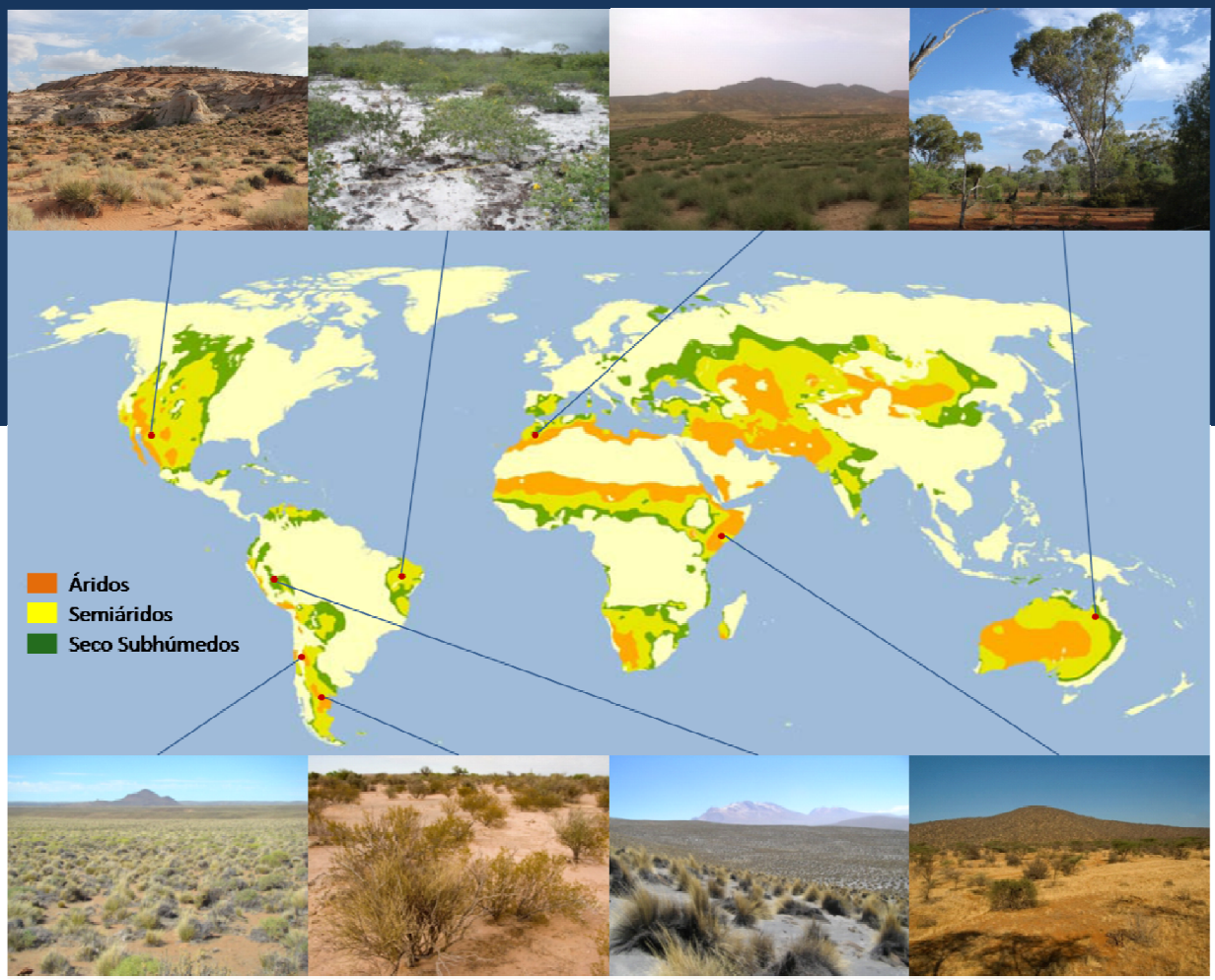
EFECTOS DEL CAMBIO CLIMÁTICO SOBRE LA DINÁMICA DEL NITRÓGENO EN ZONAS ÁRIDAS A DISTINTAS ESCALAS ESPACIALES



TESIS DOCTORAL
MANUEL DELGADO BAQUERIZO

UNIVERSIDAD PABLO DE OLAVIDE
FACULTAD DE CIENCIAS EXPERIMENTALES
DEPARTAMENTO DE SISTEMAS FÍSICOS,
QUÍMICOS Y NATURALES
ÁREA DE ECOLOGÍA

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Manuel Delgado Baquerizo

Sevilla, 2013

EFECTOS DEL CAMBIO CLIMÁTICO SOBRE LA DINÁMICA DEL NITRÓGENO EN ZONAS ÁRIDAS A DISTINTAS ESCALAS ESPACIALES

Memoria que el Licenciado Manuel Delgado Baquerizo presenta para aspirar al Grado de Doctor por la Universidad Pablo de Olavide

Esta memoria ha sido realizada bajo la dirección de:
Dr. Antonio Gallardo Correa y Dr. Fernando Tomás Maestre Gil.

Ldo. Manuel Delgado Baquerizo
Aspirante al Grado de Doctor

Sevilla, mayo de 2013

Dr. Antonio Gallardo Correa, Catedrático de Ecología de la Universidad Pablo de Olavide y **Dr. Fernando Tomás Maestre Gíl**, Profesor Titular de Ecología de la Universidad Rey Juan Carlos

CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Efectos del cambio climático sobre la dinámica del nitrógeno en ecosistemas áridos a distintas escalas espaciales.", **son aptos para ser presentados por el Ldo. Manuel Delgado Baquerizo ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad Pablo de Olavide.**

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extendemos el presente certificado a 4 de abril de 2013

Dr. Antonio Gallardo Correa **Dr. Fernando Tomás Maestre Gíl**

Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca Predoctoral del Plan Propio de Investigación de la Universidad Pablo de Olavide

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Los trabajos de investigación presentados en esta Tesis Doctoral se realizaron en el Departamento de Sistemas Físicos, Químicos y Naturales d la Universidad Pablo de Olavide y el Departamento de Biología y Geología de la Universidad Rey Juan Carlos.

A mis padres

ÍNDICE

1. RESUMEN

A lo largo de este doctorado se llevaron a cabo una serie de experimentos de laboratorio y de campo para evaluar el impacto de distintos agentes de cambio ambiental global (en lo sucesivo cambio global) sobre el ciclo del nitrógeno en zonas áridas a distintas escalas espaciales (local, regional y global). En primer lugar llevamos a cabo un estudio observacional en 224 zonas áridas a nivel global, situadas en todos los continentes menos la Antártida, para evaluar los impactos del incremento de la aridez derivado del cambio climático sobre los ciclos biogequímicos del nitrógeno (N), carbono (C) y fósforo (P). Los resultados obtenidos indicaron que este aumento de la aridez conllevará una disminución del control biótico (p. ej. menor cobertura vegetal) y un incremento del abiótico (p. ej. mayor dominio de la meteorización mecánina) sobre los ciclos biogeoquímicos en las zonas áridas. De este modo, los nutrientes asociados a procesos biológicos como el C y N (p. ej. fotosíntesis, descomposición de materia orgánica y fijación de N atmosférico) disminuirán con el incremento de aridez, mientras que nutrientes como el P, asociados con procesos geoquímicos (p. ej. meteorización de la roca), se verán favorecidos, generando desacoples entre los ciclos biogeoquímicos del C, N y P. Debido a la fuerte dependencia estequiométrica que los seres vivos tienen sobre los ciclos biogeoquímicos del C, N y P, su desacople podría acarrear un impacto negativo sobre la producción primaria, la respiración o la descomposición de la materia orgánica a nivel global.

En segundo lugar, evaluamos el papel de la vegetación como elemento modulador de los efectos del incremento de aridez que se espera en zonas áridas en respuesta al cambio climático sobre el N total disponible y la abundancia en el suelo de genes de bacterias (AOB) y arqueas (AOA) nitrificantes a lo largo de un gradiente regional mediterráneo (desde España a Túnez). Conforme aumentó la aridez en este gradiente, disminuyeron la disponibilidad total de N y el ratio AOB: AOA. Los micrositios con vegetación favorecieron un incremento de AOB, mientras que suelos desnudos favorecieron la abundancia de AOA, más resistentes al estrés ambiental. Los resultados obtenidos indican que la vegetación podría reducir los impactos del incremento de aridez derivado del cambio climático sobre el N disponible del suelo y los microorganismos implicados en la nitrificación, debido a la acumulación de matera orgánica que ésta promueve, y a los nichos que proporciona a diferentes grupos de bacterias y arqueas nitrificantes.

Por último, evaluamos el papel de la costra biológica del suelo (CBS), comunidades dominadas por líquenes, musgos y cianobacterias, en la resistencia y resiliencia de variables del ciclo del N a cambios en temperatura, contenido de agua en suelo y en la disponibilidad de C, N y P a escala local mediante incubaciones en el laboratorio. En general, los suelos bajo CBS mostraron una mayor resistencia a los cambios en temperatura y una mayor resiliencia a las adiciones de C y N. Sin embargo, los cambios en humedad edáfica no afectaron a las variables del ciclo del N,

sugiriendo que procesos tales como la mineralización en zonas áridas pueden ser llevados a cabo en un rango amplio de humedad. Posteriormente, llevamos a cabo un experimento en cámara de cultivo para evaluar el papel modulador de la CBS sobre el ciclo del N en respuesta a pequeños pulsos de agua (1% de la capacidad de campo), similares a los producidos por los eventos de rocío. La CBS favoreció una acumulación de N total disponible en suelo en respuesta a estos pequeños pulsos de agua, siendo el mecanismo descrito en este trabajo uno de los posibles responsables del incremento de los contenidos de N típicamente observado bajo la CBS en zonas áridas.

En su conjunto, la investigación realizada en el marco de esta tesis doctoral, ha profundizado nuestro conocimiento sobre los papeles que juegan la CBS y la vegetación como moduladores de los impactos del cambio global sobre el ciclo del N en zonas áridas. Del mismo modo, concluimos que un incremento de aridez a nivel mundial podría llevar a un desacople de los ciclos del C, N y P en suelo en los ecosistemas más áridos, lo que posiblemente afectará a los procesos y servicios ecosistémicos que prestan estos ambientes. Asimismo, el trabajo realizado en esta tesis pone de manifiesto que el estudio de los impactos del cambio global requiere del entendimiento de atributos y procesos ecosistémicos ligados a distintas escalas espaciales, que van desde patrones generales ligados a escala global a los mecanismos y factores concretos que actúan a escalas regionales y locales.

2. INTRODUCCIÓN GENERAL

2.1 Zonas áridas: definición e importancia

Las zonas áridas[1] ocupan el 41% de la superficie terrestre, y se extienden por todos los continentes excepto la Antártida (Figura 2.1). La aridez es posiblemente el fenómeno climático y físico que mejor define a las zonas áridas (FAO 1989; Whitford 2002; Johnson 2006; Ward 2009). Este fenómeno conlleva un permanente déficit hídrico, debido a la alta evapotranspiración, que excede a la precipitación en estas zonas, que se caracterizan también por sus altas temperaturas, su elevada insolación, presentar una gran variabilidad en la precipitación anual y sufrir largas sequías (FAO 1989). El agua es así el principal limitante de la producción primaria y descomposición de la materia orgánica en las zonas áridas (Schlesinger 1996; Whitford 2002; Johnson 2006). Distintos criterios han sido usados a lo largo del tiempo para definir la aridez y las distintas zonas áridas del planeta (véase Ward 2009 para una revisión), siendo la clasificación propuesta por el programa medioambiental de Naciones Unidas (UNEP, por sus siglas en ingles: United Nations Environmental Programme), basada en el índice de aridez (IA), actualmente la más aceptada (Gao y Giorgi 2008; Ward 2009; Maestre et al. 2012a). Este índice viene dado por el cociente entre la precipitación y la evapotranspiración potencial anual media de una determinada zona y divide a las zonas áridas en cuatro grandes grupos, todos ellos caracterizados por un índice de aridez menor de 0.65 (UNEP 1992): hiper-áridas (IA<0.05), áridas (0.05<IA<0.20), semiáridas (0.20<IA<0.50) y seco-subhúmedas (0.50<IA<0.65). Además de por su déficit hídrico, las zonas áridas se caracterizan por presentar suelos poco desarrollados y pobres en nutrientes, y por una vegetación compuesta de arbustos y herbáceas perennes que se distribuyen heterogéneamente en el espacio, formando una matriz de parches de vegetación y zonas no vegetadas (e.g., Reynolds et al. 1999; Valentin et al. 1999; Maestre & Cortina 2002; Rietkerk & van de Kopel 2008).

Sus características abióticas y bióticas han generado la idea equivocada de que las zonas áridas son zonas improductivas, desprovistas de interés desde el punto de vista ecológico (Maestre et al. 2012a). Sin embargo, estas regiones albergan alrededor del 20% de las zonas con mayor diversidad de plantas, el 30% de zonas endémicas de aves (MEA 2005; White & Nackoney 2003) y juegan además un papel fundamental en la regulación global de los ciclos biogeoquímicos del carbono (C) y el nitrógeno (N; Maestre et al. 2012b). Por ejemplo, las zonas áridas son responsables de alrededor del 30% de las emisiones naturales de N a la atmosfera (N_2, N_20, NO_x, NH_3) y albergan el 27% del C orgánico global en sus suelos (Bowden 1986; MEA 2005).

[1]En la presente tesis se utiliza este término como traducción del inglés "drylands", que engloba a los lugares con valores del índice de aridez por debajo de 0.65

Más allá de su interés ecológico, las zonas áridas son de vital importancia socio-económica, ya que es el lugar en el que habita el 38% de la población global y constituyen uno de los mayores reservorios de petróleo y metales preciosos en la actualidad (Reynolds et al. 2007; OPEC 2010; White & Nackoney 2003). A pesar de su importancia ecológica y socio-económica, las zonas áridas permanecen como uno de los biomas menos estudiados sobre la faz de la tierra, sobre todo cuando las comparamos con otros, como las zonas tropicales, templadas o la tundra (Schimel 2010; Maestre et al. 2012a). El incremento de la población humana, previsto en 9 mil millones de personas para 2050 (UN 2009), hará que cada día nuestra productividad agrícola y energética dependa más de las zonas áridas debido a su gran extensión y a la cantidad de bienes y servicios que prestan (MEA 2005). Todo ello hace que conocer la respuesta de las zonas áridas a los cambios ambientales que están ocurriendo en la actualidad, como el cambio climático o las alteraciones en la abundancia de nutrientes como el N y el P promovidos por las actividades humanas, sea fundamental para entender los posibles impactos que conllevarán sobre el planeta y sus habitantes.

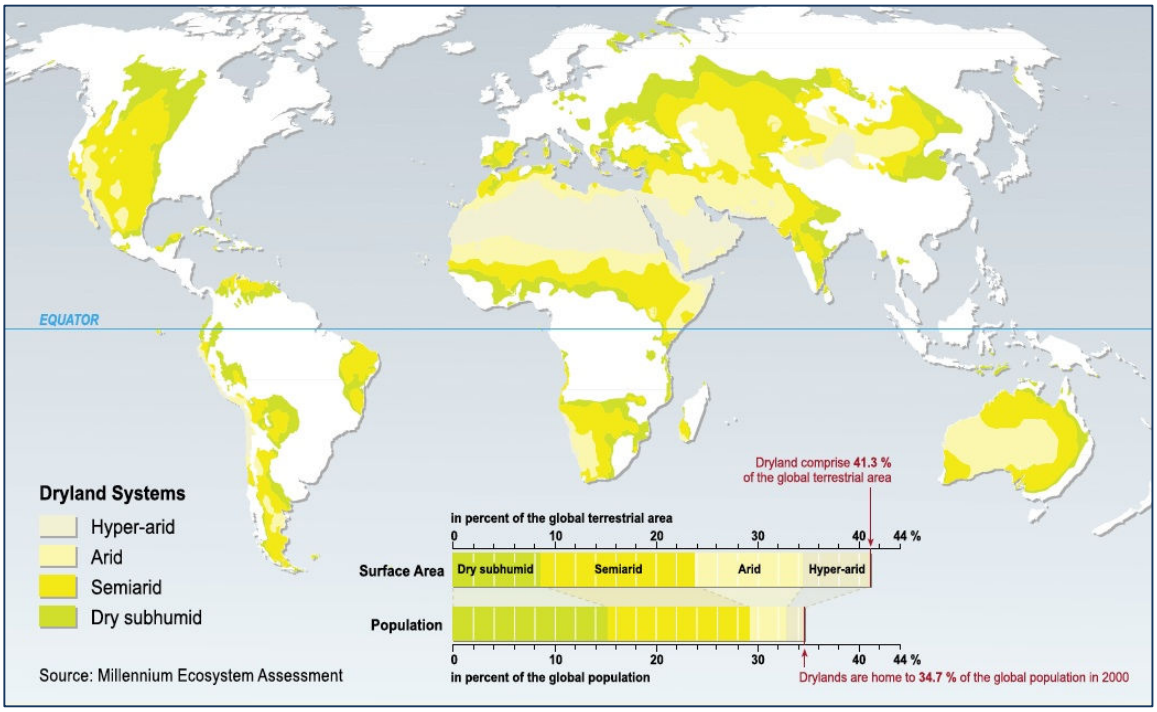

Figura 2.1. *Distribución mundial de las zonas áridas. Fuente: MEA 2005*

2.2 La costra biológica del suelo y su importancia en los ciclos biogeoquímicos

En las zonas áridas, los espacios situados entre las plantas vasculares suelen estar frecuentemente cubiertos por comunidades de costra biológica del suelo (CBS; Eldridge & Greene 1994; Belnap & Lange 2003; Castillo-Monroy & Maestre 2011; Maestre et al. 2011), las cuales están compuestas por un complejo mosaico de líquenes, bacterias, cianobacterias, algas, hepáticas, hongos y musgos. Se trata de poblaciones altamente diversas de organismos autótrofos y heterótrofos, que residen en un fino manto de aproximadamente 1 cm de espesor (Belnap & Lange 2003). Bajo tierra, las hifas de los hongos y los filamentos de cianobacterias, conforman una matriz que interacciona con las partículas del suelo, extendiéndose su influencia, desde varios milímetros a pocos centímetros de profundidad (Johnson et al. 2005). La CBS se encuentra presente en todas las zonas áridas y semiáridas frías, templadas o calidas, llegando a constituir hasta un 70% de la cobertura biótica en algunos ecosistemas (Belnap et al. 2001). No obstante y a pesar de la gran diversidad de ambientes en los que se encuentra presente, la CBS mantiene muchas similitudes en torno a su función, estructura y composición, para los distintos ecosistemas (Belnap & Lange 2003; Maestre et al. 2011). En ambientes áridos y semiáridos, la CBS, permanece seca e inactiva durante periodos secos, prehidratándose y reactivándose rápidamente tras el humedecido de la parte superficial del suelo (Belnap & Lange 2003; Belnap et al. 2004; Veste et al. 2008; Wilske et al. 2008; Rao et al. 2009; Pintado et al. 2010). Se sabe también que la CBS puede incrementar la cantidad de agua recogida por eventos de rocío, debido a la mayor rugosidad que confiere al suelo donde se desarrolla (Kidron 2000; Kidron et al. 2002; Jacobs et al. 2000; Jacobs et al. 2002; Rao et al. 2009; Chamizo et al. 2012). La CBS también afecta a otros procesos clave que determinan la humedad del suelo en zonas áridas, como la infiltración (Eldridge et al. 2000, 2010; Maestre et al. 2002; Eldridge et al. 2010; Chamizo et al. 2012).

La CBS no sólo determina en buena medida la hidrología de las zonas áridas, sino que juega también un importante papel modulando los ciclos del C y N en el suelo de estos ambientes (Belnap & Lange 2003; Delgado-Baquerizo et al. 2010; 2013; Castillo-Monroy et al. 2011b). Por un lado, la CBS actúa creando "islas de fertilidad" a pequeña escala, ya que numerosos estudios realizados en zonas áridas de todo el planeta han visto como el suelo debajo de la CBS tiene mayores contenidos en macro- (e.g. N, P) y micronutrientes (e.g. Mg, Cu, Ca) que los espacios de suelo desnudo adyacentes (Beraldi-Campesi et al. 2009; Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010; 2013; Miralles et al. 2012a; b; c; Chamizo et al. 2012).

La CBS fija importantes cantidades de C en el suelo via fotosíntesis (Elbert et al. 2012), y

los micrositios dominados por la CBS pueden ser los principales emisores de CO_2 a la atmósfera via respiración del suelo (Castillo-Monroy et al. 2011). Además, la CBS controla procesos relacionados con el ciclo del N tales como la fijación de N, la nitrificación o la denitrificación (Billings et al. 2002; Belnap & Lange 2003; Barger et al. 2005; Delgado-Baquerizo et al. 2010; Castillo-Monroy et al. 2010). Las cianobacterias que forman parte de la CBS constituyen una importante fuente de N en los ecosistemas áridos, fijando entre 0,2 y 100 kg/ha/año (Jeffries et al. 1992; Rychert y Skujins 1974) dependiendo de las condiciones de humedad y temperatura. Además, la CBS dominada por cianobacterias y cianolíquenes es capaz de fijar cantidades significativas de N atmosférico y hacerlo potencialmente disponible para las plantas vasculares, musgos y microorganismos (Belnap y Harper 1995; Belnap 2002; Porras-Alfaro et al. 2008). La CBS también afecta a la nitrificación en los suelos de ecosistemas semiáridos, aumentando el N total disponible para plantas y microorganismos en estos sistemas (Delgado-Baquerizo et al. 2010; 2013; Castillo-Monroy et al. 2010). Por ejemplo, en un estudio de campo en el área experimental de Aranjuez, situada en el centro de la Península Ibérica, los suelos bajo CBS poco desarrollada demostraron estar dominados por nitrato, mientras que aquellos dominados por CBS bien desarrollada demostraron una mayor dominancia de nitrógeno orgánico disuelto (NOD; Delgado-Baquerizo 2010). Otros estudio en esta misma zona (Delgado-Baquerizo et al. 2013) han mostrado niveles similares de N inorgánico disponible para plantas y microorganismos bajo CBS bien desarrollada y plantas vasculares como *S. tenacissima*, presentando la CBS una distribución espacial del N inorgánico más homogénea que la presente bajo *S. tenacissima*. Estos resultados sugirien que el "manto de fertilidad" formado por la CBS podría facilitar la obtención de N por parte de las raíces de las plantas (Maestre et al. 2007; Delgado-Baquerizo et al. 2013). La CBS también modula otros aspectos del ciclo del N como la desnitrificación, afectando al balance positivo de entradas y pérdidas de N del ecosistema (Billings et al. 2002, Barger et al. 2005, West & Skujins 1977). Si bien se ha avanzado notablemente en nuestro conocimiento sobre los efectos de la CBS en los ciclos biogeoquímicos en los últimos años, el papel de estos organismos en la mineralización y descomposición de la materia orgánica en respuesta a pequeños pulsos de agua similares a eventos del rocío es totalmente desconocido (Schwinning y Sala 2004), si bien es potencialmente relevante debido a la prevalencia tanto de CBS como de los aportes de agua por rocío en zonas áridas de todo el planeta (Kidron 2000; Jacobs et al. 2002; Moro et al. 2007; Zhang et al. 2009; Rao et al. 2009).

El ciclo del N engloba una serie de procesos "simples" llevados a cabo por grupos específicos de organismos –como la nitrificación– y otros más "complejos" que requieren de una mayor diversidad de organismos para poder ser llevados a cabo (como la despolimerización: producción de NOD; Schimel et al. 2005). Distintos estudios han observado cómo la CBS tiene una

comunidad de hongos específica (Porras-Alfaro et al. 2008; Bates et al. 2010; Abed et al. 2013), que se encuentra ligada a procesos tales como la descomposición de materia orgánica (Austin et al. 2004; Robertson & Groffman 2007) y la transferencia de C y N entre la CBS y las plantas vasculares (Porras-Alfaro et al. 2008), y que explicaría la mayor dominancia de NOD observada bajo comunidades de CBS (Delgado-Baquerizo et al. 2010).

La CBS puede afectar a la abundancia y diversidad de organismos del suelo tales como bacterias y hongos (Yeager et al. 2007, Bates et al. 2010, Castillo-Monroy et al. 2011c). Además, Yu et al. (2012) demostraron que comunidades bien desarrollados de CBS pueden incrementar la diversidad funcional microbiana del suelo, fomentando el consumo de compuestos aromaticos y carbohidratos en suelo. Por otro lado, otros estudios han mostrado que un incremento en el C y N incrementan y disminuyen, respectivamente, la diversidad microbiana del suelo, mientras que adiciones de P conllevan resultados inconclusos (Sharma el al. 1998; Coleman & Whitman 2005; Schimel et al. 2005). Sin embargo, poco se sabe acerca de como la CBS modula la respuesta del ciclo del N y de la diversidad funcional microbiana a cambios simultáneos en la abundancia de C, N y P.

2.3. El ciclo del nitrógeno en zonas áridas

El N es, después del agua, el factor que más frecuentemente limita la producción primaria en las zonas áridas y semi-áridas (Schlesinger 1990; Schlesinger 1996; Whitford 2002; Robertson y Groffman 2007). Las zonas áridas cubren más de un tercio de la superficie terrestre, si bien la mayoría de los estudios sobre ciclos del N, se han desarrollado en ecosistemas mésicos (Schlesinger et al. 1990; Schimel 2010), existiendo diferencias en magnitud y dominancia relativa de los procesos que dominan el ciclo del N en éstos sistemas. Por ejemplo, mientras que en los ecosistemas templados y tropicales húmedos, la disponibilidad de N procedede principalmente de la descomposición continuada de la materia orgánica (Schlesinger 1996), en las zonas áridas la fuente principal de N proviene fundamentalmente de la muerte de los microorganismos del suelo (Singh 1989; Schimel et al. 2007). En zonas áridas, las entradas de N al ecosistema se producen por fijación atmosférica de N (Rychert y Skujins 1974; Johnson et al. 2005), mientras que las salidas de N del sistema están reguladas por procesos de desnitrificación y volatilización de amonio, cuyo equilibrio viene dado por la oxidación de este último, primer paso de la nitrificación, que es llevada a cabo por ciertos grupos de heterótrofos y por arqueas y bacterias nitrificantes del suelo (Schlesinger 1996; Johnson et al. 2005; Robertson & Groffman 2007; Verhamme et al. 2011).

La disponibilidad de N en ecosistemas desérticos está controlada por factores abióticos tales

como el clima, el pH, o la textura de suelo. Los escasos e irregulares procesos de precipitación, junto a las altas temperaturas que caracterizan los ecosistemas áridos, son dos de los principales factores que regulan el ciclo del N (Austin 1998; Austin 2004; Aranibar 2004; McCulley 2009). Por ejemplo, un incremento en temperatura ha sido relacionado con una mayor mineralización y reciclado de nutrientes en suelo (Szukics et al. 2010). Las bajas precipitaciones que caracterizan a las zonas áridas limitan la descomposición de la materia orgánica en estos ecosistemas (Gallardo and Merino 1993; Schlesinger 1996), sin bien la presencia de pequeños pulsos de agua provenientes del rocío desde los meses de otoño a primavera (Kidron 2000; Jacobs et al. 2002; Moro et al. 2007), proporcionan una fuente de agua que podría ser usada por los microorganismos del suelo para llevar a cabo la descomposición y mineralización de la materia orgánica, favoreciendo el rápido reciclado de nutrientes después de la muerte microbiana (Schwinning y Sala 2004). Aunque numerosos estudios realizados en zonas áridas han demostrado la importancia de los aportes de agua provenientes del rocío, que pueden suponer hasta el 40% del total de aporte de agua anual, en lugares como Tabernas, los desiertos de Negev y del Namib y distinos desiertos en China (Kidron 2000; Jacobs et al. 2002; Moro et al. 2007; Zhang et al. 2009; Rao et al. 2009), existe un desconocimiento importante sobre los efectos directos de estos pulsos en los procesos biogeoquímicos, y en el ciclo del N en particular.

Las zonas áridas presentan normalmente suelos poco profundos, desarrollados frecuentemente sobre sustratos calizos, margosos o yesosos y con pH básico-neutros (Schlesinger 1990; IUSS Working Group WRB, 2006). Mientras que pH ácidos (menores de 6) favorecen la dominancia de amonio (debido al equilibrio químico del N con los protones del suelo) y la descomposición de materia orgánica derivada del crecimiento de comunidades de hongos, valores de pH entre 6 y 8 favorecen a los grupos de bacterias nitrificantes que llevan a cabo la nitrificación en el suelo (Anthonisen et al. 1976; Cookson et al. 2006; Nicol et al. 2008). La textura es otro factor que modula el ciclo del N en ecosistemas áridos. De este modo, zonas con mayor aridez tienden a una textura más arenosa debido al predominio de la meteorización mecánica sobre la química que se potencia con la ausencia de cobertura vegetal (FAO 1989; Vicente-Serrano et al. 2012). Un mayor contenido de arena favorece procesos aeróbicos tales como la nitrificación (FAO 1989; Robertson & Groffman 2007), sin embargo su falta de complejo de cambio en comparación con los suelos arcillosos favorecen el lavado de N tras procesos torrenciales de precipitación (FAO 1989). Otros factores, tales como el cociente C:N y la disponibilidad de N en el suelo son importantes moduladores de las tasas de mineralización e inmobilización de N en el suelo (Austin et al. 2004; Schimel & Bennet 2004; Schimel et al. 2005). De este modo, en micrositios (p. ej. suelos sin vegetación) y ecosistemas (p. ej. tundra ártica; Figura 2.2) pobres en N, o con altos ratios de C:N

(>30), el NOD producido a partir de la despolimerización de la materia orgánica podría ser la forma dominante en el suelo (Schimel & Bennet 2004). En micrositios (ej. suelo bajo vegetación) y ecosistemas (ej. suelos agricolas; Figura 2.2) ricos en N o con bajo ratio C:N, el nitrato y la nitrificación serían dominantes (Schimel & Bennet 2004; Cookson et al. 2006; Schimel et al. 2005; Robertson & Groffman 2007). Siguiendo la hipótesis de Schimel & Bennet (2004), los ecosistemas pobres en N tales como las zonas áridas (Delgado-Baquerizo et al. 2010; 2011) deberían estar dominados por NOD, si bien estudios previos de campo y laboratorio han demostrado que estos ecosistemas tienden a una dominancia en nitrato que puede ser debida a su baja relación C:N, valores de pH básico-neutros y altos contenidos en arena (Austin et al. 2004; Cookson et al. 2006).

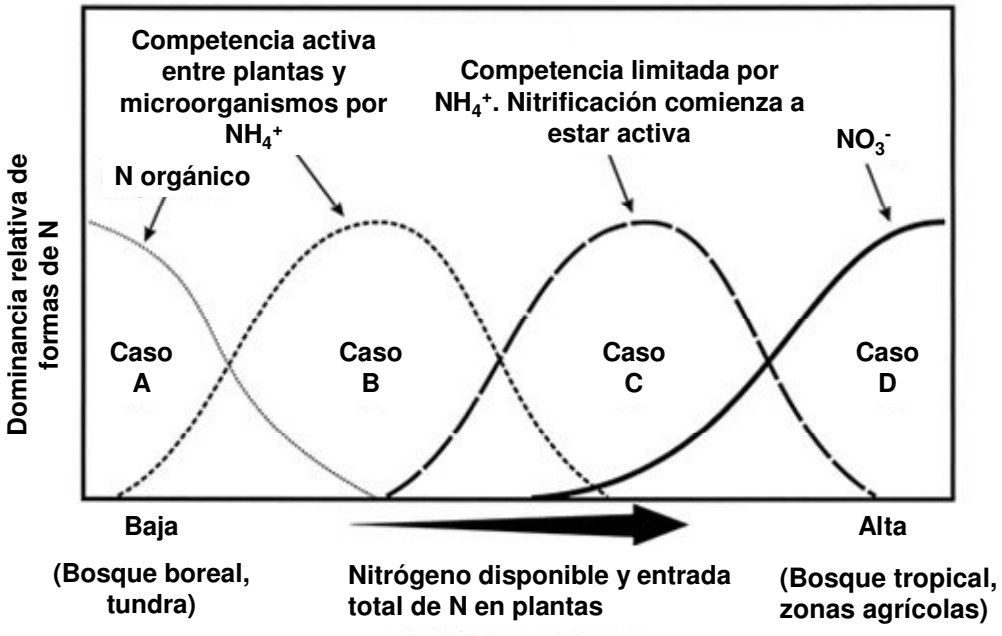

Figura 2.2. *Dominancia relativa de formas de nitrógeno en función de la fertilidad del ecosistema. Adaptada de Schimel & Bennett (2004).*

Todos los factores abióticos que modulan la concentración y dominancia de formas del ciclo del N en zonas áridas, como la disponibilidad de agua y la temperatura, están a su vez influenciados por la existencia de micrositios proporcionados por la presencia de los distintos grupos de plantas y microorganismos que se desarrollan sobre el suelo (Schimel & Bennet 2004; Schimel et al. 2005). Los ecosistemas áridos suelen poseer una vegetación dispersa y en mosaico, donde manchas discretas de vegetación se embeben en una matriz de suelo desprovisto de vegetación vascular (Reynolds et al. 1999; Valentin et al. 1999, Maestre & Cortina 2002; Rietkerk & van de Kopel 2008). Esta matriz está a su vez ocupada por la CBS. De este modo, los distintos micrositios compuestos a grandes rasgos por plantas vasculares, CBS y suelo desnudo, poseen distinta influencia sobre el ciclo del N, debido en parte a su distinta capacidad de fijar N, variaciones en la

cantidad, calidad y forma de la entrada de hojarasca o su habilidad de modular las condiciones micro-climáticas (ej. disponibilidad de agua en suelo; Belnap et al. 2004; Maestre et al. 2001; 2003; Miralles et al. 2012a;b;c; Chamizo et al. 2012). Así pues, en zonas semiáridas Mediterráneas, especies de plantas tales como *Stipa tenacissima* y *Retama sphaerocarpa* han mostrado generar verdaderas "islas de fertilidad" en estos ecosistemas, incrementando la cantidad de hojarasca, humedad y nutrientes en suelo, y disminuyendo la temperatura, en comparación con zonas de suelo desnudo (Pugnaire et al. 1996a;b; Moro et al. 1997; Maestre et al. 2001, 2003). Sin embargo, el papel modulador de la vegetación en la respuesta a impactos relacionados con el cambio climático, como el incremento de aridez, sobre el N disponible para plantas y microorganismos, así como sobre las comunidades microbianas que participan en el reciclado de este elemento, es todavía desconocido, si bien las manchas de vegetación podrían suponer verdaderos refugios para ciertos grupos de microorganismos edáficos (Goberna et al. 2007; Miralles et al. 2012a; Abed et al. 2013; Sayer et al. 2013). Del mismo modo, la CBS incrementa la resistencia física del suelo y la disponibilidad de agua bajo su cobertura (Belnap & Lange 2003; Belnap 2004; Belnap 2006; Chamizo et al. 2012), habiéndose comprobado que también afecta a la concentración y distribución espacial de distintas formas de N en el suelo (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010; 2013). Sin embargo, el efecto modulador de los distintos micrositios sobre el ciclo del N (concentración y formas de N, así como sobre las comunidades microbianas envueltas en los procesos de nitrificación el suelo) en respuesta a cambios ambientales tales como incremento de temperatura y la disminución del contenido de agua en suelo, han sido escasamente estudiados (Szukics et al. 2010; Delgado-Baquerizo et al. 2011).

