WA x RE	2.19	0.15	0.01	0.93	control - WA x RE	-2.26	**0.03**	-0.14	0.89
	change in BSC	2.70	0.11	0.59	0.45	WA - RE	-0.93	0.36	-4.06	**0.00**
						WA - WA x RE	-0.08	0.94	-1.97	0.06
						RE - WA x RE	-0.85	0.40	-2.10	**0.05**

TABLE S4. Linear mix model of the effect of climate change treatments on *nosZ* and *pmoA* functional genes under low and high biocrust cover, and pairwise comparisons between treatments by differences of least square means. WA = warming, RE = rainfall exclusion and BSC = biocrust cover.

		Low BSC		High BSC			Low BSC		High BSC	
		F	*P*	F	*P*		t	*P*	t	*P*
nosZ	RE	1.14	0.30	0.28	0.60	control - WA	0.21	0.84	-1.58	0.13
	WA	0.18	0.68	0.03	0.87	control - RE	1.74	0.10	-1.17	0.26
	change in BSC	3.22	0.10	2.68	0.12	control - WA x RE	-0.79	0.44	-0.15	0.89
	WA x RE	0.33	0.57	0.80	0.38	WA - RE	-1.51	0.15	-0.42	0.68
						WA - WA x RE	0.99	0.34	-1.44	0.17
						RE - WA x RE	-2.54	**0.02**	1.03	0.32
pmoA	rainfall exclusion	7.82	**0.01**	0.05	0.82	control - Wa	-1.88	0.06	-1.12	0.28
	warming	0.02	0.88	0.10	0.76	control - RE	-0.11	0.91	-1.00	0.33
	change in BSC	0.07	0.79	1.11	0.31	control - WA x RE	-2.19	**0.03**	-0.57	0.58
	WA x RE	0.68	0.41	0.00	0.97	WA - RE	-1.76	0.08	-0.12	0.90
						WA - WA x RE	0.29	0.77	-0.55	0.59
						RE - WA x RE	-2.08	**0.04**	0.43	0.67

FIGURE S1. Detailed view of experimental plots from the warming (a), rainfall exclusion (b) and warming + rainfall exclusion (c) treatments. Photo credits: Felipe Gutierrez.

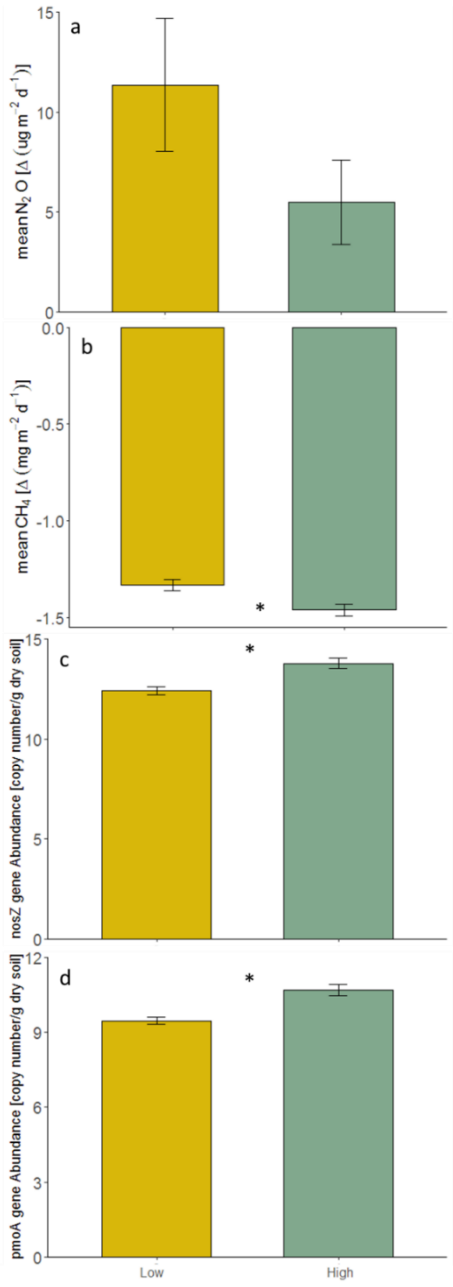

FIGURE S2. Averaged (throughout the study period) N_2O (a) and CH_4 (b) fluxes and log-transformed averaged abundances of *nosZ* (c) and *pmoA* genes (d) in areas with low (left), and high (right) initial biocrust cover. Asterisk indicate significant differences in comparisons between low and high biocrust cover by student's t test (p<0.05). Data represent means ± s.e. (n= 7 in gases fluxes and n= 5 in gene abundances).

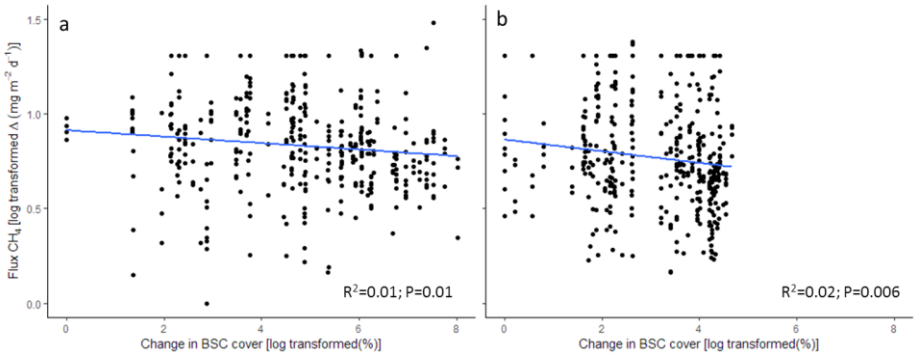

FIGURE S3. Linear regressions between the change in biocrust (BSC) cover and CH_4 fluxes for areas with low (left), and high (right) initial biocrust cover.

Capítulo 3/ Chapter 3

Nitrogen deposition influences greenhouse gases fluxes in a semiarid shrubland

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Abstract

Nitrogen (N) deposition is a key driver of soil-atmosphere greenhouse gases exchange. We simulated four levels of N-deposition to evaluate its effects on nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) fluxes. Increasing N-deposition lead to a consistent increase in N_2O emissions, likely due to increases in soil microbial nitrification and/or denitrification processes. However, only intermediate levels of N-deposition reduced CH_4 uptake and tended to reduce CO_2 emissions, suggesting the existence of tipping points between N saturated and unsaturated conditions. Our study provides novel insight into the response of GHGs to long-term N-deposition in semi-arid shrublands, forecasting increases in N_2O emissions, and decreases in CH_4 uptake rates, with likely consequences to the on-going climate change.

Keywords

Carbon dioxide, global change, methane, nitrous oxide, N-deposition

Introduction

The atmospheric concentration of greenhouse gases (GHG) has increased in the last decades contributing to the on-going climate change (Pachauri & Meyer, 2014). While carbon dioxide (CO_2) fluxes and dynamics have been widely studied in the last years, other important greenhouse gases, such as nitrous oxide (N_2O) or methane (CH_4), have received less attention (but see Soussana et al., 2007; Snyder et al., 2009).

Nitrogen (N), along with water, is a main limiting factor for plants and microbes in semiarid ecosystems (Fenn *et al.*, 2003). In these areas, an increase in N deposition due to human activities might be an important source of N increasing soil fertility (Chapin *et al.*, 1986). However, in excess, anthropogenic N deposition can cause imbalances in the N cycle, eutrophication, and soil acidification (Bobbink *et al.*, 2010; Ochoa-Hueso *et al.*, 2013), and influence soil organic matter decomposition and microbial activity (Lo Cascio *et al.*, 2017). Previous studies suggest that N deposition can be a key driver of GHG fluxes (Mosier et al., 1991; Bowden et al., 2000), but our knowledge about how different levels of N deposition can affect the exchange of GHG between the soil and the atmosphere in semiarid ecosystems is still limited. Because of the water and nutrient limiting nature of semiarid ecosystems, GHG fluxes are expected to be low (Dalal & Allen, 2008). However, the global extension of these ecosystems, which might increase in the coming decades due to the forecasted increase in aridity associated to the ongoing climate change, can make semiarid regions a relevant source/sink for GHG fluxes (Austin *et al.*, 2004). Herein, we aimed to understand, under field conditions, the effects of 8 years of simulated N deposition on N_2O, CH_4 and CO_2 fluxes in a Mediterranean semiarid shrubland.

Material and methods

We conducted this study in the natural reserve El Regajal-Mar de Ontígola (Central Spain; 40°00'24.772''N - 3°37'12.788''W; 580 m.a.s.l.). The climate is Mediterranean semi-arid. Average annual temperature and rainfall are 15 °C and 358 mm, respectively. Soils are rich in calcium carbonate with a sandy clayish structure. The dominant perennial vegetation is *Quercus coccifera* L. and *Rosmarinus officinalis* L. Open areas among vegetation patches are partially covered with a well-developed biocrust community. The background annual N deposition in the study area is 6.4 kg N $ha^{-1}yr^{-1}$ (Ochoa-Hueso *et al.*, 2013). See Ochoa-Hueso, Hernandez, Pueyo, & Manrique, 2011 for a full description of the study site.

In October 2007, to simulate nitrogen deposition equivalent to those predicted in the Mediterranean region by 2050 (Phoenix *et al.*, 2006), we established six experimental blocks consisting of four 2.5 x 2.5 m plots (one control [0 kg N $ha^{-1}yr^{-1}$] and three N-fertilised plots [10, 20 and 50 kg N $ha^{-1}yr^{-1}$]). We simulated a wet N deposition event once a month spraying 2 L of NH_4NO_3, except for July and August, when we did not fertilize to simulate the typical summer drought, and September, when a three-month total N load in 2 L was applied to simulate the N availability peak that occurs after the summer drought (Fenn *et al.*, 2003). From 2011 onwards, N applications were done every three months.

From April 2015 to May 2016, we estimated soil-atmosphere N_2O, CH_4 and CO_2 fluxes using the static chamber method (Bowden *et al.*, 2000). Soil temperature and moisture were continuously monitored using automated sensors (EC-5, Decagon Devices Inc., Pullman, WA, USA). Greenhouse gases (GHG) fluxes were estimated from the linear rate of change in gas concentration, soil surface area and chamber volume (Durán *et al.*, 2013b). Soil NO_3^--N and NH_4^+-N were analysed colorimetrically as explained in Durán et al., 2013a. We

tested the effect of the different levels of N fertilisation on GHG fluxes within a repeated measures general linear mixed effects framework. To test those differences between levels of N-fertilisation, we used differences of least squares means. We examined the relationship between N_2O fluxes and N-fertilisation doses and soil N availability with linear regressions. All analyses were performed in R (R Core Team, 2017). Data associated with this study are available in Figshare (Lafuente et al., 2019b). For more details on the methods, see Appendix.

Results and discussion

The three studied gas fluxes (i.e. N_2O, CH_4 and CO_2) showed a high temporal variability throughout the study period independently of the treatment (Figure 1). Nitrous oxide fluxes ranged on average from 10 to 30 μg $m^{-2}d^{-1}$ (Figure 1a). Despite that the high intra-annual variability did not allow us to detect significant differences, N_2O emissions showed a strong positive correlation with increasing N-fertilisation (R^2=0.95; P=0.02; Figure 2a). Also, we found a significant and positive relationship between soil N availability (i.e. NO_3^--N+NH_4^+-N) and N_2O emissions (R^2=0.89; P=0.04), which has been traditionally linked to increased N_2O emissions (Snyder *et al.*, 2009; Homyak *et al.*, 2016). Literature based on field experiments with multiple doses of N describe positive responses in N_2O fluxes to increasing N inputs (e.g. Shcherbak et al., 2014). Here, after 8 years of field experiment simulating N-deposition with four doses of N, we detected a consistent increase in N_2O emissions, particularly in the 50 kg N $ha^{-1}yr^{-1}$ treatment (~168%) (Figure 2a). In semiarid ecosystems, N is the second factor limiting productivity only after water availability (Robertson & Groffman, 2007). Our results suggest that forecasted increases in N-availability via N-deposition is likely to increase soil microbial nitrification and/or denitrification processes, enhancing N emissions from Mediterranean semiarid systems (Dalal

& Allen, 2008).

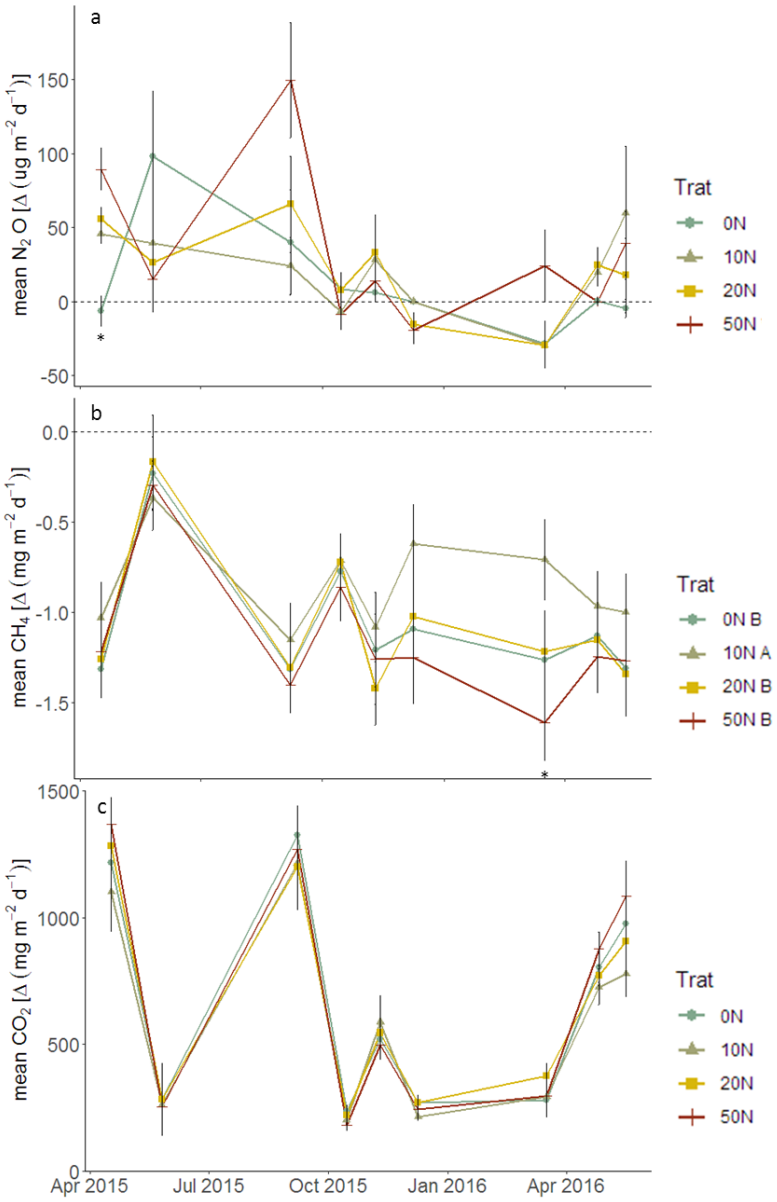

FIGURE 1 Temporal variation in N2O (a), CH4 (b), and CO2 (c) fluxes throughout the duration of the experiment. Data are means ± s.e. (n = 6). Capital letters in legend, in each panel, show significant differences among fertilisation treatments. 0N = 0 kg N ha-1yr-1, 10N = 10 kg N ha-1yr-1, 20N = 20 kg N ha-1yr-1, 50N = 50 kg N ha-1yr-1. Asterisks indicate significant differences among N doses (P<0.05) in specific dates.