2.4. Cambio ambiental global y ciclos biogeoquímicos en zonas áridas

El incremento de temperatura, los cambios en precipitación y el desacople en los ciclos biogeoquímicos del C, N y P, son alguno de los fenómenos ambientales derivados de las actividades humanas sobre el planeta (p. ej. agricultura, quema de combustibles fósiles, ganadería etc.; Solomon et al. 2007; Finzi et al. 2011; Peñuelas et al. 2012). Las características biofísicas propias de las zonas áridas (p. ej. baja cobertura vegetal, escasez de nutrientes y agua) hacen a estos sistemas altamente vulnerables al cambio ambiental global (en lo sucesivo cambio global), y en particular al cambio climático (Schlesinger 1990; Körner 2000; Reynolds et al. 2007; Maestre et al. 2012a; Vicente-Serrano et al. 2012). Aunque existen discrepancias en los modelos actuales de cambio climático a nivel global, la mayoría de los modelos existentes sugieren un incremento en extensión de las zonas áridas a nivel mundial, así como un incremento de la aridez dentro de sus regiones bioclimáticas (zonas áridas, semiáridas y secas-subhumedas; Gao y Giorgi, 2008; Dai

2012; Maestre et al. 2012a). Del mismo modo, se espera un incremento de temperatura media de unos 3ºC en las regiones áridas para finales del siglo XXI (Solomon et al. 2007). Aunque los cambios en la disponibilidad de agua son los más discutidos por estos modelos, sugiriendo cambios del ± 25% en la precipitación media anual dependiendo de la región donde nos encontremos, se espera un incremento de los eventos extremos de precipitación y sequía en las regiones áridas (Maestre et al. 2012a). Además, una disminución generalizada a nivel global del 15% de humedad en el suelo ha sido predicha en los ecosistemas terrestres para finales de siglo (Dai 2013). Tanto el impacto derivado de las actividades humanas como el causado por el incremento de aridez podría tener implicaciones muy importantes en el acoplamiento de los ciclos biogeoquímicos a nivel global. Un mayor impacto humano suele estar relacionado como una mayor entrada de N –por deposición atmosférica, abonado y ganadería– y de C, debido a la quema de combustibles fósiles (Finzi et al. 2011; Peñuelas et al. 2012). Un incremento en el N disponible proveniente de estas fuentes podría incrementar la producción neta y descomposición de la materia orgánica en ecosistemas pobres en N, como los situados en las zonas áridas (Cookson et al. 2006; Delgado-Baquerizo et al. 2011). Sin embargo, esto solo sería posible siempre que no existieran otros nutrientes limitando estos procesos, tales como el P, clave en la producción de adenosin tri-fosfato (ATP), y que puede limitar todo el metabolismo energético (Finzi et al. 2011). Las entradas de P al sistema vienen dadas por la meteorización mecánica de la roca madre (Walker & Syers 1976; Vitousek 2004), y su movilidad a nivel global esta mucho más limitada que las de C y N, que al disponer de formas gaseosas, son abundantes a nivel atmosférico (Peñuelas et al. 2012). Por el contrario, el aumento de aridez podría disminuir la abundancia de nutrientes ligados a procesos biológicos como la descomposición o producción primaria (N y C), mientras que podría incrementar la abundancia de otros ligados a procesos físico-químicos, como el P. Al mismo tiempo, los incrementos de N y temperatura derivados de las actividades humanas (Finzi et al. 2011; Peñuelas et al. 2012) podrían incrementar la mineralización del N en el suelo, resultando en un aumento de las formas inorgánicas en suelo a través de procesos como la nitrificación, que afectaría a la toma preferencial de distintas formas de N por parte de las plantas (Nordin et al. 2001; Warren 2009; Paulding et al. 2010).

Los distintos agentes de cambio global acarrean efectos a distintas escalas espaciales. Factores como el incremento de aridez podrían, sin embargo, tener un impacto a nivel regional y global sobre la disponibilidad de N y los microorganismos relacionados con su ciclo. A estas escalas, la vegetación podría jugar un papel regulador sobre el impacto del incremento de aridez, generando islas de fertilidad y refugio para grupos concretos de microorganismos relacionados con el ciclo del N. Por último, y debido a la fuerte dependencia estequiometrica del C, N y P en los

seres vivos a nivel global, su desequilibrio podría acarrear un impacto negativo sobre la producción primaria, la respiración o la descomposición de la materia orgánica (Finzi et al. 2011; Peñuelas et al. 2012). La mayoría de las publicaciones que estudian la importancia del cambio global sobre los ciclos biogeoquímicos en zonas áridas se centran en regiones concentras (p. ej. Zavaleta et al. 2003; Reed et al. 2012), siendo difícil extrapolar sus resultados a otras zonas del planeta. A pesar de la importancia de las zonas áridas para la población humana, no existe en la actualidad ningún estudio a escala global que haya evaluado como un incremento en la aridez podría afectar al balance entre C, N y P en los suelos de estos ambientes.

A escala local, cambios en la temperatura, el contenido de agua del suelo y en los ratios de C, N y P podrían conllevar alteraciones en los procesos que determinan la disponibilidad de determinadas formas de N en microsities específicos, como los que crea la presencia de CBS (ej. Delgado-Baquerizo et al. 2010). De hecho, distintos estudios sugieren que el cambio climatico podría reducir las tasas fotosinteticas de líquenes (Maphangwa et al. 2012) y musgos (Grote et al. 2010), lo que reduciría su crecimiento y dominancia dentro de la CBS (Zelikova et al. 2012; Reed et al. 2012; Escolar et al. 2012). Además, se ha visto que cambios en los patrones de precipitación disminuyen la abundancia de otros componentes de la CBS, como las cianobacterias (Johnson et al. 2012). Estudios recientes han mostrado que alternaciones en los patrones de precipitación y la consiguiente sustitución de musgos por cianobacterias, genera perdidas de la fertilidad y incrementa la dominancia de nitrato en suelo en zonas del suroeste de EEUU (Reed et al. 2012; Zelikova et al. 2012). Sin embargo, el papel que juega la CBS como modulador de las respuestas del ciclo del N al cambio global es prácicamente desconocido.

2.5. Estructura y objetivos de la tesis

El estudio de las causas e impactos derivados del cambio global requiere del entendimiento de atributos y procesos ecosistémicos ligados a distintas escalas espaciales (Levin 1992). La existencia de factores específicos que ocurren a pequeña escala, como los eventos de rocío, podrían determinar la respuesta del ciclo de N al cambio global en zonas áridas al cambio global (Figura 2.3). El estudio de los impactos del cambio global sobre este ciclo a pequeña escala requiere el establecimiento de estudios detallados de áreas concretas, lo que imposibilita la extrapolación de los resultados obtenidos a escalas superiores debido a la falta de representación de factores ambientales tales como el grado de la cobertura vegetal o el tipo de suelo (Figura 2.3).

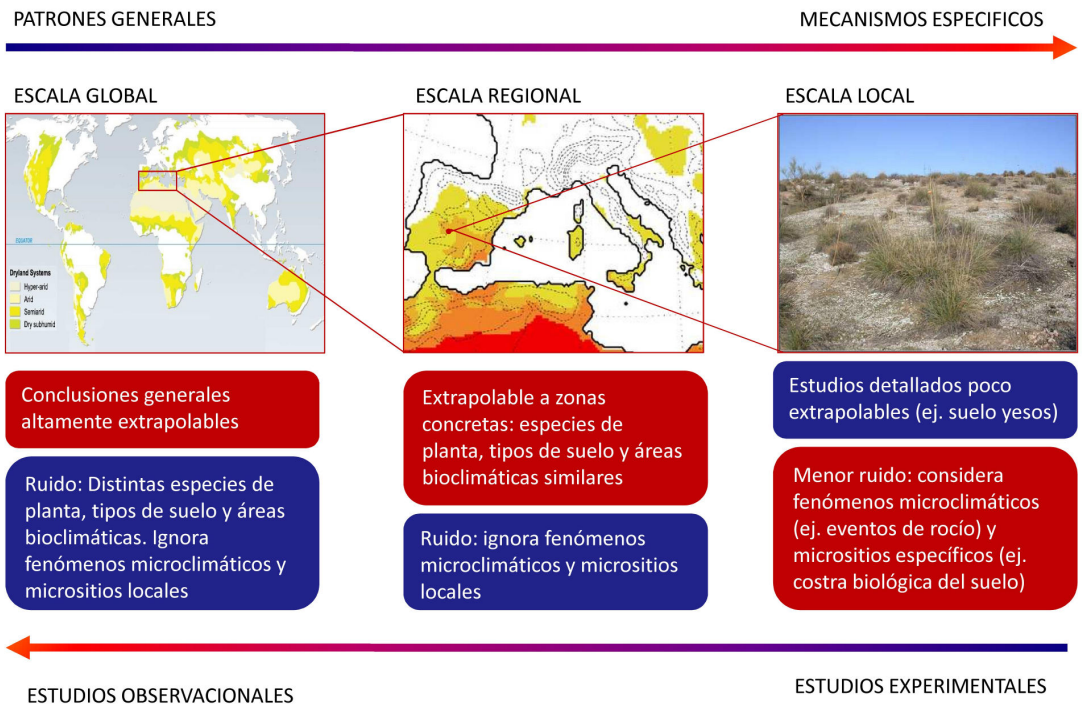

Figura 2.3. *Esquema donde se engloban las distintas ventajas (rojo) y desventajas (azul) de las distintas escalas espaciales estudiadas en esta tesis.*

Por otro lado, los análisis llevados a cabo a escalas globales y regionales son útiles a la hora de sugerir patrones generales en respuesta a factores asociados al cambio climático, sin bien no permiten detectar los mecanismos concretos que están detrás de los patrones observados, debido en parte a la presencia de ruido (distintos tipos de ecosistema, tipo de suelo, regiones biogeográficas y bioclimáticas, microclimas, etc.). Todo ello hace que no exista una única escala a la cual los impactos derivados del cambio climático sobre el funcionamiento del ecosistema puedan ser descritos (Levin 1992). Aí pues, señalar los factores locales que determinan la respuesta del ciclo del N al cambio global nos ayudará a explicar los detalles que generan ruido a escalas superiores. A lo largo de esta tesis doctoral se han llevado a cabo una serie de experimentos de laboratorio y estudios observacionales considerando distintas escalas espaciales (local, regional y global), con el objetivo de evaluar el impacto del cambio global sobre el ciclo del nitrógeno en zonas áridas. Este análisis pluriescalar nos permitirá esclarecer el papel de distintos factores ecológicos propios de cada escala espacial en respuesta a los impactos provenientes del cambio global sobre el ciclo del N.

El objetivo **general** de esta tesis es evaluar como el cambio climático afectará a variables clave del ciclo de N a distintas escalas espaciales en zonas áridas. En particular, esta tesis analiza cómo los distintos micrositios característicos de estos ecosistemas (suelo desnudo, plantas vasculares y CBS) modulan la respuesta de variables clave del ciclo del N a distintos agentes de

cambio global, tales como el incremento de temperatura, aumento de aridez y cambios en la disponibilidad de nutrientes. La presente tesis está dividida en tres grandes bloques, dedicado cada uno de ellos a las tres grandes escalas espaciales abordadas en la misma. Los distintos capítulos que se incluyen en cada bloque se presentan a continuación.

Bloque I. Escala global: Evaluando el efecto de la aridez sobre los ciclos del C, N y P a nivel global.

Este bloque está compuesto por el **capítulo 1 (Aridity decouples soil C, N and P biogeochemical cycles in global drylands)**, en el que se evalúa, utilizando una red de 224 parcelas establecida en zonas áridas de todo el planeta en el marco del proyecto BIOCOM (Maestre et al. 2012b; Figura 2.4), el posible impacto del incremento de aridez pronosticado para final del siglo XXI sobre los ciclos biogeoquímicos del C, N y P a **escala global**, discutiendo a su vez las implicaciones de dicho impacto en el funcionamiento del ecosistema.

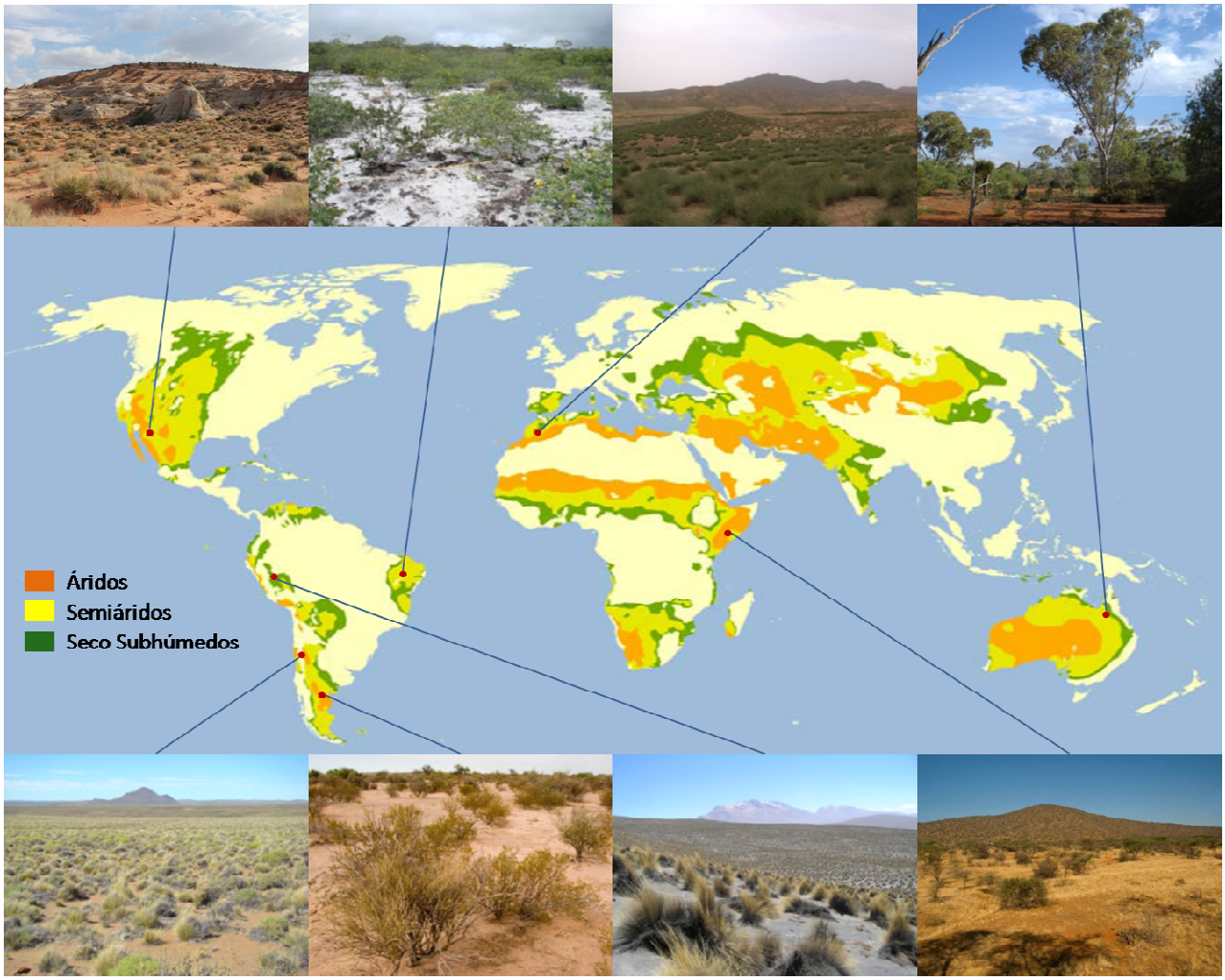

Figura 2.4. *Localización aproximada y fotografías de ocho de las 224 zonas muestreadas para el análisis global presentado en el capítulo 1. Adaptada de Maestre et al. (2012a).*

Bloque II. Escala regional: Evaluando el efecto modulador de la vegetación sobre el ciclo del N en respuesta al incremento de aridez.

En el bloque II se estudia el papel que las plantas vasculares tienen como moduladores de la respuesta del ciclo del N al incremento de aridez pronosticado para el final del siglo XXI en una serie de formaciones dominadas por *Stipa tenacissima* muestreadas a lo largo de un gradiente mediterráneo que va desde España hasta Túnez (Figura 2.5). En el **capítulo 2** (**Aridity modulates N availability in arid and semiarid Mediterranean grasslands**) evaluamos el impacto de la aridez sobre la disponibilidad de N en los espartales muestreados a lo largo de este gradiente, y discutimos el papel de las plantas vasculares sobre la disponibilidad de N. En el **capítulo 3** (**Vascular plants mediate the effects of aridity and soil properties on ammonia-oxidizing bacteria and archaea**) analizamos cómo la aridez y la presencia de *S. tenacissima* afectan a la abundancia de bacterias y arqueas nitrificantes en los ecosistemas estudiados.

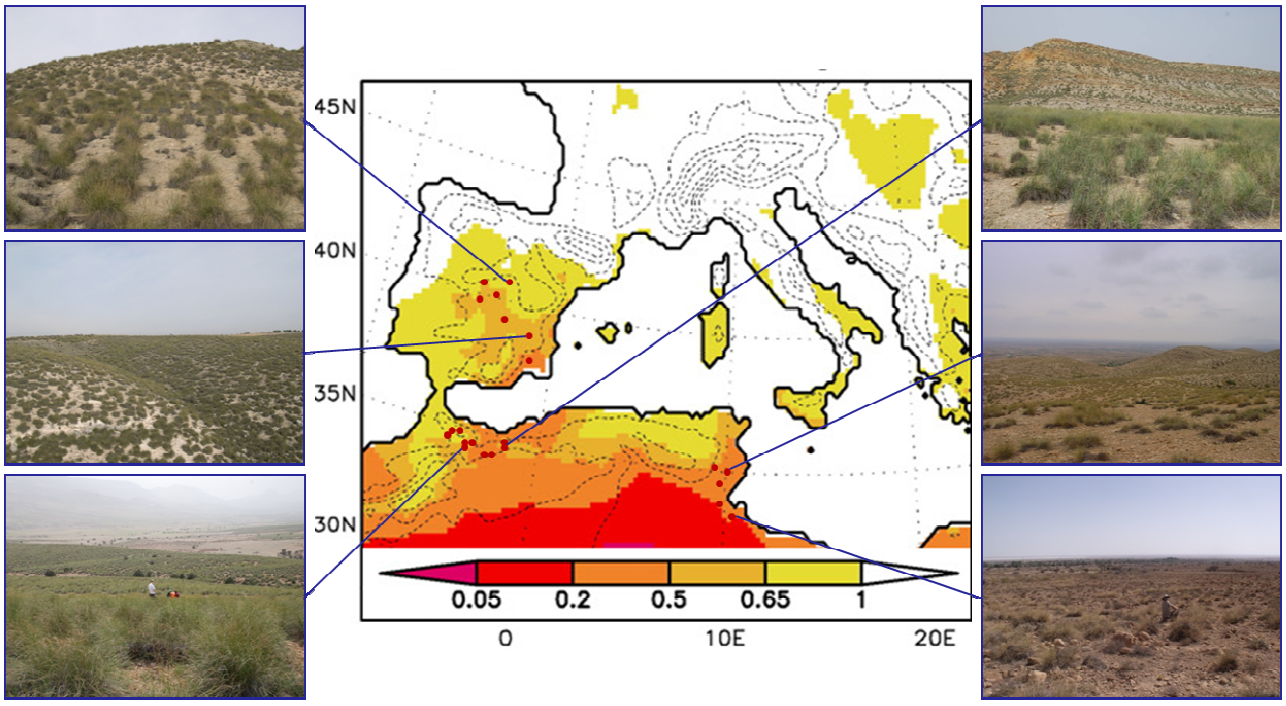

Figura 2.5. *Localización aproximada y fotografías de algunos de los espartales muestreados a lo largo de un gradiente regional Mediterráneo desde España a Túnez. En el mapa, modificado de Gao & Giorgi (2008), se observa el índice de aridez de la UNEP (1992), que incluye zonas hiper-áridas (IA<0.05), áridas (0.05<IA<0.20), semiáridas (0.20<IA<0.50) y seco-subhúmedas (0.50<IA<0.65).*

Bloque III. Escala local: Evaluando el efecto modulador de la CBS sobre el ciclo del N en respuesta a cambios microclimáticos.

En este último bloque se evalúa cómo la CBS modula la resistencia y resiliencia de variables del ciclo del N a escala local en respuesta al incremento de temperatura, a cambios en humedad del suelo y a variaciones en la disponibilidad de nutrientes edáficos (C, N y P).

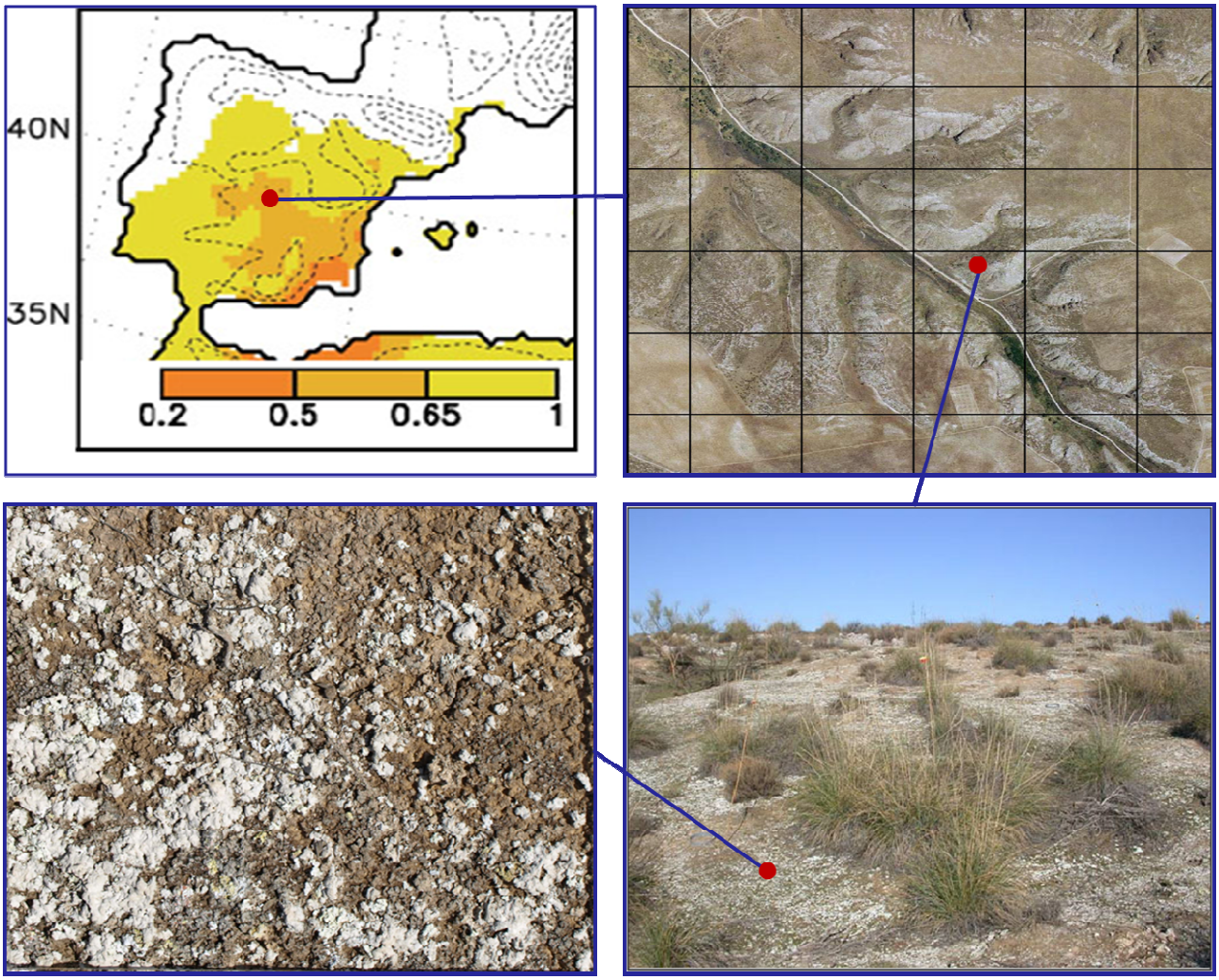

Figura 2.6. *Localización y fotografías de la estación experimental de Aranjuez. En la figura superior izquierda se muestran isolíneas correspondientes al índice de aridez (UNEP, 1992), que incluye zonas hiper-áridas (IA<0.05), áridas (0.05<IA<0.20), semiáridas (0.20<IA<0.50) y secas-subhúmedas (0.50<IA<0.65).*

En el **capítulo 4** (**Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions**) evaluamos cómo la CBS afecta a la diversidad funcional microbiana y la resiliencia de variables asociadas al ciclo del N frente a cambios cambios en los contenidos de C, N y P en el suelo. En el **capítulo 5** (**Biological soil crusts increase the resistance of soil nitrogen dynamics to changes in temperatures in a semi-arid ecosystem**) estudiamos cómo la CBS afecta a la resistencia de variables asociadas al ciclo del N a cambios en la

temperatura y el contenido de agua en suelo. En el **capítulo 6** (**Biological soil crusts promote N accumulation in response to dew events in dryland soils**) se analiza cómo la CBS afecta a la disponibilidad de N en respuesta a pequeños pulsos de agua, similares a los encontrados en ecosistemas áridos durante eventos de rocío. Todos los estudios de este bloque se han realizado con muestras de suelo y CBS provenientes de la estación experimental de Aranjuez (Figura 2.6), situada en el centro de la Península Ibérica.

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1. BLOQUE I: ESCALA GLOBAL

CAPÍTULO 1

Aridity decouples soil C, N and P biogeochemical cycles in global drylands

Manuel Delgado-Baquerizo, Fernando T. Maestre, Antonio Gallardo, Matthew A. Bowker, Matthew Wallenstein, Jose Luis Quero, Victoria Ochoa, Beatriz Gozalo, Miguel García-Gómez, Santiago Soliveres, Pablo García-Palacios, Miguel Berdugo, Enrique Valencia, Cristina Escolar, Tulio Arredondo, Claudia Barraza-Zepeda, Donaldo Bran, José Antonio Carreira, Mohamed Chaieb, Abel A. Conceição, Mchich Derak, Adrián Escudero, David J. Eldridge, Carlos I. Espinosa, Juan Gaitán, Gabriel Gatica, Susana Gómez-González, Elizabeth Gusman, Julio R. Gutiérrez, Adriana Florentino, Estela Hepper, Rosa M. Hernández, Elisabeth Huber-Sannwald, Mohammad Jankju, Jushan Liu, Rebecca L. Mau, Maria Miriti, Jorge Monerris, Kamal Naseri, Zouhaier Noumi, Abelardo Ospina, Vicente Polo, Aníbal Prina, Eduardo Pucheta, Elizabeth Ramírez, David A. Ramírez-Collantes, Roberto Romão, Matthew Tighe, Cristian Torres-Díaz, Eugene D. Ungar, James Val, Wanyoike Wamiti, Deli Wang, Eli Zaady

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The biogeochemical cycles of carbon (C), nitrogen (N) and phosphorus (P) are interlinked with primary production, respiration, and decomposition in terrestrial ecosystems[1]. The increase in aridity predicted for the 21st Century in many drylands worldwide[2,3,4] may threaten the balance among these cycles, differentially affecting the availability of essential nutrients depending on the degree to which they are under biological versus geochemical control[5,6,7]. We conducted a study to evaluate the relationships between aridity and soil C, N and P availability in 224 dryland sites worldwide. We observed a negative effect of aridity on the concentration of soil organic C and total N, but a positive effect on available P. Aridity was negatively related to plant cover, which may favor the dominance of physical (i.e. wind-blown sands that abrade exposed rock surfaces) over biological (i.e. litter decomposition) processes. Our results suggest that the predicted increase in aridity with climate change will reduce the concentration of N and C in global drylands, which are primarily under biological control, but will increase that of P, which is mainly derived from minerals in rocks and sediments. These changes will uncouple the C, N and P cycles in drylands, and will hinder the provision of key services provided by these ecosystems.

Biogeochemical cycles are biologically coupled, from molecular to global scales, due to the conserved elemental stoichiometry of plants and microorganisms that drive the cycling of C, N and P[1]. The availability of C and N is primarily linked to biological processes such as photosynthesis, atmospheric N fixation and subsequent microbial mineralization[5,6,7]. However, available P for plants and microorganisms[8] is derived mainly from mechanical rock weathering, and to a lesser extent from organic matter decomposition[5,6,7]. The importance of biological versus geochemical control of nutrient cycling has been shown to change with ecosystem development[6,7]. For example, during the earliest stages of ecosystem succession, a relative prevalence of geochemical control on nutrient cycling means that P is made available by mechanical rock weathering, but N and C are scarce, leading to a disparity in the C, N and P cycles relative to plant nutrient requirements[6,7,9]. Climatic controls on ecosystem development and biogeochemical cycles are particularly relevant in arid, semi-arid, and dry-subhumid ecosystems (drylands) because their biological activity is mainly driven by water availability[10,11,12]. Drylands cover about 41% of Earth's land surface and support over 38% of the global human population[13], constituting the largest terrestrial biome on the planet[14]. The increase in aridity predicted for the late 21st century in many regions worldwide will expand the total area of drylands globally[2,3,4]. These changes are predicted to exacerbate processes leading to land degradation and desertification, which already threaten the livelihood of over 250 million people living in the world´s drylands[13,14]. For example, a worldwide decrease in soil moisture

ranging from 5% to 15% has been predicted for the 2080-2099 period[4]. Of particular concern is that the biogeochemical cycles of C, N and P could become uncoupled under rapid climate change because of the different degrees of control exerted on these elements by biological and geochemical processes[1,15,16,17]. As the global human population continues to grow, we will increasingly rely on marginal lands – particularly drylands – for the production of food, wood and biofuels, and to offset the emission of greenhouse gases[13-16]. These ecosystem services can be greatly and negatively impacted by the decoupling of the biogeochemical cycles of C, N and P in soils[13-16]. Despite their extent and importance for human populations, it is largely unknown how predicted increases in aridity may influence such cycles[14,16], and no global field studies have been conducted on this topic yet[18].

We evaluated how aridity affects the balance between C, N and P in soils collected from 224 dryland sites from all continents except Antarctica. As aridity is a fundamental driver of biological and geochemical processes in drylands[5,12,13], we predicted that increasing aridity would reduce biological activity[16,17] and therefore the availability in nutrients under more strict biological control (C and N)[16], but would favor the relative dominance of nutrients linked to geochemical processes (P)[1,6,15,16], causing a stochiometric imbalance in the nutrient cycles associated with C and N limitation in drylands[15]. We selected organic C and total N as surrogates of C and N availability because they were highly related to other available C and N forms for plants and microorganisms such as dissolved carbohydrates, amino acids and inorganic-N (see Appendix S1). We used phosphate because it is an universal source of P for plants and microorganisms that plays a key role in the processes of C and N fixation in drylands. It also has a non-biological primary origin whose main source is primary minerals in rocks and sediments[1,6,7,8,15]. Negative quadratic relationships were observed between aridity and both organic C and total N concentrations (Fig. 1a, c). However, aridity was positively related to phosphate (Fig. 1e). Similarly, a negative quadratic relationship was observed between aridity and the N:P and C:P ratios (Fig. 1b, d). The ratio C:N decreased linearly with increasing aridity (Fig. 1f). Phosphate concentrations were highest in the most arid sites, possibly as the result of the physical and mechanical disintegration of rocks and subsequent accumulation of P due to low productivity[5,7,19]. Mechanical rock weathering is the main P input into terrestrial ecosystems, but N is either absent or uncommon in primary minerals, and inputs therefore are largely derived from atmospheric N fixation and/or deposition[6,7,18,]. While biological weathering rates should decline with increasing aridity, mechanical rock weathering may increase with aridity, releasing P-bearing minerals[7,15,19].

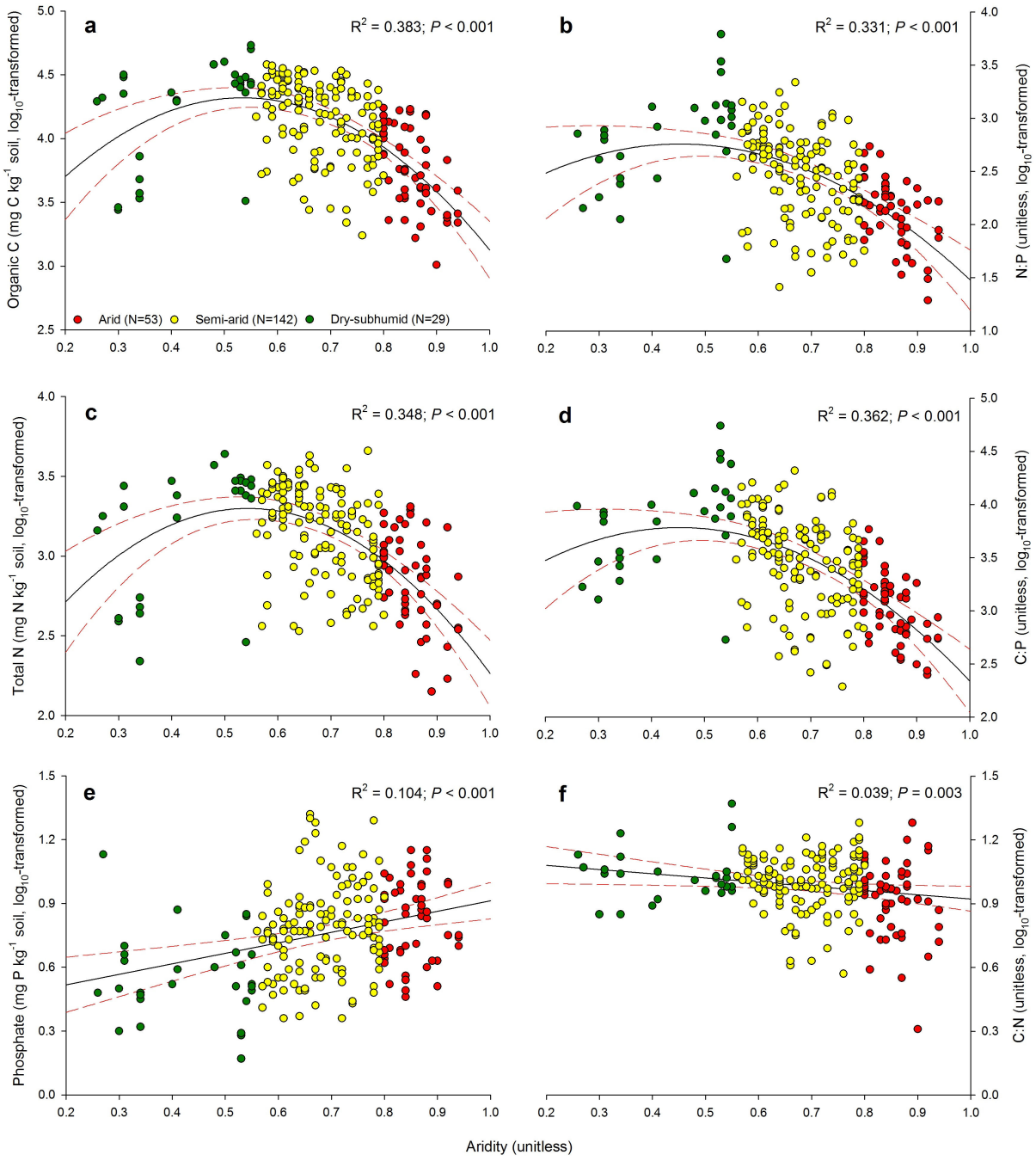

Figure 1. *Relationships between aridity (1-aridity index) and the concentration of organic carbon (C), total nitrogen (N), phosphate (P) and the ratios N:P, C:P and C:N in our study sites. The solid and dashed lines represent the fitted quadratic regressions and their 95% confidence interval, respectively.*

To clarify the effects and relative importance of aridity on the availability of C, N and P, we generated a structural equation model based on the known effects and relationships among key drivers of organic C, total N and phosphate (Fig. 1; see Appendix S2 for rationale). We included phosphatase activity in this model, which is the enzyme responsible for releasing phosphate from organic sources, and is considered a surrogate of biological P demand (organic P in the soil can be used by plants through the action of phosphatases[8,20]). Our model explained 47%, 24% and 57% of the variance in the organic matter component (first component from a PCA with organic C and total

N, Appendix S3), phosphate and phosphatase activity, respectively. Aridity had a negative direct effect in the organic matter component and phosphatase activity, but a positive effect on phosphate content (Fig. 2). In addition, aridity was also the most important predictor of these variables (Fig. 3a,b,c; Appendix S3).

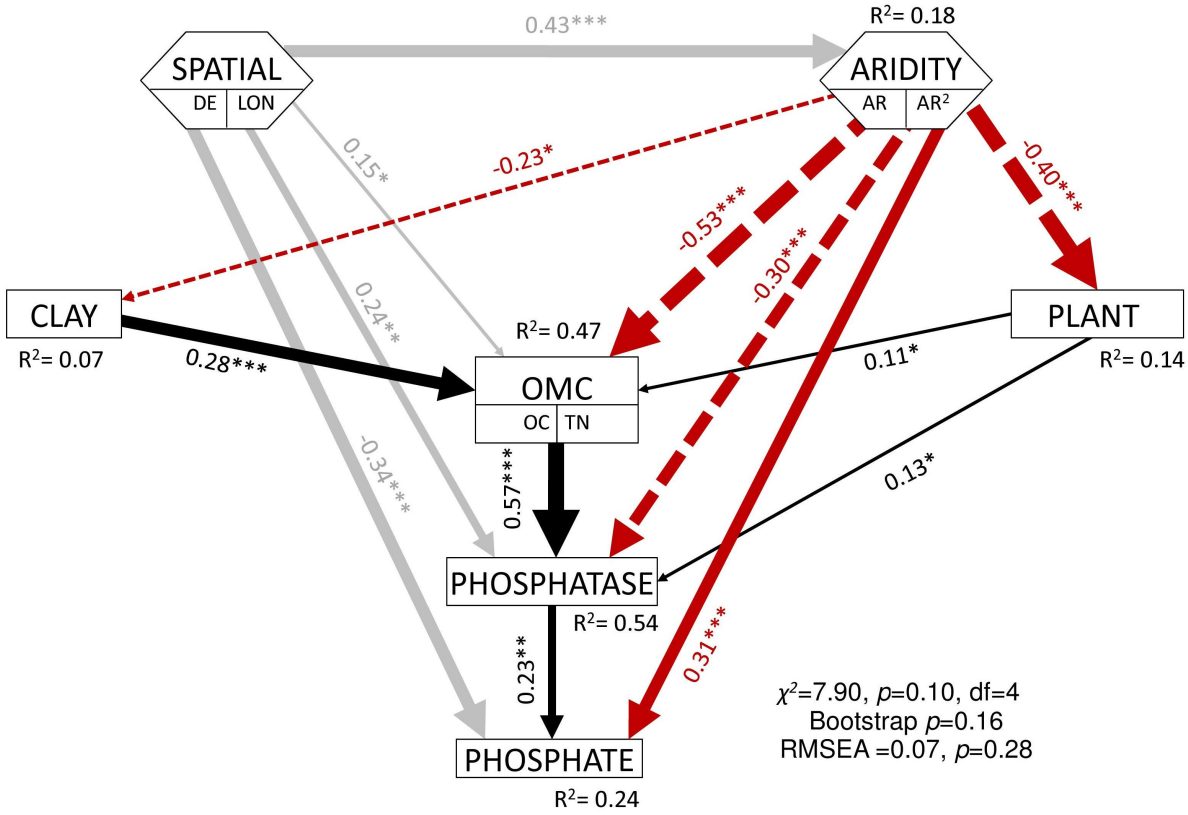

Figure 2. *Global structural equation model, depicting the effects of aridity (ARIDITY, associated paths in red), percentage of clay (CLAY), plant cover (PLANT), and the spatial co-ordinates of the study sites (composed by distance from equator [DE] and longitude [LON], associated paths in grey) on the organic matter component (OMC, first axis from a principal component analysis conducted with soil organic carbon [OC] and total nitrogen [TN], see Appendix S1), the concentration of phosphate and the activity of phosphatase. Numbers adjacent to arrows are standardized path coefficients, analogous to regression weights, and indicative of the effect size of the relationship. Continuous and dashed arrows indicate positive and negative relationships, respectively. The width of arrows is proportional to the strength of path coefficients. The proportion of variance explained (R^2) appears above every response variable in the model. Goodness-of-fit statistics for each model are shown in the lower right corner. There are some differences between the a priori model and the final model structures owing to removal of paths with coefficients close to zero (see a priori model in Supplementary Fig. S1); these modifications make only trivial changes in the models. Hexagons are composite variables. Squares are observable variables. Significance levels are as follows: *P < 0.05, ** P < 0.01 and *** P < 0.001. See Appendices S2 y S3 for further statistical details and rationale on the model used.*

Our findings suggest that predicted increases in aridity in drylands globally[2,3,4] will lead to severe nutrient depletion[7] in these environments, particularly in the most arid sites. For example, the increasing limitation of N in drylands, which are already poor in nutrients[5,16], could further inhibit N mineralization in soils because of the severe competition between plants and microorganisms for N, potentially leading to a negative feedback on nutrient availability[21] (Supplementary Fig. 2, 3). The observed decrease in the N:P ratio with increasing aridity is similar to what would be expected during long-term ecosystem development[6,7]. Although this progression has been described at the geological time scale (thousands to millions of years), and changes in aridity occur at the ecological time scale (tens to thousands of years), both processes may share the same biogeochemical signatures, such as N and P accumulation mediated by shifts in the relative importance of biological and geochemical processes[6,7]. Thus, in more extreme arid systems, phosphate accumulates by geochemical processes, but N is only slowly incorporated by N fixation[6,7], being also limited by low C (energy) availability[1,15]. In the less arid sites (dry-subhumid ecosystems), where N and C are already available for plant and microorganisms, P becomes available through the activity of extracellular phosphatase enzymes (which require N investment)[7], coupling P availability to biological processes[22].

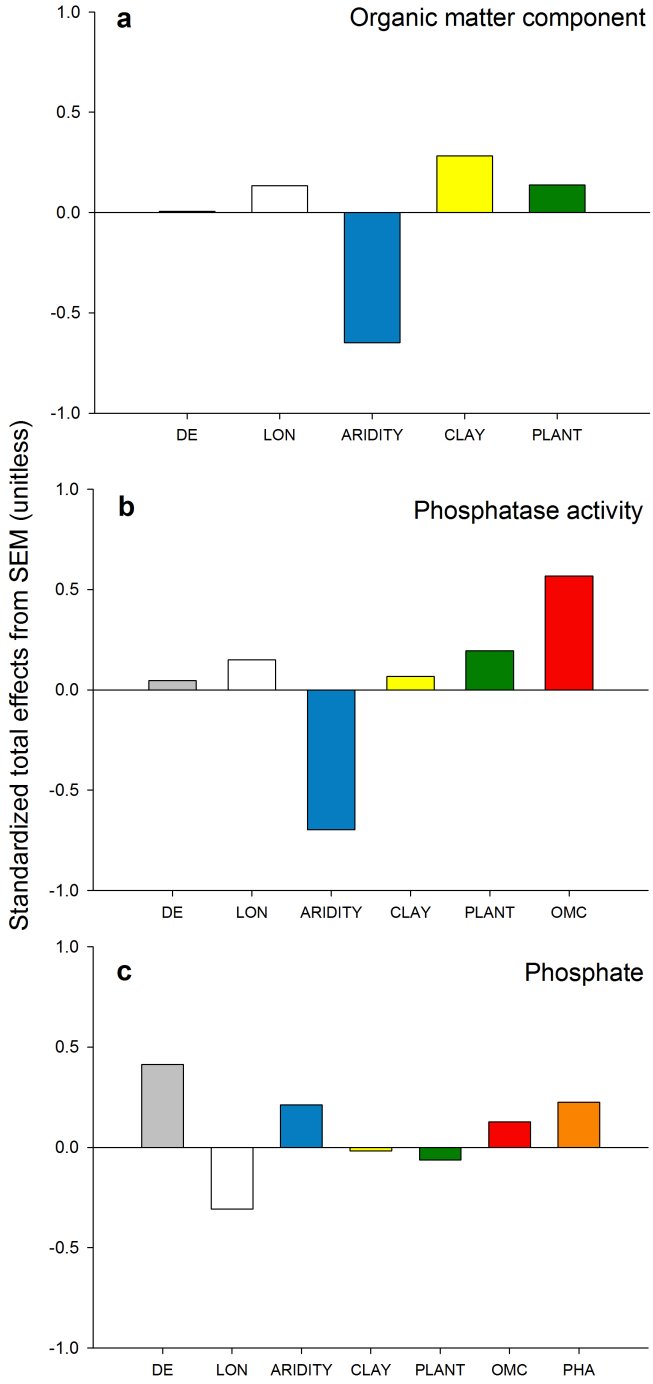

Figure 3. *Standardized total effects (direct plus indirect effects) derived from the structural equation modeling, including the effects of aridity (ARIDITY), percentage of clay (CLAY), plant cover (PLANT), distance from equator (DE) and longitude (LON) on the organic matter component (OMC, first axis from a principal component analysis conducted with soil organic carbon and total nitrogen, see Appendix S1), phosphate and phosphatase activity.*

Predicted increases in aridity will also likely reduce plant cover in drylands (Fig. 2)[17], favoring the dominance of physical (e.g wind-blown sands that abrade exposed rock surfaces)[19] over biological (i.e. litter decomposition or N fixation) processes, distorting soil C, N and P cycles[1].

Carbon and N become uncoupled with P in response to increasing aridity (Fig. 1). Under arid and semi-arid conditions, the limited availability of labile C and available N may unbalance C, N, and P availability, constraining plant and microbial activity and diversity[1,15]. This may have an important negative effect on primary production and organic matter decomposition[1,14,22], even when P is available. In addition, the observed decrease in phosphatase activity and the increase of P observed in coarser, sandier soils with increasing aridity suggests that key abiotic and biotic processes, such as soil formation and organic matter decomposition, may be reduced with increasing aridity[19]. The decrease in the C:N ratio with increasing aridity observed here agrees with experimental studies showing that drought periods decouple C and N cycles in drylands[23]. While organic C and total N are strongly correlated in the studied sites (Pearson´s r = 0.92), these results suggest that future climatic conditions may promote N losses in drylands, particularly if increases in aridity reduce vegetation cover in these ecosystems (Fig. 2). The imbalance observed in the C, N and P cycles with increasing aridity may have other important consequences for drylands. For example, an increase in aridity that reduces N availability will limit the capacity of plant primary productivity to buffer human-induced increases in atmospheric CO_2 concentrations, because the rate of photosynthesis is proportional to the amount and activity of the N-rich enzyme ribulose bisphosphate carboxylase oxygenase in leaves[1,23]. This may contribute to a warmer world by the end of the 21st century, by limiting the capacity of plants and microorganisms to fix CO_2 derived from human activities[1,15,24]. In addition, decreases in the supply of N relative to that of P may have a short-term effect by differentially constraining the growth rates of plant species based on their stoichiometry[15,25]. Such reductions may also have long-term evolutionary effects by selecting plants and microorganisms with different levels of N in their nucleotides, potentially altering ecosystem structural and functional traits[15,26].

Our results indicate that biogeochemical cycles in drylands can be particularly fragile in the face of rapid climate change, especially in the transition areas from semi-arid to arid climates. Carbon, N and P availability appeared to be more resistant to changes in aridity in the transition from dry-subhumid to semi-arid than in that from semi-arid to arid areas, where we observed substantial and abrupt declines in organic C and total N, but an increase in phosphate (Fig. 1a, c, e). Similarly, we observed the same abrupt decrease in N:P and C:P ratios from semi-arid to arid sites, which was not observed in the C:N ratio (Fig. 1b, d, f). Evaluation of critical global transitions and tipping points are of major importance to assess the effects of global change on ecosystems, and is an active area of current research[27]. The abrupt changes observed in the C:P and N:P ratios in the transitions from semi-arid to arid climates, together with the predicted increase in the proportion of

global drylands considered as arid[2], may force these systems into a long process towards the recovery of ecosystem stoichiometry.

This is the first global empirical study relating aridity to the decoupling of C, N and P biogeochemical cycles in drylands. Together, our findings suggest that the predicted increase in aridity across drylands will reduce the concentration of biologically-controlled C and N, but will increase that of P, whose primary source is rock weathering. These changes will interrupt the C, N and P cycles in drylands in a non-linear manner, and may have important impacts on biogeochemical reactions controlling key ecosystem functions (e.g., primary production, respiration and decomposition) and services from local to global scales[1].

METHODS SUMMARY

Field data were collected from 224 dryland sites located in 16 countries from all continents except Antarctica (see Maestre et al.[28] for full details on the study sites sampled). Locations for this study were chosen to represent a wide spectrum of abiotic (climatic, soil type, slope) and biotic (type of vegetation, total cover, species richness) features characterizing drylands worldwide. At each site, we established a 30 m × 30 m plot representative of the dominant vegetation. Within each plot, we measured plant cover using the line-intercept method along four 30 m-long transects separated 8 m from each other[28]. Soils were sampled using a stratified random procedure. At each plot, five 50 cm × 50 cm quadrats were randomly placed under the canopy of the dominant perennial plant species and in open areas devoid of perennial vegetation, and a composite sample (0-7.5 cm depth) was obtained from each of them (10-15 soil samples per site were collected, over 2600 samples were collected and analyzed in total). After field collection, soil samples were taken to the laboratory, where they were sieved, air-dried for one month and stored for laboratory analyses. The % of clay, the concentration of organic C, total N and phosphate, and the activity of phosphatase were determined as described in Appendix S1. All these variables were then averaged to obtain site-level estimates by using the mean values observed in bare ground and vegetated areas, weighted by their respective cover at each site.

We first explored the relationship of aridity with the different selected C (organic C), N (total N) and P (phosphate) variables and with the N:P, C:P and N:C ratios by using either lineal or curvilinear regressions (Appendix S3). We used as our surrogate of aridity the inverse of the aridity index (AI = P/PET[18]), which was gathered from the Worldclim global database[29] (Appendix S3). We then used structural equation modeling[30] to examine the relative importance of aridity on the organic C, total N, activity of phosphatase and phosphate (*a priori* model in Supplementary Fig. 1).

Because organic C and total N were very closely related, we could not introduce them into the same model without risking collinearity. We reduced these two variables to a single variable using principal components analysis (PCA) on the correlation matrix, then introduced the first axis of this PCA (which was highly related to organic C and total N; Pearson´s r = 0.98) as a new variable into the model (organic matter component; see Appendix S3).

We evaluated the fitting of our data to the *a priori* model (Supplementary Fig. 1) using the Chi-square test and root mean square error of approximation (RMSEA); as the residuals of some data were not normally distributed, we confirmed fit using the Bollen-Stine bootstrap test (Fig. 1). See Appendices S2 and S3 for the rationale on the selected variables and complete details of the statistical analyses conducted.

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Author Contributions

F.T.M., M.D.B and A.G designed this study. F.T.M. coordinated all field and laboratory operations. Field data were collected by all authors except A.E., A.G., B.G., E.V., M.B. and M.D.W. Laboratory analyses were done by V.O., A.G., M.B., M.D.B., E.V., and B.G. Data analyses were done by M.D.B and M.A.B. The paper was written by M.D.B, F.T.M., M.D.W and A.G, and the remaining authors contributed to the subsequent drafts.

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Supplementary materials

Appendix S1. Selection of soil C, N and P surrogates

The biogeochemical cycles of carbon (C), nitrogen (N), and phosphorus (P) are interlinked with primary production, respiration, and decomposition in terrestrial ecosystems[1-3]. All these nutrients have a large variety of forms in soils, including different qualities (labile and recalcitrant) and chemistries (organic and inorganic)[2,4,5]. Only some of them, however, are available for plants and microorganisms[2,6]. Because of the importance of the biogeochemical cycles of C, N and P on the plant and microorganism stoichiometries[1,7,8], we focused our study on the available nutrient forms for these organisms. Thus, we selected total organic C as our surrogate of the C cycle because we observed that it is a parsimonious summary of labile C sources available to soil microorganisms, as this variable is strongly correlated with the availability of other labile C sources such as dissolved carbohydrates (Pearson´s r = 0.59, $P < 0.001$) and phenols (r = 0.55, $P < 0.001$)[4,9] at the studied sites. Similarly, total N was selected as our N cycle surrogate because of its relationship to other N forms available for plants and microorganisms, such as dissolved inorganic-N (r = 0.40, $P < 0.001$) and amino acids (r = 0.41, $P < 0.001$)[2,5,6]. Finally, we selected phosphate because it is a universal source of P for plants and microorganisms, because it plays a key role in the processes of C and N fixation in drylands, and because of its non-biological primary origin, being its main source primary minerals in rocks and sediments[1,3,8,10-14]. In addition, in a subset of 37 from our 224 sites, including soils from Australia, Chile, Mexico and Spain, phosphate concentration was positively related to total P concentration (r = 0.53, $P < 0.001$). To complete our information on the P cycle, we measured the activity of phosphatase, which is the enzyme responsible for releasing phosphate from organic sources, and is considered a surrogate of biological P demand[13,14].To avoid problems associated with the use of multiple laboratories when analyzing the soils from different sites, and to facilitate the comparison of results between them, dried soil samples from all the countries were shipped to Spain for analyses.

All the analyses for organic C, available P, total P and phosphatase activity were carried out at the laboratory of the Biology and Geology Department, Rey Juan Carlos University (Mostoles, Spain). Analyses of total N were carried out at the University of Jaen (Jaen, Spain). The remaining soil analyses were carried out at the laboratory of the Department of Physical, Natural and Natural Systems, Pablo de Olavide University (Seville, Spain). Organic C was determined by colorimetry after oxidation with a mixture of potassium dichromate and sulfuric acid[15]. Total N was obtained using a CN analyzer (Leco CHN628 Series, Leco Corporation, St Joseph, MI, USA). Total P was

measured using a SKALAR San++ Analyzer (Skalar, Breda, The Netherlands) after digestion with sulphuric acid. Available P was measured following a 0.5M $NaHCO_3$ (pH: 8.5) extraction[16]. Soil extracts in a ratio of 1:5 were shaken in a reciprocal shaker at 200 rpm for 2 h. An aliquot of the centrifuged extract was used to the colorimetric determination of P inorganic available (PO_4^{-3}), based on the reaction with ammonium molybdate and development of the "Molybdenum Blue" color[17]; the pH of the extracts was adjusted with 0.1N HCl when necessary. Phosphatase activity was measured by determination of the amount of *p*-nitrophenol (PNF) released from 0.5 g soil after incubation at 37°C for 1 h with the substrate *p*nitrophenyl phosphate in MUB buffer (pH 6.5). The remaining soil variables were measured from K_2SO_4 0.5 M soil extracts in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20°C and filtered to pass a 0.45-μm Millipore filter. The filtered extract was kept at 2°C until colorimetric analyses, which were conducted within the 24 h following the extraction. Sub-samples of each extract were taken for measurements of dissolved phenols, carbohydrates (sum of hexoses and pentoses) and aminoacids according to Chantigny *et al.* (9). Inorganic-N (sum of ammonium and nitrate) concentrations were also measured for each K_2SO_4 0.5 M extract subsample by following the indophenol blue method as described in Delgado-Baquerizo *et al.* (18).

Appendix S2. Structural equation modeling approach: rationale of the variables included

Aridity is a fundamental driver of biological and geochemical processes[3,19-24] in arid, semi-arid, and dry-subhumid ecosystems (areas with a precipitation/potential evapotranspiration ratio below 0.65[19], hereafter drylands), where water availability is the most limiting resource[19-24]. Aridity modulates water availability, and as such has a substantial impact on factors such as plant productivity, microbial activity[25-26], nutrient concentration and soil enzyme activities[3,19-24]. Aridity has both direct and indirect impacts on ecosystem services, and on ecosystem processes directly related to ecosystem functioning[20,22]. For example, increasing aridity in drylands has been observed to decrease vegetation cover[22], indirectly promoting soil erosion by wind[19], which can subsequently lead to land degradation and desertification[19,20]. Wind erosion can remove silt, clay, and organic matter from the surface soil, leaving behind sand and infertile materials[19]. In addition, aridity promotes soil drying, increasing its salinity levels and enhancing soil erosion, which remove fine, nutrient-rich particles such as clay[19,20,23,24].

The cover of perennial vascular plants is also a key driver of ecosystem structure and functioning in drylands, as this variable largely determines processes such as plant facilitation, litter production and decomposition, and biological N fixation, as well as the ability of landscapes to

retain water and nutrients[27-33]. Therefore, plant cover is closely related to nutrient availability in dryland soils[3,27-30].

Clay plays an important role on the retention of water and nutrients at the soil surface, where microbial activity is greatest, and can also modify local pH[19,33-38]. The activity of phosphatase was included in our structural equation model (SEM) because extracellular enzymes are proximate agents of organic matter decomposition, and their assessment can be used as indicators of microbial nutrient demand[13,14]. The activity of extracellular enzymes, which are produced by both plants and microorganisms[13,14], is known to be negatively affected by factors linked to aridity such as low water availability and soil salinity[13,14,38-40]. Enzyme activities have been observed to be associate with clay abundance in soil[38-39]. Phosphatase is the enzyme responsible for releasing phosphate from organic sources, and is considered a surrogate of biological P demand[13,14]. Phosphate is a universal source of P for plants and microorganisms[14]. As biochemical energy (ie ATP, nicotinamide adenine trinucleotide), P has a major role in the C and N fixation in drylands, and is non-biological in origin, being derived from rocks and sediments[1]. Carbon and N are primarily linked to biological processes such as photosynthesis, atmospheric N fixation and subsequent microbial mineralization[3], and are considered as key elements for enzyme production[3,13]. Finally, we included the spatial location (latitude and longitude) of each site in our structural equation model (Fig. S1) to account for the spatial autocorrelation present in our data (see Maestre *et al.*[41] for a related approach).

Appendix S3. Statistical and numerical analyses

We first explored the relationship of aridity with the different selected C (organic C), N (total N) and P (phosphate) variables, and with the N:P, C:P and N:C ratios, by using either lineal or curvilinear (quadratic) regressions. Among these, the function that minimized the Second-order Akaike Information[42] was chosen in each case. All the nutrient ratios were log transformed to achieve normality before conducting these analyses. The aridity index (AI = precipitation/potential evapotranspiration)[19-20] of each site was calculated using data interpolations provided by Worldclim[43-44]. To facilitate the interpretation of our results, we used the inverse of the aridity index (1-AI) as our surrogate of aridity. This index increases with decreasing annual mean precipitation in our database (Pearson´s r=0.91, $P < 0.001$).

To determine the relative importance of aridity on the selected soil nutrients differentially linked to biological (C and N) versus geochemical (P) control, we used Structural Equation Modeling (SEM)[45]. Overall, SEM has emerged as a synthesis of path analysis, factor analysis, and

maximum-likelihood techniques, and has been thoroughly used in the ecological sciences a causal inference tool[45-47]. It can test the plausibility of a causal model, which is based on *a priori* information regarding the relationships among the particular variables of interest. Some data manipulation was required prior to modeling. We checked the bivariate relationships between all variables to ensure that a linear model was appropriate. We identified some curvilinear relationships among our variables. Several variables showed a curvilinear relationship with latitude, such that areas more close to the equator tended to be different from areas farther from the equator. This was simply handled by expressing latitude as distance from the equator (i.e. the absolute value of latitude). Because longitude has an arbitrary 0, this transformation did not apply to longitude. We found that organic C, total N and the activity of phosphatase were curvilinearly influenced by aridity, and that these relationships were well described by a second-order polynomial. In order to introduce polynomial relationships into our model, we calculated the square of aridity and introduced it into our model using a composite variable approach described below. We also examined the distributions of all of our endogenous variables, and tested their normality. Clay, organic C, total N, activity of phosphatase and phosphate were log-transformed to improve normality. Because organic C and total N were very closely related (r = 0.92), we could not introduce them into the same model without risking collinearity. Attempts to construct a latent variable including organic C and total N were not successful. Thus, we reduced these two variables to a single variable using principal components analysis on the correlation matrix, then introduced this new variable into the model (organic matter component). We interpret this variable as organic C and N, as both variables were very highly correlated with the PCA axis (Pearson´s r= 0.98); although some of the total N is certainly in mineral form[46], this very close relationship indicates that total N is under tight control of organic matter in the studied drylands. Then, we established an *a priori* model (Fig. S1), based on the known effects and relationships among the drivers of C, N and P availability, which are explained in Appendix S2. This model included: spatial structure (latitude and longitude), aridity, percentage of plant cover and clay, organic matter component (total N and organic C), activity of phosphatase and phosphate (Fig. S1).

When these data manipulations were complete, we parameterized our model using our dataset and tested its overall goodness of fit. There is no single universally accepted test of overall goodness of fit for structural equation models, applicable in all situations regardless of sample size or data distribution. Most modelers circumvent this problem by using multiple goodness of fit criteria. We used the Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when $RMSEA\ 0 \leq RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$). Additionally, and because some variables were

not normal, we confirmed the fit of the model using the Bollen-Stine bootstrap test (the model has a good fit when $0.10 <$ bootstrap $P \leq 1.00$)[48]. Our *a priori* model attained an acceptable fit by all criteria, and thus no post hoc alterations were made.

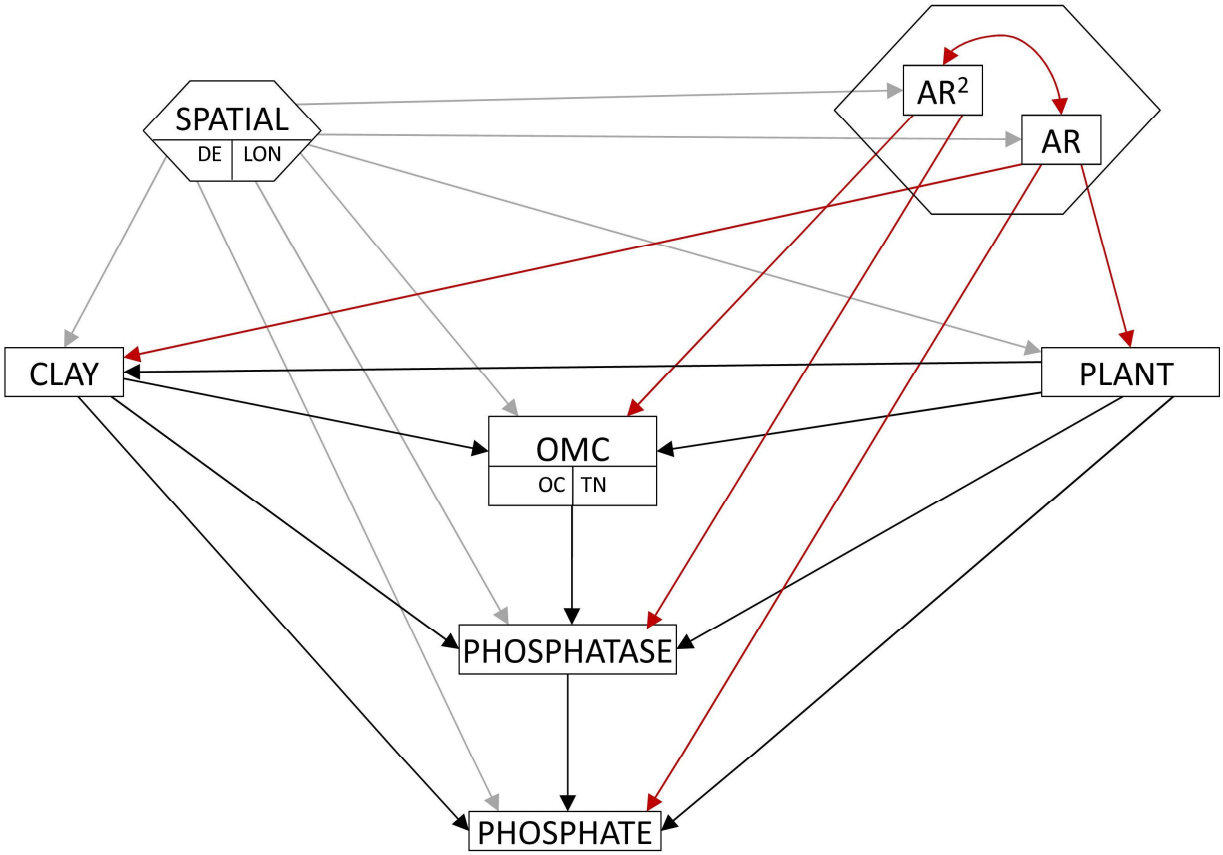
After attaining a satisfactory model fit, we introduced composite variables into our model. The use of composite variables does not alter the underlying SEM model, but collapses the effects of multiple conceptually-related variables into a single composite effect, aiding interpretation of model results[45]. Distance from the equator and longitude were included as a composite variable, because together they determine the spatial proximity of plots. A separate composite was constructed for each response variable. We also used composite variables to model the non-linear response of the organic matter component (first axis from a PCA with organic C and total N) and phosphatase activity to aridity. As previously mentioned, both aridity and its square are introduced as variables in the model (Fig. S1). Because one is mathematically derived from the other, they are allowed to covary. In cases where a non-linear fit is desired, the effects of aridity and aridity square on a given response are composited. The resulting effect has no interpretable sign, because the relationship may be positive over some portion of the data and negative over other portions. In cases, where a simple linear effect of aridity was desired (e.g. phosphate), we simply included a single path from aridity and did not invoke aridity square (Fig. S1).

With a reasonable model fit, and composite variables constructed, we were free to interpret the path coefficients of the model, and their associated *P* values. A path coefficient is analogous to a partial correlation coefficient, and describes the strength and sign of the relationships between two variables[45]. Since some of the variables introduced were not normally distributed, the probability that a path coefficient differs from zero was tested using bootstrap tests[44]. Bootstrapping is preferred to the classical maximum-likelihood estimation in these cases, because in bootstrapping, probability assessments are not based on an assumption that the data match a particular theoretical distribution. Thus, data are randomly sampled with replacement in order to arrive at estimates of standard errors that are empirically associated with the distribution of the data in the sample[45].

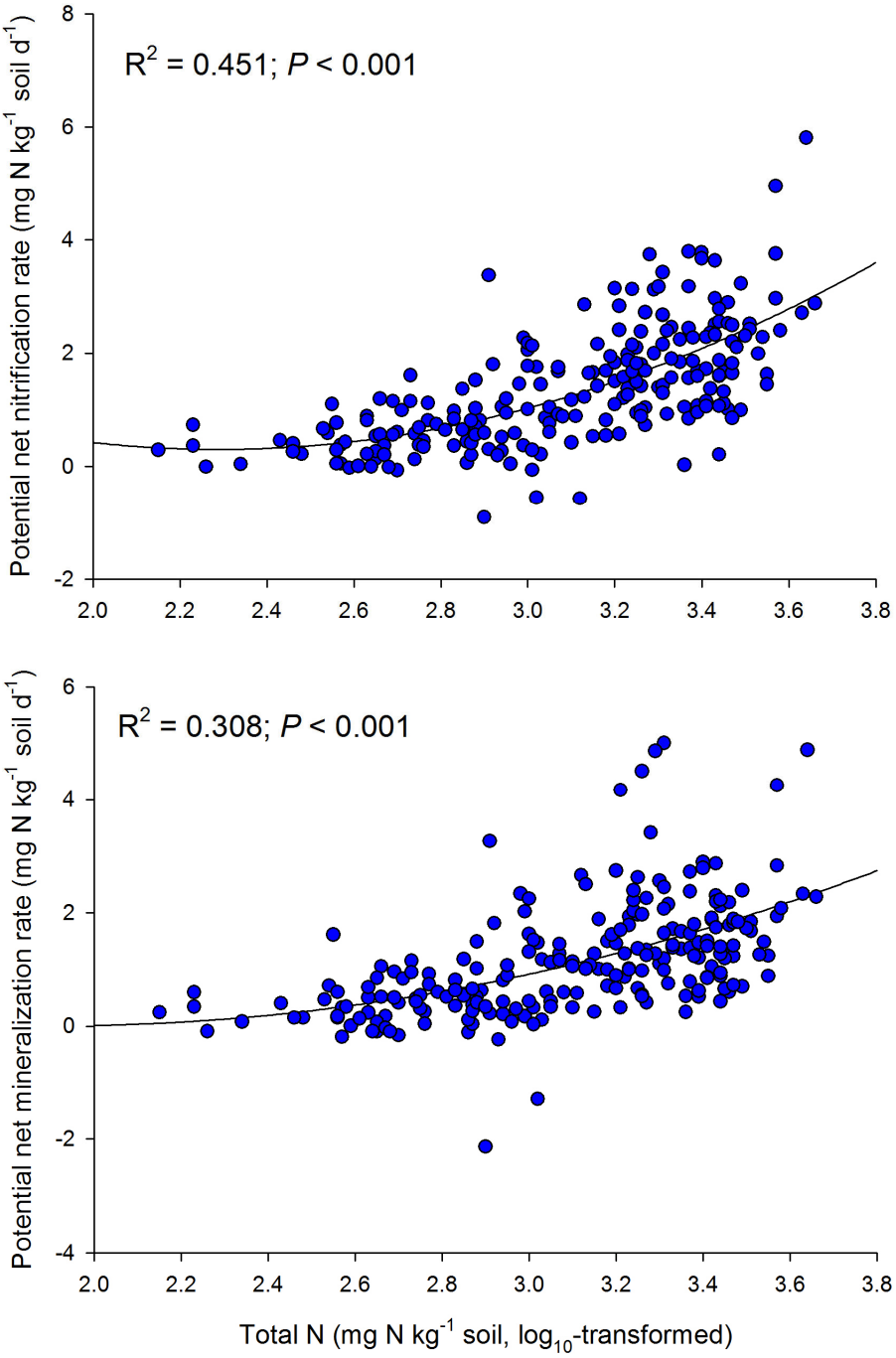
Another important capability of SEM is its ability to partition direct and indirect effects that one variable may have on another, and estimate the strengths of these multiple effects. Unlike regression or ANOVA, SEM offers the ability to separate multiple pathways of influence and view them as a system[45-46]. Thus, SEM is useful for investigating the complex networks of relationships found in natural ecosystems[46].

To aid final interpretation in light of this ability of SEM, we calculated the standardized total effects of aridity, % of clay and plant cover, spatial (distance to equator and longitude) on the organic matter component, but also the effect of organic matter component on phosphatase and phosphate activity. The net influence that one variable has upon another is calculated by summing all direct and indirect pathways between the two variables. If the model fits the data well, the total effect should approximate be the bivariate correlation coefficient for that pair of variables[45-46].

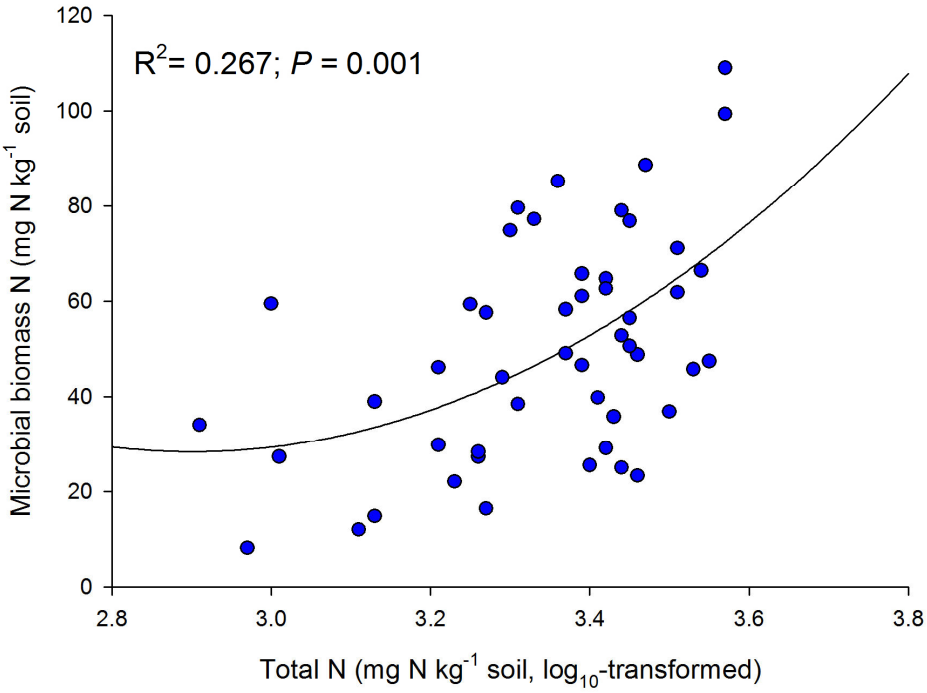
All the SEM analyses were conducted using AMOS 18.0 (Amos Development Co, Crawfordville, FL, USA). The remaining statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).



Supplementary figure 1. *A priori structural equation model (SEM) used in this study. We included in this SEM aridity (AR, compositive variable formed by AR and AR^2), plant cover (in %, PLANT), percentage of clay (CLAY), distance from equator (DE) and longitude (LON) as a composite variable (SPATIAL), activity of phosphatase, organic matter component (OMC, first component from a PCA conducted with organic C [OC] and total N [TN]) and phosphate. We built our SEM by taking into account all these relationship, as explained in Appendix S2. There are some differences between the a priori model and the final model structures owing to removal of paths with coefficients close to zero (see Fig. 1). Hexagons are composite variables. Squares are observable variables.*



Supplementary figure 2. *Relationships between total N and the potential net nitrification (upper graph) and mineralization rates (lower graph) measured at our study sites. Air-dried soil samples were re-wetted to reach 80% of field water holding capacity and incubated in the laboratory for 14 days at 30º C (49). Potential net nitrification and ammonification rates were estimated as the difference between initial and final nitrate and ammonium concentrations, respectively by following Delgado-Baquerizo and Gallardo (50). The solid line denotes the quadratic model fitted to the data (R^2 and p values shown in each panel).*



Supplementary figure 3. *Relationships between the total N and microbial biomass N in a subset of 49 from our 224 sites. All air-dried soil samples were adjusted to 55% of their water-holding capacity previous to the analyses of microbial biomass N as described in Zornoza et al. (51). Microbial biomass N was determined using the fumigation-extraction method following Brookes et al. (52). Non-incubated and incubated soil subsamples were fumigated with chloroform for 5 days. Non-fumigated replicates were used as controls. Fumigated and non-fumigated samples were extracted with K_2SO_4 0.5 M in a ratio 1:5 and filtered through a 0.45-µm Millipore filter. Microbial biomass N concentration was estimated as the difference between total N of fumigated and unfumigated digested extracts as described in Delgado-Baquerizo et al. (53) and then divided by a Kn (fraction of biomass N extracted after the CHC_{13} treatment) of 0.54[52]. The solid line denotes the quadratic model fitted to the data (R^2 and p values shown in the graph).*

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4. BLOQUE II: ESCALA REGIONAL

CAPÍTULO 2

Aridity modulates N availability in arid and semiarid Mediterranean grasslands

Manuel Delgado-Baquerizo, Fernando T. Maestre, Antonio Gallardo, José L. Quero, Victoria Ochoa, Miguel García-Gómez, Cristina Escolar, Pablo García-Palacios, Miguel Berdugo, Enrique Valencia, Beatriz Gozalo, Zouhaier Noumi, Mchich Derak, Matthew D. Wallenstein.