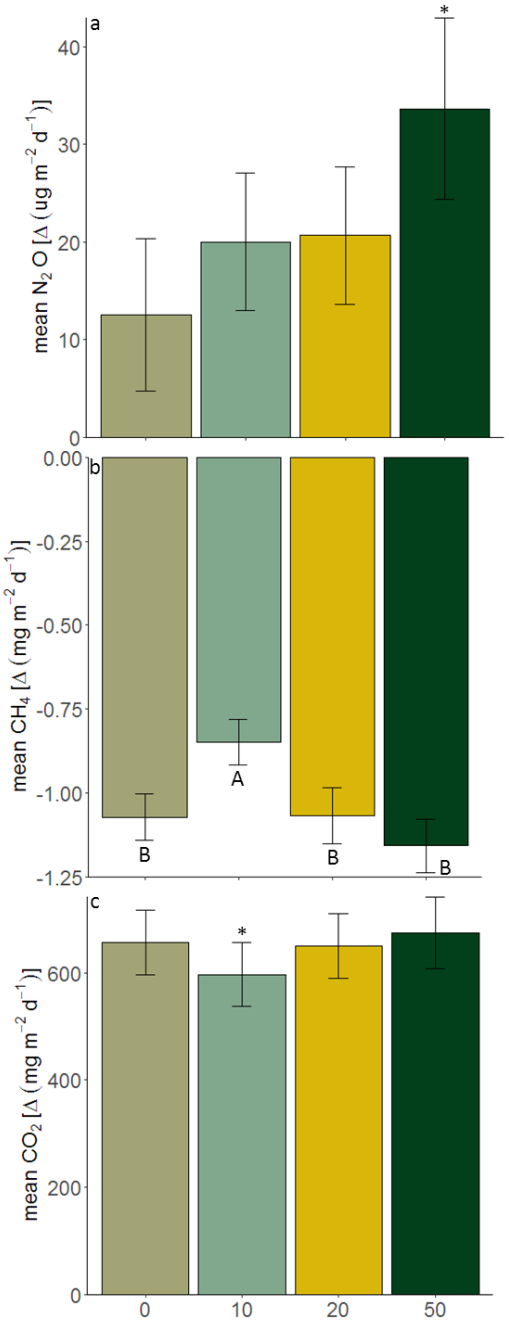

FIGURE 2 Averaged N_2O (a), CH_4 (b) and CO_2 (c) fluxes estimated under different N-deposition concentrations (0 kg N $ha^{-1}yr^{-1}$, 10 kg N $ha^{-1}yr^{-1}$, 20 kg N $ha^{-1}yr^{-1}$, 50 kg N $ha^{-1}yr^{-1}$). Different letters indicate significant differences among treatments (P<0.05). Asterisks indicate marginally significant differences among N doses (P<0.06). Data represent means ± s.e. (n=6).

As expected, our experimental site was a net CH_4 sink (Potter *et al.*, 1996), and fluxes ranged from -0.85 to -1.16 mg $m^{-2}d^{-1}$ (Figure 1b). Whereas the lowest fertilisation treatment (i.e. 10 kg N $ha^{-1}yr^{-1}$) significantly reduced the methane uptake, higher doses of N fertilization (i.e. 20-50 kg N $ha^{-1}\,yr^{-1}$) did not differ significantly from the values of the control plots (Figure 2b). Mosier et al. (1991), observed that increases in N turnover with fertilization were linked to reductions in soil CH_4 uptake. Our results from a field experiment simulating scenarios of realistic future levels of N deposition suggest a non-linear response of soil-atmosphere CH_4 fluxes to N-deposition, with intermediate levels (10 kg N $ha^{-1}yr^{-1}$) of N-deposition having the greatest impact on the capacity of semiarid ecosystems to act as sinks of atmospheric CH_4. A possible explanation for this lack of linearity is that type II metanotrophic bacteria (favoured by low N availability) were excluded by moderate N inputs and substituted by type I metanotrophs (favoured by high N availability; Hanson & Hanson, 1996). However, more long-term studies focused on specific biotic and abiotic drivers would be necessary to fully mechanistically understand and forecast CH_4 fluxes under increasing N-deposition.

Carbon dioxide fluxes ranged from 597 to 675 mg $m^{-2}\,d^{-1}$ (Figure 1c). Similar to CH_4, we observed a non-linear response to the different N doses. The 10 kg N $ha^{-1}yr^{-1}$ treatment tended to reduce CO_2 emissions (P = 0.06), but higher amounts of N-fertilization (i.e. 20-50 kg N $ha^{-1}\,yr^{-1}$) did not differ on CO_2 efflux from the unfertilised plots (Figure 2c). Our results are consistent with Micks, et al (2004) and Ambus & Robertson (2006), who observed no changes in soil respiration to long term fertilisations in forests and grasslands, and suggest that factors other than N, such as C or water availability, may limit soil respiration rates in these systems. Further, the detected differences between the 10 and the 20-50 kg N $ha^{-1}\,yr^{-1}$ treatments for both CH_4 and CO_2 fluxes may reveal the

existence of a worth-exploring threshold between unsaturated and saturated N conditions, which may determine differences in the microbial communities, and the C fluxes between the soil and the atmosphere.

Our study provides novel insight into the response of GHGs to continuous N deposition in semi-arid Mediterranean shrublands. Our results, obtained after 8 years of N fertilization, suggest that projected increases in N deposition could lead to increases in the emissions of N_2O from these ecosystems, and decreases in their capacity to consume atmospheric CH_4, (at least with intermediate levels of N deposition), with likely consequences to the on-going climate change.

Acknowledgements

We thank Ana Ros for her help with lab analyses and the owner of "Finca El Regajal" for their kind allowance to work in their property. A. L. is supported by a FPI fellowship from the Spanish Ministry of Economy and Competitiveness (BES-2014-067831). J.D acknowledges support from the Fundação para Ciência e Tecnologia (IF/00950/2014) and the FEDER (PT2020 Partnership Agreement and COMPETE 2020; UID/BIA/04004/2013). This research was supported by the Spanish Ministry of Economy and Competitiveness (CGL2013-44661-R and AGL2015-64582-C3-3-R), and the Comunidad de Madrid and European Structural and Investment Funds (AGRISOST-CM S2013/ABI-2717).

Supplementary material

Greenhouse gas exchange measurements

In each of the 24 established plots, we inserted 5 cm into the ground a polyvinyl chloride (PVC) ring (diameter = 20 cm, height = 7 cm). We used the static chamber method (Bowden *et al.*, 2000) to estimate soil-atmosphere N_2O, CH_4 and CO_2 fluxes. From April 2015 to May 2016 we carried out nine sampling campaigns, approximately once a month. Immediately before each measurement, we placed a 20 cm diameter and 9 cm high PVC chamber on top of each of the 24 permanent rings and sealed it with a rubber band. Each chamber was covered with reflective material for thermal isolation during the measurement and had a sampling port in the top centre of the camber for the air sampling. We collected gas samples from chamber's headspace using needle and polypropylene syringes at 0, 30 and 60 min after chamber closure, and we transferred the air to 22 ml pre-vacuumed vials and kept them at room temperature until analyses. We used a HP-6890 gas chromatograph (GC), equipped with a headspace autoanalyser (HT3; Agilent Technologies, Barcelona, Spain), a ^{63}Ni electron capture detector (for N_2O estimation) and a flame-ionization detector fitted with a methaniser (for CH_4 and CO_2 detection). The carrier gas used was helium.

We used the linear rate of change in gas concentration, soil surface area and chamber volume during the 60 min period (Durán et al., 2013), to estimate N_2O, CH_4 and CO_2 fluxes (reported as changes in milligrams or micrograms (for N_2O) per square metre per day [$\Delta(mg\ m^{-2}d^{-1})$]). More than 90% of the N_2O, CH_4 and CO_2 fluxes were linear (R^2 >0.7). We discarded those non-linear rates and estimate missing values using the missForest algorithm in the R package missForest, which iteratively fills missing values in all columns of a data frame based on predictions from random forest models (Stekhoven,2013). For the iteration we included the averaged soil temperature and moisture matching the

treatment, date and time of the sampling. We estimated 1.8%, 2.3% and 1.8% of the N_2O, CH_4 and CO_2 rates, respectively.

Statistical analyses

We tested the effect of the different levels of N fertilisation on GHG fluxes within a repeated measures general linear mixed effects framework with the *lmer* in the R package *lmer4* (Bates 2015). To test those differences between levels of N-fertilisation, we used differences of least squares means using the function *difflsmeans* in the R package *lmerTest* (Kuznetsova, 2017). We performed all analyses using R software version 3.4.0 (R Core Team, 2017).

Capítulo 4/ Chapter 4

Global drivers of methane oxidation and denitrifying gene distribution in drylands

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Abstract

Aim: Soil *pmoA*- and *nosZ*-carrying microorganisms are major drivers of methane and nitrous oxide fluxes. However, most studies on these organisms have been conducted in mesic ecosystems so little is known about the factors driving their distribution in drylands, the largest biome on Earth. We conducted a global survey to evaluate the role of climate- and soil-related variables as predictors of the richness, abundance and community structure of bacteria carrying *pmoA* and *nosZ* genes.

Location: 80 dryland ecosystems from six continents.

Time period: February 2006 - December 2011

Major taxa studied: Methanotrophic (carrying the *pmoA* gene) and denitrifiying (carrying the *nosZ* gene) bacteria.

Methods: We used data from a standardized field survey and structural equation modelling to evaluate the direct and indirect effects of climatic (aridity, rainfall seasonality, mean annual temperature) and soil (organic carbon, pH and texture) variables on the total abundance, richness and community structure of microorganisms carrying *pmoA* and *nosZ* genes.

Results: Taxa related to *Methylococcus capsulatus* or *Methylocapsa* sp., often associated with more mesic environments, were common in global drylands. The abundance and richness of methanotrophs were not associated with climate or soil properties. However, variables such as mean annual temperature, rainfall seasonality, organic C, pH and sand content were highly correlated with their community structure. Aridity and soil variables such as sand and pH were correlated with the abundance, community structure and richness of the *nosZ* bacterial community.

Main conclusions: Our study provides novel insights on drivers of the abundance, richness and community structure of soil microorganisms carrying *pmoA* and *nosZ* genes in drylands worldwide, which are of paramount importance to forecast changes in the soil:atmosphere exchange of greenhouse gases under ongoing climate change.

Key words

Abundance, community structure, *denitrifiers,* drylands, *methanotrophs, richness*.

Introduction

Microbial communities are the main biotic drivers of methane (CH_4) and nitrous oxide (N_2O) emissions from soils (Dalal & Allen, 2008). For instance, aerobic soils are the only biological sink for atmospheric CH_4, and about two thirds of the CH_4 found in the atmosphere derives from microbial metabolism (Dalal & Allen, 2008; Conrad, 2009). Methanogens produce CH_4 under anaerobic conditions (e.g. rice fields, lake sediments or wetlands) through a process called methanogenesis (Le Mer & Roger, 2001). As such, CH_4 production in drylands (arid, semi-arid and dry-subhumid ecosystems), environments characterized by having low and infrequent rainfall regimes, has been traditionally considered to be low (Castaldi, Ermice, & Strumia, 2006). Conversely, methanotrophs possess the methane monooxygenase encoded by the *pmoA* gene, which oxidises CH_4 under aerobic conditions, therefore potentially reducing atmospheric CH_4 concentrations. Because of their aerobic requirements, we expect methanotrophs to be important member of microbial communities in these ecosystems (Safriel & Adeel, 2005). Alternatively, NH_4^+, competes with CH_4 for the methane monooxigenase producing hydroxylamine, which is further metabolised to nitrite (King & Schnell, 1994). However, this alternative in natural systems appear to be very minor (Stein, Roy, & Dunfield, 2012; Nazaries, Murrel, Millard, Baggs, & Singh, 2013). On the other hand, microbial processes such as nitrification and denitrification are responsible for over two-thirds of the soil N_2O emissions to the atmosphere (Dalal & Allen, 2008; Butterbach-Bahl, Baggs, Dannenmann, Kiese, & Zechmeister-Boltenstern, 2013). Nitrifying microorganisms transform NH_4^+ to NO_3^-, releasing N_2O to the atmosphere as a by-product (Bremner, 1997). Denitrification (i.e. the reduction of NO_3^- to NO, N_2O and finally to N_2) is a multi-step process carried out by different microorganisms and their enzymes, including the nitrous oxide reductase (*nosZ*) that catalyses the last step of this process.

Many studies have explored what are the main abiotic and biotic drivers of carbon dioxide (CO_2) fluxes (the major anthropogenic greenhouse gas [GHG]), but much less is known about the factors controlling CH_4 and N_2O fluxes, which have global warming potentials 28 and 265 times greater than that of CO_2, respectively (Nakicenovic & Swart, 2000). The traditional consensus that GHGs fluxes are likely to be small in drylands has resulted in a bias in which the vast majority of knowledge about the exchange of CH_4 and N_2O between the soil and the atmosphere, and about its associated microbial ecology, comes from studies carried out in mesic ecosystems (Oertel, Matschullat, Zurba, Zimmermann, & Erasmi, 2016). However, drylands cover 45% of the land surface (Prăvălie, 2016), and sustain over 40% of human population (Reynolds *et al.*, 2007). Further, these numbers are likely to increase substantially in coming decades due to climate change-driven increases in aridity (Huang, Yu, Guan, Wang, & Guo, 2015) and projected human population growth rates (United Nations, 2017). In addition, recent evidence suggests that CH_4 and N_2O fluxes in drylands might be relevant at the global scale, given both the reported process rates and their extent worldwide (e.g. Martins, Nazaries, Macdonald, Anderson, & Singh, 2015; Weber, et al., 2015; Hu Trivedi, He, & Singh, 2017).