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Abstract

While much is known about the factors that control each component of the terrestrial nitrogen (N) cycle, it is less clear how these factors affect total N availability, the sum of organic and inorganic forms potentially available to microorganisms and plants. This is particularly true for N-poor ecosystems such as drylands, which are highly sensitive to climate change and desertification processes that can lead to the loss of soil nutrients such as N. We evaluated how different climatic, abiotic, plant and nutrient related factors correlate with N availability in semiarid *Stipa tenacissima* grasslands along a broad aridity gradient from Spain to Tunisia. Aridity had the strongest relationship with N availability, suggesting the importance of abiotic controls on the N cycle in drylands. Aridity appeared to modulate the effects of pH, plant cover and organic C (OC) on N availability. Our results suggest that N transformation rates, which are largely driven by variations in soil moisture, are not the direct drivers of N availability in the studied grasslands. Rather, the strong relationship between aridity and N availability could be driven by indirect effects that operate over long time scales (decades to millennia), including both biotic (e.g. plant cover) and abiotic (e.g. soil OC and pH). If these factors are in fact more important than short-term effects of precipitation on N transformation rates, then we might expect to observe a lagged decrease in N availability in response to increasing aridity. Nevertheless, our results suggest that the increase in aridity predicted with ongoing climate change will reduce N availability in the Mediterranean basin, impacting plant nutrient uptake and net primary production in semiarid grasslands throughout this region.

Key words: pH; Organic C; N mineralization; N transformation rate; C:N ratio

Introduction

Nitrogen (N) is, after water, the most important factor limiting plant growth, net primary production and microbial decomposition in drylands [1-2]. Thus, it is important to develop a predictive understanding of the factors controlling the amount of available N within an ecosystem, which includes both the inorganic (nitrate and ammonium) and organic forms that can be assimilated by plants and microorganisms [3-4]. The amount of available N in an ecosystem is controlled by the cumulative effects of microbially-driven N inputs through N-fixation, by the mineralization of N from organic matter, and by N-losses through leaching and gas emissions [5-6].

Although temperature and moisture are strong drivers of N transformation rates in soils [7-8], the net effect of climate on N availability remains unclear [5-6]. For example, N mineralization

and decomposition increase with temperature and soil moisture, but high rainfall levels may also enhance denitrification by promoting anaerobic conditions and nitrate leaching [5, 8, 9-10]. Some studies have shown that increases in mean annual precipitation enhance the concentration of available N in the field [11-12], but others have found the opposite [9-10], or have reported inconsistent responses [13-14]. Studies focusing on the effects of temperature on N dynamics have also shown inconsistent results [2]. Some experiments show that increases in temperature enhance N availability [8, 15], but others found no significant effects of temperature on the concentration of N in soils [16]. The contradictory results observed to date may be due to differences in plant species, soil types and ecosystems studied, which may determine the effects of temperature and precipitation on soil N availability.

Environmental variables such as plant cover, soil pH, texture and organic matter are also major drivers of N cycling [6, 12, 17]. The uptake of N by plants, as well as the litter and root exudates they produce, affects N concentration in soils [2, 18]. Sandy soils typically exhibit lower denitrification rates, but higher N losses after rainfall events, than clay soils [5, 19]. Plants typically increase the amount of litter mass and soil water moisture under their canopies compared to adjacent bare soil microsites, affecting N mineralization and depolymerization processes [20-21]. Acidic soil pH and high organic C concentrations may favor fungal communities, which are involved in depolymerization and decomposition processes, increasing the amount of available N [6, 19, 22]. Finally, the C:N ratio modulates the immobilization and mineralization of N [3, 22].

Despite the emergence of new paradigms for soil N cycling that emphasize the importance of both organic and inorganic N forms [3-4, 18], and the growing literature on the topic, we lack an integrated understanding of the most important determinants of N availability. This is particularly true for N-poor ecosystems such as drylands (arid, semiarid and dry-subhumid ecosystems; [22-24]). In addition, these areas are more open in terms of their N cycling relative to more humid systems, where most studies have been performed, because they support high N losses relative to N turnover [25]. Drylands are of paramount importance for the Earth system, as they cover about 41% of Earth's land surface and support over 38% of the total global human population [26]. Improving our knowledge of the factors driving N availability in drylands can greatly enhance our ability to understand how ongoing climate change may affect their functioning [17], and can help to establish "early warning" signals of the onset of desertification processes [26], which often start with the loss of soil nutrients such as N [27].

This study had two main objectives. First, we aimed to evaluate the relationships between N availability (measured as the sum of extractable inorganic and organic N forms) and climatic (aridity), abiotic (soil pH and sand content), plant (average interdistance between plant patches) and nutrient related (organic carbon, N mineralization rate, N transformation rate and the C:N ratio) factors in arid and semiarid grasslands dominated by the tussock grass *Stipa tenacissima* L. along a broad aridity gradient from Spain to Tunisia. They are one of the most widespread and representative ecosystems of the semiarid regions of the Mediterranean basin [28], which is considered highly vulnerable to climate change and desertification [29]. Our second objective was to evaluate whether *S. tenacissima* tussocks modify the relative importance of climate, abiotic, plant and nutrient related factors as potential modulators of N availability. This species is able to create "resource islands" [20; 30-31] and modify microclimate, infiltration and soil water availability dynamics [32-34] as compared to adjacent bare ground areas. As such, they can potentially alter how climate and other factors affect N availability.

Methods

Study area

This study was carried out in 22 sites located in Spain, Morocco and Tunisia (See map S1), which cover most of the geographic distribution range of *S. tenacissima* grasslands in the Mediterranean basin [28]. All of the sites were located in areas of arid or semiarid climate; total annual precipitation and mean temperature ranged from 141 mm to 465 mm, and from 12.5ºC to 20ºC, respectively (Table S1). Slope and elevation ranged between 1º and 22º, and between 172 m a.s.l. and 1427 m a.s.l., respectively (Table S1). All sites were located on calcareous soils, and on south-facing slopes. Perennial vegetation cover ranged between 8% and 64%, and was in all cases an open steppe dominated by *S. tenacissima*, with shrub species such as *Quercus coccifera* L., *Rosmarinus officinalis* L. and *Thymus vulgaris* L. in Spain, *Cistus clusii* and *Helianthemum apenninum* L. (Mill.) in Morocco, and *Artemisia herba-alba Asso* and *Hammada scoparia* (Pomel) Iljin in Tunisia.

Sampling design and measurements

At each site, we established a 30 m × 30 m plot representative of the dominant vegetation. In the upper left corner of each plot, we located a 30 m transect oriented downslope for the vegetation survey. Three parallel transects of the same length, spaced 8 m apart across the slope, were added. Along each transect, we collected a continuous record of vegetated patches and bare ground areas

(i.e. devoid of vascular vegetation). From these transects, we obtained total plant cover, the average interdistance between plant patches and the number of plant patches per 10 m of transect. Vegetation surveys were carried out during 2005 and 2006 (Spain) and in 2010 (Morocco and Tunisia).

We sampled soils in Spain and North Africa (Morocco and Tunisia) during 2006 and 2010, respectively, using a stratified random procedure. Five 50 cm × 50 cm quadrats were randomly placed at each of two microsites: bare ground areas and *S. tenacissima* tussocks (Bare and Stipa microsites hereafter). A composite sample consisting of five 145 cm^3 soil cores (0–7.5 cm depth) was collected from each quadrat, bulked and homogenized in the field. After collection, soils were transported to the laboratory and air-dried at room temperature for four weeks. Previous studies have found that soil biochemical properties are minimally affected by air-drying in semiarid Mediterranean soils [35]. No specific permissions were required for our field activities, except for the site located in Djebel Bou-Hedma Biosphere Reserve and National Park (Tunisia). The required authorization to work at this site was obtained from the Ministère de l'Agriculture, Direction générale des forêts, Tunisia's authority that manages the Djebel Bou-Hedma Biosphere Reserve and National Park. The study did not involve handling or collection of endangered species.

Organic C (OC) was determined following Anderson and Ingramm [36]. Total N was measured with a CN analyzer (Leco CHN628 Series, Leco Corporation, St Joseph, MI, USA). Total available N was colorimetrically determined from K_2SO_4 extracts as the sum of ammonium, nitrate and dissolved organic nitrogen following Delgado-Baquerizo *et al.* [37]. Potential net N transformation (production of available N) and mineralization (production of inorganic-N) rates were measured by determination of the total available and mineral N before and after incubation in the laboratory at 80% of water holding capacity and 30ºC for 14 days [23]. Soil pH was measured in all the soil samples with a pH meter, in a 1: 2.5 mass: volume soil and water suspension. One composite sample each per microsite (Bare and Stipa) and plot were analyzed for sand, clay and silt content according to Kettler *et al.* [38].

The UNEP (1992) aridity index (AI = P/PET, where P is annual precipitation and PET is annual potential evapotranspiration) of each site was gathered from the Worldclim global database [39-41], as described in Maestre *et al.* [17]. The AI decreases as aridity increases, and to avoid confusing readers we used the inverse of AI in this work. Thus, aridity = 1-AI. This index is strongly related to both annual average rainfall (R^2=-0.98) and temperature (R^2=0.80) in our study sites.

Statistical analyses

Due to the low number of study sites as compared to number of climatic, abiotic, plant and nutrient variables studied, and the significant correlations between some of the variables from each group (Table S2), we reduced the dimensionality of our dataset by selecting one representative variable for each group (climatic, abiotic, plant and nutrient). We first conducted correlation analyses between the total available N and the different climatic (aridity), abiotic (pH and sand content), plant (total plant and bare cover, average interdistance between plant patches, plant patch area and number of plant patches per 10 m of transect) and nutrient (OC, C:N ratio, potential net mineralization and potential net N transformation rate) variables studied in both Stipa and Bare microsites (Table S3). We then retained for further analyses those variables significantly related to available N in both Stipa and Bare microsites. Aridity, pH and OC were retained as the climatic, abiotic and nutrient variables for subsequent analyses. All of the plant variables were significant related to N availability (Table S3). Thus, we carried out a principal component analysis (PCA) to unify these variables into a single plant component. The first component of this PCA (plant-ax1), which explained the 83.3% of the variance and was the only axis with an eigenvalue higher than 1 (4.167), was retained for further analyses. This component was negatively related to coverage of bare ground and the average plant patch interdistance ($r = -0.983$ and -0.889, respectively $P < 0.01$ in both cases), but positively related to the *Stipa tenacissima* coverage, plant patch area and number of plant patches per 10 m of transect, respectively ($r = 0.932$, 0.894 and 0.861, respectively, $P < 0.01$ in all cases).

With this reduced set of independent variables (aridity, pH, OC and plant-ax1), we then used a multi-model inference approach based on information theory and ordinary least squares regression [42] to evaluate their relative importance as drivers of N availability (dependent variable). We ranked all the models that could be generated with our independent variables according to the second-order Akaike information criterion (AIC_c), calculated as described in Fotheringham *et al.* [43]. The AIC_c of each model was then transformed to ΔAIC_c, which is the difference between AIC_c of each model and the minimum AIC_c found for the set of models compared. The ΔAIC_c values were also used to obtain the Akaike weights of each model (w_i), according to Burnham & Anderson [42]. Akaike's weights were also used to define the relative importance of each predictor across the full set of models evaluated by summing w_i values of all models that include the predictor of interest, taking into account the number of models in which each predictor appears [42]. As we were interested in evaluating whether *S. tenacissima* tussocks modified the importance

of the different drivers of N availability evaluated, multi-model analyses were carried out for Bare and Stipa microsites separately. These analyses were conducted with the software SAM 4.0 [44].

Aridity and OC, which were highly correlated, had a high explanatory power (Fig. 1) and importance (Fig. 2) among the different predictors of available N that we evaluated (Table S2). Thus, to tease out their relative importance as drivers of N availability, we used structural equation modeling [SEM, 45]). This tool has emerged as a synthesis of path analysis, factor analysis, and maximum likelihood techniques, and has been thoroughly used in the ecological sciences a causal inference tool [46-47]. It can test the plausibility of a directed, causal model like that proposed here. Another important capability of SEM is its ability to partition direct and indirect effects that one variable may have on another, and estimate the strengths of these multiple effects. Unlike regression or ANOVA, SEM offers the ability to separate multiple pathways of influence and view them as a system [45]. Thus, the use of SEM is useful for investigating the complex networks of relationships found in natural ecosystems [47]. Path coefficients were obtained using the maximum likelihood estimation technique. These coefficients are interpreted as the size of an effect that one variable exerts upon another. Because of our SEM was saturated (the number of degrees of freedom was zero), no probability level could be assigned to the chi-square statistic, making the model untestable. Thus, the free covariance weight between Aridity and the best solution was chosen through maximization of the maximum likelihood function. The goodness of fit of SEM models was checked following Schermelleh-Engel *et al.* [48]. SEM models were separately conducted for Bare and Stipa microsites with the software AMOS 20 (IBM SPSS Inc, Chicago, IL, USA).

Results

Soil characteristics changed consistently across the aridity gradient studied. Soil pH increased with increasing aridity for both Stipa and Bare microsites ($p<0.01$; Table S2). OC decreased with increases in aridity for both microsites ($p<0.01$; Table S2), but the soil C:N ratio was not related to aridity ($p>0.05$; Table S2). The cover of *Stipa*, and both the number and area of plant patches were negatively related to aridity ($p<0.01$; Table S2), while the average interdistance between consecutive plant patches and the cover of bare ground areas increased concomitantly with aridity ($p<0.01$; Table S2). Total N availability decreased with aridity for both Stipa ($p<0.01$) and Bare ($p<0.01$) microsites ($p<0.01$; Table S3; Fig.1). N availability was correlated with both soil and plant characteristics. For example, N availability was positively correlated with pH and OC in both Stipa and Bare microsites ($p<0.01$;Table S3; Fig.1). The relationship between N availability and sand content was only significant in the Stipa microsites ($p=0.01$; Table S3). We did not find a

significant relationship between N availability and the C:N ratio (Table S3). The plant-ax1 was positively related to N availability in both microsites ($p<0.01$; Table S3; Fig.1).

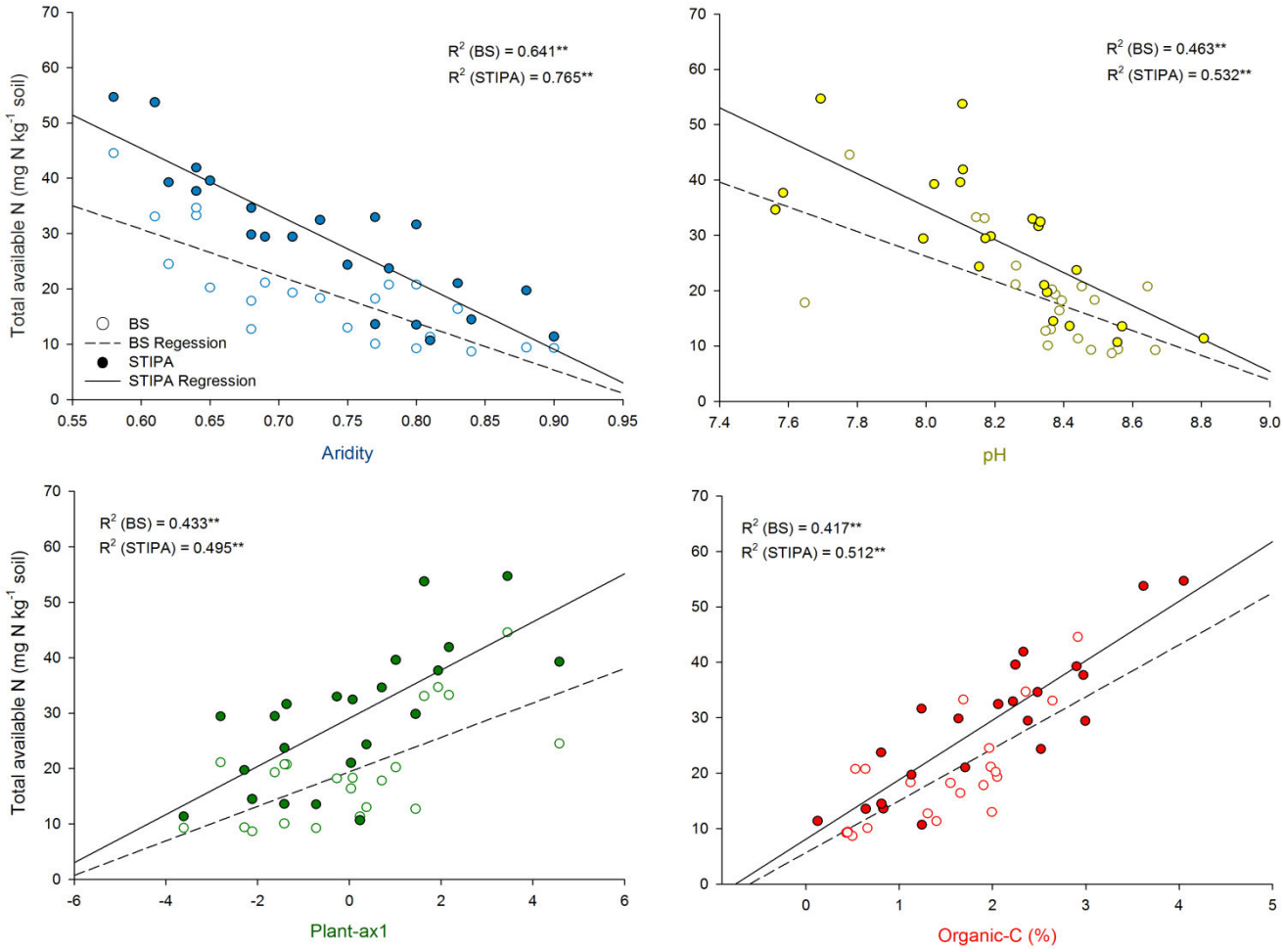

Figure 1. *Relationships between total nitrogen (N) availability and aridity, pH, plant-ax1 (first component of a PCA including the cover of bare and plant microsites, average plant patch interdistance, area of plant patches and number of plant patches per 10 m of transect) and organic carbon for both Stipa tenassicima (STIPA) and Bare ground (BS) microsites. Every data point is the average of five replicated soil samples. Significance levels are as follows: * $p < 0.05$,** $p < 0.01$ and *** $p < 0.001$.*

Total N availability did not appear to be driven by N supply rates, as the relationships between N availability and either potential net mineralization or N transformation rates were non-significant ($p>0.05$; Table S3). Multi-model analyses showed that aridity and OC were the most important variables affecting N availability. Aridity explained more of the variability in N availability in Bare microsites (Fig.2; Table 1), but OC was the most important predictor of N availability in Stipa microsites (Fig.2; Table 1).

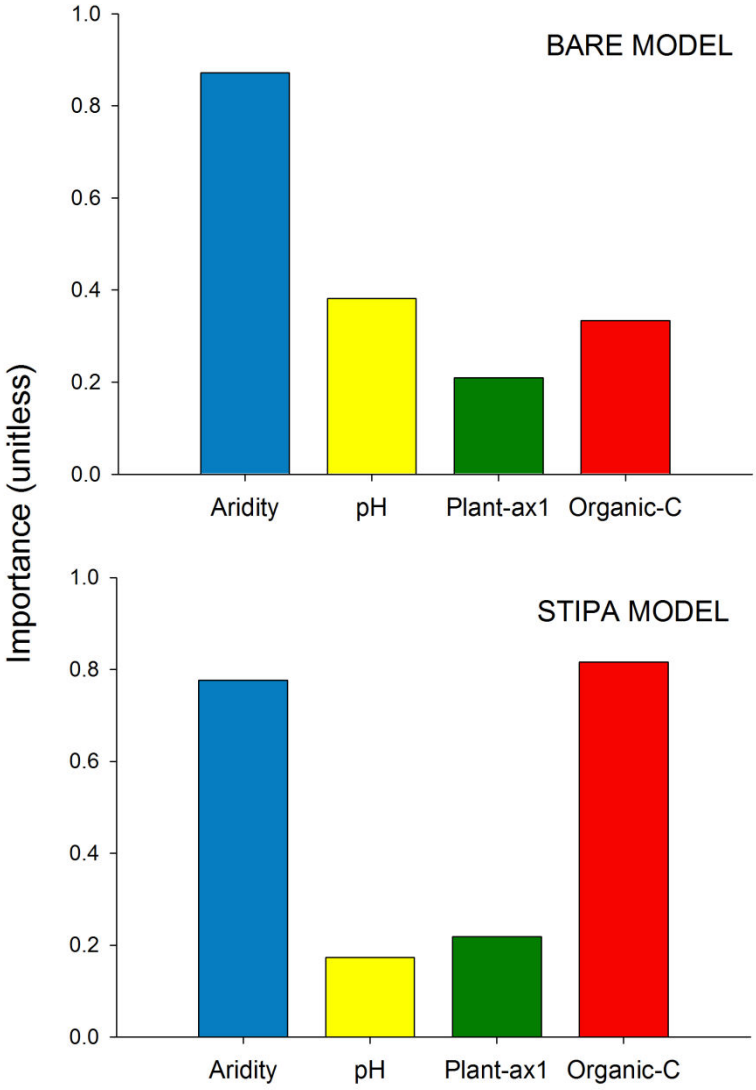

Figure 2. *Relative importance of aridity, pH, organic C, and plant-ax1 (first component of a PCA including the cover of bare and plant microsites, average plant patch interdistance, area of plant patches and number of plant patches per 10 m of transect) variables as drivers of variations in N availability. Results are shown for: i) bare ground microsites, and ii) Stipa tenacissima microsites. The height of each bar is the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears.*

Table 1. *Top eight best-fitting regression models, ranked according to their AICc value, are presented. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. Aridity, pH, plant-ax1 and organic C were included in these models. Bare = data from bare ground soils only, and Stipa = data from Stipa tenacissima soils only.*

BARE

Aridity	pH	Plant-ax1	Organic-C	R2	AICc	ΔAIC	Wi
X				0.635	146.24	0	0.31
X	X			0.673	146.89	0.64	0.22
X			X	0.658	147.82	1.58	0.14
X		X		0.638	149.09	2.85	0.07
X	X		X	0.68	149.8	3.55	0.05
X	X	X		0.674	150.17	3.92	0.04
			X	0.561	150.31	4.07	0.03
		X	X	0.605	151.01	4.77	0.04

STIPA

Aridity	pH	Plant-ax1	Organic-C	R2	AICc	ΔAIC	Wi
X			X	0.82	145.83	0	0.41
X				0.77	148.32	2.49	0.12
			X	0.76	148.51	2.68	0.11
X	X		X	0.82	149.03	3.2	0.08
X		X	X	0.82	149.09	3.27	0.08
		X	X	0.79	149.2	3.37	0.08
X	X			0.77	151	5.17	0.03
X		X		0.77	151.28	5.45	0.01

The SEMs were satisfactorily fitted to our data, as indicated by goodness-of-fit statistics (Fig.3; [48]). These analyses revealed that the effects of OC on N availability were largely modulated by aridity, which had larger total effects on N availability than OC in both Bare and Stipa microsites (Fig. 3).

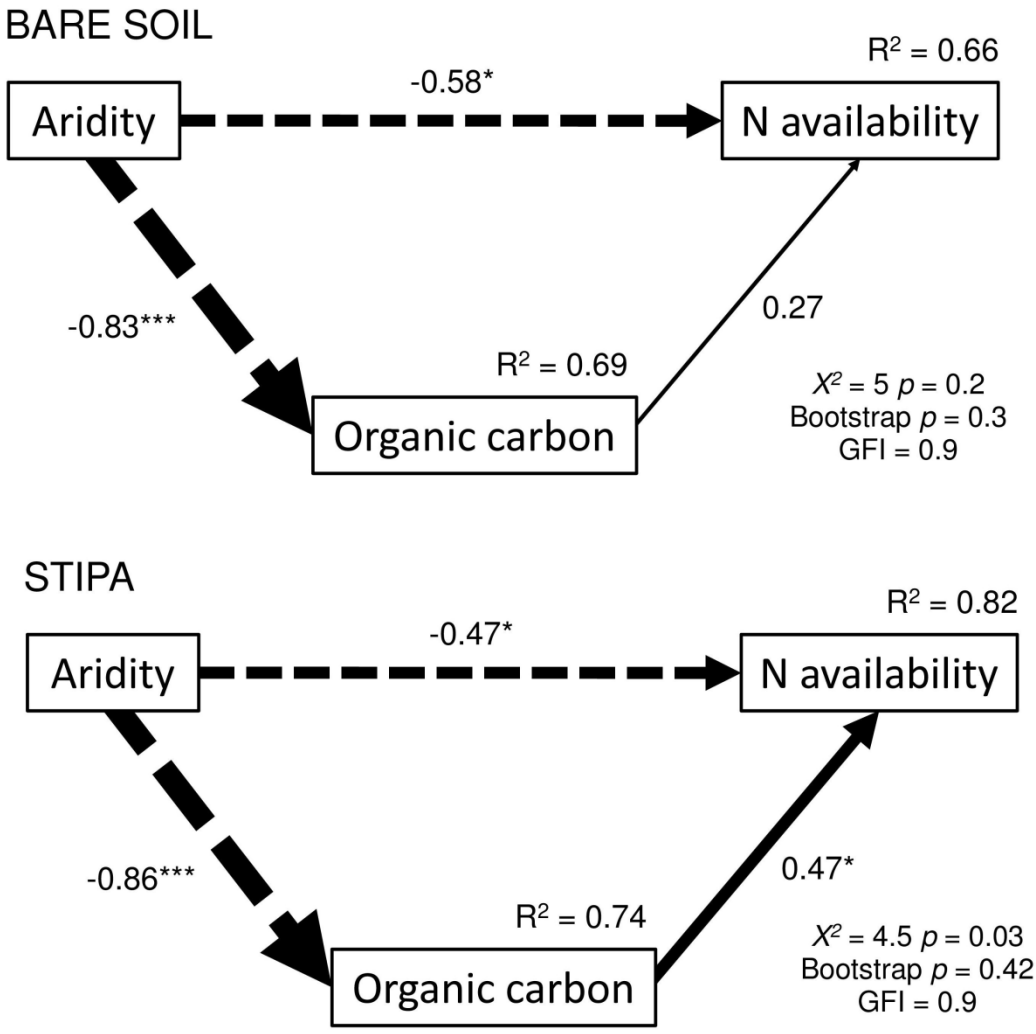

Figure 3. *Structural equation models showing the direct and indirect effects of aridity and organic carbon on the total nitrogen availability for the Stipa tenacissima (STIPA) and bare ground (BARE SOIL) microsites. Continuous and dashed arrows indicate positive and negative relationships, respectively. Width of arrows is proportional to the strength of path coefficients. The proportion of variance explained (R^2) appears above every response variable in the model. Goodness-of-fit statistics for each model are shown in the lower right corner. Significance levels are as follows: * $p < 0.05$,** $p < 0.01$ and *** $p < 0.001$.*

Discussion

Evaluating which abiotic and biotic factors drive the N cycle has been an important research focus over the last two decades. It has been motivated by the importance of N for the functioning of the Earth system, and by anthropogenic activities affecting N cycling in a range of ecosystems [42]. Yet, most of the studies carried out on this topic have focused on the effects of single variables, and relatively few of them have been carried out in drylands [11, 13-14].

Our results indicate that aridity is the most important driver of N availability in the studied grasslands, highlighting the importance of abiotic controls of the N cycle in drylands. This availability was strongly correlated with multiple soil and plant variables (Table S2), which are also highly related to different aspects of the N cycle [5-6]. Aridity also appears to be a strong driver of OC, which was strongly correlated with N availability in this study. Increases in OC may enhance the activity of heterotrophic fungi and bacterial communities that carry out depolymerization and mineralization processes, increasing N availability [22].

Plant and soil properties were also correlated with N availability. Reductions in aridity along the environmental gradient were associated with increases in the plant-ax1, i.e. in the cover, area and number of *S. tenacissima* patches (Table S2). As plant cover increases across the aridity gradient, N and C inputs to the soil from litter are also likely to increase, as indicated by higher decomposition rates under *S. tenacissima* canopies than on surrounding bare ground areas [49-50]. The link between plant cover and N availability is likely to be most evident in ecosystems with low organic matter content, such as those studied, where existing substrate pools in the soil are small relative to the inputs of nutrients entering the soil from plant detritus [51]. Increases in rainfall (lower aridity conditions) may favor N leaching and soil formation processes, decreasing the pH and sand content of the soil, which may increase the retention of OC and favor fungal communities involved in N mineralization and depolymerization processes [5-6]. We observed an increase in N availability with decreases in soil pH and increases in OC contents, consistent with previous studies [6, 22]. However, the relationship between N availability and OC in drylands is not straightforward because N availability should be more related to microbial biomass turnover and atmospheric inputs than to soil organic matter conent [52-54].

Other factors, such as potential net N mineralization and transformation rates, and the C:N ratio, were poorly related to N availability. These results were unexpected, as N transformation rates (which include depolymerization and N mineralization) should be strongly linked to N availability [18]. Our results suggest that potential net N transformation rates, conducted under laboratory conditions (30ºC and 80% of water holding capacity), may not be a good indicator of soil N availability in arid and semiarid ecosystems because they do not take into account the effects of drying-rewetting cycles on microbial and plant processes, including rapid microbial turnover and plant nutrient uptake and losses through leaching or gaseous emissions [23; 55]. A decrease in the C:N ratio has been suggested to increase N mineralization [22]. However, this ratio did not show a significant relationship with the amount of available N in this study. This finding suggests that the

C:N ratio may not be a good indicator of N availability in this system, possibly because this ratio includes both labile and recalcitrant C, the latter being less available for microorganisms [2, 22].

Important differences were found among microsites when modeling N availability. While aridity was the most important driver of changes in N availability in the Bare model, OC was as important as aridity in Stipa microsites. Soils at this microsite had more OC than soils in Bare microsites in most of the sites surveyed (14 out of 22 sites, Table S4). Similar results have been found in other *S. tenacissima* steppes (e.g. [20]). Increasing OC content may drive increased biomass of heterotrophic microbial communities (fungi and bacteria), which drive N mineralization and depolymerization processes, increasing N availability and favoring a fertility island effect under the canopy of *S. tenacissima* [2, 6, 20, 30-34]. The effect of *S. tenacissima* on microclimate and soils beneath their canopies, including lower temperature and higher moisture compared to bare ground areas [20, 33], may have also promoted N mineralization and depolymerization processes.

Studies aiming to evaluate how N availability changes along regional climatic gradients have been carried out in drylands from the USA [14], Argentina [12] and South Africa [11]. However, none of them considered both organic and inorganic N availability. Our results indicate that aridity is the most important factor modulating total N availability in Mediterranean *S. tenacissima* grasslands. In addition, four different organic (DON and amino acids) and inorganic (ammonium and nitrate) N forms were positively related to N availability but negatively related to aridity, showing that decreasing N availability with increasing aridity may affect both organic and inorganic N forms available to plants and microorganisms (Figure S1 and S2). This suggests that changes in precipitation regimes are likely to affect N availability, albeit the temporal scale of this response is uncertain. Climate change models predict reductions in rainfall and increases in temperature throughout the semiarid and arid areas of the Mediterranean basin [29]. While soil moisture is a proximate driver of N transformation rates, our results suggest that N transformation rates are not the most important drivers of N availability. Rather, the strong relationship between aridity and N availability could be driven by indirect effects that operate over longer time scales (decades to millennia) including both biotic (e.g. plant cover) and abiotic (e.g. soil OC and pH). If these factors are in fact more important than short-term effects of precipitation on N transformation rates, then we might expect to observe a lagged decrease in N availability in response to increasing aridity. If N availability does decrease in response to climate change, it will have important harmful effects on plant nutrition and net primary production within *S. tenacissima* grasslands, and will also negatively impact other key supporting (e.g. soil conservation, [50]), provisioning (grazing, [56])

and cultural (e.g. game hunting, [57]) ecosystem services provided by this ecosystem throughout the Mediterranean basin.

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CAPÍTULO 3

Vascular plants mediate the effects of aridity and soil properties on ammonia-oxidizing bacteria and archaea

Manuel Delgado-Baquerizo, Antonio Gallardo, Matthew D. Wallenstein, Fernando T. Maestre

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Abstract

An integrated perspective of the most important factors driving the abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in natural ecosystems is lacking, especially in drylands. We evaluated how different climatic, abiotic, and nutrient-related factors determine AOA and AOB abundance in bare and vegetated microsites from grasslands throughout the Mediterranean Basin. We found a strong negative relationship between the abundance of AOA genes and soil fertility (availability of C, N and P). Aridity and other abiotic factors (pH, sand content and electrical conductivity) were more important than soil fertility in modulating the AOA:AOB ratio. AOB were more abundant under vegetated microsites, while AOA, highly resistant to stressful conditions, were more abundant in bare ground areas. These results suggest that AOA may carry out nitrification in less fertile microsites, while AOB predominate under more fertile conditions. Our results indicate that the influence of aridity and pH on the relative dominance of AOA and AOB genes is ultimately determined by local-scale environmental changes promoted by perennial vegetation. Thus, in spatially heterogeneous ecosystems such as drylands, there is a mutual exclusion and niche division between these microorganisms, suggesting that they may be functionally complementary.

Key words

Organic C; pH; ammonium; aridity index; *amoA* genes.

Introduction

Prior to the recent discovery of archaeal ammonia oxidizers, it was assumed that the critical process of autotrophic nitrification was performed exclusively by bacteria. Many recent studies have detected variants of the ammonia monooxygenase (*amoA*) gene associated with ammonia oxidizing archaea (AOA) in a variety of environments, often in greater abundance than those associated with ammonia oxidizing bacteria (AOB; Leininger et al., 2006). The physiology of AOA differs substantially from AOB. For example, AOA are thought to be better adapted to water-stress environments such as drylands (Adair & Schwartz, 2008), and may be able to function under more stressful conditions than AOB (Valentine, 2007). In addition, AOA microorganisms have been showed to preserve a high level of ammonia-oxidizing activity under low ammonium conditions (Verhamme et al., 2011; Martens-Habbena et al., 2009).

The potential for AOA to perform nitrification in stressful aquatic and terrestrial environments, such as thermal hot springs (You et al., 2009) and drylands (Adair & Schwartz, 2008), challenges traditional assumptions about the types of environments where this process occurs and is important. Since Leininger et al. (2006) suggested that AOA were the dominant ammonia-oxidizing prokaryotes in soils, other studies have showed similar or lower abundance of AOA regarding AOB in these environments (Jia & Conrad, 2009; Di et al., 2009; 2010; Xia et al., 2011). The abundance of AOA and AOB seems to be modulated by climate (i.e. temperature and water availability; Szukics et al., 2010), nutrient conditions (i.e. Wessén et al., 2010, 2011; Verhamme et al., 2011), and by other abiotic factors such as soil texture, pH and salinity (Moin et al., 2009; Nicol et al., 2008; Gubry-Rangin et al., 2011; Wessén et al., 2011). However, field and lab studies carried out to date have shown inconsistent responses of both AOA and AOB to variations in environmental conditions. For example, increases in both AOB and AOA gene abundances have been observed with increasing temperature in some studies (Szukics et al., 2010), but others have found the opposite or have reported inconsistent responses (Rasche et al., 2011; Jung et al., 2011). Similarly, contradictory responses of both AOA and AOB genes have been observed with changes in pH (He et al., 2007; Nicol et al., 2008), soil nitrogen (Rasche et al., 2011; Wessen et al., 2010) and soil carbon (C; He et al., 2007; Shen et al., 2008). The inconsistent results observed to date might be caused by differences in plant communities, soil types and climates studied, which mask the effects of these environmental variables on the distribution of AOA and AOB. Most studies conducted so far have also focused on single environmental variables that have been manipulated in the lab or under field conditions (i.e. Nicol et al., 2008; Verhamme et al., 2011). Although some studies have studied the abundance of AOA and AOB in soils along natural gradients in factors such as pH (Gubry-Rangin et al., 2011), C to N ratios (Bates et al., 2011), salinity (Moin et al., 2009) or temperature (Fierer et al., 2009), we lack an integrated perspective on the most important environmental drivers modulating their abundance under natural conditions, particularly in drylands (López et al., 2003; Adair & Schwartz, 2008, Gleeson et al., 2008; Bastida et al., 2009).

Drylands are of paramount importance for the Earth system, as they cover about 41% of Earth's land surface and support over 38% of the total global population (Reynolds et al., 2007). In addition, these ecosystems are highly heterogeneous, typically having sparse plant coverage with open spaces located between plant canopies (Maestre & Cortina, 2002, Schlesinger et al., 1996), which may provide different potential niches for AOA and AOB microorganisms. However, the effects of vegetated microsites on the abundance of AOA and AOB microorganisms have not, to our knowledge, been studied before.