The abundance of *pmoA* and *nosZ* genes is strongly related to GHG fluxes, and have been used to predict them (Nazaries, Pan, et al., 2013; Powell, Welsh, & Hallin, 2015; Martins, et al., 2017). Although the major ecological drivers of CH_4 oxidation and denitrification rates (e.g., soil moisture and texture) are starting to be assessed (Butterbach-Bahl et al., 2013; Nazaries, Murrell, et al., 2013), much less is known about the distribution and drivers of the richness, abundance and community structure of *pmoA*- and *nosZ*-carrying microorganisms (i.e. methanotrophs and denitrifiers, respectively) across the globe.

Global-scale studies conducted in recent years have emphasized the role of climatic factors (e.g. aridity), and of soil properties (e.g., pH) as main

drivers of soil microbial communities both in drylands (Maestre *et al.*, 2015a) and elsewhere (Delgado-Baquerizo *et al.*, 2018b). However, to the best of our knowledge, no similar studies have been conducted on *pmoA*- and *nosZ*-carrying microorganisms to date. To fill this knowledge gap, this study aims to identify major biotic and abiotic factors that affect the abundance, richness and/or community structure of functional genes involved in CH_4 (*pmoA*) and N_2O (*nosZ*) fluxes in dryland soils globally. We did so using a dataset including 80 dryland sites from six continents. Given the potential importance of functional genes as modulators of CH_4 and N_2O fluxes (Martins, Macdonald, Anderson, & Singh, 2016), a better understanding of what drives the abundance, richness and community structure of the soil microorganisms containing these genes across large environmental gradients is of paramount importance to predict GHG fluxes in global drylands under climate change.

Material and Methods

Study sites

This study was carried in 80 dryland ecosystems from 12 countries (Argentina, Australia, Chile, China, Iran, Israel, Mexico, Morocco, Spain, Tunisia, USA and Venezuela: Map S1). The locations surveyed encompass a wide variety of the biotic and abiotic conditions that can be found in drylands worldwide. Study sites had an aridity index (AI = mean precipitation/potential evapotranspiration) ranging from 0.06 to 0.50, mean annual temperature and precipitation values ranging from -1.8 °C to 24.2 °C, and from 67 mm to 766 mm, respectively, and included major vegetation types (grasslands, shrublands, savannahs and dry forests). Field sampling took place between February 2006 and December 2011 in 30 m x 30 m plots representative of the vegetation found at each site following a standardized protocol (see Maestre et al, 2012 for more details). In short, to ensure that we capture the spatial heterogeneity and to avoid bias in the sampling, five 50 cm x 50 cm quadrats were randomly placed under the

canopy of the dominant perennial species and in open areas devoid of perennial vascular vegetation at each plot; when more than one dominant plant species was present, five additional quadrats were established under the canopy of the co-dominant perennial species. At each sampling quadrat, a composite topsoil sample (five 145 cm^3 soil cores 0-7.5 cm depth) was collected, bulked and homogenised in the field and taken to the laboratories of Rey Juan Carlos University (Spain). These samples were used to obtain a composite sample per microsite (vegetated and open areas) and site. Field samples were sieved (2 mm mesh) and split into two: a portion was air dried and used to analyse soil physico-chemical properties, and the other was frozen and stored at -20°C for molecular analyses.

Environmental and soil properties

Standardized climate data (mean annual temperature and rainfall seasonality) for all sites were obtained from Worldclim (www.worldclim.org), a high resolution (30 arc seconds or ~ 1km at equator) database generated from a high number of climate observations and topographical data (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). We estimated the degree of aridity of each site by obtaining its aridity index (precipitation/potential evapotranspiration) from Trabucco & Zomer (2009). For clarity, we used aridity [1- AI] so that higher values indicate increasing aridity (Delgado-Baquerizo et al, 2013). Soil organic C was determined by colorimetry after oxidation with a mixture of potassium dichromate and sulphuric acid (Anderson & Ingram, 1993). Soil pH was measured with a pH meter, in a 1:2.5 (mass:volume, soil:water) suspension. Soil sand content was estimated according to Kettler, Doran, & Gilbert (2001).

Functional microbial communities

We extracted soil total genomic DNA from 0.5 g of frozen soil using the PowerSoil DNA Isolation kit (MOBIO Laboratories, Inc. USA) following the

manufacturer's protocol, except for modifications in the cell lysis step (we used a tissue homogenizer (Precellys 24- dual. Bertin technologies, France) at 4500 rpm for 45 s, twice). The abundance of functional genes related to methanotrophs (*pmoA*) and N_2O reducing bacteria (*nosZ*) was determined with real-time quantitative PCR (q-PCR) using 96-well plates on a BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). These genes were quantified in duplicate (and then pooled, standard approach; Lammel, Feigl, Cerri, & Nüsslein, 2015; Martins et al., 2015; Wen et al., 2018) using the pmo189f/pmo650r (Bourne, McDonald, & Murrel, 2001) and nosZ2f/nosZ2r (Henry, Bru, Stres, Hallet, & Philippot, 2006) primers, respectively (Table S1). We used a well-established approach to study methanotrophs (Nazaries et al., 2011, Nazaries, Karunaratne, Delgado-Baquerizo, Campbell, & Singh, 2018) and denitrifiers (Singh, Tate, Thomas, Ross, & Singh, 2011) using T-RFLP (Terminal Restriction Fragment Length Polymorphism). Soil DNA samples were amplified with PCR using specific primers for methanotrophs and N_2O reducing bacteria (Table S2). The PCR products were purified using a FavoPrep GEL/PCR purification kit (FAVORGEN Biotech Corporation, Taiwan) following the manufacturer's instructions. The PCR purified products were digested with HhaI and MspI restriction enzymes (for *pmoA* and *nosZ*, respectively) in an 11 µl reaction mixture containing 9.4 ng μl^{-1} of PCR product, BSA, 10x NH_4 buffer and 0.5 ng μl^{-1} restriction enzyme (New England Biolabs, UK). Samples were incubated at 37ºC for 3h, which was followed by a deactivation at 95ºC for 10 min. After digestion, 2 µl of each sample was mixed with 0.3 µl of Genescan-600 LIZ size marker and 10 µl of Hi-Di formamide (Applied Biosystems, Warrington, United Kingdom). Prior to fragment analysis, samples were denatured at 95°C for 5 min and then chilled on ice for 5 min. A fragment size analysis was carried out with an Applied Biosystems 3500 Genetic Analyser. Terminal restriction fragments (T-RFs) generated by the sequencer were analysed using GeneMapper™ 4.0 (Applied Biosystems, Warrington, United Kingdom) and raw

data originated from GeneMapper™ were processed with the T-REX online software (Culman, Bukowski, Gauch, Cadillo-Quiroz, & Buckley, 2009). To control data quality, noise was filtered (peak area above the fluorescence noise), T-RFs aligned (2 bp as clustering threshold) and T-RF present in less than three sites were discarded. We estimated the community structure (size clades within a community, i.e. number of base pairs within a T-RF) and richness (number of clades) according to the number of unique T-RFs and their abundance based on the q-PCR analyses. A total of 116 (*pmoA*) and 118 (*nosZ*) operational taxonomic units (OTUs) were found in the 160 soil samples (80 sites x 2 microsites) analysed.

Statistical analyses

We used structural equation modelling (SEM, Grace, 2006) to evaluate the direct and indirect effects of geographical location (latitude and longitude), aridity, mean annual temperature, rainfall seasonality, microsite (open/vegetated areas) and soil properties (pH, sand content and organic C) as predictors of the abundance, richness and the community structure of *pmoA* and *nosZ* genes (see Fig. S1 for our *a priori* model). As a preliminary step to create an expression of community structure compatible with SEM, we used a three dimensional non-metric multidimensional scaling (NMDS) ordination, based on Bray-Curtis distance, to summarize the structure of the community of methanotrophs (Fig. S2a) and *nosZ* carrying denitrifiers (related to N_2O reducing bacteria; Fig. S2b; Paliy & Shankar, 2016). NMDS was performed in R with the vegan package (Oksanen *et al.*, 2013). We included the three axes obtained from NMDS analyses in the SEM. We expressed the geographical latitude as decimal degrees and decomposed longitude in its sine and cosine to account for the spatial autocorrelation of our data. We included microsite as a binary variable (1 and 0 denote samples coming from under the canopy of the dominant vegetation and plant interspaces, respectively) to capture the variability

between vegetated microsites and plant interspaces microsites (Ochoa-Hueso *et al.*, 2017). The remaining variables were transformed using natural logarithms (i.e. rainfall seasonality, *pmoA* and *nosZ* gene abundances), the square root (i.e. soil organic C and *pmoA* gene richness) and exponential (pH) transformations to improve normality. All variables (except microsite) were centred prior to SEM analysis to facilitate the interpretation of parameter estimates. By default, structural equation modelling is based on an underlying linear model, an assumption that must be checked. Overall, the relationships expressed in our model were well-approximated linearly, except that we observed a quadratic relation between aridity and the abundance of *nosZ* carrying denitrifiers (Fig. S3a). To include this curvilinear relationship in our otherwise linear model, we used the method suggested by Grace (2006). Briefly, aridity and its square (having first been centred to zero) were entered into the model as predictors, and their effects on the response of interest were pooled using a composite variable.

To test the goodness of fit of our SEMs, we used the chi-squared test (χ^2; the model has a good fit when χ^2/df is low, i.e., *c*. ≤ 2, and *P* is high, traditionally > 0.05), the root-mean-square error of approximation (RMSEA; the model has a good fit when RMSEA is indistinguishable from zero, and *P* is high, traditionally > 0.05), as well as the Bollen-Stine bootstrap tests (Schermelleh-Engel, Moosbrugger, & Müller, 2003). All indices suggested adequate model fit (Figs. 1 and 2), so we were free to interpret the path coefficients of the model and their associated *P*-values. A path coefficient is analogous to the partial correlation coefficient or regression weight, and describes the strength and sign of the relationships between two variables (Grace, 2006). The probability that a path coefficient differs from zero was tested using bootstrap tests, as our data were not always normally distributed (Schermelleh-Engel *et al.*, 2003; Kline, 2011). We calculated the standardized total effects of all drivers on the selected functional gene attributes (Grace, 2006). The net influence that one variable had

upon another was calculated by summing all direct and indirect pathways (effects) between two variables. All SEM analyses were conducted using AMOS 24.0 (IBM SPSS, Chicago, IL, USA).

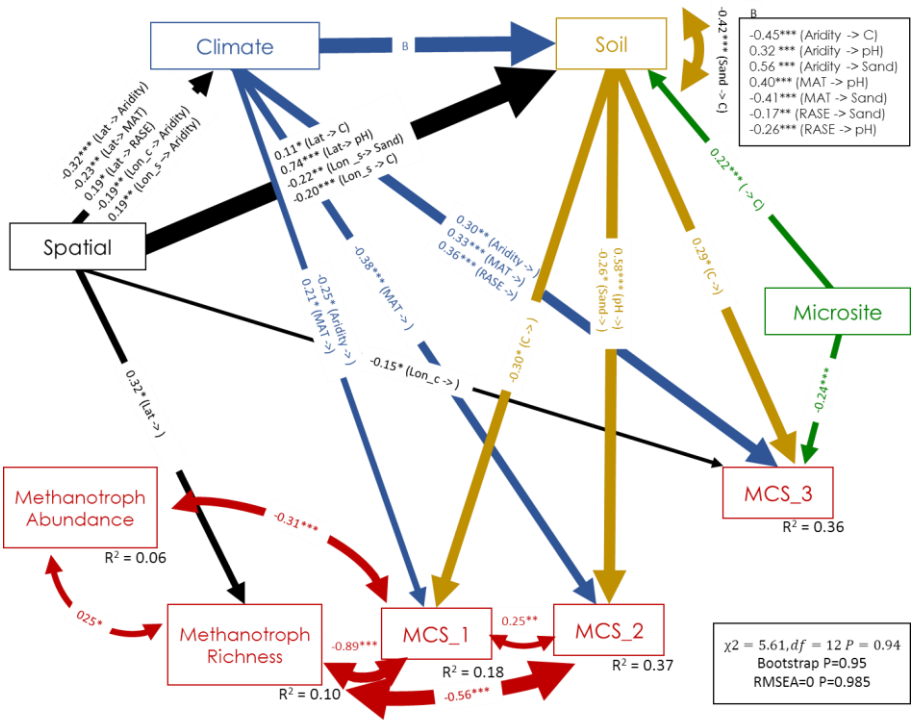

FIGURE 1 Structural equation model, describing the effects of spatial location (latitude [Lat], longitude sine [Lon_s] and longitude cosine [Lon_c]), climate (aridity, mean annual temperature [MAT] and rainfall seasonality [RASE]), soil properties (organic carbon [C], pH and sand content [Sand]) and microsite (open/vegetated areas) on methanotrophic bacteria abundance, richness and community structure. MCS = NMDS ordination axis for Methanotrophic bacteria community structure. The components within spatial location, climate and soil properties are included in the model as independent observed variables, but in this figure are grouped for simplicity. Numbers within the arrows show standardised path coefficients and indicate the effect size of the relationship among variables. Arrow widths are proportional to the strength of the relationship. Only significant relationships are shown (P < 0.05) *P < 0.05, **P < 0.01, ***P < 0.001. The goodness of fit of the model is shown in the bottom right hand corner of the figure (df, degrees of freedom; RMSEA, root mean square error of approximation).

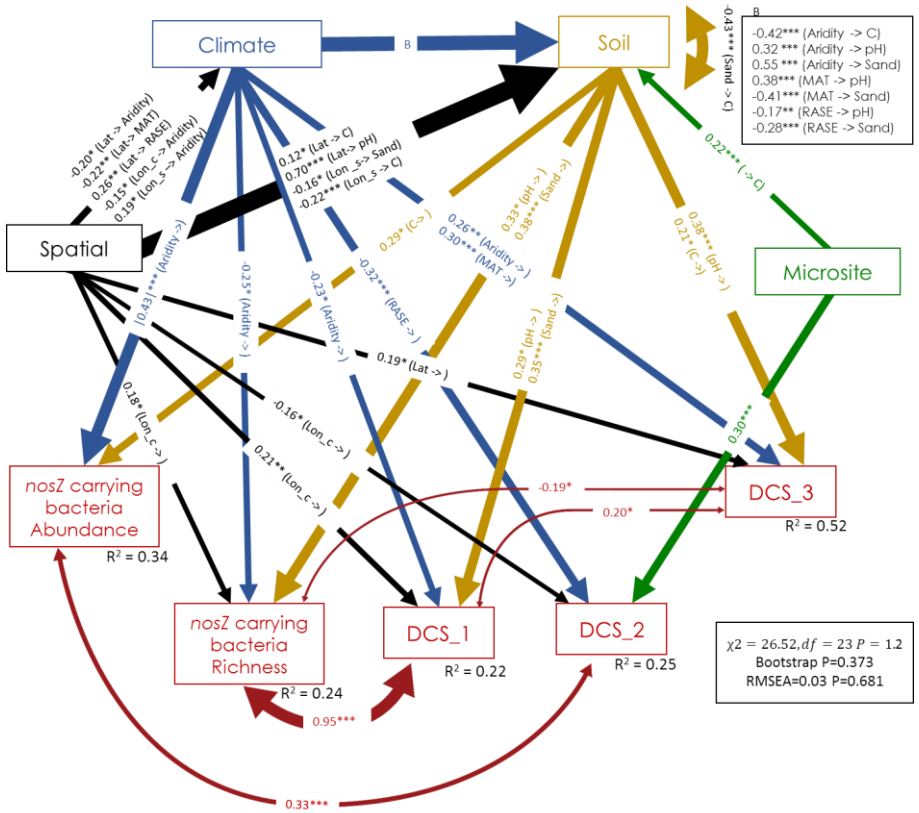

FIGURE 2 Structural equation model, describing the effects of spatial location, climate, soil properties and microsite on *nosZ* carrying bacteria abundance, richness and community structure. DCS = NMDS ordination axis for *nosZ* carrying bacteria community structure. The path coefficient for the direct effect of climate on *nosZ* carrying bacteria is expressed as an absolute value because the relationship is curvilinear, rendering the sign uninterpretable. Rest of caption as in Figure 1.

Differences across vegetation types (grasslands, shrublands and open forests) and aridity classes (arid and semi-arid), regardless of the microsite considered were compared with one-way ANOVA; the Tukey's post-hoc HSD test was used to assess significant differences among them. These analyses were performed in R (R Core Team, 2017). Data associated with this study are available in Figshare (Lafuente et al., 2019c).

Results

Dominant taxa of pmoA- and nosZ- carrying bacteria in drylands

Seven (79, 86, 35, 245, 27, 83 and 31) and six (32, 73, 107, 35, 47 and 39) dominant T-RFs accounted for >75% relative abundance of *pmoA* and *nosZ* genes, respectively, across global drylands (Fig. 3).

The first and second axes of the NMDS for methanotrophs (MCS_1) were positively (Spearman's ρ = 0.34) and negatively (ρ = -0.68) correlated with T-RF_31, respectively, which is related to USCα, a distant relative of *Methylocapsa* sp (a type-II methanotroph). The second methanotroph NMDS axis (MCS_2) was also positively correlated with T-RF_79 (Spearman's ρ = 0.41), which is related to type-II methanotrophs associated with the Methylocystaceae family (Nazaries, Pan, et al., 2013). The third methanotroph NMDS axis (MCS_3) was positively correlated with T-RF_79 and T-RF_ 245 (ρ = 0.46 and ρ = 0.49, respectively); the later belongs to type-I methanotrophs and is closely related to *Methylococcus capsulatus* (Nazaries *et al.*, 2011). Similarly, the first axis of the NMDS for *nosZ* carrying denitrifiers (DCS_1) was positively correlated to T-RF_88 and T-RF_132 (ρ = 0.66 and ρ = 0.54, respectively); these fragments were present in more than 70% of the samples analysed. The second denitrifier NMDS axis (DCS_2) was positively correlated with T-RF_471, T-RF_73, T-RF_107 and T-RF_331 (ρ = 0.66, ρ = 0.54, ρ = 0.53, and ρ = 0.51, respectively), fragments present in at least 86% of the samples analysed, and negatively correlated with T-RF_35 (ρ = -0.57), which was found in all the soil samples evaluated. The third denitrifier NMDS axis (DCS_3) was negatively correlated with T-RF_104 (ρ = -0.45). These affiliations are based on previous studies using the exact same protocol we used (Nazaries et al., 2011, Nazaries, Pan, et al., 2013). Unfortunately, to our knowledge there is no published work that provides information to relate these fragments to specific taxonomical groups for *nosZ* genes.

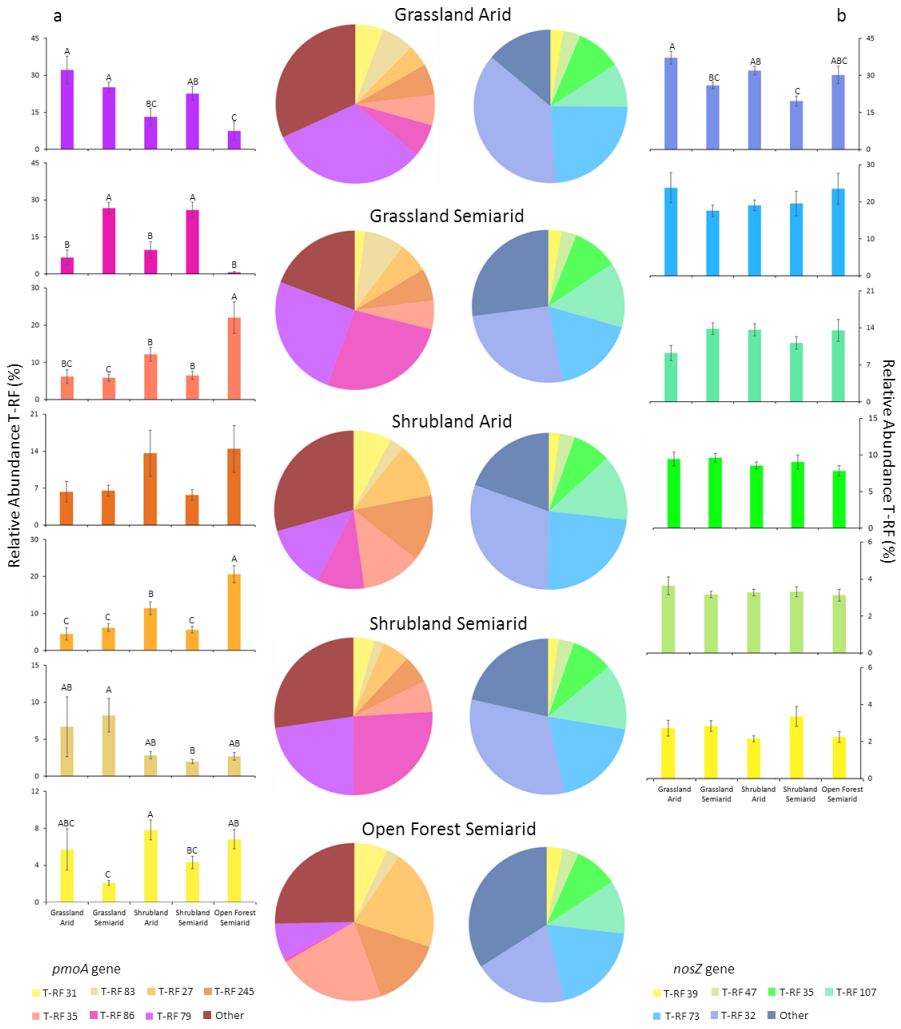

FIGURE 3 Relative abundance of major T-RFs of the *pmoA* (a) and *nosZ* (b) genes across major vegetation and aridity categories [n =12 (11 for *nosZ*), 56 (53 for *nosZ*), 26 (22 for *nosZ*), 52 and 14 for arid grasslands, semiarid grasslands, arid shrublands, semiarid shrublands and semiarid open forest sites, respectively]. Letters over the bars indicate significant differences across vegetation biomes and aridity classes (p-value <0.05, post-hoc test after ANOVA).

Effects of vegetation type and climate on dominant taxa on pmoA- and nosZ-carrying bacteria

On average, we found $5.1x10^7$ and $5.8x10^8$ copies of *pmoA* and *nosZ*, respectively. Changes in total abundance and richness of *pmoA*, and in richness of *nosZ*, genes were not detectable across either vegetation types or aridity classes (Fig. S4). However, we detected an increased *nosZ* gene abundance in semiarid grasslands and shrublands (Fig. S4). The dominant *pmoA* gene T-RFs were highly variable across vegetation types and aridity classes considered (Fig. 3a), in contrast with the *nosZ* gene, where the dominant T-RFs were T-RF_32 and T-RF_73, regardless of the vegetation types and aridity classes considered. The abundance of the dominant T-RFs across vegetation types and aridity classes was very consistent, and we only detected changes in the abundance of T-RF_32 (Fig. 3b). In semiarid open forests, T-RF_32 was less abundant than in arid grasslands and semiarid shrublands (Fig. 3b).

Direct and indirect effects of climate and soil properties on pmoA- and nosZ-carrying bacteria

Increases in aridity showed a quadratic relation with *nosZ* abundance (Fig. S3a). Increases in soil organic C and pH were linearly associated with increases in *nosZ* abundance (Fig. S3b). Increases in sand content were linearly associated with reductions in *nosZ* abundance (Fig. S3c).

Our structural equation modelling (SEM) provided a comprehensive view of the major ecological predictors of the richness, abundance and community structure of *pmoA*- and *nosZ*- carrying bacteria. It was able to explain only 6% of the variance of the abundance of methanotrophic bacteria, and 10% of their richness, but it was more successful explaining their community structure (18- 37% of NMDS axes; Fig. 1). Neither the abundance nor the richness of the *pmoA* gene were directly explained by any of the soil or climate variables evaluated, nor by the microsite (vegetation vs. bare soil) considered.

The only detectable, albeit weak, effect was a positive effect of latitude on *pmoA* gene richness, indicating a higher richness for methanotrophs in the Northern Hemisphere. Climate and soil were roughly equal predictors of the community structure of methanotrophs. Specifically, we found that the MCS_1 axis (potentially associated with genera *Methylocapsa*) was directly and positively associated with mean annual temperature, and directly and negatively related to the amount of soil organic C. In other words, taxa related to this axis (see Table S3) might prefer locations with higher temperature and low soil C. Aridity had a direct negative effect on the MCS_1 axis, and an indirect positive effect on this axis by reducing the content of soil organic C. The MCS_2 (potentially associated with taxa *Methylocystaceae*) axis was directly and positively associated with soil pH, and directly and negatively related to mean annual temperature and soil texture. Thus, taxa related to this axis (see Table S3) might prefer locations with lower temperature and high pH. Mean annual temperature also indirectly and positively influenced the MCS_2 axis via increases in soil pH. The MCS_3 axis (potentially associated with taxa *Methylocystaceae* and *Methyloccoccus capsulatus*) was directly and positively associated with aridity, mean annual temperature, rainfall seasonality and soil organic C, but was negatively associated with the proximity of vegetation. This means that taxa related to this axis might prefer both unvegetated surfaces with higher temperature and rainfall seasonality and soils with higher amounts of organic C. Aridity also indirectly and negatively influenced the MCS_3 axis via reductions in the amount of soil organic C. The standardised total effects highlighted soil variables as major drivers of MCS_1 (organic C and sand content, Fig. 4) and MCS_2 (soil pH and texture, Fig. 4); mean annual temperature, rainfall seasonality and soil organic C were major predictors of MCS_3 (Fig. 4).

Our SEM explained 34% of the variance of the abundance of *nosZ* carrying denitrifiers, 24% of their richness and 22-52% of their structure (NMDS axes, Fig. 2). Aridity and soil variables were equally informative determinants of

the abundance and richness of the *nosZ* gene. The abundance of this gene was positively associated with soil organic C, indicating that *nosZ* carrying denitrifiers might prefer soils with higher organic C content. Aridity directly and curvilinearly influenced the total abundance of *nosZ* (i.e. intermediate aridity values were associated with the greatest *nosZ* abundance). Further, aridity had an indirect negative effect associated with this variable via its negative effect on soil organic C (Fig. 5). The richness of *nosZ* was directly and positively influenced by soil properties (pH and soil texture) and directly and negatively associated with aridity. Put simply, the richness of *nosZ* was higher in coarser soils with higher pH levels. Aridity also had an indirect effect on the richness of *nosZ* gene through its direct positive effects on soil pH and texture and direct negative effect on organic C (Fig. 5).

The structure of *nosZ* carrying bacterial communities was influenced by both climatic and soil variables, being aridity, soil pH and sand content the most influential drivers of the NMDS axes. The DCS_1 axis was directly and negatively associated with aridity, and positively associated with soil pH and texture. In other words, taxa related with this axis might prefer coarser soils with higher pH levels. Aridity also had an indirect effect on DCS_1 through its positive effect on soil pH and texture (Fig. 5). The DCS_2 axis was directly and negatively associated with rainfall seasonality, and positively associated with vegetation, suggesting that taxa related to this axis might prefer vegetated sites with lower rainfall seasonality. Rainfall seasonality also had a positive indirect effect on DCS_2 through its direct negative effect on soil texture (Fig. 5). The DCS_3 axis was directly and positively associated with aridity, mean annual temperature, soil pH and organic C. In simple terms, taxa related to this axis might prefer warmer and more arid locations, as well as basic soils with higher organic C content. Also, mean annual temperature was indirectly and negatively associated with DCS_3 through its positive effect on soil pH (Fig. 5). The standardised total effects showed aridity and soil organic C as the major drivers

of *nosZ* abundance (Fig. 4). Soil variables (soil texture and pH) were the major drivers of *nosZ* richness (Fig. 5) and of DCS_1 (Fig. 4). Vegetation and rainfall seasonality were the major drivers of DCS_2 (Fig. 4), and mean annual temperature was the major driver of DCS_3 followed by soil pH, aridity and soil organic C (Fig. 5).