This study had two major objectives. First, we aimed to evaluate the relationships between the abundance of AOA and AOB and climate (aridity), abiotic factors (pH, sand content and electrical conductivity), and variables related to soil carbon (organic-C, hexoses and activity of β-glucosidase), nitrogen (ammonium, nitrate, dissolved organic N and total available N) and phosphorus (phosphate and phosphatase activity) in semiarid Mediterranean grasslands along a broad aridity gradient (from Spain to Tunisia). Factors such as climate (temperature and water availability; Szukics et al., 2010), pH (Nicol et al., 2008; Gubry-Rangin et al., 2011) soil texture (Wessén et al., 2011), electrical conductivity (i.e. salinity; Moin et al., 2009), and nutrients (indicators of C, N and P availability; He et al., 2007; Wessén et al., 2010; 2011) were included in our study because they have been shown be correlated with the abundance of AOA and AOB in terrestrial ecosystems. Second, we aimed to evaluate the relative importance of climate, abiotic factors and soil nutrient variables as potential modulators of AOA and AOB abundance in contrasting microsites (vegetated and bare ground areas). We hypothesized that because AOB have been observed to be stimulated under high ammonium conditions (Verhamme et al., 2011), and AOA have been suggested to be adapted to low nutrient availability and energy-limiting conditions (Valentine et al., 2007; Verhamme et al., 2011), AOA would be more abundant than AOB under less fertile conditions (lower nutrient content and higher aridity). Moreover, AOA should dominate in bare ground areas because of their apparent adaptation to high temperatures, low water availability and energetically stressful conditions (Valentine, 2007; Adair & Schwartz, 2008; You et al., 2009), while AOB may prefer vegetated microsites, typically characterized by higher availability of water and nutrients (Maestre et al., 2001, 2003; Cortina & Maestre, 2005). Improving our knowledge of the factors driving the abundance of AOA and AOB in soils may help us to clarify the contribution of these organisms to the accumulation of NO_3^- often reported in drylands (Cookson et al., 2006), and thus to achieve a better understanding of the role of these microorganisms in the N cycle.

Materials and Methods

Study site

This study was conducted in 16 *Stipa tenacissima* L. grasslands from Spain, Morocco and Tunisia (Fig. S1; Table S1). The area sampled covers the core of the geographic distribution of this vegetation type in the Mediterranean Basin, which spans from Spain to Lybia (Lé Houerou 2001). Mean annual precipitation (MAP) and temperature (MAT) of the study sites varied from 141 mm to 465 mm, and from 13ºC to 20ºC, respectively (Table S2). The content of sand and electrical

conductivity of the sites studied ranges from 33.5% to 80.5% and from 63.5 to 238.9 µS cm^{-1}, respectively. As typically found in drylands (FAO, 1998), the range of pH found at our sites is narrow (from 7.56 to 8.57), which may restrict the prokaryotic communities at these sites to specific lineages (Gubry-Rangin et al., 2011). Perennial vegetation cover ranged between 8% and 64%, and was in all cases an open steppe dominated by *Stipa tenacissima* (Fig. S1), with shrub species such as *Quercus coccifera L., Rosmarinus officinalis L.* and *Thymus vulgaris L.* in Spain, *Cistus clusii* and *Helianthemum apenninum L.* (Mill.) in Morocco, and *Artemisia herba-alba Asso* and *Hammada scoparia* (Pomel) Iljin in Tunisia. Detailed information on the characteristics of each site can be found in Tables S1 and S2.

Sampling design and measurements

We established a 30 m × 30 m plot representative of the vegetation found at each site. We obtained mean annual temperature and precipitation data from the WorldClim global database (Hijmans et al., 2005). The UNEP (1992) aridity index (AI = P/PET, where P is annual precipitation and PET is annual potential evapotranspiration) of each site was gathered using data from WorldClim as described in Maestre et al. (2012). The AI decreases as aridity increases, and for clarity we used the inverse of AI in this work. Thus, aridity = 1-AI. This index is strongly related to both annual average rainfall (R^2 = -0.98) and temperature (R^2 = 0.74) in our study sites.

Soil sampling was carried out during the summer season of 2006 (Spain) and in 2010 (Morocco and Tunisia). Five composite soil samples from the top 7.5 cm of the mineral soil profile were collected at each plot from two microsites: bare ground areas devoid of vascular vegetation (hereafter Bare) and under the canopy of *S. tenacissima* (hereafter Stipa), totaling 160 soil samples. Soils were transported to the laboratory and sieved (2 mm mesh). A portion of this soil was frozen at -80ºC for molecular analysis, while the rest was air-dried at room temperature for four weeks for physio-chemical analyses (pH, texture, electrical conductivity, organic C, activity of β-glucosidase, hexose content, total available, phosphate and phosphatase activity). Previous studies have found that these properties are hardly affected by air-drying in semiarid Mediterranean soils (Zornoza et al., 2006, 2009). Organic C was determined following Anderson & Ingramm (1993). The activity of β-glucosidase was assayed following Tabatabai (1982). Total available N (sum of ammonium, nitrate and DON) and hexoses were colorimetrically analyzed from K_2SO_4 0.5 M soil extracts using a 1:5 soil: extract ratio as described in Delgado-Baquerizo et al., (2013) and Chantigny et al., (2006), respectively. Phosphate was determined by colorimetry from a 0.5M $NaHCO_3$ extraction (Bray & Kurtz 1945). Phosphatase activity was measured following Tabatabai & Bremner (1969). Soil pH

was measured for all of the soil samples with a pH meter in a 1:2.5 mass: volume soil and water suspension. One composite sample per microsite and site were analyzed for sand, clay and silt content according to Kettler et al., (2001). Electrical conductivity was determined by using a conductivity meter in the laboratory.

qPCR analysis

Soil DNA was extracted from 0.5 g of defrosted soil sample using the MoBio Powersoil DNA Isolation Kit (Carlsbad, USA) according to the instructions provided by the manufacturer and stored at −20°C. We performed quantitative PCR reactions in triplicate using 96-well plates on an iCycler iQ thermal cycler (BioRad). The *amoA* genes of AOB and AOA were amplified using the primers *amoA1F* (GGGGTTTCTACTGGTGGT) / *amoA2R* (CCCCTCKGSAAAGCCTTCTTC) and *Arch-amoAF* (STAATGGTCTGGCTTAGACG) /*Arch amoAR* (GCGGCCATCCATCTGTATGT), respectively, as described previously by Rotthauwe et al., (1997) and Francis et al., (2005). The 25 µl reaction mixture contained: 12.5 µL FastStart Universal SYBR Green Master (Rox), 0.75 µL (10 mM) each primer, 1 µL BSA, 1-10 ng template DNA and ultraclean water to volume. The cycling conditions were 95ºC for 10 min, followed by 35 cycles of 95ºC 60 s; 55ºC 45 s and 72ºC 60 s for both primer sets. Standards were run in triplicate in each assay, and our standard calibration curve was developed using a serial 10^{-3} and 10^{-9} dilution from 30 ng μl^{-1}. We generated melting curves for each run to verify product specificity by increasing the temperature from 55ºC to 95ºC. Efficiencies for all quantification reactions were higher than 90%, with R^2 values ranging from 0.90 to 0.99. Results were expressed as number of copies of genes g $soil^{-1}$. The total amount of the AMO gene (*amoA*) was calculated as the sum of AOA and AOB genes.

qPCR standard curve preparation

The *amoA* bacterial and archaeal primers described above were used to amplify *amoA* genes from DNA extracted from composite soil samples. In parallel, both PCR products were cloned into *E. coli* using a TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. One specific clone was selected for AOA and AOB cultures in order to generate the standard curves. Plasmid DNA was extracted with a Plasmid Mini Kit (Invitrogen), and the insert was sequenced using M13F and M13R primers to check that AOA and AOB were correctly inserted into their respective plasmids (sequences from selected AOA and AOB clones are available in Table S3). These sequences were compared to known *amoA* genes in the Genbank database (http://www.ncbi.nlm.nih.gov) using BLAST. BLAST analysis showed that the sequences were > 99% similar to known AOA (i.e. Uncultured archaeon clone E1-76 ammonia monooxygenase gene)

and AOB (i.e. *Nitrospira* sp.) genes. We also checked the length of the insert AOA and AOB amplicon in their respective plasmids with PCR using the AOA, AOB and M13 primers and conducting electrophoresis in agarose gels in order to check the integrity of the fragment.

Statistical analyses

Due to the low number of study sites as compared to the number of climatic (aridity), abiotic (pH, sand content and electrical conductivity), and nutrient (organic-C, hexoses, activity of β-glucosidase, total available N, ammonium, nitrate, DON, phosphate and phosphatase activity) variables studied, and to the significant relationships between some of the variables from each group (Table S4), we reduced the dimensionality of our dataset. To accomplish this, we conducted a principal component analysis (PCA) with abiotic and nutrient variables separately to reduce them to an abiotic and a nutrient component, respectively. These analyses were carried out separately for the Stipa and Bare microsites. We kept the first component from the PCAs for further analyses, which had an eigenvalue higher than 1 and explained 75% and 64% of the variance from the PCA for the nutrient (nutrient-ax1) and abiotic (abiotic-ax1) variables in the Stipa microsite, and 62% and 58% of this variance in the Bare microsite, respectively. The nutrient-ax1 was positively related to all of the nutrient variables evaluated in this study (Table S4). The abiotic-ax1 component was negatively related to pH and sand content, but positively related to electrical conductivity.

We used univariate linear regression analyses to examine the relationship between AOA and AOB with the climatic (aridity), abiotic (abiotic-ax1) and nutrient (nutrient-ax1) variables. AOA, AOB and AOA:AOB were log-transformed to normalize them prior to regression analyses. Next, we used a multi-model inference approach based on information theory and ordinary least squares (OLS) regression to evaluate the relative importance of climatic (aridity), abiotic (abiotic-ax1) and nutrient (nutrient-ax1) variables on the AOA, AOB and AOA: AOB ratio (Burnham & Anderson, 2002). This approach does not rely on hypothesis testing for fitting models, but instead uses information theory to assess the probability that a given model is the most appropriate description of the observed data. Multi-model inference approaches are recommended when dealing with observational data collected along environmental gradients, as in this study (Chatterjee and Price, 2001; de Albuquerque et al., 2011; Maestre et al. 2012). We calculated the relative importance of aridity, abiotic factors (abiotic-ax1) and nutrient variables (nutrient-ax1) as predictors of the abundance of AOA and AOB, and of the AOA:AOB ratio as the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which

each predictor appears (Burnham & Anderson, 2002). We conducted separate analyses for Bare and Stipa microsites.

We tested for differences between *amoA* organisms (AOA and AOB), microsites (Bare and Stipa) and sites on the abundance of *amoA* genes using a three-way ANOVA approach. We evaluated differences between microsites and sites on the AOA:AOB ratio using a two-way ANOVA. In these analyses, we considered the *amoA* organisms (AOA and AOB) and the microsite (Bare and Stipa) as fixed factors, and site as a random factor. When significant interactions between factors were found, data were divided into subsets based on one of the factors of the interaction, and then were subjected to two-way or one-way ANOVA as appropriate.

Multi-model analyses were conducted using SAM 4.0 (Rangel et al., 2010); other statistical analyses were carried out with SPSS 15.0 Statistics Software (SPSS Inc., Chicago, IL, USA).

Results

Both AOA gene abundance and the AOA:AOB ratio were positively related to aridity and to the abiotic-ax1 (lower pH and sand content, but higher electrical conductivity) in the Bare microsites along the gradient studied (Fig. 1; Table S5). The nutrient-ax1 was negatively related to AOA gene abundance and the AOA:AOB ratio in the Bare microsite, and to AOA in both Bare and Stipa microsites, along this gradient (Fig.1). AOB gene abundance was significant negatively related to nutrient-ax1 in the Stipa microsite (Fig. 1; Table S5); a positive trend between both variables was observed in the Bare microsite (Fig. 1; Table S5).

Soil nutrients (nutrient-ax1 component) were the most important factor modulating AOA gene abundance in both Stipa and Bare microsites (Fig. 2). However, contradictory results were found when analyzing the importance of abiotic, nutrient and climatic variables (Fig. 2). Aridity was positively correlated to the AOA:AOB ratio in the Bare microsite, but this ratio did not show significant relationships with any of the variables measured in Stipa microsites (Fig. 2; Table S5; Table 1). The abiotic component always explained more variance of the abundance of AOA and AOB, and of the AOA:AOB ratio, in Bare than in Stipa microsites (Fig. 2).

Significant differences between sites were found for both AOA and AOB gene abundances in Bare and Stipa microsites ($p<0.01$; Fig. 3; Table S6). However, significant *amoA* organisms (AO) x microsite (MI) x site (SI), AO x MI and AO x SI interactions were found when analyzing these data ($p<0.01$; Table S6; Fig. 3). In Bare microsites, differences between *amoA* organisms and sites were

observed ($p<0.01$), although the interaction AO x SI suggested that differences between AOA and AOB varied depending on the site considered ($p<0.01$; Table S6; Fig. 3). In Stipa microsites, AOB were more abundant than AOA in all the sites ($p<0.01$; Table S6; Fig. 3). A higher concentration of AOA was also observed in Bare microsites across the different sites ($p<0.01$; Table S6; Fig. 3). Differences between microsites and sites in the abundance of AOB were also observed ($p<0.01$). The MI x SI interaction found for this variable, however, indicated that differences between Bare and Stipa microsites were site-dependent ($p<0.01$; Table S6; Fig. 3).

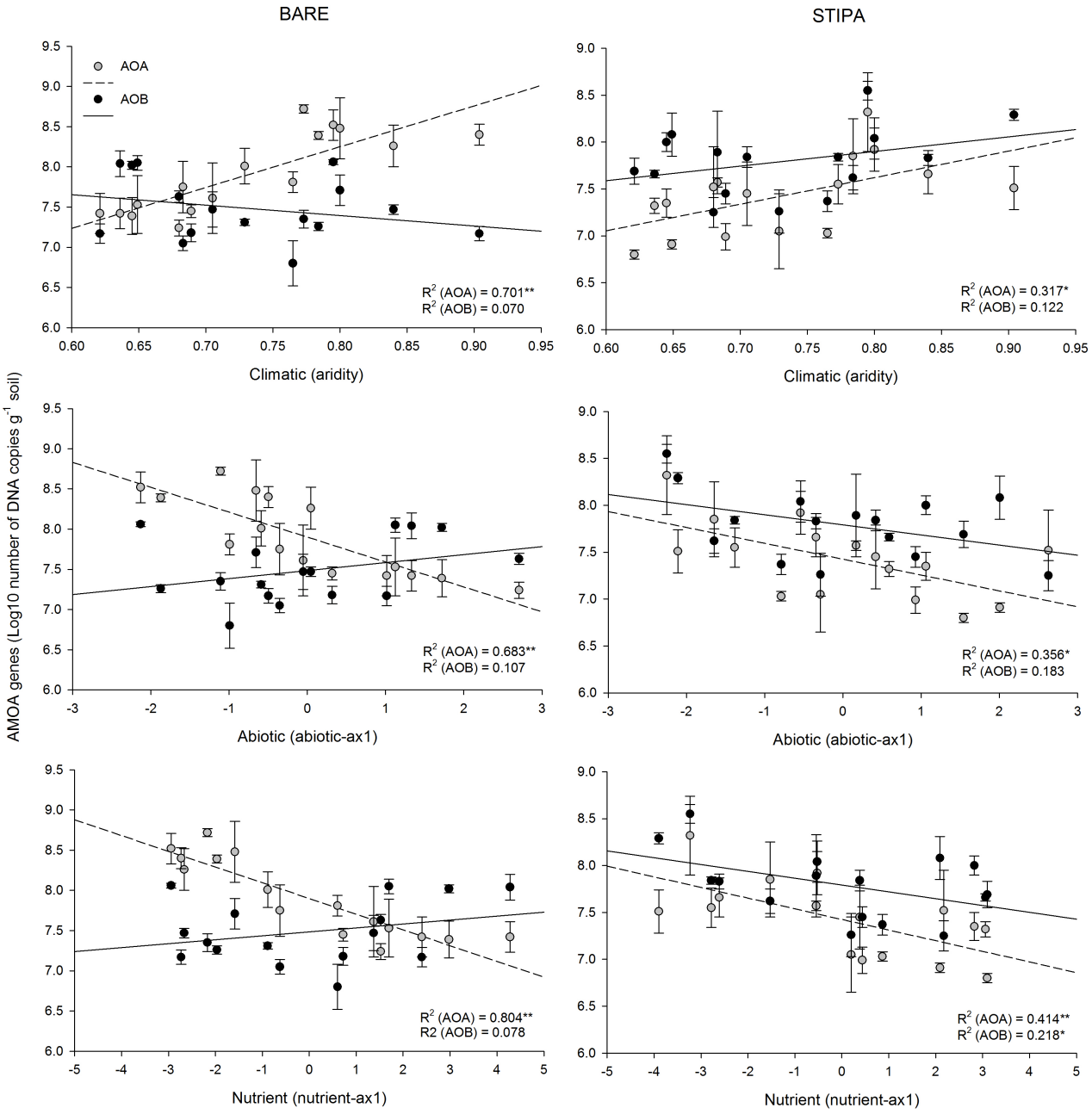

Figure 1. *amoA gene abundance (AOA and AOB) in relation to aridity, abiotic factors (abiotic-ax1) and nutrient variables (nutrient-ax1) in both Stipa tenacissima (STIPA) and bare ground (BARE) areas. Results of linear regression analyses (R^2 and fitted lines, when significant) are shown; * P < 005, ** P < 001, *** P < 0001.*

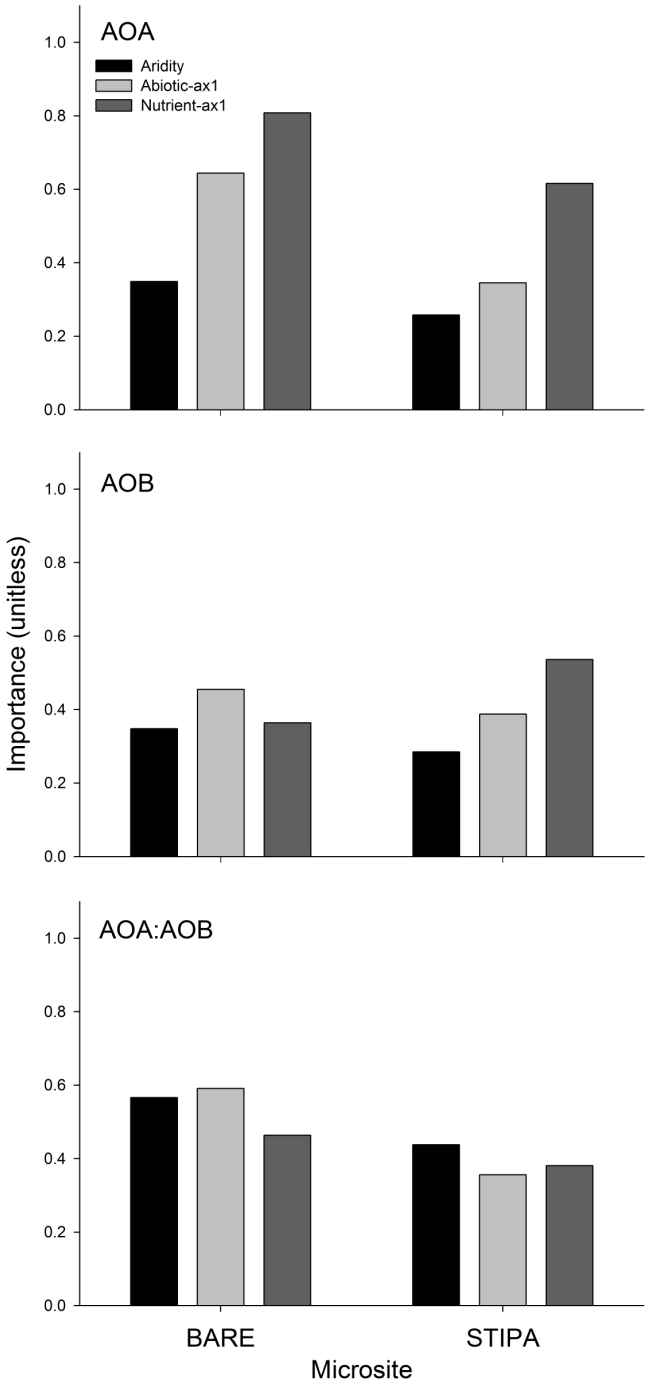

Figure 2. *Relative importance of aridity, abiotic factors (abiotic-ax1) and nutrient variables (nutrient-ax1) as predictors of the abundance of AOA and AOB, and of the AOA:AOB ratio in Stipa tenacissima (STIPA) and bare ground (BARE) areas. The height of each bar is the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears (see table 1).*

Table 1. *Top five best-fitting regression models for the AOA, AOB and AOA:AOB ratio, ranked according to their AICc value, are presented. Each column represents a different predictor variable (aridity, abiotic-ax1 and nutrient-ax1); shaded cells indicate that the variable has been included in the model. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. ΔAICc are difference between the AICc of each model and that of the best model. Wi means Akaike weights. BARE = models conducted with bare ground data. STIPA = models conducted with Stipa tenacissima data.*

BARE						
AOA						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.85	2.97	0.00	0.37
			0.81	3.56	0.59	0.28
			0.84	4.44	1.47	0.18
			0.86	5.78	2.81	0.09
			0.82	6.27	3.29	0.07
AOB						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.11	21.00	0.00	0.34
			0.08	21.52	0.52	0.26
			0.07	21.66	0.66	0.24
			0.11	24.66	3.56	0.06
			0.11	24.56	3.60	0.06
AOA:AOB						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.73	8.15	0.00	0.33
			0.64	9.01	0.87	0.21
			0.70	9.86	1.71	0.14
			0.61	10.07	1.92	0.13
			0.59	10.99	2.84	0.08
STIPA						
AOA						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.42	15.10	0.00	0.45
			0.36	16.62	1.52	0.21
			0.32	17.55	2.45	0.13
			0.42	17.47	3.37	0.08
			0.42	18.71	3.61	0.07
AOB						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.22	15.83	0.00	0.38

			0.19	16.52	0.69	0.27
			0.12	17.68	1.86	0.15
			0.24	18.94	3.12	0.08
			0.22	19.38	3.56	0.06
AOA:AOB						
Aridity	**abiotic-ax1**	**nutrient-ax1**	**R^2**	**AICc**	**ΔAIC**	**Wi**
			0.04	-16.47	0.00	0.32
			0.02	-16.18	0.29	0.27
			0.01	-15.92	0.55	0.24
			0.05	-13.16	3.31	0.06
			0.06	-12.91	3.56	0.05

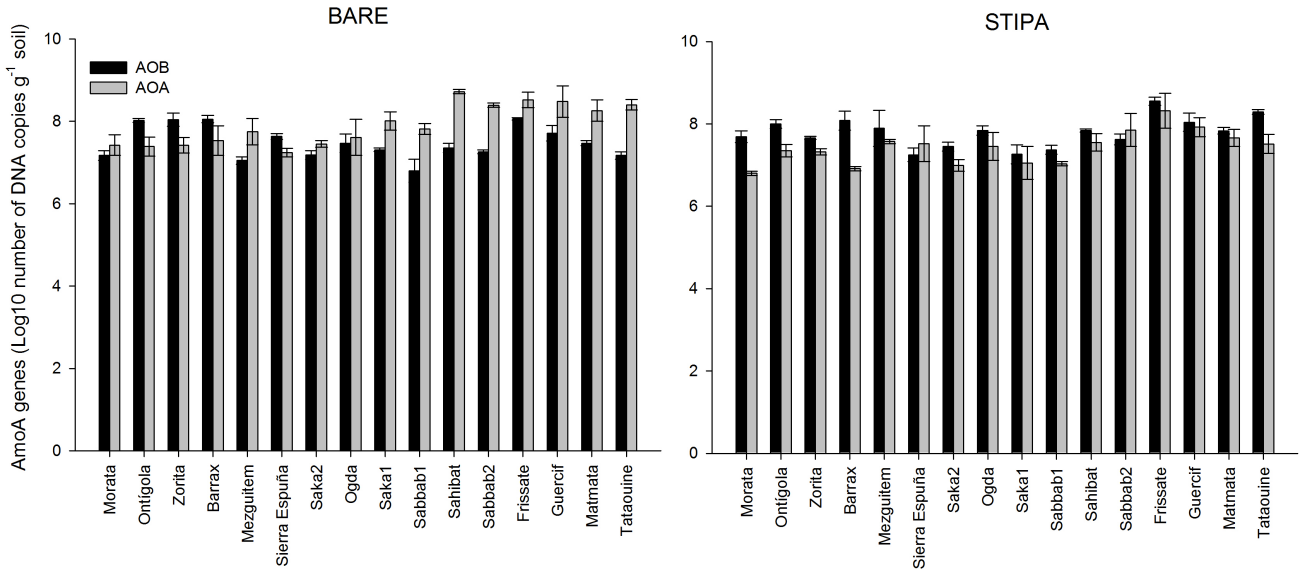

Figure 3. *amoA gene abundance (AOA and AOB) in both Stipa tenacissima (STIPA) and bare ground (BARE) areas for the different study sites sorted from less (left) to more aridity (right). Data represent means ± SE (n = 5).*

Discussion

The relative abundance of AOA and AOB should be related to the rate at which nitrite is produced through nitrification (Verhamme et al., 2011), since their growth is primarily coupled to autotrophic ammonia oxidation (although some AOA may be non-autotrophic (Mußmann et al., 2011)). Thus, it is important to understand the factors that drive their abundance in soils. The present study complements previous work that focused on single nutrient variables, and strongly suggests that AOA are generally inhibited by increasing soil fertility conditions in drylands. We found that the

abundance of AOA was inversely related to the nutrient-ax1, an aggregate index of organic C, hexoses, β-glucosidase activity, ammonium, DON, total available N and phosphatase activity (Table S4), in both Bare and Stipa microsites across the studied aridity gradient, where low nutrient concentrations are typical (Zak et al., 2003; Delgado-Baquerizo et al., 2011). In addition, a decrease in the AOA:AOB ratio was observed with increasing carbon (i.e. organic-C), nitrogen (i.e. ammonium) and phosphorus (i.e. activity of phosphatase) variables in the Bare microsites (Table S4). These results suggest that AOA may outcompete AOB microorganisms under oligotrophic conditions due to their high resistance to water and nutrient stress (Adair & Schwartz, 2008; Verhamme et al., 2011), hence carrying out nitrification under conditions that have long been assumed to be unfavorable. Since ammonium is the primary substrate for both AOA and AOB, we might expect that both groups would both respond positively to increases in ammonium. However, this may not be the case in natural systems because they have to compete for ammonium with heterotrophic microbes and plants. Previous studies have shown that AOB increase in response to ammonium fertilization (Verhamme et al. 2011), but it has been unclear if this pattern translates to natural fertility gradients. We observed a trend of increasing AOB with increasing nutrient availability in Bare microsites, suggesting that AOB are able to compete for ammonium against heterotrophic microbes. Additionally, AOB were always more abundant than AOA in Stipa microsites (Table S6), suggesting that AOB are also better competitors for ammonium than AOA in the face of competition with plants. However, the negative relationship observed between both AOA and AOB and the nutrient-ax1 –highly related to organic C and phosphatase activity (Table S4)– in Stipa microsites suggests that both AOA and AOB can be outcompeted by heterotrophic microbes and plants as fertility increases. Thus, nitrification rates might decrease with increasing fertility despite the corresponding increase in substrate availability.

Other abiotic factors besides fertility might also affect the abudance of AOA and AOB, and thus the potential for nitrification in any environment. In our study, AOA and AOB tended to increase with increasing pH in the Stipa microsites, although our study sites have limited and alkaline pH range (7.56 to 8.57). Other studies have shown that AOB increase and AOA decrease as pH increases (from pH 4.9 to 7.5; Nicol et al., 2008). These contradictory findings might suggest that unique AOA ecotypes are found in dryland soils that are better adapted to high pH than those found in acid and neutral soils. Other factors such as sand content were positively related to the abundance of AOA (but not AOB) in both Bare and Stipa microsites. Similarly, Wessén et al. (2011) observed a decrease in AOA with increasing clay % (usually inversely related to sand content), but these authors did not find any relationship between clay and AOB abundance. Increasing sand content reduces the ability of soils to retain nutrients (FAO, 1989), but increases soil aeration, which may

favor AOA growth. Electrical conductivity, highly linked to salinity, was negatively related to AOA, but not to AOB abundance. This result suggests that salinity concentration may modulate AOA growth, as suggested by others (Mosier et al., 2008; Moin et al., 2009). In the Bare microsites, AOA were more abundant than AOB under the most arid conditions (Table S6). Overall, abiotic-ax1, together with aridity, were the most important factors modulating the AOA:AOB ratio, a response consistent with studies showing that abiotic factors such as pH, salinity and sand content may be important factors distinguishing the niches of AOA and AOB microorganisms (Nicol et al., 2008; Moin et al., 2009; Wessén et al., 2011).

While it is clear that both abiotic factors and substrate competition can affect the abundance of AOA and AOB, plants might also impact their abundance by altering abiotic conditions. Soils under *S. tenacissima* receive higher litter inputs, and have lower temperature and higher moisture content than soils located in adjacent bare ground areas (Maestre et al., 2001, 2003). Environmental changes induced by *S. tenacissima* may promote increases AOB dominance under its canopy, while AOA, more resistant to abiotic stress, dominate in Bare microsites (Valentine, 2007; Adair & Schwartz, 2008; You et al., 2009). In addition, *S. tenacissima* may act as islands of fertility, where AOA may be outcompeted by AOB. These results are consistent with previous reports of differences in the abundance of different microbial groups between Stipa and Bare microsites (Maestre et al., 2009). Thus, the nitrate accumulation reported in this study (Table S2) and observed in many other arid and semiarid ecosystems (Hook & Burke, 1995; Bennett & Adams, 1999; Cookson et al., 2006) could be the result of the activity of AOA and AOB in different microsites (vegetated and bare ground areas). In a previous study under controlled conditions, Verhamme et al. (2011) related nitrification rates to AOA and AOB abundance in soils. The generally weak relationship observed in our soils between nitrate and both AOA and AOB abundance suggests that other nitrogen transformations, such as denitrification, could be taking place at our sites, making it difficult to interpret the actual contribution of AOA and AOB to standing pools of nitrate. The modulating effects of *S. tenacissima* tussocks on the relative dominance of two physiologically different groups of microorganisms, such as AOA and AOB, suggests that the microsites typically found in these ecosystems (e.g. vegetated patches and bare ground areas) may provide different niches to other physiologically contrasting groups of microorganisms, which may be involved in diverse processes that affect the overall functioning of these ecosystems. Given the importance of both abiotic and plant-mediated drivers of AOA and AOB abundance, climate change could have a strong impact on nitrification rates. The increase in aridity predicted for the Mediterranean basin (Gao & Georgi, 2008) could affect the AOA:AOB ratio by increasing the abundance of AOA, potentially relegating AOB organisms to the microsites provided by *S. tenacissima*. In addition,

increasing aridity may lead to an increase and decrease in the sand content and nutrient availability, respectively (FAO 1989), affecting the abundance of AOA, hence the AOA:AOB ratio in drylands

In conclusion, we showed that increases in overall soil fertility inhibit AOA abundance in arid and semi-arid Mediterranean grasslands, where low nutrient concentrations are typical. Thus, AOA may only be competitive under oligotrophic conditions because of their high resistance to low nutrient conditions. Abiotic factors such as aridity and pH modulate the relative dominance of AOA genes, but their influence is ultimately determined by local-scale environmental changes promoted by perennial vegetation, which result in different niches for microorganisms within a given site. Although the actual contribution of AOA and AOB microorganisms to the nitrification remains unknown, this study showed that in spatially heterogeneous ecosystems such as drylands, there is a mutual exclusion and niche division between these microorganisms, suggesting that they may be functionally complementary. Thus, the amount of nitrate observed in this study in the less fertile conditions suggests that the basal nitrate accumulation reported in many arid and semiarid ecosystems may be related to the relative abundance of AOA and AOB in different microsites.