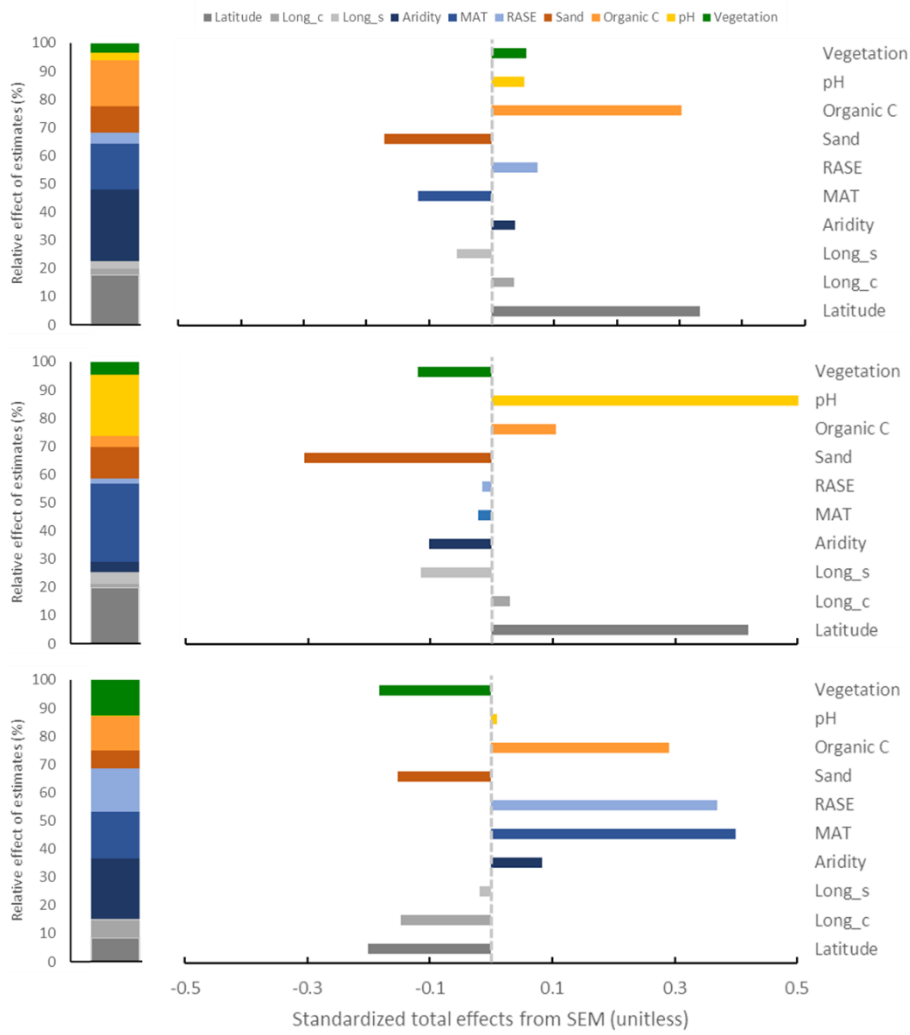

FIGURE 4 Standardised total (direct plus indirect effects) and absolute effects of the estimates derived from the structural equation modelling, including latitude [Lat], longitude cosine [Lon_c]), longitude sine [Lon_s], aridity, mean annual temperature [MAT], rainfall seasonality [RASE], sand content, organic carbon, pH and microsite (open/vegetated areas) on MCS_1 (a), MCS_2 (b) and MCS_3 (c) of methane-oxidising bacteria.

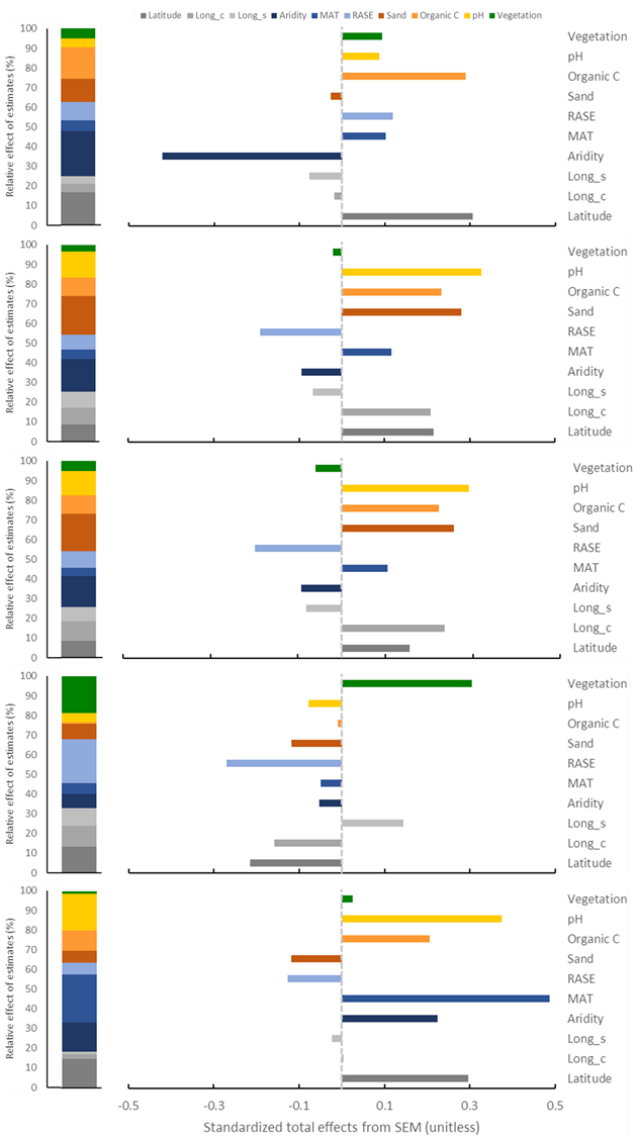

FIGURE 5 Standardised total effects (direct plus indirect effects) and absolute effects of the estimates derived from the structural equation modelling, including latitude [Lat], longitude cosine [Lon_c]), longitude sine [Lon_s], aridity, mean annual temperature [MAT], rainfall seasonality [RASE], sand content, organic carbon, pH and microsite (open/vegetated areas) on the abundance (a), richness (b), DCS_1 (c), DCS_2 (d) and DCS_3 (e) of *nosZ* carrying bacteria.

Discussion

Understanding the main ecological drivers of the abundance, richness, and community structure of soil methanotrophs and *nosZ* carrying denitrifiers is crucial to improve our understanding of how global change will affect the distribution of these functionally important microbial communities in drylands worldwide. Our study provides the first global assessment of bacterial taxa carrying *pmoA* and *nosZ* genes in drylands. Moreover, our findings provide novel insights of the ecological predictors of these organisms at a global scale, which can be used to model the abundance and richness of methanotrophs and *nosZ* carrying denitrifiers under global change scenarios. We observed that taxa often associated with more mesic environments (e.g. T-RFs 31 [*Methylocapsa* sp.] and 245 [*Methylococcus capsulatus*], Nazaries et al, 2011, 2018), are also common in drylands across the globe. We also found that climate (i.e. mean annual temperature, rainfall seasonality or aridity) and soil properties (i.e. organic C, pH or sand content) were important predictors of the community structure of both *pmoA* and *nosZ* genes. Our results suggest that many of the effects of climate on the community structure of methanotrophs and *nosZ* carrying denitrifiers are driven indirectly by changes in soil variables such as organic C, pH and texture. Furthermore, our modelling approach was more effective in explaining the richness, abundance and community structure of the *nosZ*- than the *pmoA*- carrying bacteria. Despite the known effects of climate on soil microorganisms, we could not detect any significant direct effect of climate on methanotrophic bacteria comparable to the strong effects observed for *nosZ* carrying denitrifiers, suggesting that methanotrophs have lower sensitivity to key climatic drivers than *nosZ* carrying denitrifiers (Martins *et al.*, 2017).

Direct and indirect effects on methanotrophic bacteria

Despite the commonly held assumption that climate is a critical determinant of the abundance of methanotrophs (Mohanty, Bodelier, & Conrad, 2007), we did

not find strong direct effects of either temperature or aridity on the abundance and richness of the *pmoA* gene (Fig. 1). These results suggest that, unlike in more mesic ecosystems (Le Mer & Roger, 2001), methanotrophic bacterial abundance and richness are not well predicted by averaged annual climatic parameters (aridity index and temperature) and that, in drylands, these organisms could be largely driven by more stochastic events (e.g. water pulses, Martin*s* et al., 2015) not properly captured by climatic interpolations. With regard to community structure, some of the most dominant *pmoA* T-RFs observed in our study (i.e. T-RFs 31 and 245) have also been described as dominant T-RFs in more mesic and humid systems (Nazaries et al., 2011, 2018; Nazaries, Pan, et al., 2013). These results indicate that methanothrophs can succeed in a wide range of environmental conditions. Especially, T-RF_245, potentially associated with *Methylococcus capsulatus*, which is widely described in mesic ecosystems (Bourne *et al.*, 2001; Nazaries *et al.*, 2011), was also one of the most abundant T-RFs observed in our study (Fig. 3). Such a finding highlights the global distribution of this methanotroph taxon and suggests that methanotrophic communities might be consistently abundant in drylands, confirming that the low and infrequent rainfall regimes characterizing these ecosystems support the maintenance of methanotrophic communities (Striegl, MCSonnaughey, Thorstenson, Weeks, & Woodward, 1992). Despite the consistent high abundance of T-RF_245 in drylands, this taxon was associated with more arid sites with higher temperatures (Fig. 1), which matches our current knowledge on T-RF_245 (a relative to *Methylococcus capsulatus*), a thermophile (growth > 45ºC) methanotroph from the Methylococcaceae family (Nazaries, Murrell et al., 2013).

In addition to some direct effects of climate on the methanotrophic community, we found that some effects of climate were indirect and driven by changes in soil properties. Different levels of soil organic C, pH and texture associated with changes in aridity and mean annual temperature were major

environmental predictors of the structure of methanotrophic communities. Soil properties and climate are expected to be among the major predictors of methanotrophs (Le Mer & Roger, 2001; Sullivan, Selmants, & Hart, 2013). The negative effect of soil organic C on the MCS_1 axis could be related to the existence of high affinity methanotrophs, which are capable of using atmospheric CH_4 as a carbon source (Bender & Conrad, 1992). We expect these taxa to be major constituents of the methanotrophic community in dryland soils, although further work is needed to support this assumption. However, in addition to CH_4, methane-oxidising bacteria are able to facultatively use organic C sources (Sullivan, Selmants, & Hart, 2013). Supporting previous research suggesting that soil pH is a strong driver of bacterial abundance and diversity (Fierer & Jackson, 2006; Maestre et al., 2015), we observed a significant association of pH with the MCS_2 axis describing the methanotrophic community structure, which was correlated to T-RF_31 and T-RF_79, potentially related to the family Methylocystaceae. This family encompasses several acidophilic species (growth at a pH of 3.8-5.5), which can at least partially explain our results. Finally, atmospheric CH_4 consumption occurs in the first centimetres of the soil profile, which makes it highly dependent on physical factors controlling gas diffusion (Koschorreck & Conrad, 1993). Indeed, our SEM pointed towards soil texture and climate as major drivers of the methanotrophic community structure, highlighting the importance of soil aeration for these microorganisms.

Direct and indirect effects on nosZ carrying bacteria

Unlike for methanotrophs, our SEM provided detailed information on the major drivers of the abundance and richness of *nosZ* carrying denitrifiers. For instance, we found a strong effect of aridity on the abundance of the *nosZ* gene (due to the second order polynomial relation between aridity and *nosZ* gene abundance, this path has an interpretable sign), and a negative effect of aridity

on its richness. Aridity also showed a strong influence on the community structure of *nosZ* carrying denitrifiers. In drylands, aridity is a major driver of nitrogen availability, as increasing aridity reduces organic matter, nitrogen availability and soil microbial activity associated with N cycling (Delgado-Baquerizo *et al.*, 2013a, 2016b). Our study shows an interesting relationship between aridity and *nosZ* carrying denitrifiers at the global scale, which might result in potential implications for the capacity of drylands to exchange N_2O with the atmosphere in a changing world. As aridity continues to increase worldwide due to climate change (Hu *et al.*, 2017), the changes in the abundance and decrease in the richness of *nosZ* gene with aridity may reduce the potential of drylands to support complete denitrification of N_2O to N_2. Interestingly, unlike a study that has observed a negative impact of increasing soil temperature on the abundance of *nosZ* genes in a boreal–temperate ecotone (Martins *et al.*, 2017), we could not detect such an effect in global drylands. We hypothesize that water availability restrictions imposed in drylands overcome the known effects of temperature on the abundance of *nosZ* gene found in more mesic areas.

Soil properties were also important drivers of the abundance, richness and community structure of *nosZ* carrying denitrifiers, and indirectly expressed the effects of climate on *nosZ* genes. Denitrification processes, which produce N_2O or N_2 as by-product, have been shown to be highly dependent on soil properties (Andersen & Petersen, 2009). Accordingly, in our global study, the abundance of the *nosZ* gene was strongly driven by soil organic C, a common surrogate of soil fertility (Delgado-Baquerizo et al., 2017; Fig. 5), suggesting this microbial functional group is nutrient limited in global drylands and respond positively to increases in organic C contents. Soil pH and climate-related variables (i.e. aridity), which are also known to alter denitrifier activity and determine the capacity of N_2O reductase to assemble (Liu, Mørkved, Frostegård, & Bakken, 2010), were also positively and negatively, respectively, related to the richness (but not to the abundance) of the *nosZ* gene. Below optimal pH (6.0),

the assemblage of the *nosZ* protein is impaired (Liu *et al.*, 2010), consequently, higher pH might enhance the richness of *nosZ* gene as shown in our SEM. Sand content and pH were also major drivers of the structure of the *nosZ* carrying denitrifying community (Fig. 2). Denitrification rate is reduced with decreasing pH (Burford & Bremner, 1975). However, not all denitrification steps are equally affected by soil pH, and thus N_2O production could increase under lower pH values (Burford & Bremner, 1975). On the other hand, denitrification products shift towards N_2 production as pH increases (Dalal & Allen, 2008). Our results are in agreement with Zeng et al., (2017), who also observed a strong positive effect of soil pH on the N_2O reducing bacterial community.

Concluding remarks

Here we report, for the first time, the direct and indirect effects of climate- and soil-related variables on the abundance, richness and community structure of two functional genes related to methanotrophy and denitrification in global drylands. First, we demonstrate that *pmoA* and *nosZ* associated microbial communities are widely distributed in global drylands, highlighting the importance of including methanotrophs and *nosZ* carrying denitrifiers in dryland biogeochemical models. Second, we provide strong observational evidence that both climate- (i.e. aridity, mean annual temperature and rainfall seasonality), and soil-related (i.e. soil organic C, pH and soil texture) variables are important predictors of the abundance and richness of *nosZ* gene and of the community structure of methanotrophs and *nosZ* carrying denitrifiers. Finally, we show that *nosZ carrying* denitrifiers might be more sensitive than *pmoA* carrying bacteria to the known drivers of microbial abundance and richness.

Climatic models forecast widespread increases in aridity by the end of the 21st century, which will increase the extension of drylands worldwide (Huang et al, 2016). Here we demonstrate that these changes could alter methanotrophic and *nosZ* carrying denitrifying communities. Increases in aridity

associated with climate change are expected to reduce vegetation cover (Delgado-Baquerizo *et al.*, 2013a) (Ulrich *et al.*, 2014), reducing organic C inputs into the soil and thus soil organic C contents (Delgado-Baquerizo *et al.*, 2013a). We found a negative effect of microsite on the methanotrophic bacterial community structure, suggesting that the expected reduction in vegetation cover due to climate change will directly and indirectly alter the structure of methane-oxidising bacterial communities, which might affect the net CH_4 exchange to the atmosphere. Our results also point towards changes in the *nosZ* carrying denitrifying community due to forecasted increases in aridity and reductions in soil organic C with climate change. These changes are likely to result in a reduced capacity of dryland soils to carry out the final step of the denitrification (reduction of N_2O to N_2), favouring net N_2O emissions to the atmosphere. Together, our results provide a better understanding of the environmental factors driving variation in the abundance, richness and structure of *nosZ* and *pmoA* genes in dryland soils, which is of paramount importance to forecast changes in the fluxes of greenhouse gases in the future.

Acknowledgements

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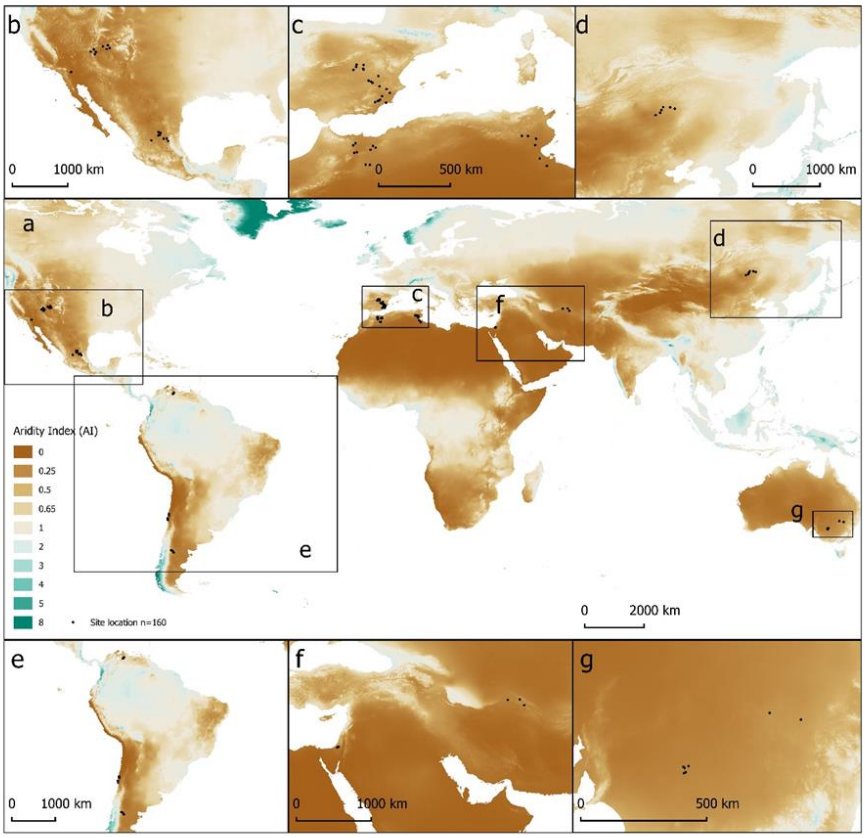
647038 [BIODESERT]) and by the Spanish Ministry of Economy and Competitiveness (BIOMOD project, ref. CGL2013-44661-R). Work on soil microbial diversity in BKS lab is funded by Australian Research Council.

Supporting information



MAP S1 Map of the 80 drylands surveyed in this study (a) and close-up maps of their location in North America (b), Mediterranean Basin (c), China (d), South America (e), Middle East (f), and Australia (g). The background indicates values of the aridity index (precipitation/potential evapotranspiration).

TABLE S1 Gene target, primer details and thermal cycling conditions used in the quantitative PCR analyses conducted.

Gene	Primer	Sequence (5' to 3')	Amplicon size (bp)	Reference	Thermal conditions
nosZ	nosZ2F	CGC RAC GGC AAS AAG GTS MSS GT	267	Henry et al., 2006	95 °C - 300 s 95 °C - 30 s 60 °C - 30 s 72 °C – 60 s 40 cycles
	nosZ2R	CAK RTG CAK SGC RTG GCA GAA			
pmoA	pmo189F	GGN GAC TGG GAC TTC TGG	500	Bourne et al., 2001	95 °C - 180 s 95 °C - 15 s 53 °C - 15 s 72 °C - 30 s 40 cycles
	pmo650R	ACG TCC TTA CCG AAG GT			

TABLE S2 Spearman correlations between NMDS axis and T-RFs for both genes *pmoA* (MCS) and *nosZ* (DCS). Red colour indicates significant relations.

T-RF	*MCS_1*	*MCS_2*	*MCS_3*	*T-RF*	*DCS_1*	*DCS_2*	*DCS_3*
27	**-0.4474**	**-0.6972**	0.0062	*27*	**-0.2299**	0.1002	0.0811
31	**-0.3371**	**-0.6785**	0.0863	*32*	-0.0125	**0.4491**	**-0.2990**
35	**-0.4798**	**-0.7316**	0.0726	*35*	-0.0043	**0.5689**	-0.0598
40	**-0.3619**	**-0.5426**	-0.0546	*39*	-0.1182	**0.4570**	**0.2022**
43	-0.1278	**-0.5008**	-0.0637	*43*	**-0.3528**	**0.2148**	-0.1555
47	-0.1537	**-0.5554**	0.1250	*47*	**-0.3012**	**0.4810**	**-0.1678**
50	**-0.3062**	0.1388	**-0.5921**	*50*	**-0.4740**	0.0927	**-0.1627**
55	**-0.2969**	**-0.5828**	-0.0427	*54*	-0.0886	-0.0204	**0.3438**
58	0.1001	**-0.3145**	-0.1104	*57*	**-0.4958**	**0.3211**	0.0624
63	0.1045	**0.1634**	**-0.2162**	*61*	**-0.3328**	**0.1979**	0.0014
67	**0.2562**	**-0.2690**	-0.1527	*65*	**-0.6142**	**0.3122**	**0.1839**
71	0.0174	**-0.3294**	0.1324	*69*	**-0.2080**	0.0617	**0.3745**
75	**0.1941**	-0.1494	**0.6057**	*73*	**0.3451**	**-0.5391**	**-0.2417**
79	**0.3381**	**0.4146**	**0.4552**	*76*	**-0.1645**	**0.1738**	-0.0484
83	**-0.3731**	0.0209	**-0.2484**	*79*	**-0.4852**	0.1054	-0.1548
86	**0.2428**	**0.7052**	**-0.4208**	*83*	**-0.5663**	**0.1666**	**0.2102**
90	0.0674	**-0.3883**	**0.2729**	*88*	**-0.6649**	0.1364	-0.0005
94	**0.2488**	**-0.2163**	-0.0390	*92*	**-0.5429**	0.0693	**0.2516**
97	**0.1617**	**-0.2790**	0.1142	*96*	**-0.4667**	0.0637	-0.0857
101	**0.2550**	**-0.2335**	-0.0569	*100*	**-0.4900**	**0.2170**	**-0.1752**
105	0.1488	**-0.1884**	-0.1038	*104*	-0.0666	-0.1141	**0.4493**
110	**0.2056**	0.1340	**-0.4334**	*107*	**0.2313**	**-0.5269**	**0.2278**
114	**0.3227**	**0.5836**	**-0.2920**	*111*	**-0.1917**	-0.0519	**0.2269**
117	**0.3362**	**-0.3841**	0.0536	*114*	**-0.4876**	-0.1546	**0.1663**
121	**0.2641**	**-0.4130**	**-0.3540**	*119*	**-0.4712**	**0.2867**	-0.0003

T-RF	*MCS_1*	*MCS_2*	*MCS_3*	*T-RF*	*DCS_1*	*DCS_2*	*DCS_3*
127	0.1528	**-0.5117**	-0.0085	*124*	**-0.4175**	0.1584	-0.1446
129	0.1057	**-0.2688**	0.1203	*128*	**-0.5868**	0.0962	-0.1159
134	**0.2554**	**-0.2357**	0.0019	*132*	**-0.5438**	**0.3427**	-0.1325
137	**0.2477**	**-0.2381**	-0.1485	*136*	**-0.6114**	-0.0910	**-0.1845**
141	**0.3113**	**-0.3094**	-0.0397	*140*	**-0.5273**	0.0092	**-0.4530**
145	**0.2813**	**-0.4163**	-0.0368	*145*	**-0.4694**	-0.0832	0.0785
148	**0.1828**	**-0.1873**	0.0923	*149*	**-0.4651**	0.0017	-0.0761
152	**0.2418**	-0.1245	0.0746	*153*	**-0.4978**	-0.0572	-0.1541
155	**0.4831**	-0.1343	0.1025	*156*	**-0.6057**	**0.3131**	**0.1990**
159	**0.2457**	**-0.3636**	-0.0312	*161*	**-0.6343**	**0.1887**	-0.0452
163	**0.3104**	**-0.2390**	-0.1270	*166*	**-0.3961**	0.0837	-0.0799
166	**0.2206**	-0.0931	**-0.2265**	*170*	**-0.4061**	**0.1606**	0.0082
170	**0.2144**	-0.1480	0.0248	*174*	**-0.5470**	0.0942	**-0.1959**
174	**0.2657**	**-0.2095**	-0.0272	*178*	**-0.4632**	**0.2149**	-0.1159
179	**0.2503**	**-0.2831**	0.1408	*182*	**-0.3773**	**-0.2124**	**-0.3789**
183	**0.2373**	**-0.2615**	0.1013	*186*	**-0.4442**	**0.1697**	-0.1504
187	**0.3591**	**-0.2975**	0.1113	*190*	**-0.4360**	-0.0760	**0.3217**
191	**0.3623**	**-0.2467**	-0.0693	*194*	**-0.4597**	**0.3042**	-0.0784
195	**0.2809**	**-0.2032**	-0.0106	*197*	**-0.5401**	-0.0775	**-0.2580**
199	**0.4642**	-0.1349	**-0.2445**	*203*	**-0.2745**	**-0.1916**	**0.3755**
205	**0.3605**	**-0.3699**	-0.0235	*207*	**-0.2229**	0.0097	0.0282
208	**0.1646**	**-0.2961**	**0.1959**	*211*	**-0.4006**	0.0621	-0.0514
212	**0.3607**	**-0.2570**	0.0095	*215*	**-0.2445**	-0.0178	**0.4675**
216	**0.3975**	-0.1303	-0.0224	*218*	**0.2411**	**-0.4749**	**0.3409**
219	**0.2951**	-0.1410	0.0866	*221*	-0.1249	-0.0090	-0.1472
224	**0.2911**	**-0.1706**	0.0155	*225*	**-0.3687**	0.0860	0.0784
228	**0.2813**	-0.1377	**0.2494**	*230*	**-0.4507**	-0.1041	0.0432

T-RF	*MCS_1*	*MCS_2*	*MCS_3*	*T-RF*	*DCS_1*	*DCS_2*	*DCS_3*
231	**0.3446**	0.0101	**0.2252**	*233*	**-0.2810**	-0.0930	-0.0381
235	**0.2877**	**-0.3087**	**0.1911**	*236*	**-0.3326**	-0.0180	**-0.1939**
238	**0.4423**	-0.1013	0.0895	*239*	**-0.2937**	**-0.2185**	**-0.1613**
245	**0.2582**	-0.0442	**0.4941**	*245*	**0.2306**	**-0.5044**	**0.3129**
247	0.1419	**-0.3545**	-0.0515	*250*	**-0.4440**	0.1559	-0.0998
253	0.1411	-0.0422	**0.2151**	*253*	**0.1847**	**-0.4392**	-0.1450
256	**0.1891**	0.0025	-0.0702	*256*	**-0.2386**	-0.0465	0.1208
260	**0.1633**	**-0.3652**	**-0.2832**	*262*	**-0.3787**	-0.1120	-0.0825
263	0.0219	**-0.2639**	**-0.2081**	*264*	**-0.1688**	-0.0832	0.0464
266	**0.1860**	-0.1268	0.0050	*270*	**-0.4374**	-0.0190	0.1489
270	**0.2308**	**-0.2035**	**-0.5126**	*274*	-0.1285	0.0088	0.1536
275	0.1091	**-0.2027**	0.0337	*278*	**-0.1686**	0.0318	**0.3191**
279	**0.3769**	-0.0643	-0.0176	*282*	**-0.3537**	0.0429	-0.0783
282	0.1094	0.0447	0.0982	*285*	**-0.2261**	-0.0518	-0.0313
286	0.1217	**0.2101**	**-0.1680**	*289*	**-0.4913**	-0.0193	0.1345
290	-0.0208	**-0.1553**	-0.0376	*292*	**-0.1796**	0.0282	**-0.1903**
292	0.0103	**-0.1763**	0.0568	*295*	**-0.2112**	-0.0644	**-0.2602**
296	0.0403	**-0.2041**	-0.0445	*299*	**-0.2979**	-0.1163	0.1278
299	0.0640	-0.0691	-0.0289	*305*	**-0.3472**	0.0625	**-0.2885**
302	0.0985	**-0.1590**	0.0309	*311*	**-0.4520**	0.1024	**-0.1928**
306	**0.2625**	-0.1221	0.1298	*315*	**-0.2144**	**-0.2027**	0.0961
309	0.1363	**-0.1901**	0.0802	*320*	**-0.3176**	-0.0784	-0.0617
314	**0.2210**	-0.1163	-0.1436	*322*	**-0.2742**	-0.0078	-0.1242
319	**0.2910**	**-0.3101**	0.0171	*328*	-0.1068	**-0.2121**	-0.0138
323	**0.2478**	**-0.1764**	0.1517	*331*	-0.0424	**-0.5130**	**-0.1847**
330	**0.3839**	0.0912	-0.0440	*338*	-0.1459	-0.1194	-0.0331
333	0.0830	**-0.2085**	**-0.1753**	*343*	**-0.1611**	-0.1019	0.0105