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Supplementary Materials

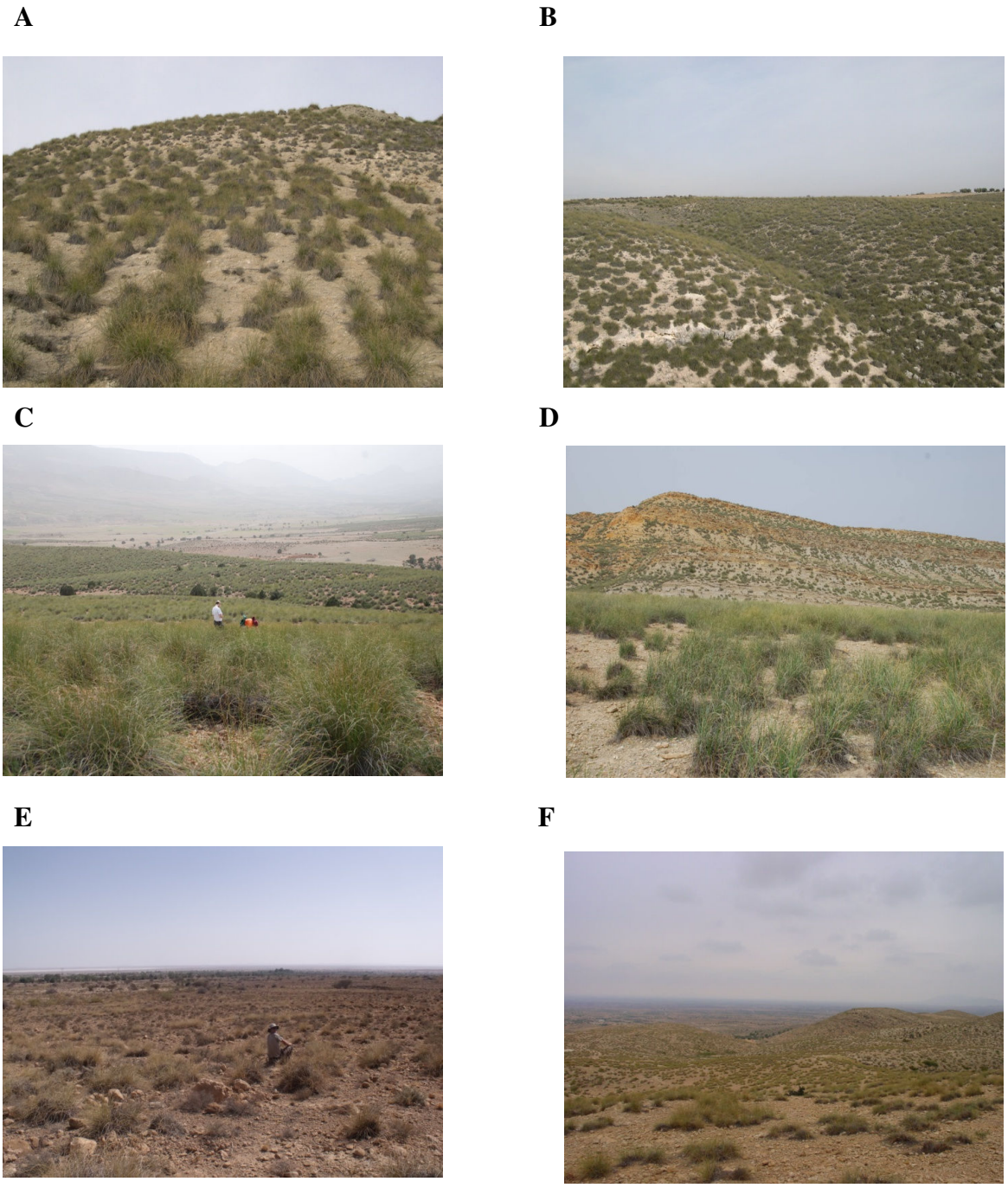


Figure S1. *Examples of Stipa tenacissima grasslands in Spain (A, B), Morocco (C, D) and Tunisia (E, F) shrubs, showing the dominance of Stipa tussocks. Photo credits: F. T. Maestre (A, B, E), C. Escolar (C, D) and J. L. Quero (F).*

Table S1. *Location, texture and % of C and N in the studied sites. TCA = Total carbon; TON = Total nitrogen.*

Site name	Country	Elevation (m)	Lattitude	Longitude	Slope (º)	Sand (%)	Clay (%)	Silt (%)	TCA (%)	TON (%)
Frissate	Morocco	1349	33°03'08 .95" N	2°25'21.32" W	1.75	81.59	3.56	14.85	1.90	0.04
Guercif	Morocco	782	33°58'35 .67" N	3°22'24.61" W	18.25	56.87	7.54	35.59	7.54	0.11
Mezguitem	Morocco	982	34°26'31 .22" N	3°35'33.05" W	14.25	54.25	4.80	40.95	8.97	0.17
Ogda	Morocco	1155	34°18'34 .82" N	1°59'57.48" W	7.00	51.51	4.80	43.69	7.54	0.26
Sabbab1	Morocco	1013	33°52'20 .90" N	3°38'02.00" W	7.25	66.28	4.45	29.27	4.68	0.16
Sabbab2	Morocco	753	33°55'57 .44" N	3°33'31.50" W	10.75	72.62	5.12	22.26	3.79	0.07
Sahibat	Morocco	1430	33°04'03 .65" N	2°43'43.35" W	5.75	67.52	3.14	29.34	3.79	0.07
Saka1	Morocco	733	34°37'59 .57" N	3°24'49.06" W	20.00	55.55	6.69	37.76	6.81	0.17
Saka2	Morocco	936	34°37'31 .97" N	3°27'53.69" W	16.50	42.78	16.21	41.00	3.03	0.21
Barrax	Spain	785	39°02'54 .74" N	2°13'49.56" W	4.50	45.53	21.16	33.31	8.89	0.21
Morata	Spain	627	40°12'31 .61" N	3°25'08.99" W	22.00	54.11	6.30	39.59	10.42	0.26
Ontígola	Spain	593	39°59'31 .33" N	3°37'08.21" W	10.25	47.64	4.49	47.87	8.99	0.23
Sierra Espuña	Spain	663	37°49'16 .16" N	1°40'25.49" W	1.25	49.95	5.77	44.28	6.64	0.21
Zorita	Spain	632	40°21'17 .25" N	2°52'38.77" W	8.00	72.69	9.28	18.03	7.95	0.32
Matmata	Tunisia	546	33º31'17 .65" N	9º58'27.69" E	22.00	65.87	5.75	28.38	2.16	0.06
Tataouine	Tunisia	303	32º59'00 .49" N	10º29'54.94 ' E	1.00	81.17	5.33	13.51	1.41	0.04

Table S2. *Values used in this study for the climatic (AI: Aridity index, MAP: Mean annual precipitation, MAT: Mean annual temperature), abiotic (SAC: Sand content; pH; CON: Electrical conductivity), carbon (C: Organic-C; HEX: Hexoses; BGL: Activity of b-glucosidase), nitrogen (NH_4^+; NO_3^-; DON, total available N) and phosphorus (PO_4^{3-}; Activity of phosphatase) variables.*

Microsite	Site name	AI	MAP (mm)	MAT (ºC)	SAC (%)	pH (H20)	CON (µS/cm)	C (%)	HEX (mg C kg^{-1} soil)	BGL (µmol PnP·g^{-1}·h^{-1})	NH_4^+ (mg N kg^{-1} soil)	NO_3^- (mg N kg^{-1} soil)	DON (mg N kg^{-1} soil)	Total available N (mg N kg^{-1} soil)	PO_4^{3-} (mg P kg^{-1} soil)	FOS (µmol PnP·g^{-1}·h^{-1})
BARE	**Frissate**	0.21	283	15.3	79.04	8.67	71.68	0.43	9.21	0.2	2.39	1.4	5.57	9.36	7.34	0.11
	Guercif	0.2	265	16	56.97	8.45	98.68	0.64	18.6	0.16	3.8	7.09	9.93	20.82	5.26	0.16
	Mezguitem	0.32	399	14.8	52.3	8.35	93.4	1.31	19.79	0.4	5.7	7.17	2.99	15.86	5.19	0.72
	Ogda	0.3	377	13.9	50.03	8.38	120.36	2.05	91.12	0.8	4.53	4.42	10.69	19.64	9.34	1.31
	Sabbab1	0.24	307	14.9	66.92	8.39	87.68	1.55	56.88	0.66	5.81	2.68	11.53	20.02	4.4	1.32
	Sabbab2	0.22	289	16.3	70.74	8.64	63.46	0.53	29.87	0.1	3.13	4.38	10.59	18.1	3.41	0.09
	Sahibat	0.23	310	14.6	74.48	8.35	93.76	0.66	14.29	0.39	2.91	3.93	1.69	8.53	5.42	0.48
	Saka1	0.27	339	15.9	55.27	8.49	107.1	1.12	22.29	0.63	5.7	5.41	3.18	14.29	4.72	0.57
	Saka2	0.31	385	14.7	41.56	8.26	105.18	1.98	27.5	0.84	7.76	10.5	2.84	21.1	4.6	0.59
	Barrax	0.35	415	13.7	33.58	8.37	177.14	2.04	43.1	1.74	9.24	4.14	9.43	22.81	16.15	1.01
	Morata	0.38	432	14	52.99	8.26	209.38	1.97	70.5	1.57	10.83	6.49	7.92	25.24	6.78	1.07
	Ontígola	0.36	412	14.5	45.66	8.15	232.04	1.69	49.2	1.9	11.1	9.57	12.88	33.55	7.67	1.3
	Sierra Espuña	0.32	378	15.1	47.95	7.65	238.96	1.9	79.04	1.31	8.56	2.09	7.41	18.06	3.07	1.17
	Zorita	0.36	405	13.6	70.48	7.56	164.4	2.35	87.28	2.32	14.98	7.56	8.32	30.86	2.36	1.3
	Matmata	0.16	221	18.7	64.93	8.54	209.02	0.5	13.49	0.2	1.6	6.18	0.87	8.65	2.01	0.13
	Tataouine	0.1	141	20	80.45	8.48	196.64	0.45	16.74	0.15	1.45	5.51	2.47	9.43	2.24	0.09

Microsite	Site name	AI	MAP (mm)	MAT (ºC)	SAC (%)	pH (H20)	CON (µS/cm)	C (%)	HEX (mg C kg^{-1} soil)	BGL (µmol PnP·g^{-1}·h^{-1})	NH_4^+ (mg N kg^{-1} soil)	NO_3^- (mg N kg^{-1} soil)	DON (mg N kg^{-1} soil)	Total available N (mg N kg^{-1} soil)	PO_4^{3-} (mg P kg^{-1} soil)	FOS (µmol PnP·g^{-1}·h^{-1})
STIPA	**Frissate**	0.21	283	15.3	81.59	8.57	82.72	0.64	12.11	0.43	2.43	2.78	7.52	12.73	6.62	0.23
	Guercif	0.2	265	16	56.87	8.33	101.14	1.24	37.73	0.89	6.81	8.38	15.64	30.83	6.05	0.91
	Mezguitem	0.32	399	14.8	54.25	8.19	147	1.64	39.2	0.81	8.18	16.56	6.23	30.97	6.75	0.79
	Ogda	0.3	377	13.9	51.51	8.17	158.82	2.38	27.71	1.54	7.28	9.6	12.54	29.42	10.22	1.4
	Sabbab1	0.24	307	14.9	66.28	8.31	121.64	2.22	57.2	1.15	10.34	11.53	12.64	34.51	5.72	1.35
	Sabbab2	0.22	289	16.3	72.62	8.44	76.8	0.81	37.47	0.59	5.68	6.48	12.16	24.32	4.47	0.69
	Sahibat	0.23	310	14.6	67.52	8.42	75.72	0.83	12.79	0.68	3.32	4.67	4.62	12.61	4.63	0.59
	Saka1	0.27	339	15.9	55.55	8.33	127.62	2.06	31.03	1.69	8.28	11.36	8.96	28.6	4.99	1.29
	Saka2	0.31	385	14.7	42.78	7.99	128.96	2.99	34.4	1.62	8.28	11.98	8.39	28.65	4.14	1.06
	Barrax	0.35	415	13.7	45.53	8.1	320.48	2.24	67.6	2.63	10.45	14.87	14.24	39.56	12.72	1.4
	Morata	0.38	432	14	54.11	8.02	291.54	2.9	107.44	2.33	15.98	10.71	13.31	40	4.89	1.69
	Ontígola	0.36	412	14.5	47.64	8.11	207.36	2.33	61.12	2.85	13.44	16.23	12.24	41.91	5.91	2.02
	Sierra Espuña	0.32	378	15.1	49.95	7.56	299.3	2.48	97.04	1.91	11.85	8.3	14.65	34.8	3.96	1.72
	Zorita	0.36	405	13.6	72.69	7.59	171.88	2.97	104.4	2.57	14.7	7.83	15.56	38.09	3.77	1.68
	Matmata	0.16	221	18.7	65.87	8.37	194.78	0.81	18.91	0.85	3.2	8.14	3.14	14.48	2.15	0.49
	Tataouine	0.1	141	20	81.17	8.81	159.12	0.13	8.06	0.2	0.88	6.34	4.25	11.47	2.5	0.07

Table S3. *DNA sequences from AOA and AOB selected clones by using generic M13F and M13R primers. These clones were used to generate standard curves.*

amoA gen	**Primer**	**Sequence**
AOB	M13F	TCTCTTTTAGGGGCGGATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCA GTGTGATGGATATCTGCAGAATTCGCCC TTCCCCTCGGGAAAGCCTTCTTCGCCGTAGGCGGTGACGTCGTTCTTCATGG TGACTCGGCCGCGGGGGCCTTTGACGTA GTAGAAAGCGGTGCAGTAGAGCTTGCCAAAGTACCACCAGACGCAGAACA TGAGCATGGAGACGAAGGCGGAGAAGAAGG CCGCGATGACGGTGGTGTGTCCGCCAAAGGTGCGCAGCGAGCCTTGTTCGA TCAGCCGTACGTATTCAGGCATGCCGGTG CGTACATATAGGAAGCCGGTGTAGTCAGCCAGGGAGAGCAATACGCCTTCG GCTACCAGCGGCAGGTGCGTCGGTCCAAA AATGGGCCAGTTGCCCGGGTAGAACAGCAGGCCGAATGCGCCGCCGCCGAC CAGTGCGGTGATCATCCAGTTACGGGTGA GCAGCAGGACGGTGTCCATGATGAGGGCGCCAGGTATCATGGTGGAGGGG AAGACGAAGTTGATGGGGTAGTGCGACCAC CAGTAGAAACCCCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTG GATCCGAGCTCGGTACCAAGCTTGGCGTA ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCA CACAACATACGAGCCGGAAGCATAAAGT GTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGC GCTCACTGCCCGCTTTCCAGTCGGGAAAC CTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGT TTGCGTATTGGGCGCTCTTCCGCTTCCTC GCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT CACTCAAAGGCGGTAATACGGTTATCCA CAGAATCAGGGGGATAACGCAGAAAGAACATGTGAGCCAAAAGGCCCAGC AAAAGGCCCAGGAAACCGTAAAAAGGGCG
AOB	M13R	TTTTTTGTTTCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAAT TCGCCCTTGGGGTTTCTACTGGTGGTCG CACTACCCCATCAACTTCGTCTTCCCCTCCACCATGATACCTGGCGCCCTCA TCATGGACACCGTCCTGCTGCTCACCCG TAACTGGATGATCACCGCACTGGTCGGCGGCGGCGCATTCGGCCTGCTGTTC TACCCGGGCAACTGGCCCATTTTTGGAC CGACGCACCTGCCGCTGGTAGCCGAAGGCGTATTGCTCTCCCTGGCTGACTA CACCGGCTTCCTATATGTACGCACCGGC ATGCCTGAATACGTACGGCTGATCGAACAAGGCTCGCTGCGCACCTTTGGC GGACACACCACCGTCATCGCGGCCTTCTT CTCCGCCTTCGTCTCCATGCTCATGTTCTGCGTCTGGTGGTACTTTGGCAAGC TCTACTGCACCGCTTTCTACTACGTCA AAGGCCCCCGCGGCCGAGTCACCATGAAGAACGACGTCACCGCCTACGGCG AAGAAGGCTTTCCCGAGGGGAAGGGCGAA TTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGG CCCAATTCGCCCTATAGTGAGTCGTATTA CAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTT ACCCAACTTAATCGCCTTGCAGCACATC CCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTT CCCAACAGTTGCGCAGCCTGAATGGCGAA TGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGC GCAGCGTGACCGCTACACTTGCCAGCGCC CTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGG CTTTCCCCGTCAAGCTCTAAATCGGGG GGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA AACTTGATTAGGGGTGAT

AOA	M13F	TCCTTTTGGGCGATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGT GATGGATATCTGCAGAATTCGCCCTTGT AATGGTCTGGCTTAGACGAACAACGCACTATCTGTTCATAGTAGTAGTTGCT GTGAATAGCACTCTGCTGACAATTAATG CAGGAGACTACATCTTCTACACTGACTGGGCTTGGACGTCGTTTGTAGTATT TTCGATATCACAATCGACAATGCTTGCA GTAGGAGCAATTTACTACATGCTCTTTACAGGAGTTCCAGGTACAGCGACA TATTACGCAACCATTATGACAATTTATAC ATGGGTTGCAAAAGGAGCTTGGTTTGCATTAGGATACCCGTATGACTTCATC GCAGTACCAGTATGGATACCTTCAGCAA TGTTGTTAGACCTTACGTATTGGGCCACGAGGCGCAATAAGCACGCCGCTA TACTTATCGGCGGTACTTTGATCGGTCTC TCAATCCCACTCTTCAACATGATAAACTTACTGCTGGTAAGAGATCCCCTGG AAGTAGCATTCAAGTATCCTCGACCGAC ATTGCCACCTTACATGACGCCTATAGAACCTCAGGTCGGTAAGTTCTACAAC AGTCCCGTAGCCTTGGGAGCTGGCGCTG GGGCAGTGCTAACTGTGCCTATAGCAGCGTTAGGTGCGAAACTAAACACGT GGACATACAGATGGATGGCCGCAAGGGCG AATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAG CTTGGCGTAATCATGGTCATAGCTGTTTC CTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA GCATAAAGTGTAAAGCCTGGGGTGCCTAA TGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGT CGGGAAACCTGTCGTGCCAGCTGCATTA ATGAATCGGCCAACGCGCGGGGAGAGGCGGGTTTGCGTATTGGGCGCTCTT TCCGCTTTCCTCGCTCACTGAACT
AOA	M13R	ATTTTGTACGAGCTCGGATCACTAGTAACGGCCGCCAGTGTGCTGGAATTCG CCCTTGCGGCCATCCATCTGTATGTCCA CGTGTTTAGTTTCGCACCTAACGCTGCTATAGGCACAGTTAGCACTGCCCCA GCGCCAGCTCCCAAGGCTACGGGACTGT TGTAGAACTTACCGACCTGAGGTTCTATAGGCGTCATGTAAGGTGGCAATG TCGGTCGAGGATACTTGAATGCTACTTCC AGGGGATCTCTTACCAGCAGTAAGTTTATCATGTTGAAGAGTGGGATTGAG AGACCGATCAAAGTACCGCCGATAAGTAT AGCGGCGTGCTTATTGCGCCTCGTGGCCCAATACGTAAGGTCTAACAACATT GCTGAAGGTATCCATACTGGTACTGCGA TGAAGTCATACGGGTATCCTAATGCAAACCAAGCTCCTTTTGCAACCCATGT ATAAATTGTCATAATGGTTGCGTAATAT GTCGCTGTACCTGGAACTCCTGTAAAGAGCATGTAGTAAATTGCTCCTACTG CAAGCATTGTCGATTGTGATATCGAAAA TACTACAAACGACGTCCAAGCCCAGTCAGTGTAGAAGATGTAGTCTCCTGC ATTAATTGTCAGCAGAGTGCTATTCACAG CAACTACTACTATGAACAGATAGTGCGTTGTTCGTCTAAGCCAGACCATTAC AAGGGCGAATTCTGCAGATATCCATCAC ACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCCTATAGTG AGTCGTATTACAATTCACTGGCCGTCGTT TTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTG CAGCACATCCCCCTTTCGCCAGCTGGCG TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCT GAATGGCGAATGGACGCGCCCTGTAGCGG CGCATTAAGCGCGGCGGGTGTGGTGGGTTACGCGCAGCGTGACCGCTACAC TTGCCAGCGCCCTAGCGCCCGCCTCCTTT

Table S4. *Pearson correlations coefficients between abiotic-ax1 and nutrient-ax1 with the original abiotic and nutrient variables respectively, for the bare soil Bare and Stipa microsites. SAC= sand content; CON= electrical conductivity; BGL= activity of b-glucosidase; DON= dissolved organic N; FOS: activity of phosphatase. Significance levels are as follows: * p < 0.05 and ** and p < 0.01.*

Microsites	**BARE**	**STIPA**
Abiotic variables	**Abiotic-ax1**	**Abiotic-ax1**
SAC (%)	-0.626**	-0.821**
pH (H20)	-0.802**	-0.845**
CON (µS/cm)	0.831**	0.829**
Nutrient variables	**Nutrient-ax1**	**Nutrient-ax1**
Organic-C (%)	0.942**	0.917**
Hexoses (mg C kg^{-1} soil)	0.838**	0.889**
BGL (µmol PnP·g^{-1}·h^{-1})	0.942**	0.929**
NH_4^+ (mg N kg^{-1} soil)	0.947**	0.979**
NO_3^- (mg N kg^{-1} soil)	0.33	0.598*
DON (mg N kg^{-1} soil)	0.550*	0.779**
Total available N (mg N kg^{-1} soil)	0.893**	0.963**
FOS (µmol PnP·g^{-1}·h^{-1})	0.901**	0.967**
PO_4^{3-} (mg P kg^{-1} soil)	0.28	0.29

Table S5. *Pearson correlations coefficients between ammonium oxidising bacteria (AOB), archaea (AOA) and the AOA:AOB ratio with the climatic (aridity), abiotic (sand content, pH, and electrical conductivity) and nutrient (organic-C, hexoses: HEX, activity of b-glucosidase: BGL, ammonium: NH_4^+, nitrate: NO_3^-, dissolved organic N: DON, total available N, phosphate: PO_4^{3-} and activity of phosphatase: FOS) variables for the bare soil Bare and Stipa microsites. Significance levels are as follows: * p < 0.05 and ** and p < 0.01.*

	BARE			**STIPA**		
	AOA	**AOB**	**AOA:AOB**	**AOA**	**AOB**	**AOA:AOB**
Aridity	0.838**	-0.27	0.768**	0.564*	0.35	0.3
Sand content (%)	0.719**	-0.14	0.614*	0.564*	0.4	0.24
pH (H_20)	0.684**	-0.29	0.667**	0.4	0.540*	-0.07
Electrical conductivity (µS/cm)	-0.515*	0.29	-0.548*	-0.528*	-0.13	-0.44
Organic-C (%)	-0.933**	0.15	-0.761**	-0.712**	-0.572*	-0.25
Hexoses (mg C kg^{-1} soil)	-0.754**	0.14	-0.626**	-0.5	-0.42	-0.14
BGL (µmol PnP·$g^{-}1·h^{-1}$)	-0.811**	0.47	-0.865**	-0.650**	-0.31	-0.42
NH_4^+ (mg N kg^{-1} soil)	-0.827**	0.35	-0.804**	-0.624**	-0.47	-0.25
NO_3^- (mg N kg^{-1} soil)	-0.32	-0.01	-0.23	-0.604*	-0.24	-0.44
DON (mg N kg^{-1} soil)	-0.37	0.29	-0.44	-0.24	-0.28	0
Total available N (mg N kg^{-1} soil)	-0.732**	0.33	-0.723**	-0.596*	-0.4	-0.28
FOS (µmol PnP·$g^{-1}·h^{-1}$)	-0.835**	0.1	-0.654**	-0.605*	-0.516*	-0.18
PO_4^{3-} (mg P kg^{-1} soil)	-0.24	0.41	-0.42	-0.16	0.26	-0.42

Table S6. *Summary results of the three-way ANOVA analyses carried out with AMO organisms (AOA and AOB), and of the two-way ANOVA analyses conducted with the AOA:AOB ratio. In these analyses, study site (SI) was considered as a random factor, while microsite (Bare and Stipa; MI) and AMO organisms (AOA and AOB; AO) were considered as fixed factors. When significant interactions between factors were found, separate two-way ANOVA and one-way ANOVA analyses were conducted for each site.*

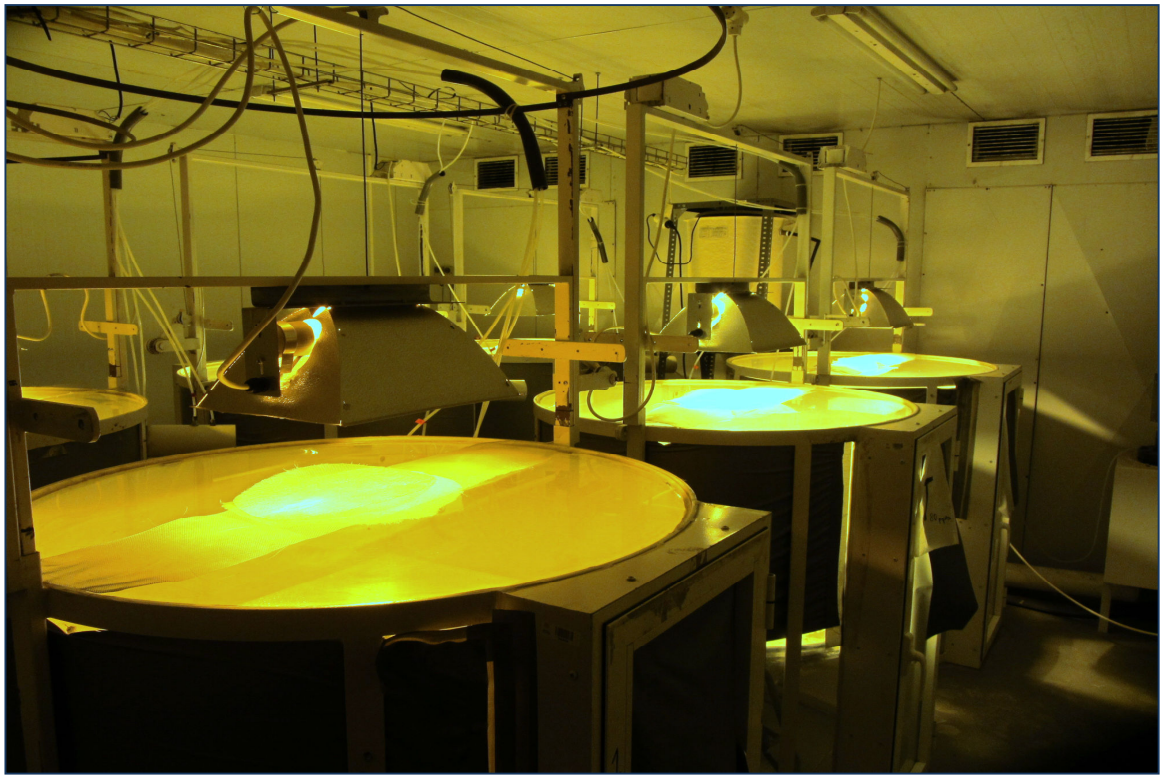
VARIABLE	**Level**	**Factor**	**DF**	**MS**	**F**	**p**
AMOA		AO	1	0.378	1.967	0.16
		MI	1	0.74	3.854	0.05
		SI	15	1.149	5.983	<0.001
		AO x MI	1	8.308	43.246	<0.001
		AO x SI	15	0.478	2.491	<0.01
		MI x SI	15	0.283	1.472	0.12
		AO x MI x SI	15	0.545	2.839	<0.01
		RES	175	0.192		
Interaction AO x SI x MI	MI (BARE)	AO	1	2.907	19.611	<0.001
		SI	15	0.722	4.873	<0.001
		AO x SI	15	1.043	7.033	<0.001
		RES	92	0.148		
New interaction AO x SI	MI (BARE) x SI (Frissate)	AO	1	0.249	5.309	0.069
		RES	5	0.047		
	MI (BARE) x SI (Guercif)	AO	1	0.631	2.466	0.177
		RES	5	0.256		
	MI (BARE) x SI (Mezguitem)	AO	1	0.184	0.72	0.435
		RES	5	0.256		
	MI (BARE) x SI (Ogda)	AO	1	0.033	0.093	0.771
		RES	6	0.356		
	MI (BARE) x SI (Sabbab1)	AO	1	2.656	13.385	<0.05
		RES	6	0.198		
	MI (BARE) x SI (Sabbab2)	AO	1	2.218	303.683	<0.001
		RES	5	0.007		
	MI (BARE) x SI (Sahibat)	AO	1	3.342	110.053	<0.001
		RES	5	0.03		
	MI (BARE) x SI (Saka1)	AO	1	0.7	7.263	<0.05
		RES	6	0.096		
	MI (BARE) x SI (Saka2)	AO	1	0.086	3.968	0.14
		RES	3	0.022		
	MI (BARE) x SI (Barrax)	AO	1	1.888	5.477	<0.05

		RES	8	0.345		
	MI (BARE) x SI (Morata)	AO	1	0.048	0.443	0.535
		RES	5	0.108		
	MI (BARE) x SI (Ontígola)	AO	1	0.872	12.407	<0.05
		RES	5	0.07		
	MI (BARE) x SI (Sierra Espuña)	AO	1	0.275	10.79	<0.05
		RES	5	0.026		
	MI (BARE) x SI (Zorita)	AO	1	0.963	6.581	<0.05
		RES	7	0.146		
	MI (BARE) x SI (Matmata)	AO	1	0.876	5.091	0.05
		RES	8	0.172		
	MI (BARE) x SI (Tataouine)	AO	1	3.465	54.482	<0.001
		RES	8	0.064		
	MI (STIPA)	AO	1	5.482	22.769	<0.001
		SI	15	0.781	3.243	<0.001
		AO x SI	15	0.137	0.571	0.889
		RES	83	0.241		
	AO (AOA)	MI	1	5.988	18.679	<0.001
		SI	15	0.841	2.623	<0.01
		MixSI	15	0.454	1.415	0.162
		RES	77	0.321		
	AO (AOB)	MI	1	2.462	27.001	<0.001
		SI	15	0.865	9.486	<0.001
		MixSI	15	0.364	3.988	<0.001
		RES	98	0.091		
New interaction MI x SI	AO (AOB) x SI (Frissate)	MI	1	0.414	17.935	<0.001
		RES	6	0.023		
	AO (AOB) x SI (Guercif)	MI	1	0.13	0.642	0.449
		RES	7	0.202		
	AO (AOB) x SI (Mezguitem)	MI	1	0.476	1.562	0.28
		RES	4	0.305		
	AO (AOB) x SI (Ogda)	MI	1	0.556	3.557	0.101
		RES	7	0.156		
	AO (AOB) x SI (Sabbab1)	MI	1	0.949	4.528	0.087
		RES	5	0.21		
	AO (AOB) x SI (Sabbab2)	MI	1	0.191	4.836	0.07
		RES	6	0.04		
	AO (AOB) x SI (Sahibat)	MI	1	0.526	20.54	<0.001

		RES	7	0.026		
	AO (AOB) x SI (Saka1)	MI	1	0.106	0.688	0.434
		RES	7	0.154		
	AO (AOB) x SI (Saka2)	MI	1	0.083	2.069	0.224
		RES	4	0.04		
	AO (AOB) x SI (Barrax)	MI	1	0.018	0.164	0.698
		RES	7	0.112		
	AO (AOB) x SI (Morata)	MI	1	0.531	8.044	<0.05
		RES	6	0.066		
	AO (AOB) x SI (Ontígola)	MI	1	0.005	0.19	0.678
		RES	7	0.026		
	AO (AOB) x SI (Sierra Espuña)	MI	1	0.407	6.738	<0.05
		RES	6	0.06		
	AO (AOB) x SI (Zorita)	MI	1	0.201	4.014	0.085
		RES	6	0.05		
	AO (AOB) x SI (Matmata)	MI	1	0.276	12.261	<0.05
		RES	7	0.023		
	AO (AOB) x SI (Tataouine)	MI	1	2.922	106.607	<0.001
		RES	7	0.027		
AOA:AOB		MI	1	16.217	41.511	<0.001
		SI	15	0.754	1.929	0.035
		MI x SI	15	0.825	2.112	0.02
		RES	67	0.391		
INTERACTION MI x SI	SI (Frissate)	MI	1	1.91	6.593	0.05
		RES	5	0.29		
	SI (Guercif)	MI	1	0.992	3.211	0.123
		RES	6	0.309		
	SI (Mezguitem)	MI	1	0.696	1.728	0.319
		RES	2	0.403		
	SI (Ogda)	MI	1	0.449	0.561	0.495
		RES	4	0.801		
	SI (Sabbab1)	MI	1	2.085	13.926	<0.05
		RES	3	0.15		
	SI (Sabbab2)	MI	1	5.532	4.785	0.08
		RES	5	1.156		
	SI (Sahibat)	MI	1	5.315	60.366	<0.01
		RES	5	0.088		
	SI (Saka1)	MI	1	2.309	2.954	0.161
		RES	4	0.781		

SI (Saka2)	MI	1	0.625	17.02	0.026
	RES	3	0.037		
SI (Barrax)	MI	1	0.123	0.161	0.709
	RES	4	0.764		
SI (Morata)	MI	1	1.365	18.242	<0.05
	RES	2	0.075		
SI (Ontígola)	MI	1	0.042	0.275	0.692
	RES	1	0.153		
SI (Sierra Espuña)	MI	1	0.36	0.398	0.573
	RES	3	0.904		
SI (Zorita)	MI	1	0.115	1.193	0.311
	RES	7	0.096		
SI (Matmata)	MI	1	1.844	9.143	<0.01
	RES	7	0.202		
SI (Tataouine)	MI	1	8.69	64.993	<0.01
	RES	6	0.134		
MI (BARE)	MI	15	1.754	6.251	<0.001
	RES	36	0.281		
MI (STIPA)	MI	15	0.286	0.551	0.889
	RES	31	0.518		

5. BLOQUE III: ESCALA LOCAL



CAPÍTULO 4

Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions

Manuel Delgado-Baquerizo, Lourdes Morillas, Fernando T. Maestre, Antonio Gallardo

Plant and Soil **(en segunda revisión).**

Abstract

Aims Human activities are unbalancing nutrient cycles in natural ecosystems. However, our knowledge about how these changes will affect the soil microbial functional diversity and the nitrogen (N) cycle is still scarce in drylands, the biggest biome on Earth. Communities dominated by lichens, mosses and cyanobacteria (biocrusts) influence multiple processes from the N cycle such as N fixation and mineralization rates. We evaluated how biocrusts modulate the effects of different N, carbon (C) and phosphorus (P) additions on theN availability,, the dominance of different available N forms and the microbial functional diversity in dryland soils.

Methods Soil samples from bare ground (BG) and biocrust-dominated areas were gathered from the center of Spain and incubated during seven or 21 days under different combinations of N, C and P additions (N, C, P, N + C, N + P, P + C, and C + N + P).

Results The relative dominance of dissolved organic N (DON) and the microbial functional diversity were higher in biocrust than in BG microsites when C or P was added. Changes in the C to N ratio, more than N availability, seem to modulate N transformation processes in the soils studied. In general, biocrusts increased the resilience to N impacts (N, C+N, N+P, C+N+P) of the total available N, ammonium, nitrate and DON when C was present.

Conclusions Our results suggest that biocrusts may buffer the effects of changes in nutrients ratios on the microbial functional diversity and DON dominance in dryland soils. These organisms may thus have an important role increasing the resilience of the N cycle to unbalances in C, N and P derived from human activities.

Key words: Mineralization; Dissolved organic N; Shannon-Weaver diversity index; Carbon; Phosphorus.

Introduction

Human activities are changing the ratios of nitrogen (N), carbon (C) and phosphorus (P) in natural ecosystems at an unprecedented rate in the Earth´s history (Finzi et al. 2011). For instance, an increase in the C and N to P ratio is currently happening in most terrestrial ecosystems because of C and N fertilization derived from human activities (Peñuelas et al. 2012). However, our knowledge about how these changes modulate key soil processes in arid, semi-arid and dry-subhumid ecosystems (drylands hereafter) is still scarce (Hooper et al. 2005; Schimel 2010; Finzi et al. 2011),

even when this biome covers 41% of Earth's land surface and supports over the 38% of the global human population (Reynolds et al. 2007).

Nitrogen is, after water, the most important factor limiting net primary production and organic matter decomposition in drylands (Schlesinger 1996; Robertson and Groffman 2007). The N cycle includes "narrow" processes carried out for a specific groups of microorganisms, such as nitrification, and "aggregated" procedures as depolymerization (production of dissolved organic N [DON] and ammonification, which are carried out by a larger and diverse group of microorganisms (Wall et al. 2005; Schimel et al. 2005; Cookson et al. 2006). Some authors have found that augments in N and C availability decrease and increase microbial diversity, respectively, while increases in P had negligible effects on this variable (Sharma el al. 1998; Coleman and Whitman 2005; Schimel et al. 2005). Thus, a decrease in functional diversity may limit N transformation processes such as depolymerization in soils (Robertson and Groffman 2007). Very few studies have evaluated how simultaneous changes in C, N and P availability affect the dominance of N forms (NH_4^+, NO_3^- and DON), particularly in N-poor ecosystems such as drylands (Cookson et al. 2006; Qiu et al. 2008; Delgado-Baquerizo et al. 2011; Delgado-Baquerizo and Gallardo 2011). A shift in the dominance of N form derived from changes in the C, N and P ratio may affect N uptake by plants due to their different preferences for different N forms (Nordin et al. 2001; Warren 2009; Paulding et al. 2010). For example, while early-successional species show preference by nitrate, the uptake of ammonium and organic N is quantitatively more significant for late-successional species (Chapin et al. 1993; Houlton et al. 2007).

Of special consideration when studying drylands are communities of mosses, lichens, and cyanobacteria living on the soil surface (biocrusts hereafter), which occupy open spaces located between plant canopies. Biocrusts are considered key players in the N cycle in drylands, as they affect N fixation (Belnap 2002), nitrification (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2013a), and gaseous N losses (Barger et al. 2005). In addition, biocrusts can also affect the abundance and diversity of soil organisms such as bacteria and fungi (Yeager et al. 2007, Bates et al. 2010, Castillo-Monroy et al. 2011a). However, much less is known on how biocrusts and changes in nutrient ratios jointly impact the functional diversity of heterotrophic microbial communities. Biocrusts can also confer physical protection to the soil (e.g. Belnap 2006), and increase the resistance of specific ecosystem functions, such as N mineralization, to global change impacts such as changes in temperature and soil water content (Delgado-Baquerizo et al. 2013a; Reed et al. 2012). Biocrusts have also shown to enhance processes such as C fixation, atmospheric N fixation and phosphatase activity, suggesting an important role in the stoichiometry of the C, N

and P cycles in drylands (Castillo-Monroy et al. 2011a; Bowker et al. 2011; Maestre et al. 2012; Elbert et al. 2012).

Given the multiple roles that biocrusts play in N cycling, and the large areas covered by these organisms (Belnap and Lange 2003), explicitly considering them when evaluating N transformation processes can greatly improve our knowledge on the N cycle in drylands. In this study, we evaluated the effects of biocrusts on the response of N availability, the relative dominance of N forms (ammonium, nitrate and DON), and the functional diversity of microbial communities to multiple N, C and P additions (N, C, P, N + C, N + P, P + C, and C + N + P) in soils from a semi-arid site located in Central Spain, where strong effects of biocrusts on different aspects of the N cycle have already been observed (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010, 2013a, 2013b).

We tested the following hypotheses: i) at high C availability, biocrusts may promote DON dominance and increase the soil microbial functional diversity, because of their association with heterotrophic communities that carry out organic matter decomposition (Bates et al. 2010); ii) the C:N ratio modulates N dynamics regardless the N availability. Thus, at the same N concentration, but different C availabilities, the N form dominance will shift from nitrate (when only N is added) to ammonium and DON (when both C and N are added; Cookson et al. 2006); iii) the heterotrophic functional diversity of biocrust-dominated soils may be limited by C because N fixation is frequently associated with biocrust-forming organisms (Belnap 2002), and most C sources coming from biocrusts, particularly from lichens, are recalcitrant forms of C (Cornelissen et al. 2007); iv) biocrusts will confer resilience to the N cycle facing imbalances in the C:N:P ratio. We expect so because biocrusts have been showed to enhance the availability of C, N and P in soils where present (Castillo-Monroy et al. 2011a, Bowker et al. 2011, Maestre et al. 2012, Elbert et al. 2012), and thus may help to maintain the functional diversity of soil microbial communities and the dominance of processes that control the N cycle.

Methods

Sampling design

Soils for this study were collected from the Aranjuez experimental station, located at the centre of the Iberian Peninsula (40º02’ N – 3º 37’W; 590 m a.s.l.; 8º slope facing SE). The climate is Mediterranean semi-arid, with average annual rainfall and temperature of 388 mm and 14 ºC, respectively. Perennial plant cover is lower than 40%, and is dominated by the perennial grass *Stipa*

tenacissima L. Open areas between plant patches contain a well developed biocrust community dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt (see Castillo-Monroy et al. 2010 for a full species checklist). The soil is classified as Xeric Haplogypsid (USDA 2003), and its main properties are shown in Table S1.

Soil sampling was carried out during Spring of 2010. Five soil samples from the top 4 cm of the mineral soil profile were collected under each of two microsites: well-developed biocrusts dominated by *Diploschistes diacapsis*) and bare ground areas devoid of vascular vegetation and visible biocrust components (cover of mosses and lichens < 5%; BG hereafter). Soil samples were taken at distances higher than 5 m. Previous studies conducted at our study area shown a small-scale spatial dependence (lower than 20 cm) for N in biocrust microsites (Delgado-Baquerizo et al. 2013b). As such, the samples collected are assumed to be independent. Soils were transported to the laboratory and air-dried at room temperature for four weeks. Previous studies have found that soil biochemical properties are hardly affected by air-drying in semiarid Mediterranean soils (Zornoza et al. 2009), which otherwise are under dry conditions most of the year (e.g., Maestre et al. 2002; see also Castillo-Monroy et al. 2011b for moisture data for our study area).

Nutrient treatments, N availability and relative dominance of N forms

Soil samples (2.5 gr of air-dried soil) from BG and biocrust-dominated microsites were preconditioned before the addition of nutrients. To recover their microbial activity, we incubated them at 20ºC and 60% of water holding capacity during seven days (Qiu et al. 2008). These soils were then treated with 0.5 ml of an amendment solution, which contained alone or in combination, the following ingredients: 100 mg N kg^{-1}, 2.323 mg C kg^{-1} and 20 mg P kg^{-1} (Qiu et al. 2008). The nutrients amended were within the range concentrations of available soil N, C and P typically found in drylands (Jalali 2007; Vu et al. 2008; Delgado-Baquerizo et al. 2013a). Thus, soil samples were incubated at 20ºC under different nutrient additions in a factorial design (Control, N, C, P, C+N, N+P, C+P, and C+N+P), and analyzed after seven and 21 days. The last period was chosen to make sure that the initial effect of the added ammonium on the N transformation processes and dominance forms had disappeared. Samples were mixed by vibration after nutrient additions, and soil containers were covered with gas-permeable thin plastic film prior to incubations.

Incubated (Control, N, C, P, C+N, N+P, C+P, and C+N+P) and non-incubated soil samples were extracted with K_2SO_4 0.5 M in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm

for 1 h at 20ºC, and filtered to pass a 0.45-µm Millipore filter (Jones and Willett, 2006). The filtered extract was kept at 2ºC until colorimetric analyses, which were conducted within the 24 h following the extraction. Sub-samples of each non-incubated (air-dry) extract were taken for measurements of glucose (Chantigny et al. 2006) and P (Allen et al. 1986). Ammonium (NH_4^+-N), Nitrate (NO_3^--N) and DON concentrations were also measured for each non-incubated air-dried, K_2SO_4 extract subsample. The availability of N was estimated from K_2SO_4 extracts as the sum of DON, NH_4^+-N and NO_3^--N before and after the seven and 21 days incubation period for each nutrient treatment evaluated (Delgado-Baquerizo et al. 2011). The relative dominance of N forms (DON, $^-$ NH_4^+-N and NO_3^--N) were calculated after the seven and 21 days incubation period as described in Delgado-Baquerizo et al. (2013a). All results were expressed on a dry soil basis.

Microbial functional diversity

The functional diversity of soil heterotrophic microbial communities was analyzed with the MicroResp® technique (Campbell et al. 2003). This method is based on obtaining community level physiological profiles (CLPP) using 15 carbon sources that differ in structural complexity, and has been successfully used with dryland soils (Oren and Steinberger 2008; García-Palacios et al. 2011). We used amino acids (L-alanine, L-lysine, arginine, L-cysteine and N-acetyl-glucosamine), carbohydrates (D-fructose, D-galactose, D-glucose, L-arabinose and D-trehalose), and carboxylic acids (citric acid, L-malic acid, oxalic acid and amino butyric acid) for MicroResp® analyses. This method does not provide information on the taxonomical or phylogenetical diversity of the soil microbial community, but is commonly used to interpret changes in heterotrophic functional diversity because different carbon sources correspond to the catabolic attributes of diverse soil microbial functional groups (Zak et al. 1994; Øvreås, 2000; García-Palacios et al. 2011). Prior to MicroResp® analyses, soil samples were incubated at 50% SWC and 20ºC under the different treatments using two incubation periods (seven and 21 days). MicroResp® plates were set up following García-Palacios et al. (2011). They were incubated for 6 h and read at 570 nm. Then, the results were calculated on the basis of the 16^{th} substrate (water), which represents the basal respiration. In this study we compared the samples from the same soil type and were interested in the relative differences between treatments more than in the absolute CO_2 rates; thus we expected any artifacts promoted by the emission of abiotic CO_2 to similarly affect all the treatments evaluated (García-Palacios et al. 2011). The Shannon-Weaver Diversity Index (H') was calculated to determine heterotrophic microbial functional diversity by using the CO_2 responses to the different C sources as (Shannon and Weaver 1963):

$$H' = -\sum_{i=1}^{S} pi \cdot \ln pi$$

where pi is the ratio of the activity of a particular C substrate and the sum of activities of all C substrates (Zak et al. 1994).

Statistical and numerical analyses

To evaluate the effects of the different nutrient additions, we calculated the absolute increment (A_i) in the total available N, the relative dominant of N forms (NH_4^+-N, NO_3^--N and DON) and the microbial functional diversity (H´) for each treatment (C, N, P, C+N, N+P, C+P and C+N+P) relative to the control (incubated soils with no nutrients addition) in both biocrust and BG microsites for the seven and 21 days incubation periods. As our data did not follow ANOVA assumptions (normality and homogeneity of variances), the effects of incubation period (TI: seven and 21 days), microsite (MI: BG and biocrust soils) and nutrient treatment (TR: N, C, P, C+N, N+P, C+P, C+N+P), on these increments were tested by using the semi-parametric PERMANOVA approach (Anderson 2001); with all these factors being fixed. When significant interactions between TI and TR factors were found, we conducted separate PERMANOVA analyses for the different TI levels. We also tested the differences between microsite and incubation period in the concentration of ammonium, nitrate and DON for the controls (non-nutrient treatment) and between biocrust and BG microsites in the different forms of N (ammoniun), C (glucose) and P (phosphate) before nutrient additions by using PERMANOVA, with biocrust presence/absence as a fixed factor. PERMANOVA analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK).

We also evaluated how biocrusts affected the resilience of the soil N variables to N additions (N, CN, NP and CNP) using the Orwin and Wardle (2004) resilience index. This index was calculated for N availability, ammonium, nitrate and DON using the following equation:

$$RL = \frac{2 \cdot |Do|}{(|Do| \cdot |Dx|)} - 1$$

where Do is the difference between the control and the disturbed (extracted N in the control + added N) soil at the end of the disturbance and Dx is the difference between the control and the disturbed

soil at the time point chosen to measure resilience (seven and 21 days). This index has the advantage to be standardized by the control, being bounded between −1 (less resilience) and +1 (maximal resilience); it remains bounded even when extreme values are encountered (Orwin and Wardle 2004).

Finally, we checked how the A_i in microbial functional diversity relates to A_i in N availability, the dominant N forms and to the N resilience by using Spearman´s correlation coefficient. Correlation analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Before the incubation, the amounts of hexoses and NH_4^+ (which were used as C and N sources for the amended soils) in biocrust and BG soils had similar values (Table S1). However, biocrust soils had a higher PO_4^{3-} concentration than BG soils ($P = 0.03$; Table S2).

After incubation, neither microsite nor incubation period affected differences in NH_4^+-N nor NO_3^--N in the control treatments (incubated soils without nutrients addition; Table 1; Table S3). A TR × TI interaction ($P < 0.01$; Table 1; Table S2) was observed when analyzing DON data. This variable was higher under BG after seven days of incubation ($P = 0.01$; Table 1; Table S2) but no differences in DON concentrations were found between microsites after 21 days of incubation ($P > 0.05$; Table S3). Microbial functional diversity was lower in biocrust than in BG soils ($P < 0.001$), but significant differences in this variable between incubation periods were not observed ($P = 0.51$, Table 1, Table S3).

Table 1. *Concentration of inorganic (ammonium and nitrate) and organic N forms and microbial functional diversity index (H´) in the control treatment for both biocrust and bare ground (BG) microsites after the different incubation periods (seven [T7] or 21 [T21] days). Units for all variables are mg kg^{-1} soil, except when indicated. Data represent means (SE), n = 5. Differences between microsites (BG and biocrust) and incubation periods (T7 and T21) were analysed by using a two-way PERMANOVA. MI = microsite, RES = residuals, TI= period of incubation. PERMANOVA results are the pseudo-F (P value). P values below 0.05 are in bold.*

	T7		**T21**		**PERMANOVA results**		
	BG	**Biocrust**	**BG**	**Biocrust**	**TI**	**MI**	**Mi x TI**
NH_4^+	2.62 (0.61)	4.75 (0.96)	2.4 (0.49)	1.87 (0.01)	0.88 (0.34)	2.66 (0.12)	0.55 (0.49)
NO_3^-	15.31 (2.12)	19.11 (0.2)	16.39 (3.79)	15.91 (1.41)	0.07 (0.78)	1.44 (0.24)	0.02 (0.90)

DON	12.09 (3.78)	3.85 (1.42)	5.54 (0.6)	6.28 (0.23)	0.01 (0.97)	4.56 (0.06)	11.94 (**<0.001**)
Available N	31.06 (5.48)	29.08 (1.31)	24.33 (3.82)	29.3 (3.50)	0.37 (0.56)	0.02 (0.89)	1.28 (0.28)
H´ (bits)	2.41 (0.04)	1.71 (0.30)	2.34 (0.08)	1.90 (0.10)	0.49 (0.50)	20.08 (**<0.001**)	0.01 (0.99)

In general, the immobilization of N was higher in biocrust than in BG soils (Fig. 1). Significant differences between biocrust and BG microsites were only observed after seven days of incubation (P = 0.01; Table S4). Overall, the addition of C promoted a decrease in the total amount of available N (Fig. 1). Differences between nutrient addition levels on this variable were observed for both incubation periods, despite the significant TR × TI interaction found (P < 0.001; Table S4).

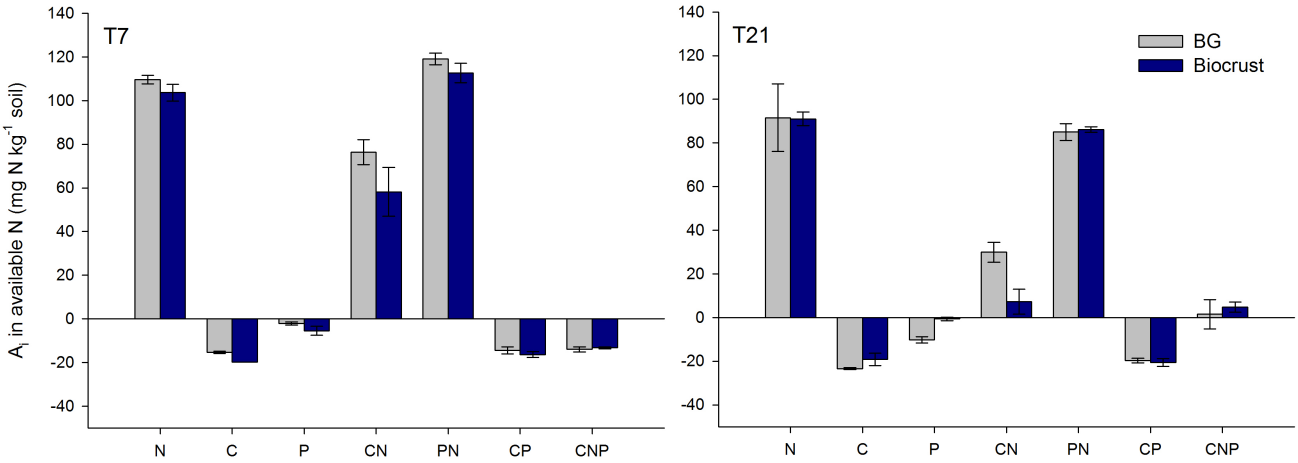


Figure 1. *Increment (A_i) in the available N (measured as the sum of NH_4^+-N, NO_3^--N and DON) under the different nutrient treatments for biocrust and bare ground soils. T7 and T21 indicate results obtained seven and 21 days after the beginning of the incubations.*

The A_i in DON dominance was higher in biocrust than in BG soils (P = 0.02; Table S4; Fig. 2). Significant differences between biocrust and BG soils were not observed either for the A_i in NH_4^+-N or NO_3^--N dominance (P > 0.05; Table S4; Fig. 1). The A_i in DON dominance was higher in C (C and C+P) treatments for both incubation periods, despite the significant TR × TI interaction observed (P < 0.001; Table S4). Similarly, the A_i in NH_4^+-N dominance was highest in the C+N treatment in both incubation periods regardless of the significant TR × TI interaction observed (P < 0.001; Table S4; Fig. 2). Nitrate was the dominant N form for both BG and biocrust microsites in N (N and N+P) treatments after 21 days of incubation only (Fig. 2). Despite the significant TR × TI interaction observed (P < 0.001; Table S4; Fig. 2), significant differences in A_i in the NO_3^--N dominance were found between treatments in both incubation periods (P < 0.01, Table S4). The dominance of nitrate dominance was higher in C (C, C+P) treatments for BG soils in the seven days

incubation period ($P < 0.001$; Table S4; Fig. 2), but differences between microsites were not observed after 21 days of incubation ($P = 0.19$; Table S4; Fig. 2).

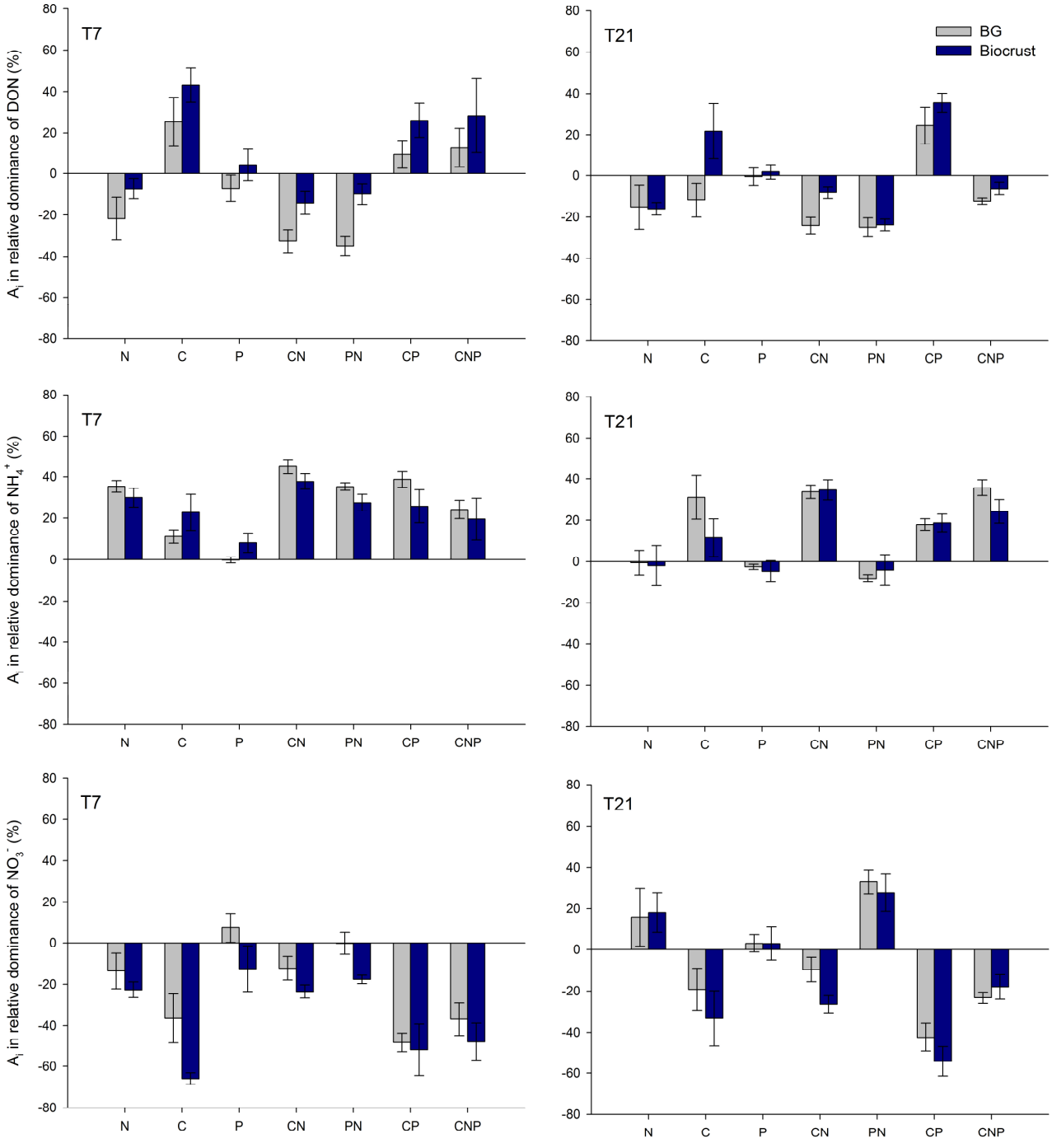


Figure 2. *Increment (A_i) in the relative dominance of different N forms (DON, ammonium and nitrate), calculated as the difference in the DON, NH_4^+-N and NO_3^--N concentrations between the seven (T7) and 21 (T21) days incubations, under the different nutrient treatments for biocrust and bare ground soils.*

Nutrient additions always decreased H´ in BG soils, with the exception of the C+N+P treatment (Fig. 3). However, in biocrust soils, the addition of C increased H´ for all cases after one week; after three weeks such an increase was observed only in the C, C+N+P and N+P treatments (Fig. 3). A significant TR × TI interaction was found when evaluating the A_i in the microbial functional diversity (H´; Table S4). Even so, significant differences between treatments were observed in this variable for both incubation periods ($P < 0.001$, Table S4).

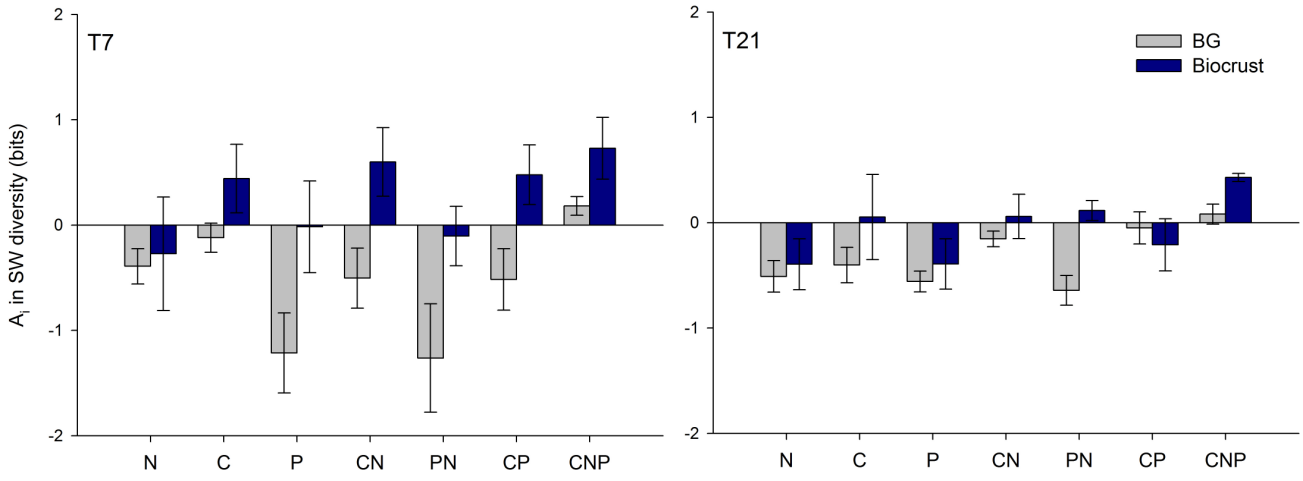


Figure 3. *Increment (A_i) in the Shannon-Weaver (SW) diversity index obtained from the Microresp results for the different nutrient treatments for biocrust and bare ground soils. T7 and T21 indicate results obtained seven and 21 days after the beginning of the incubations.*

Biocrust soils had a higher DON resilience than BG soils after N additions ($P<0.01$; Fig. 4). Regarding total available N, differences between microsites were only observed after 21 days of incubation ($P = 0.03$; Table S5), when biocrust soils showed a higher total available N resilience than BG soils in the C+N treatment (Fig. 4). Non-significant differences between BG and biocrust soils were found when analyzing the resilience of nitrate and ammonium to nutrient additions. However, a trend to increase the resilience of both ammonium and nitrate was observed in the biocrust soils amended with C+N (Fig. 4; Table S5). A significant TR × TI interaction was found when evaluating the resilience of total available N (Table S5). Nevertheless, this variable was highest in the C+N+P treatment at both incubation periods (Fig. 4). The highest resilience of the DON was found in the C+N+P treatment for both microsites ($P < 0.01$; Fig. 4). Significant differences between incubation periods were not observed for this variable ($P > 0.05$; Table S5). The highest resilience in both NH_4^+-N, and NO_3^--N was found for the C+N+P treatment in BG and biocrust microsites (Fig. 4). Significant differences between microsites were not observed for any incubation period ($P > 0.05$, Table S5). A significant TR × TI interaction was found when

evaluating the resilience of ammonium and nitrate (Table S5). Even so, significant differences between treatments were observed when evaluating the resilience of NH_4^+-N, and NO_3^--N (P < 0.01, Table S5).

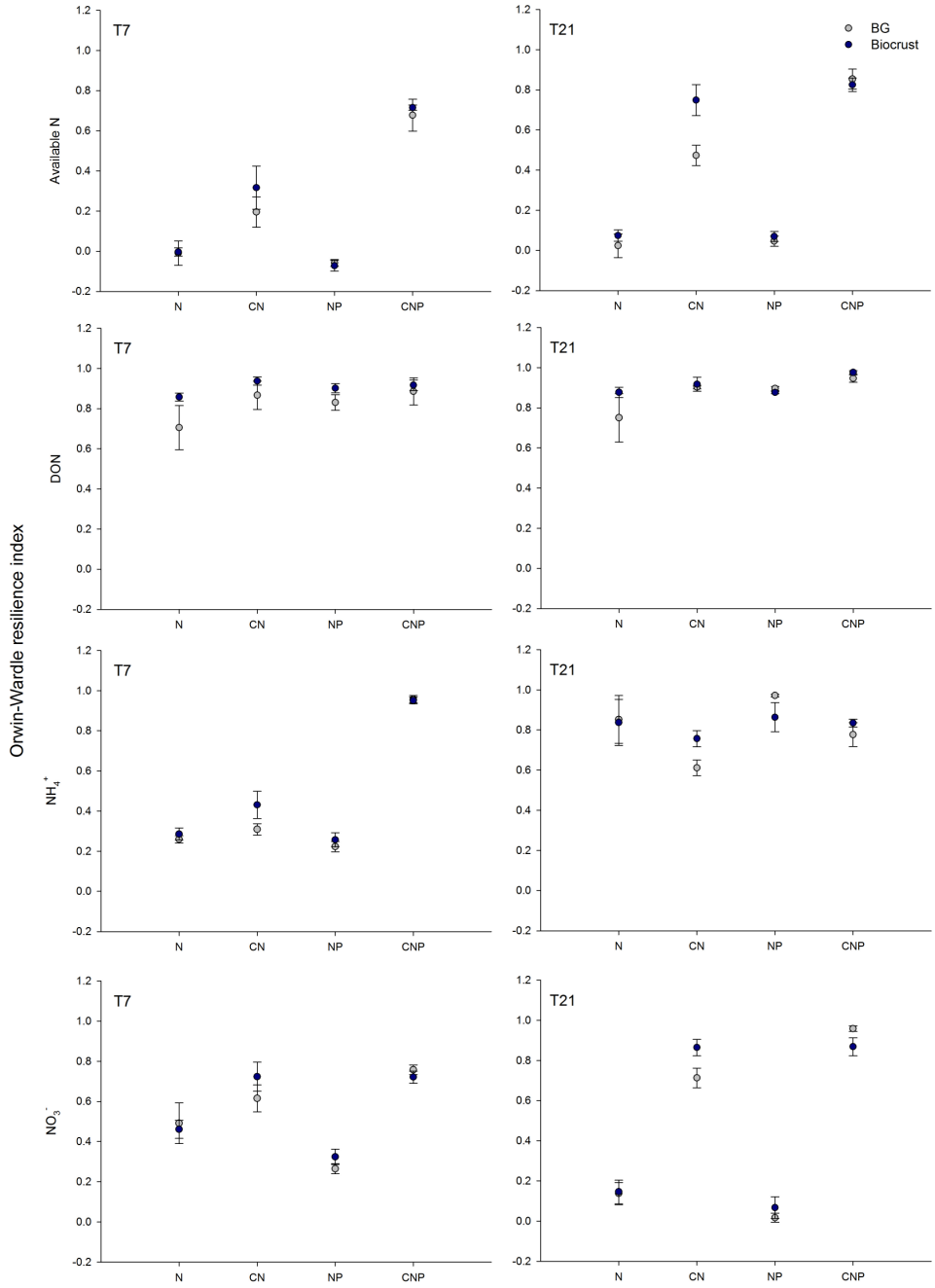


Figure 4. *Resilience in the N variables studied: available N, ammonium, nitrate and DON to the different N treatments (N, C+N, C+P and C+N+P) for biocrust and bare ground soils. T7 and T21 indicate results seven and 21 days after the beginning of the incubations.*

The A_i in H´ was positively related to the dominance of NH_4^+-N in the BG microsite after 21 days of incubation. However, the A_i in H´ was negatively related to the dominance of NO_3^--N in both microsites for the seven days incubation period, a response that was observed only in BG soils after

21 days of incubation (Table 2). The A_i in H´ was negatively and positively related to N availability and DON dominance, respectively, in both microsites, but only after seven days of incubation (Table 2). In general, the A_i in H´ was positively related to the total available N, ammonium and nitrate resilience in both microsites, but only a positive trend was observed when analyzing the relationship with the resilience of DON (Table 2).

Table 2. *Correlation coefficient (Spearman´s ρ) between the absolute increment (A_i) in microbial functional diversity (Shannon´s diversity index; H´) and the A_i in avalable N (n=35), in the dominance of N forms (DON, NH_4^+ and NO_3^-; n=35) and in the resilience of total available N, DON, NH_4^+ and NO_3^- facing N additions (N, CN, NP and CNP; n=20) for both biocrust and bare ground (BG) microsites in the two incubation periods (seven [T7] or 21 [T21] days). Significance levels are as follows: *p < 0.05 and **p<0.01.*

	T7		**T21**	
	Biocrust	**BG**	**Biocrust**	**BG**
Available N	-0.377*	-0.324*	0.014	-0.27
Relative dominance of DON	0.332*	0.343*	0.096	-0.116
Relative dominance of NH_4^+	-0.179	0.026	0.149	0.495**
Relative dominance of NO_3^-	-0.349*	-0.384*	-0.214	-0.319*
Total available N resilience	0.502*	0.417*	0.363	0.762**
DON resilience	0.302	0.38	0.302	0.267
NH_4^+ resilience	0.525*	0.685**	0.083	-0.33
NO_3^- resilience	0.415*	0.335	0.22	0.620**

Discussion

The addition of N had a negative impact on the microbial functional diversity of biocrust and BG microsites. However, when C and P were added, biocrust soils showed a higher increase in the dominance of DON and microbial functional diversity compared to BG soils. An increase in the availability of C may promote the activity of heterotrophic communities, which play an important role in the decomposition and depolymerization processes, increasing the dominance of DON under biocrust soils (Schimel and Bennett 2004; Cookson et al. 2006; Robertson and Groffman 2007). In addition, the A_i in H´ was positively and negatively related to the A_i in DON and nitrate dominance in both microsites, respectively. These results support the idea that "aggregated" processes such as depolymerization may require a larger and diverse group of heterotrophic microorganisms to be carried out, so the lack of diversity in some microbial groups (e.g. fungi), may limit organic matter decomposition and N transformation processes (Schimel et al. 2005; Cookson et al. 2006). Biocrust maintain abundant and rich fungal communities underneath them (Bates et al. 2010), which are

linked to the decomposition of soil organic matter (Austin et al. 2004), and thus may explain their higher functional diversity and DON production when C is available (Table S1; Bates et al., 2010; Delgado-Baquerizo et al. 2013c).

The increase in nitrate dominance was matched with a lower microbial functional diversity when N was added in both microsites. Thus, an increase in the N deposition derived from human activities (Finzi et al. 2011; Peñuelas et al. 2012) may result into a lower functional diversity under biocrusts, decreasing the dominance of "aggregated" processes such as depolymerization and increasing the dominance of "narrow" processes such as nitrification (Schimel et al. 2005, Cookson et al. 2006). This fact may derive into a more inorganic control of the N cycle (Schlesinger et al. 1990), which may affect the uptake of organic N by plants (Warren 2009). The addition of P resulted in a lower impact than that of N and C on the functional diversity of biocrust soils, also favoring the DON dominance comparing to the BG microsite. Our results suggest that P may be an essential nutrient in biocrust-dominated soils, limiting highly energetic processes such as organic matter decomposition or N-fixation, where ATP, and therefore P, works as currency (Belnap and Lange 2003; Bottomley and Myrold 2007). The higher microbial functional diversity observed in biocrust compared to BG soils for most of the nutrient treatments evaluated here suggests that biocrusts may confer a higher protection to microbial communities against changes in ratios C, N and P derived from human activities such as fertilization and atmospheric N deposition.

Changes in the labile C: N ratios (from glucose and ammonium, respectively), more than N availability itself, seem to modulate N transformation processes in both biocrust and BG microsites. Residual ammonium from the N treatment (addition of N alone) was still present after incubating the soils for seven days, influencing the dominance of N forms. However, after incubating the soils for 21 days, NO_3^--N was the dominant N form in the N and N+P treatments, NH_4^+-N was dominant in the C+N and C+N+P treatments, and DON was the dominant N form in the C and C+P treatments. Besides, a decrease in the total available N was observed when C was added, and with an increase in the microbial functional diversity, suggesting that the addition of C may promote the immobilization of N by heterotrophic microbial communities. All these facts support the hypothesis that the availability of C relative to that of N (Cookson et al. 2006; Robertson and Groffman 2007), more than N availability by itself (Schimel and Bennett 2004), may be modulating the N dominance form in drylands when nutrients are in easily available forms for plants and microorganisms. In this way, the nitrate accumulation typically found in drylands may be due to the low C:N ratio characterizing these systems (Hook and Burke 1995; Cookson et al. 2006; Castillo-Monroy et al. 2010).

We also evaluated how biocrusts modulate the resilience of N variables to N additions (N, C+N, N+P and C+N+P). Total available N, NH_4^+-N, NO_3^--N and DON were more resilient to joint additions of C and N in biocrust than in BG soils. This fact may have important implications at the global scale (Elbert et al. 2012), since biocrusts may provide a higher resilience than bare ground areas to changes in the nutrient ratios, and terrestrial ecosystems are currently facing an increase in the C: P and N: P ratios derived from human C and N fertilization (Peñuelas et al. 2012). In general, a higher microbial functional diversity, which was linked to C treatments and to the presence of biocrusts, was matched with a higher resilience in the N variables studied. This fact suggests that by increasing heterotrophic microbial diversity, biocrusts may provide soils a higher capacity to recover from processes such as N deposition. Overall, the resilience of N cycle to N additions was always higher in the C+N+P treatment, suggesting that any limitation in these nutrients may affect the ability of the system to absorb N impacts such as N deposition (Ochoa-Hueso et al. 2011; Peñuelas et al. 2012). However, we must explicitly highlight that extrapolations from controlled experiments such as that employed here to the field should be made with caution given the complexity of biogeochemical processes in natural environments colonized by biocrusts. Even when considering the limitations of our experimental approach, our results highlight the important role of biocrusts in the response to human-induced nutrient inputs in drylands, and pave the way for future field studies aiming to understand how these organisms will modulate nutrient cycling responses to ongoing global change.

Concluding remarks

Our results suggest that biocrusts modulate soil N dynamics and the functioning of the microbial communities in response to changes in the availability of C, N and P. Thus, biocrusts promoted an increase in the DON dominance and microbial functional diversity when C or P was added. Changes in the ratios of labile C to N, more than N availability, seems to modulate nitrification processes in the dryland soils studied. Biocrusts may have an important role in increasing the resilience of the N cycle to C:P and N:P imbalances derived from C and N human fertilization, such as N deposition and increases in atmospheric CO_2 concentration.

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Supplementary material

Table S1. *Main soil characteristics (pH, texture, C and N) and initial concentration for carbon, nitrogen and phosphorus variables extracted in biocrust and bare ground (BG) microsites for the top 10 cm of soil. Units for all variables are mg kg^{-1} soil, except when indicated.*

	Biocrust	BG
pH[1]	7.4±0.07	7.2±0.06
Silt (%)[1]	30.0±2.75	38.0±1.09
Clay (%)[1]	6.3±0.00	6.3±0.00
Sand (%)[1]	63.7±2.75	55.7±1.09
C (%)[1]	14.0±1.10	9 .00±1.10
N (%)[1]	1.4±0.20	0.8±0.08
NH_4^+-N	2.07±0.27	2.32±0.38
$N0_3^-$-N	12.24±0.84	12.42±1.95
DON	6.89±0.84	8.43±0.72
Hexoses-C	7.5±1.34	6.55±0.74
$P0_3^-$-P	0.42±0.04	0.16±0.02
C:N ratio[1]	17.32 ± 1.03	14.4 ± 0.71
Fungal:bacterial ratio[2]	0.02 ± 0.01	0.003 ± 0.001

[1] Data from Castillo-Monroy et al (2010).

[2] Bacterial 16S and fungal 18s rRNA genes were measured using quantitative PCR according to Evans and Wallenstein (2011).

Table S2. *Summary results of the PERMANOVA analyses carried out with ammonium (NH_4^+), hexoses and phosphate in the non-incubated (air-dry) soils. MI = microsite, RES = residuals. P values below 0.05 are in bold.*

	Factor	Source	df	MS	Pseudo-F	*P*
NH_4^+		MI	1	39.72	0.19	0.65
		RES	8	210.07		
Hexoses		MI	1	65.94	0.24	0.67
		RES	8	273.4		
Phosphate		MI	1	3859.9	23.97	**0.03**
		RES	8	161.02		

Table S3. Summary results of the PERMANOVA analyses carried out with ammonium (NH_4^+), nitrate ($N0_3^-$), dissolved organic nitrogen (DON) and functional diversity index (H´) in the control treatments (incubated soils with no nutrients addition) . MI = microsite, RES = residuals, TI= period of incubation. *P* values below 0.05 are in bold.