T-RF	*MCS_1*	*MCS_2*	*MCS_3*	*T-RF*	*DCS_1*	*DCS_2*	*DCS_3*
339	0.1428	**-0.2691**	**-0.2314**	*348*	**-0.3345**	0.0413	-0.1257
345	**0.3828**	-0.1348	0.0885	*351*	**-0.1646**	-0.0527	-0.1063
348	**0.1803**	-0.1393	-0.0140	*355*	**-0.2641**	0.1024	-0.0906
352	**0.3117**	-0.0998	-0.1156	*359*	**-0.2158**	-0.0358	0.0125
360	**0.2992**	**0.3507**	0.0238	*363*	-0.1327	-0.0138	-0.1350
363	**0.1704**	-0.0795	**-0.1564**	*365*	**-0.3069**	0.0863	-0.1012
367	**0.1769**	-0.0802	-0.1166	*368*	**-0.1652**	-0.1010	-0.0051
370	**0.1813**	-0.1259	0.1166	*374*	-0.0694	**-0.1772**	**-0.2947**
373	0.0998	-0.0712	-0.0554	*379*	**-0.3100**	**-0.1669**	0.0432
377	0.0830	**-0.2177**	-0.0253	*383*	-0.0342	-0.0238	-0.1167
380	0.0174	-0.1428	**-0.1857**	*385*	-0.0647	**0.1643**	-0.1257
387	**0.1856**	-0.0046	**0.1553**	*391*	-0.1444	**0.2147**	**0.3351**
391	0.0603	**-0.2041**	**-0.2160**	*397*	-0.0891	**0.2405**	**0.4175**
400	-0.0138	-0.0445	0.0408	*399*	-0.1155	**0.2052**	**0.3696**
406	0.0576	0.0011	**0.1808**	*411*	-0.1227	-0.1134	0.0457
417	0.0908	-0.1154	-0.0663	*415*	**-0.1697**	-0.0583	-0.0553
424	0.1300	0.0243	**0.1923**	*421*	-0.1408	-0.0096	-0.0038
429	**0.1849**	**-0.2488**	0.0199	*429*	0.0309	**-0.5183**	0.0011
432	0.1351	**-0.1835**	-0.0181	*435*	-0.0066	-0.0152	0.0333
435	0.0883	0.0402	-0.0517	*438*	**-0.2687**	0.0488	0.0062
444	0.1028	**-0.1618**	0.0279	*442*	-0.1429	-0.0918	0.0196
459	0.0862	**-0.1661**	**0.1984**	*446*	**-0.2217**	-0.1125	**0.1803**
474	0.0521	-0.0317	-0.0664	*449*	-0.1499	-0.0245	**0.1833**
480	**0.2758**	**-0.1978**	0.0781	*458*	-0.1084	0.0275	0.0915
486	-0.0174	-0.0408	0.1172	*463*	-0.1382	0.0381	0.0389
490	**0.2062**	0.0228	0.0550	*471*	**0.2169**	**-0.6593**	0.0418
502	**0.4907**	**0.3254**	**0.1831**	*480*	-0.0511	0.1007	0.1445

T-RF	*MCS_1*	*MCS_2*	*MCS_3*	*T-RF*	*DCS_1*	*DCS_2*	*DCS_3*
504	0.0449	**0.1888**	0.0747	*493*	-0.0124	-0.0254	0.0786
508	0.0824	**-0.3179**	**-0.2743**	*501*	**-0.1630**	**0.2468**	**0.3613**
558	0.1551	0.0914	**-0.2483**	*509*	0.0038	-0.1402	0.1297
569	-0.0675	**-0.2910**	**-0.2659**	*534*	-0.0292	0.0105	-0.0724
575	**0.3178**	0.0345	**-0.2276**	*542*	**-0.1718**	0.0936	**-0.2056**
				547	-0.1420	-0.0756	-0.1591
				569	-0.0124	-0.0254	0.0786

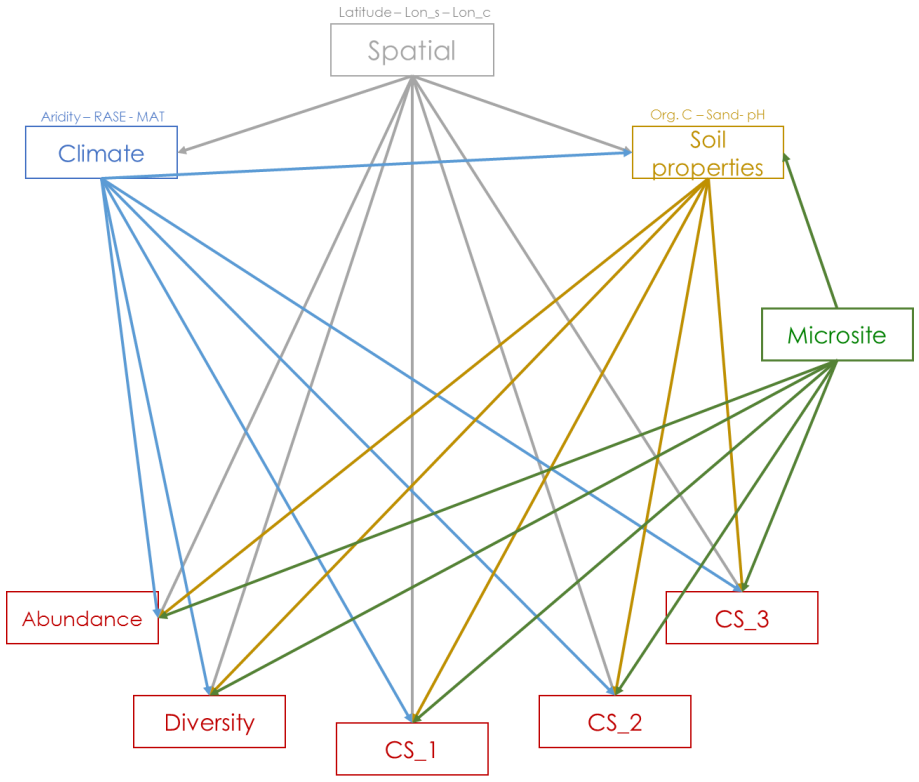


FIGURE S1 A priori structural equation model (SEM) used in this study. We included spatial coordinates (latitude, longitude sine and longitude cosine), climate (aridity, rainfall seasonality [RASE] and mean annual temperature [MAT]), soil properties (organic carbon, sand content and pH), and microsite (binary variable, 0 for open and 1 for vegetated sites) as potential drivers of the abundance, richness and community structure (NMDS axis) of *nosZ* carrying bacteria and methanotrophs.

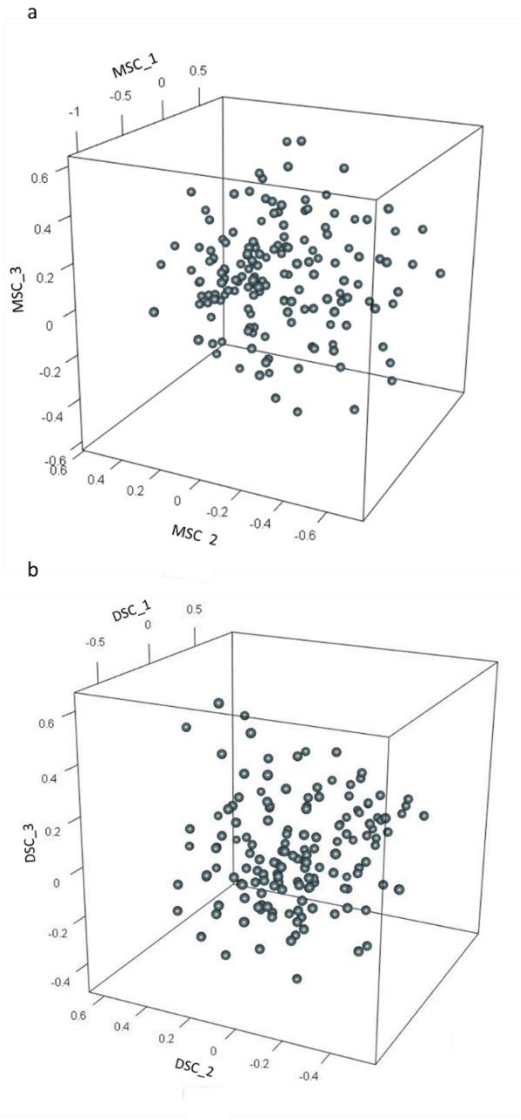


FIGURE S2 Ordination diagram of non-metric multidimensional scaling (NMDS) for methanotrophs (a) and denitrifiers carrying *nosZ* gene (b). Each data point represents one soil sample. We introduced the three axis MSC_1-3 and DSC_1-3 in the structural equation models to summarise the community structure of methanotrophs and denitrifiers carrying *nosZ* gene, respectively.

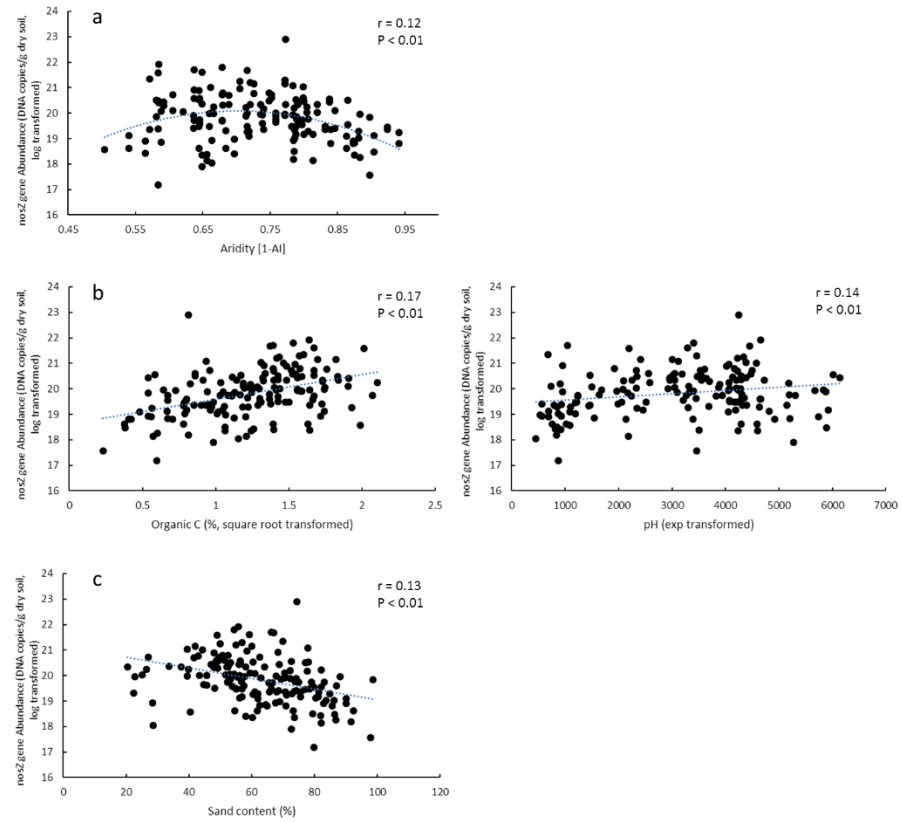


FIGURE S3 Bivariate relationships between some of the studied variables.

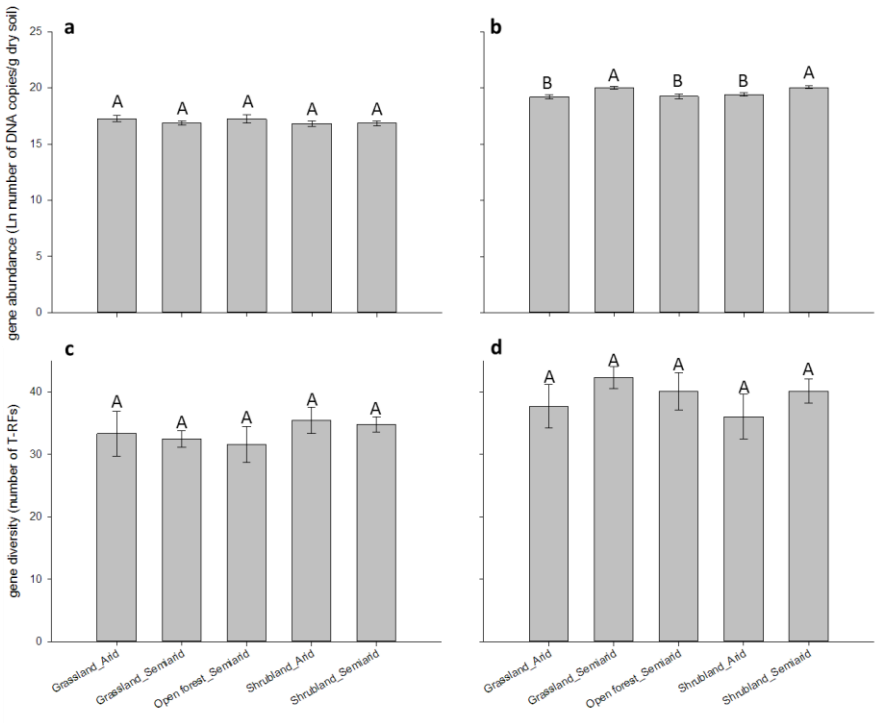


FIGURE S4 Total abundance (A and B) and richness (C and D) of *pmoA* and *nosZ* genes respectively, across the major vegetation types and aridity categories [n =12, 56, 26, 52 and 14 for *pmoA* gene and n =12 (11 for *nosZ* richness), 53 (55 for *nosZ* richness), 22, 51 (52 for *nosZ* richness) and 13 (14 for *nosZ* richness) for *nosZ* genes in arid grasslands, semiarid grasslands, arid shrublands, semiarid shrublands and semiarid open forest sites, respectively]. Letters over the bars indicate significant differences across vegetation biomes and aridity classes ($p<0.05$). Data are means ± SEM.

Discusión general

Esta tesis aborda cómo distintos aspectos del cambio ambiental global (cambio climático y deposición de nitrógeno) afectan a aspectos clave relacionados con la hidrología y el intercambio de gases de efecto invernadero entre el suelo y la atmósfera en ecosistemas áridos a distintas escalas espaciales (local y global). A escala local, los resultados indican que cambios en la temperatura y precipitación debidos al cambio climático, así como un aumento en la deposición de N, podrían dar lugar a un aumento en las emisiones de N_2O de los suelos de regiones áridas. Los cambios en temperatura y precipitación, debidos al cambio climático, redujeron la capacidad de fijar CH_4, de los suelos de las regiones áridas a escala local. Sin embargo, la simulación a escala local de niveles intermedios de deposición de N (i.e. 10 kg N $ha^{-1}yr^{-1}$), redujo la fijación de CH_4 y las emisiones de CO_2, de los suelos de las regiones áridas mediterráneas. Por el contrario, la simulación de niveles altos de deposición de N (i.e. 20-50 kg N $ha^{-1}yr^{-1}$) revirtió el efecto en los flujos de CH_4 y CO_2. Nuestros resultados apuntan la importancia de la costra biológica para entender las respuestas de los ecosistemas áridos al cambio climático. Así, estas comunidades aumentaron las ganancias de agua del suelo tras los pulsos de lluvia, redujeron notablemente las emisiones de N_2O bajo los tratamientos de exclusión de lluvia y la combinación de calentamiento con exclusión de lluvia, aumentaron la fijación de CH_4 bajo los tratamientos de calentamiento y la combinación de calentamiento con exclusión de lluvia, y aumentaron la abundancia de genes relacionados con la oxidación del metano y la desnitrificación. A escala global, variables climáticas como la temperatura media anual, la estacionalidad de las lluvias o la aridez, y variables edáficas como el C orgánico, el pH o el contenido en arenas del suelo fueron importantes predictores de la estructura de las comunidades de microorganismos desnitrificantes portadores del gen *nosZ* y de microorganismos metanotrofos.

La costra biológica modula los impactos del cambio climático en la hidrología del suelo y los flujos de gases de efecto invernadero

Debido al efecto del contenido en agua del suelo en la difusión de gases y la actividad de los microorganismos, conocer la dinámica de humectación y desecación del suelo es clave para comprender los flujos de gases de efecto invernadero en zonas áridas (Firestone & Davidson, 1989; Singh *et al.*, 1997; Le Mer & Roger, 2001; Chapuis-Lardy *et al.*, 2007; Horvath *et al.*, 2008; Grote *et al.*, 2010; Thomey *et al.*, 2011; Delon *et al.*, 2015).

Tras ocho años de simulación de cambio climático (aumento de la temperatura media anual de 2.5 °C y reducción del 33% de la precipitación total), se vio que tanto la humectación como la desecación del suelo, mostraron respuestas variables en el tiempo a los pulsos de lluvia. Los resultados del **capítulo 1** indican que las ganancias de agua en el suelo dependen tanto de la cantidad como de la intensidad del pulso de lluvia, existiendo una interacción entre ambos atributos, de manera que si la intensidad del pulso es muy alta las ganancias de agua son menores de lo esperado. Además, cuanto mayor sea la humedad inicial del suelo, menores serán las ganancias de agua después de los eventos de lluvia.

Las costras biológicas son un componente clave en las zonas áridas, pudiendo llegar a cubrir el 70% de la superficie del suelo (Belnap *et al.*, 2001) y además juegan un importante papel regulando su hidrología (Chamizo *et al.*, 2016a). Chamizo et al. (2012), así como Kidron & Yair (1997), observaron que en los suelos dominados por costra biológica, la escorrentía era menor que en suelos desnudos. Además, (Faist *et al.*, 2017) observaron que la capacidad para reducir la escorrentía era mayor en costras con un mayor grado de desarrollo. En el **capítulo 1** de esta tesis se observó un efecto positivo significativo de las comunidades de costra biológica con un alto grado de desarrollo sobre la capacidad de los suelos de absorber agua tras los pulsos de lluvia, tal como se ha comprobado en estudios anteriores realizados en zonas semiáridas (Cantón

et al., 2004; Chamizo *et al.*, 2012b, 2013, 2016a,b). Por el contrario, las costras biológicas también favorecieron la desecación del suelo una vez finalizados los pulsos de lluvia, lo que sugiere que la evaporación es mayor. Este resultado se debe posiblemente a la presencia de cianobacterias y líquenes como *Toninia sedifolia*, abundantes en la zona de estudio (Cano-Díaz *et al.*, 2018), y que favorecerían el oscurecimiento de la superficie y, por lo tanto, las pérdidas de agua por evaporación (Rutherford *et al.*, 2017).

Los resultados obtenidos en el **capítulo 1** demuestran que las dinámicas de humectación y secado del suelo pueden verse alteradas por los efectos del cambio climático, particularmente por el aumento de temperatura. En primer lugar, los tratamientos de cambio climático, y en particular el calentamiento, afectaron de manera significativa a las comunidades de costra biológica, que han ido reduciendo su grado de desarrollo desde el montaje del experimento en 2008 hasta el momento de los muestreos de esta tesis (Escolar *et al.*, 2012; Ladrón de Guevara *et al.*, 2014; Maestre *et al.*, 2015b). Además, y de manera similar a lo observado en otros estudios (Liancourt *et al.*, 2012; León-Sánchez *et al.*, 2016), bajo el tratamiento de calentamiento (~2.5 °C) las parcelas ganaron menos agua y se secaron más comparación con otros tratamientos.

Pese a la reducción de cobertura de costra inducida por los tratamientos de cambio climático, los resultados del **capítulo 2** ponen de manifiesto un fuerte efecto del legado de las comunidades de costra biológica. Éstas son capaces de modular, años después de sufrir los efectos del cambio climático y reducir su grado de desarrollo, los efectos del cambio climático en los flujos de gases de efecto invernadero (GEI). Por ejemplo, la exclusión de lluvia y la combinación de calentamiento y exclusión de lluvia provocaron una drástica reducción en las emisiones de N_2O, y un aumento de la fijación de CH_4 atmosférico, pero únicamente en las parcelas que tenían niveles altos de desarrollo de costra biológica al inicio del experimento.

La temperatura y la humedad del suelo figuran entre los principales

factores reguladores de los flujos de GEI (Dijkstra & Morgan, 2012; Zhou *et al.*, 2016). Un aumento de la temperatura del suelo puede aumentar el metabolismo de los microorganismos, aumentando las flujos de N_2O y CH_4 a la atmósfera (Dalal *et al.*, 2003; Dalal & Allen, 2008). Sin embargo, en las zonas áridas donde el agua ya es un factor limitante, reducciones en la humedad del suelo (como consecuencia del cambio climático o de la cobertura de costra biológica), pueden reducir el metabolismo microbiano y los flujos de N_2O y CH_4 a la atmósfera (Austin *et al.*, 2004; Chapuis-Lardy *et al.*, 2007). En la línea de lo que apuntan estos estudios, en el **capítulo 2** de esta tesis observamos una drástica reducción de las emisiones de N_2O a la atmósfera (Nielsen & Ball, 2015) y un aumento en la captación de CH_4 atmosférico (Galbally *et al.*, 2008; Dijkstra *et al.*, 2013; Sullivan *et al.*, 2013) bajo los tratamientos de exclusión de lluvia y su combinación con el calentamiento. Estos efectos podrían deberse a una reducción de la humedad del suelo causada directamente por nuestros tratamientos de cambio climático, así como por el aumento de la desecación promovido por la costra biológica observado en el **capítulo 1**.

La disponibilidad de sustrato es otro factor clave en la regulación de los flujos de GEI (Merbold *et al.*, 2014; McDaniel *et al.*, 2019). En las mismas parcelas de estudio, Delgado-Baquerizo et al. (2014) vieron una acumulación de N inorgánico en los tratamientos de exclusión de lluvia y calentamiento sólo en zonas con poco desarrollo de la costra biológica. La alta disponibilidad de formas inorgánicas de N y condiciones aeróbicas favorecen la nitrificación frente a la desnitrificación contribuyendo al aumento de los flujos de N_2O (Weier *et al.*, 1993; Dalal *et al.*, 2003; Delgado-Baquerizo *et al.*, 2016b). Mientras que en zonas áridas, comunidades de costra biológica bien desarrolladas se relacionan con una menor acumulación de N inorgánico (Castillo-Monroy *et al.*, 2010; Delgado-Baquerizo *et al.*, 2010, 2014) lo que reduciría los flujos N_2O a la atmósfera. Por otro lado, la acumulación de NH_4^+ en el suelo (poca costra biológica) lleva a la inhibición de la oxidación del CH_4 atmosférico lo que podría explicar el efecto

modulador de la costra biológica frente al cambio climático observado en esta tesis doctoral (King & Schnell, 1994).

Los resultados mostrados en el **capítulo 3** muestran que al aumentar la deposición de N lo hacen también las emisiones de N_2O. Este aumento podría deberse a una mayor disponibilidad de N en el suelo (i.e. NO_3^--N y NH_4^+-N; Homyak et al., 2014; Shcherbak et al., 2014; Snyder et al., 2009), que favorece los procesos de nitrificación y/o desnitrificación (Dalal & Allen, 2008). Sin embargo, la respuesta de los flujos de CH_4 y CO_2 suelo-atmósfera a niveles crecientes de deposición de N no fue lineal. Los flujos de CH_4 y CO_2 con 10 kg N ha^{-1} yr^{-1} disminuyeron, pero a partir de 20 kg N ha^{-1} yr^{-1} volvieron a aumentar. Estos resultados, podrían indicar la existencia de un umbral de saturación de N entre 10 y 20 kg N ha^{-1} $año^{-1}$ (Nilsson & Grennfelt, 1988; Fenn *et al.*, 2008, 2010; Bobbink *et al.*, 2010). La alta disponibilidad de N podría estar relacionada con cambios en las comunidades de microorganismos, lo que a su vez afectaría a los flujos de GEI dando lugar al umbral observado en esta tesis doctoral (Hanson & Hanson, 1996).

Efecto del cambio climático y variables abióticas en genes relacionados con la oxidación del metano y la desnitrificación a distintas escalas espaciales

Las comunidades de costra biológica tienen un efecto muy importante sobre la fertilidad del suelo (Weber *et al.*, 2016; Ferrenberg *et al.*, 2017), lo que podría favorecer un aumento de la abundancia microbiana (Maestre *et al.*, 2011; Barger *et al.*, 2016). A escala local la presencia de costra biológica con un alto grado de desarrollo inicial dejó un importante legado aumentando la abundancia de genes relacionados con la reducción del N_2O y la oxidación del CH_4, lo que a su vez podría estar relacionado, al menos parcialmente, con la reducción de los flujos de N_2O y el aumento en la fijación de CH_4 que se observó en el **capítulo 2** bajo los tratamientos de calentamiento y exclusión de lluvia. Sin

embargo, y contrariamente a lo observado a otro estudio anterior en un bosque australiano (Martins *et al.*, 2016), no se encontró una relación significativa entre la abundancia general de genes y la funcionalidad. En el **capítulo 2** especulamos que esta falta de relación entre la abundancia del gen *pmoA* y el flujo de CH_4 podría deberse a la competencia entre el NH_4^+ del suelo y el CH_4 por la enzima metano monooxigenasa (King & Schnell, 1994).

El estudio a escala global cuyos resultados conforman el **capítulo 4** indica que la aridez es un factor clave en las comunidades de desnitrificantes portadores del gen *nosZ*. Específicamente, aumentos en la aridez están claramente relacionados con una reducción de la riqueza y abundancia de estos microorganismos y con cambios en su composición. En zonas áridas la disponibilidad de carbono y nitrógeno está fuertemente controlada por el grado de aridez, ya que al aumentar ésta disminuyen tanto la materia orgánica como la actividad microbiana asociada con la disponibilidad del nitrógeno (Hooper & Johnson, 1999; Robertson & Groffman, 2007; Delgado-Baquerizo *et al.*, 2013a, 2016b; Wang *et al.*, 2014). Los resultados mostrados en el **capítulo 4** muestran como a mayor carbono orgánico en el suelo mayor abundancia del gen *nosZ*. Estos resultados sugieren que el C orgánico es un factor determinante de la abundancia de los microorganismos desnitrificantes portadores de este gen. Los microorganismos metanotrofos son abundantes en las zonas áridas de todo el mundo (Striegl *et al.*, 1992). Sin embargo, y contrariamente a la creencia generalizada de que el clima es un factor crítico determinante de su abundancia (Mohanty *et al.*, 2007), los modelos de ecuaciones estructurales usados en el **capítulo 4** muestran como en ecosistemas áridos la abundancia y riqueza de metanotrofos podría estar determinada por factores más estocásticos (como los pulsos de lluvia; Martins et al., 2015) que no están adecuadamente reflejados en parámetros como la aridez o la temperatura media anual. En cambio, la composición de las comunidades de metanotrofos sí se vio directamente afectada por variables climáticas (aridez, temperatura media anual y

estacionalidad de lluvias) tanto de forma directa como indirectamente a través de su efecto sobre el C orgánico (Sullivan *et al.*, 2013), el pH (Fierer & Jackson, 2006) o el contenido en arenas del suelo. Este último resultado destaca la importancia de la aireación del suelo y difusión de gases a la hora de estructurar las comunidades de microorganismos metanotrofos (Le Mer & Roger, 2001).

General conclusions

1. Simulated climate change, and warming in particular, affects the soil hydrology of biocrust-dominated ecosystems both directly, by increasing evaporation and indirectly, by reducing the cover of lichen-dominated biocrusts and by altering biocrusts ability to control water gains and losses after rainfall events.
2. Experimental increase in soil temperature and reduction of soil moisture consequence of our climate change treatments increased the rate of N_2O emissions and reduced the CH_4 sink capacity of soils in a semiarid ecosystem from central Spain. These responses, however, were modulated by the degree of development of biocrusts.
3. The abundance of *nosZ* and *pmoA* carrying bacteria communities showed marked seasonal patterns in our climate change experiment. However, biocrusts consistently increased the abundance of both functional genes.
4. Projected increases in N deposition in semi-arid ecosystems could lead to increases in the emission of N_2O. In addition, intermediate levels of N deposition would decrease the capacity of these soils to oxidise atmospheric CH_4 and emit CO_2 to the atmosphere. These changes have the potential to influence the atmospheric concentrations of these greenhouse gasses and therefore the ongoing climate change.
5. Methane oxidising bacteria and *nosZ* carrying denitrifiers are widely distributed in global drylands. Both climate- (i.e. aridity, mean annual temperature and rainfall seasonality) and soil-related (i.e. soil organic C, pH and soil texture) variables were important predictors of the abundance and richness of *nosZ* gene and of the composition of methane oxidising bacteria and *nosZ* carrying denitrifiers.

6. Increasing aridity and the reduction in soil organic C associated with ongoing climate change could alter methane oxidising bacteria and *nosZ* carrying denitrifiers. These changes are likely to affect net CH_4 exchange to the atmosphere and result in a reduced capacity of dryland soils to carry out the final step of the denitrification (reduction of N_2O to N_2), favouring net N_2O emissions to the atmosphere.

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