	Factor	Source	df	MS	Pseudo-F	*P*
NH_4^+		TI	1	4.81	0.87	0.38
		MI	1	14.57	2.66	0.12
		TIxMI	1	3.02	0.55	0.49
		Res	14	5.47		
		Total	17			
NO_3^-		MI	1	3.92	0.07	0.78
		TI	1	73.81	1.44	0.25
		MIxTI	1	0.84	0.02	0.9
		Res	12	51.21		
		Total	15			
DON		TI	1	0	0	0.98
		MI	1	13.15	4.5659	0.06
		TIxMI	1	34.4	11.944	**<0.001**
		Res	14	2.88		
		Total	17			
	TI (7)	MI	1	169.66	4.37	**0.01**
		Res	6	38.76		
		Total	7			

	TI (21)	MI	1	1.37	1.42	0.31
		Res	8	0.96		
		Total	9			
Available N		MI	1	27.56	0.37	0.56
		TI	1	1.74	0.02	0.89
		MIxTI	1	95.92	1.28	0.28
		Res	16	74.55		
		Total	19			
H´		TI	1	0.02	0.47	0.51
		MI	1	0.89	20.08	**<0.001**
		TIxMI	1	0.01	0.01	0.99
		Res	15	0.04		
		Total	18			

Table S4. *Summary results of the PERMANOVA analyses carried out with absolute increment in the total available N, the relative dominant of N forms (NH_4^+-N, NO_3^--N and DON) and the H´ for each treatment (C, N, P, C+N, N+P, C+P and C+N+P) relative to the control treatment (incubated soils with no nutrients addition) in both biocrust and BG microsites for the 7 and 21 days incubation periods. MI = microsite, RES = residuals, TI= period of incubation. P values below 0.05 are in bold.*

	Factor	Source	df	MS	Pseudo-F	*P*
Available N		TI	1	4226.21	41.18	**<0.001**
		MI	1	282.63	2.75	0.09
		TR	7	42672.01	415.76	**<0.001**
		TIxMI	1	156.99	1.53	0.22
		TIxTR	7	1954.11	19.04	**<0.001**
		MIxTR	7	266.96	2.61	**0.02**
		TIxMIxTR	7	33.64	0.33	0.9431
		Res	113	102.64		
		Total	144			
Interaction TI x TR	TI (7)	MI	1	427.52	5.92	**0.01**
		TR	7	27953.01	387.27	**<0.001**
		MIxTR	7	86.68	1.21	0.313
		Res	56	72.18		
		Total	71			
	TI (21)	MI	1	9.23	6.96E-002	0.81
		TR	7	1706	128.77	**<0.001**
		MIxTR	7	211.21	1.5933	0.15
		Res	57	132.56		

		Total	72			
DON		TI	1	1183.71	4.05	**0.04**
		MI	1	6372.41	21.8	**<0.001**
		TR	6	7140.51	24.43	**<0.001**
		TIxMI	1	446.96	1.53	0.2161
		TIxTR	6	1433.31	4.9	**<0.001**
		MIxTR	6	214.43	0.73	0.6276
		TIxMIxTR	6	190.27	0.65	0.685
		Res	112	292.31		
		Total	139			
	TI (7)	MI	1	5097.41	13.28	**<0.001**
		TR	6	5266.81	13.72	**<0.001**
		MIxTR	6	45.06	0.12	0.99
		Res	56	383.81		
		Total	69			
	TI (21)	MI	1	1722	8.57	**<0.001**
		TR	6	3307	16.46	**<0.001**
		MIxTR	6	359.64	1.79	0.1212
		Res	56	200.8		
		Total	69			
NH_4^+		TI	1	5490.91	34.25	**<0.001**
		MI	1	393.09	2.45	0.12
		TR	6	2809.81	17.52	**<0.001**
		TIxMI	1	21.21	0.13	0.72
		TIxTR	6	1532.71	9.56	**<0.001**
		MIxTR	6	61.064	0.38	0.89
		TIxMIxTR	6	316.83	1.97	0.07
		Res	112	160.33		
		Total	139			
	TI (7)	MI	1	115.84	0.85	0.36
		TR	6	1550.91	11.36	**<0.001**
		MIxTR	6	204.28	1.5	0.19
		Res	56	136.54		
		Total	69			
	TI (21)	MI	1	298.45	1.62	0.21
		TR	6	2791.61	15.16	**<0.001**

		MIxTR	6	173.61	0.94	0.48
		Res	56	184.12		
		Total	69			
NO_3^-		TI	1	11773.01	37.26	**<0.001**
		MI	1	3600.01	11.39	**<0.001**
		TR	6	10146.01	32.11	**<0.001**
		TIxMI	1	662.91	2.09	0.15
		TIxTR	6	1297.01	4.1	**<0.001**
		MIxTR	6	212.67	0.67	0.67
		TIxMIxTR	6	152.01	0.48	0.81
		Res	112	315.91		
		Total	139			
	TI (7)	MI	1	3676.31	12.82	**<0.001**
		TR	6	4062.71	14.17	**<0.001**
		MIxTR	6	178.87	0.62	0.72
		Res	56	286.68		
		Total	69			
	TI (21)	MI	1	586.67	1.69	0.19
		TR	6	7380.61	21.39	**<0.001**
		MIxTR	6	185.82	0.54	0.77
		Res	56	345.12		
		Total	69			
H´		TI	1	0.06	0.17399	0.68
		MI	1	10.26	27.6	**<0.001**
		TR	6	2.07	5.5822	**<0.001**
		TIxMI	1	2.55	6.8818	**0.01**
		TIxTR	6	0.31	0.83129	0.55
		MIxTR	6	0.34	0.92235	0.47
		TIxMIxTR	6	0.28	0.75752	0.6
		Res	112	0.37		
		Total	139			
	TI (7)	MI	1	11.53	20.57	**<0.001**
		TR	6	1.75	3.13	**<0.001**
		MIxTR	6	0.41	0.74	0.61
		Res	56	0.56		
		Total	69			

	TI (21)	MI	1	1.28	7.02	**<0.001**
		TR	6	0.63	3.44	**<0.001**
		MIxTR	6	0.21	1.14	0.35
		Res	56	0.18		
		Total	69			

Table S5. *Summary results of the PERMANOVA analyses carried out with the resilience of ammonium (NH_4^+), nitrate ($N0_3^-$), dissolved organic nitrogen (DON). MI = microsite, RES = residuals, TI= period of incubation. P values below 0.05 are in bold.*

	Factor	Source	df	MS	Pseudo-F	*P*
Available N		TI	1	0.57	39.295	**<0.001**
		MI	1	0.06	4.7084	**0.03**
		TR	3	2.7	186.27	**<0.001**
		TIxMI	1	0.08	0.57818	0.45
		TIxTR	3	0.01	5.8456	**<0.001**
		MIxTR	3	0.04	3.098	**0.03**
		TIxMIxTR	3	0.09	0.65913	0.56
		Res	64	0.01		
		Total	79			
	TI (7)	MI	1	0.01	0.79	0.38
		TR	3	1.2	66.23	**<0.001**
		MIxTR	3	0.01	0.53	0.67
		Res	32	0.01		
		Total	39			
	TI (21)	MI	1	0.06	5.7	0.0231
		TR	3	1.592	145.44	**<0.001**
		MIxTR	3	0.04	4.11	**0.01**
		Res	32	0.01		
		Total	39			
DON		TI	1	0.01	1.5	0.23
		MI	1	0.06	5.18	**0.02**
		TR	3	0.06	5.03	**<0.001**
		TIxMI	1	0.01	0.76	0.38
		TIxTR	3	0.02	0.18	0.91
		MIxTR	3	0.01	1.11	0.35
		TIxMIxTR	3	0.01	0.14	0.94

		Res	64	0.01		
		Total	79			
NH_4^+		TI	1	2.51	165.07	**<0.001**
		MI	1	0.02	1.28	0.27
		TR	3	0.53	35.32	**<0.001**
		TIxMI	1	0.01	0.16	0.69
		TIxTR	3	0.68	44.72	**<0.001**
		MIxTR	3	0.02	1.76	0.16
		TIxMIxTR	3	0.01	0.66	0.59
		Res	64	0.01		
		Total	79			
	TI (7)	MI	1	0.01	3.12	0.08
		TR	3	1.12	193.85	**<0.001**
		MIxTR	3	0.01	1.34	0.28
		Res	32	0.01		
		Total	39			
	TI (21)	MI	1	0.01	0.16	0.69
		TR	3	0.02	3.93	**0.02**
		MIxTR	3	0.01	1.18	0.34
		Res	32	0.02		
		Total	39			
NO_3^-		TI	1	0.1	7.99	**<0.001**
		MI	1	0.01	1.24	0.27
		TR	3	2.02	157.35	**<0.001**
		TIxMI	1	0.06	0.01	0.95
		TIxTR	3	0.33	25.96	**<0.001**
		MIxTR	3	0.03	2.65	0.06
		TIxMIxTR	3	0.01	0.19	0.91
		Res	64	0.01		
		Total	79			
	TI (7)	MI	1	0.01	0.44	0.51
		TR	3	0.39752	25.14	**<0.001**
		MIxTR	3	0.01	0.8	0.51
		Res	32	0.01		
		Total	39			
	TI (21)	MI	1	0.01	0.9	0.35

	TR	3	1.9617	197.51	**<0.001**
	MIxTR	3	0.02	2.39	0.09
	Res	32	0.01		
	Total	39			

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CAPÍTULO 5

Biological soil crusts increase the resistance of soil nitrogen dynamics to changes in temperatures in a semi-arid ecosystem

Manuel Delgado-Baquerizo; Fernando T. Maestre, Antonio Gallardo

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Abstract

Aims

Biological soil crusts (BSCs), composed of mosses, lichens, liverworts and cyanobacteria, are a key component of arid and semi-arid ecosystems worldwide, and play key roles modulating several aspects of the nitrogen (N) cycle, such as N fixation and mineralization. While the performance of its constituent organisms largely depends on moisture and rainfall conditions, the influence of these environmental factors on N transformations under BSC soils has not been evaluated before.

Methods

The study was done using soils collected from areas devoid of vascular plants with and without lichen-dominated BSCs from a semi-arid *Stipa tenacissima* grassland. Soil samples were incubated under different temperature (T) and soil water content (SWC) conditions, and changes in microbial biomass-N, dissolved organic nitrogen (DON), amino acids, ammonium, nitrate and both inorganic N were monitored. To evaluate how BSCs modulate the resistance of the soil to changes in T and SWC, we estimated the Orwin and Wardle Resistance index.

Results

The different variables studied were more affected by changes in T than by variations in SWC at both BSC-dominated and bare ground soils. However, under BSCs, a change in the dominance of N processes from a net nitrification to a net ammonification was observed at the highest SWC, regardless of T.

Conclusions

Our results suggest that the N cycle is more resistant to changes in T in BSC-dominated than in bare ground areas. They also indicate that BSCs could play a key role in minimizing the likely impacts of climate change on the dynamics of N in semi-arid environments, given the prevalence and cover of these organisms worldwide.

Key words: Semiarid ecosystem; N depolymerization rate; N mineralization rate; DON.

Introduction

Arid, semi-arid and dry-subhumid ecosystems (commonly referred to as drylands) are a key terrestrial biome, covering 41% of Earth's land surface and supporting over 38% of the total global population (Reynolds et al. 2007). These areas are highly vulnerable to global change and desertification (Körner 2000; Reynolds et al. 2007). Despite their importance and extent, the global change literature is dominated by work carried out in other ecosystems (Schimel 2010), and there are important gaps in our knowledge on how global change will impact key soil processes in drylands.

The availability of resources for primary producers and soil microorganisms largely controls ecosystem performance and its capacity to respond to global change (Finzi et al. 2011). Nitrogen (N) is, after water, one of the most important factors limiting primary production in dryland ecosystems (Whitford 2002). Biological soil crusts (BSCs) are communities dominated by mosses, lichens and cyanobacteria, which constitute a key biotic component of these areas (Belnap and Lange 2003). These crusts have been found to play a prevalent role in important aspects of the N cycle, such as N fixation (Belnap 2002), nitrification (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010), and gaseous N losses (Barger et al. 2005).

Climate change is expected to cause important changes in the temperature and rainfall dynamics of drylands, including a higher frequency of intense precipitation events, increases in extreme high temperatures, decreases in extreme low temperatures, heat waves and drought (IPCC, 2007). The performance of BSC constituents, such as lichens and associated microorganisms, depends up to a large degree on soil water content (SWC) and temperature (T) conditions (Lange 2003; Del Prado and Sancho 2007; Maestre et al. 2009). Thus, expected changes in rainfall and temperature can strongly affect the functioning of BSC-forming organisms (Belnap et al. 2008; Grote et al. 2010) and the ecosystem processes that are affected by them, such as soil respiration (Maestre et al. 2010; Castillo-Monroy et al. 2011a). However, the resistance (*sensu* Orwin and Wardle 2004) of soils located under BSCs to changes in temperature and soil water content has never been tested before. Indeed, traditional laboratory studies of N mineralization have been carried out under optimal SWC and T conditions, but only a few studies have studied the influence of the physiological range of temperature and SWC on net N mineralization and depolymerization (dissolved organic nitrogen [DON] production) rates (Schimel and Bennett 2004; Szukics et al. 2010; Bregliani et al. 2010). Depolymerization, rather than ammonification, seems to be a key

controller of the N cycle, but the effects of soil temperature and moisture on the production of DON are still poorly known (Schimel and Bennett 2004; Bregliani et al. 2010).

In this study, we evaluated how different combinations of T (5ºC-30ºC) and SWC (30%-80% of water holding capacity [WHC]) affected key variables from the N cycle (ammonium, nitrate, inorganic-N, dissolved organic nitrogen [DON]; aminoacids, and N in the microbial biomass), as well as the relative dominance of the N transformation rates, in soils from microsites differing in the degree of BSC development (bare ground and well-developed BSCs areas). We also applied the Orwin and Wardle Resistance Index (2004) to evaluate how BSCs modulate changes in these variables in response to variations in T and SWC. Previous studies have suggested that nitrification rates should increase with augments in T until moderate SWC values (30-60%; Szukics et al. 2010; Bregliani et al. 2010), while ammonification might be less limited by lower temperatures (Szukics et al. 2010). However, the influence of these environmental factors on N transformation rates under BSCs has been scarcely studied before (Grote et al. 2010). We hypothesize that the influence of BSCs on the organic and inorganic N forms will determine a different response to changes in temperature and SWC to that found in areas without well-developed BSCs. Thus, an increase in the microbial metabolic rates should be expected with increases in SWC and T, decreasing the organic N and increasing the inorganic N as a result of increasing both decomposition and mineralization rates. Drawing upon previous studies (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010), we tested the hypothesis that BSCs, which confer physical protection to the soil (e.g. Belnap 2006), can also increase the resistance of the N cycle to changes in SWC and T. Thus, microbial communities under BSCs may slow down the alteration of the N dynamics under future climatic scenarios.

Methods

Study site

Soils for this study were collected from the Aranjuez experimental station, located at the centre of the Iberian Peninsula (40º02' N – 3º 37'W; 590 m a.s.l.; 8º slope Racing SE). The climate is Mediterranean semi-arid, with average annual rainfall and temperature of 349 mm and 14.5 ºC, respectively (1986-2012 period). Perennial plant cover is lower than 40%, and is dominated by the perennial grass *Stipa tenacissima* L. Open areas between plant patches contain a well developed BSC 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. (see Castillo-Monroy et al. 2010 for a full checklist).

The soil is classified as Xeric Haplogypsid (USDA 2003), and has a fine texture dominated by the presence of gypsum. The main soil properties of the study area are shown in Table 1.

Sampling design and laboratory analyses

Soil sampling was carried out during spring of 2008, the most biologically active season at the study area (Castillo-Monroy et al. 2011a). Five soil samples from the top 4 cm of mineral soil profile were collected under each of two microsites: well-developed BSCs (cover of lichens and mosses > 75%; see Appendix B of Castillo-Monroy et al. 2010) and bare ground areas (BG) devoid of vascular vegetation and visible components of BSCs (cover of mosses and lichens < 5%; BG hereafter; see Appendix B of Castillo-Monroy et al. 2010). Cyanobacteria are present at both microsites, but we have not quantified them. Nonetheless, the biotic communities are very different between BSC and BG microsites, and given the higher degree of BSC development in the former microsite, we expect that cyanobacteria should be much more abundant there (Yeager et al. 2004, Maestre et al. 2006). Soils were transported to the laboratory and air-dried at room temperature for four weeks. Previous studies have found that the biochemical properties are hardly affected by air-drying in semiarid Mediterranean soils (Zornoza et al. 2009), which otherwise are under dry conditions most of the year (e.g., Maestre et al. 2002).

Air-dried soil samples were incubated in the laboratory for 14 days by using different T (5 ºC, 10 ºC, 20 ºC and 30ºC) and SWC (30%, 50% and 80% of WHC) levels in a factorial design for both BSC and BG microsites. Five replicates were used for each combination of treatments. The incubation was performed in a closed chamber to keep SWC constant during the incubation time. The chamber was closed with polyethylene film allowing gas exchange, but avoiding water losses. Soil samples were incubated in dark conditions. Incubated and initial (air-dried) soil samples were extracted with K_2SO_4 0.5 M in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20ºC and filtered to pass a 0.45-µm Millipore filter (Jones and Willett 2006). The filtered extract was kept at 2 ºC until colorimetric analyses, which were conducted within the 24 h following the extraction. Sub-samples of each non-incubated (air-dried) extract were taken for measurements of phenols, pentoses and hexoses according to Chantigny et al (2006). Incubated and non-incubated samples were analyzed from K_2SO_4 extract subsamples following the same methods for all measurements, including inorganic N, DON, and amino acid concentrations (Chantigny et al 2006; Jones and Willett 2006; Delgado-Baquerizo et al. 2011). Ammonium concentration in the extract was directly estimated by colorimetry (indophenol blue method) using a microplate reader (Sims et al. 1995). Nitrate was first reduced to NH_4^+ by keeping overnight 250 µl of extract with ca.

20 µg of Devarda alloy in a microplate. The supernatant was transferred to other microplate and analyzed colorimetrically as explained above. Nitrate concentration (NO_3^-) in the extracts was calculated as the difference between Devarda-incubated and unincubated samples. Inorganic-N is expressed as the sum of ammonium and nitrate. DON in the extracts was first oxidized to NO_3^- with $K_2S_2O_8$ in an autoclave at 121 ºC for 55 min, and then reduced to NH_4^+ with Devarda alloy (Sollins et al. 1999). DON values were calculated as total dissolved N minus inorganic N. Ammonium, nitrate, inorganic-N, DON and amino acid concentration were also determined for each incubated K_2SO_4 extract subsample.

Potential net depolymerization, ammonification and nitrification rates were estimated as the difference between air-dried and incubated DON, NH_4^+-N and NO_3^--N concentrations for each combination of T and SWC divided by the number of days of incubation (Delgado-Baquerizo and Gallardo 2011). The sum of ammonification and nitrification rates was defined as the N mineralization rate (production of inorganic-N N), and the sum of this rate and depolymerization was defined as the N transformation rate (production of total available N). The relative dominance of potential net depolymerization, ammonification and nitrification rates was calculated as a percentage relative to the sum of these three metabolic rates (Castillo-Monroy et al. 2010; Delgado-Baquerizo and Gallardo 2011). All results were expressed on a dry soil basis.

In parallel, the N in microbial biomass (MB-N) was determined using the fumigation-extraction method following Brookes et al. (1985). Non-incubated and incubated soil subsamples were fumigated with chloroform for 5 days. Non-fumigated replicates were used as controls. Fumigated and non-fumigated samples were extracted with K_2SO_4 0.5 M in a ratio 1:5 and filtered through a 0.45-µm Millipore filter. Total N in the extracts was converted to NO_3^--N using the persulphate oxidation technique (Sollins et al. 1999), and the concentration was estimated by the colorimetric method described above. Microbial biomass-N concentration was estimated as the difference between total N of fumigated and unfumigated digested extracts divided by a Kn (fraction of biomass N extracted after the CHC_{13} treatment) of 0.54 (Brookes et al. 1985).

Statistical and numerical analyses

To evaluate how BSCs affected the resistance of the soil to changes in T and SWC, the Orwin and Wardle Resistance Index (2004) was calculated for all N variables using the following equation:

$$RS = 1 - \frac{\left(2 \cdot \left(D_0\right)\right)}{\left(\left(C_0\right) + \left(D_0\right)\right)}$$

where (D_0) is the difference between the control (C_0) and the disturbed (P_0) soil at the end of the disturbance. Disturbance is used here as the period of time that the soils were exposed to different treatments of T and SWC (14 days in our case). This index has the advantage to be standardized by the control, being bounded between -1 (less resistance) and +1 (maximal resistance); it remains bounded even when extreme values are encountered (Orwin and Wardle 2004). We evaluated how the resistance index was influenced by the different treatments separately for BSC and BG soils.

Our data did not meet ANOVA assumptions (normality and homogeneity of variances). Thus, differences between BSC and BG soils in each initial (air-dried) soil variable were tested by using the semi-parametric PERMANOVA approach developed by Anderson (2001), with BSC presence/absence as a fixed factor. PERMANOVA uses permutation tests to obtain *P* values, does not rely on the assumptions of traditional parametric ANOVA, and can handle experimental designs such as employed here. The effects of T, SWC and microsite (BSC vs. BG) on the different variables evaluated were also evaluated using PERMANOVA; in these analyses, all the factors were considered fixed. When significant interactions between factors were found, separate PERMANOVA analysis were conducted for the different factor levels. However, we interpreted the main effect in the presence of interaction when the analysis suggested that interaction only moderated the strength of the main effect and did not flip over its direction (ordinal interaction; Reinard 2006). When significant differences between SWC levels were not detected, the data were pooled among SWC levels for visualization purposes. PERMANOVA analyses were carried out using 99999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK). *P* values were not adjusted for multiple testing because this approach is considered overly conservative (Gotelli and Ellison 2004).

Results

N concentrations (before and after incubation)

Before the incubation, BSC soils had higher values of NH_4^+-N, NO_3^- -N and amino acids than BG soils (P<0.05; Table 1, Appendix 1). No significant differences were found in any of the other variables evaluated (P>0.05; Appendix 1).

Table 1. *Main soil characteristics of soils under biological soil crusts (BSC) and in bare ground areas (BG). Phenols were expressed as equivalents of 2-hydroxybenzoic acid, hexoses as equivalents of glucose, pentoses as ribose equivalents, and amino acids as equivalents of leucine. Data are means ± SE (n = 5).*

	BSC	**BG**
pH	7.4±0.1	7.2±0.1
Silt (%)	30.0±2.7	38.0±1.1
Clay (%)	6.3±0.0	6.3±0.0
Sand (%)	63.7±2.7	55.7±1.1
C:N ratio	10,11±0.7	11.24±0.1
NH_4^+-N (mg N kg^{-1} soil)*	11.97±1.4	1.86±0.1
$N0_3$_N (mg N kg^{-1} soil)*	55.28±5.4	21.42±2.5
DON (mg N kg^{-1} soil)	43.04±7.9	35.27±2.1
Aminoacids (mg kg^{-1} soil)**	19.59±3.0	5.57±1.7
MB-N (mg N kg^{-1} soil)	14.63±6.0	3.31±1.7
Phenols (mg kg^{-1} soil)	26.6±5.1	24.93±4.1
Hexoses (mg kg^{-1} soil)	145.3±24.6	202.8±39.8
Pentoses (mg kg^{-1} soil)	138.5±39.8	115.8±33.1

* Significant differences between microsites in the initial soil variables concentrations are as follows: * $p < 0.05$ and ** and $p < 0.01$. pH, soil texture and the C:N ratio data were not analyzed.

After the incubation, NH_4^+-N , NO_3^- -N, DON, amino acids, and MB-N had higher values under BSCs than in BG microsites (P<0.01; Appendix 1; Fig. 1 and Fig. 2). The concentration of NH_4^+-N significantly decreased with increases in T at both BSC and BG microsites (P<0.01; Appendix 1; Fig. 1). However, significant T × SWC, T × MI, SWC × MI and T × SWC × MI interactions were found for this variable (P<0.01; Appendix 1). In the BG samples, neither temperature nor SWC affected the concentration of NH_4^+-N (Appendix 2); however, in the BSC samples, this variable responded to changes in WHC only in the 30ºC treatment (Appendix 2). At this temperature, NH_4^+-N decreased in the 50% of WHC treatment, and increased in the highest SWC treatment (80% of WHC; P<0.01; Appendix 2). The MB-N was higher in the 50% of WHC treatment at both BSC and BG microsites (P<0.01; Appendix 1; Fig.1). A significant T × MI interaction was also found when analyzing this variable (P<0.01; Appendix 1). MB-N increased with T in the BG microsite (P<0.01; Appendix 2; Fig.1), but not in the BSC microsite (Appendix 2; Fig.1). Nitrate increased with temperature in both BSC and BG microsites (P<0.01; Appendix 1;

Fig.2), but did not change with SWC in any microsite (Appendix 1; Fig.2). Inorganic N was not affected by changes in either SWC or T, regardless the microsite considered (P>0.05; Appendix 1; Fig.2). DON decreased at intermediate T values in both BSC (20ºC) and BG (10ºC and 20ºC) microsites (P<0.01; Appendix 1; Fig.2), but was not affected by changes in SWC (Appendix 1, Fig. 2). The concentration of amino acids decreased with T in both BSC and BG microsites (P<0.01; Appendix 1; Fig.2), but was not affected by SWC (Appendix 1, Fig. 2).

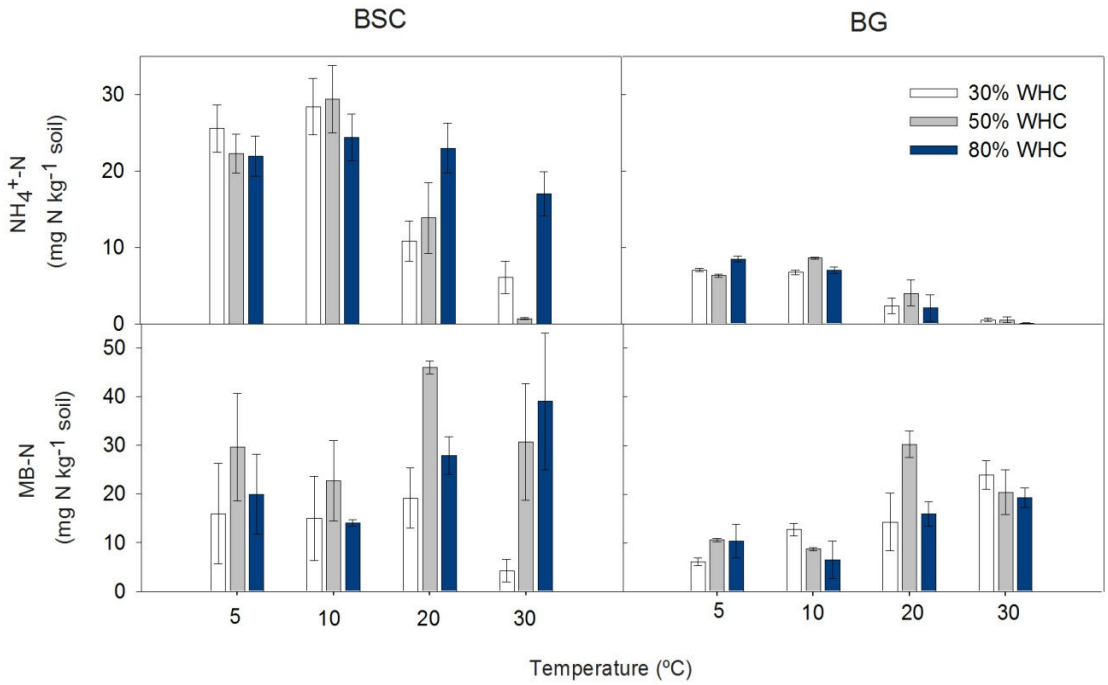

Figure 1. *Concentration of NH_4^+-N and MB-N (after incubation) under the different combinations of soil water content and temperature in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

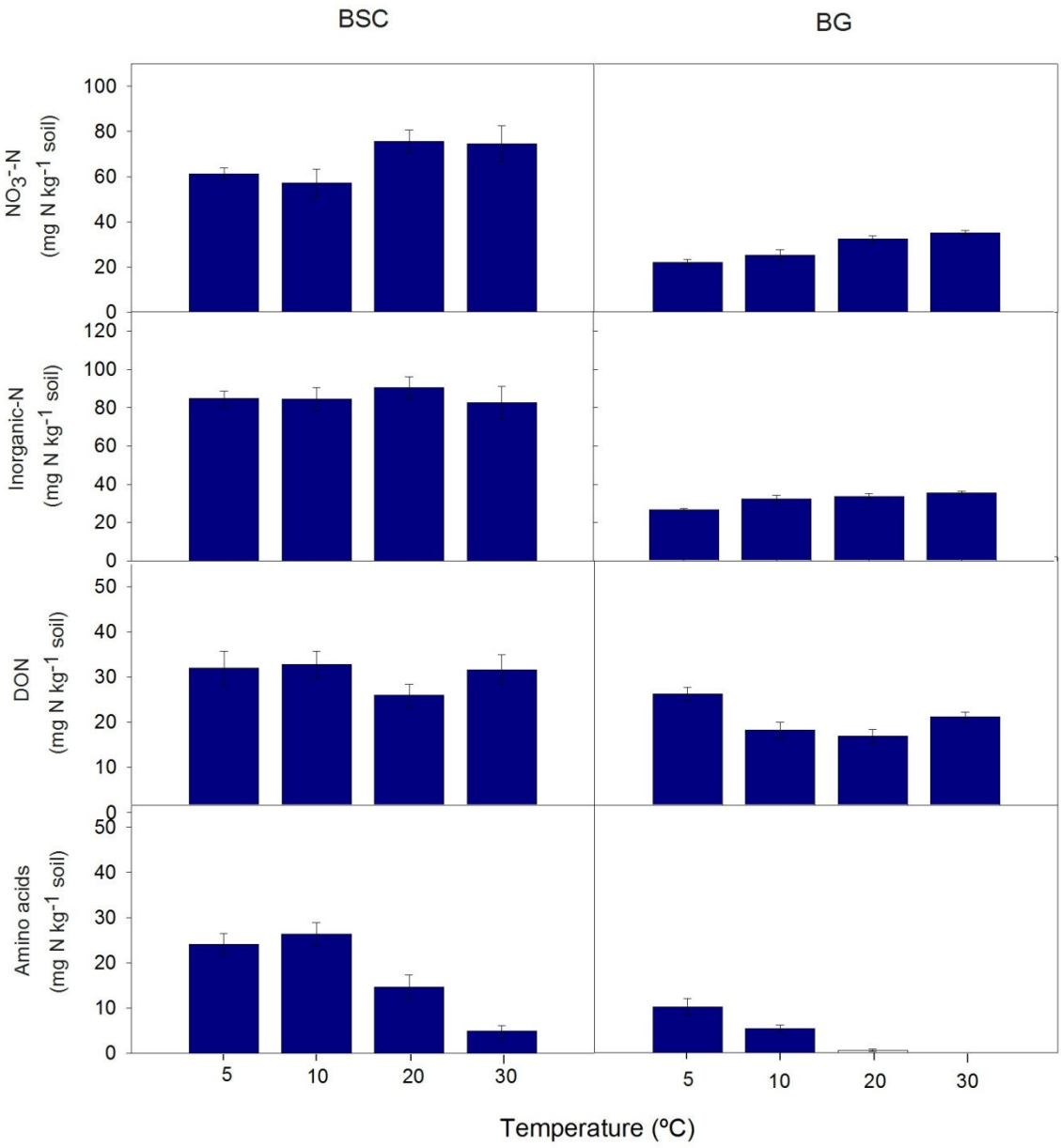

Figure 2. *Concentration of NO_3^--N, inorganic-N, DON and amino acids (after incubation) under the different temperature levels evaluated in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

N transformation rates

Variations in T and SWC did not affect the potential net mineralization rate observed in the BSC microsite (P>0.05; Appendix 2; Fig.3). This variable, however, increased with augments in T in the BG microsite (P<0.01; Appendix 2; Fig.3). BSC soils only had a higher potential net mineralization rate than BG soils at the 30% WHC (P<0.01; Appendix 2; Fig.3). Significant T × SWC × MI and T × MI interactions were found when analyzing the N transformation (production of total available N)

and potential net ammonification rates (Appendix 1, Fig. 3). When analyzing the data separately for different T values, we did not find differences in the N transformation rate between either microsite type or SWC at 5ºC (P<0.01; Appendix 2, Fig. 2). At intermediate T values (10ºC-20ºC), soils under BSC had a higher N transformation rate than BG (P<0.01; Appendix 2). At 30ºC, a significant SWC × MI interaction was found (P<0.01; Appendix 2). At this temperature, changes in the N transformation rate were not observed for the BG microsite (P>0.05; Appendix 2; Fig.3) but this variable significantly decreased under BSCs in the 80% of WHC treatment. At 30%-50% WHC, higher N transformation rates were found under BSCs (P<0.001), but no significant effects of T were found. In the 80% of WHC treatment, the N transformation rate decreased under BSCs, but not in the BG microsite (P<0.01; Appendix 2; Fig 3).

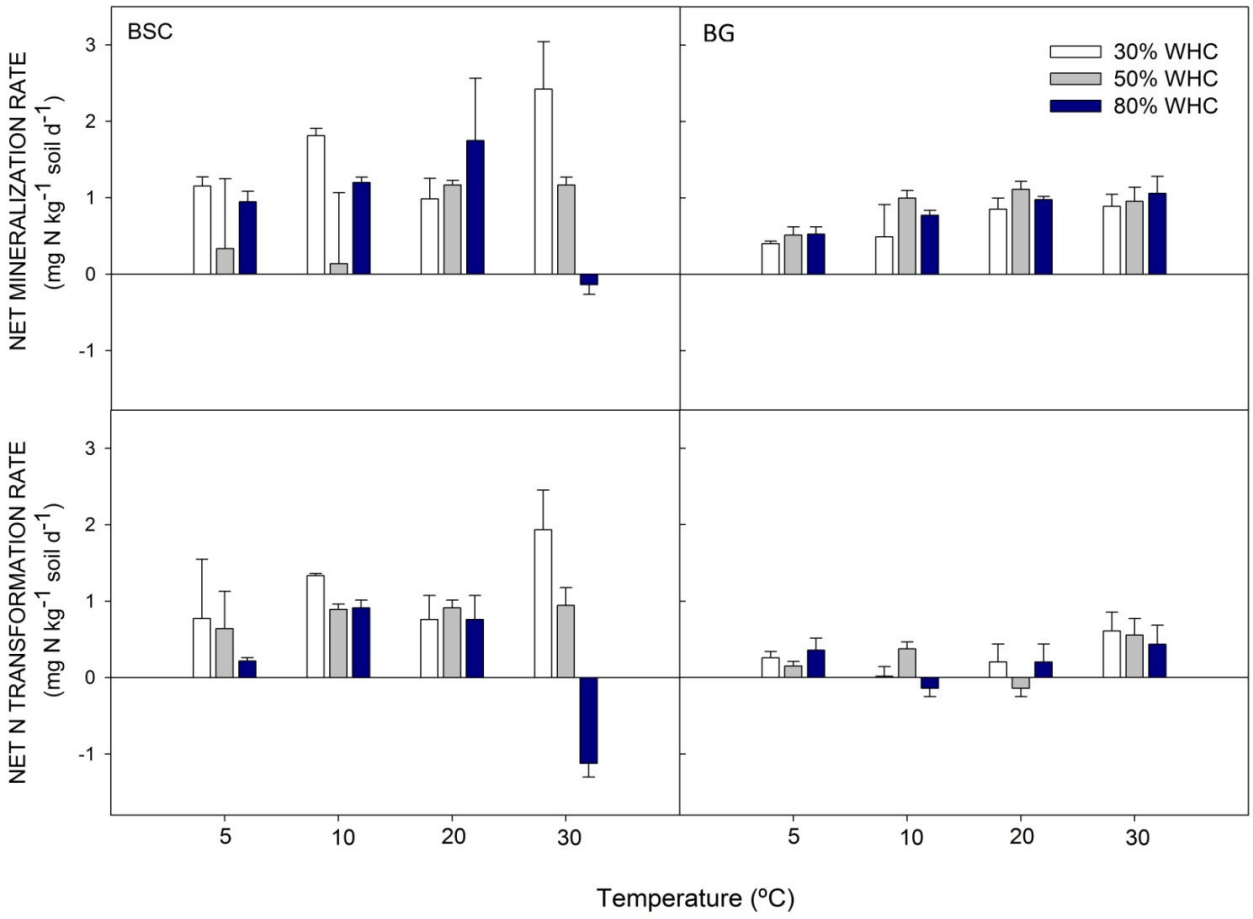

Figure 3. *Changes in the net nitrogen mineralization and transformation rates under the different combinations of soil water content and temperature in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

The dominance of ammonification decreased with temperature in both BSC and BG microsites between 30%-50% WHC (P<0.01; Appendix 2; Fig 4). For the 80% of WHC, the dominance of this process keep decreasing for the BG but did not show significant differences under BSCs (Appendix

2; Fig.4). At this SWC, the dominance of potential net ammonification was higher in the BSC than in the BG microsite (P<0.01; Appendix 2; Fig 4). The nitrification rate increased with T in both BG and BSC microsites between 30%-50% WHC (P<0.01; Appendix 1; Fig.4). Differences between microsite were not observed for these WHC (P>0.05; Appendix 2; Fig.4). However at the 80% of WHC treatment, the nitrification dominance kept increasing in the BG (P<0.01; Fig.4) but stopped increasing in the BSC microsite (P>0.05; Appendix 2). At this point, the potential net nitrification dominance was higher for the BG than for the BSC microsite (P<0.01; Appendix 2; Fig.4) In contrast, depolymerization rates were not affected by any of the treatments evaluated (P>0.05; Appendix 1).

Orwin and Wardle Resistance index

Soils under BSCs showed higher levels of ammonium resistance that those from the BG microsite (P<0.01; Fig. 5; Appendix 1 and 2). Resistance for this variable increased with T in both microsites (P<0.01; Appendix 1 and 2). Differences between SWC were observed in both BSC and BG microsites, but only at 10 ºC and 20ºC and without showing a particular pattern (P<0.01; Appendix 1 and 2).

We did not find changes with SWC for the rest of the variables studied: resistance in NO_3^--N, inorganic-N, DON, amino acids and MB-N (Appendix 1). The resistance of nitrate, inorganic-N, MB-N and amino acids was higher in the BSC than in the BG microsite (P<0.01; Fig.6; Appendix 1 and 2). The resistance of nitrate decreased with T for the BG microsite (P<0.01), but did not change under BSCs (Fig.6; Appendix 1). The resistance of inorganic N decreased with T in both microsites (P<0.01; Fig.6; Appendix 1). No significant differences between microsites were found when analyzing the resistance of DON; this variable showed its lowest values in the intermediate T (10 and 20ºC; P<0.01; Appendix 1). The resistance of MB-N and amino acids did not change with T in either BG or BSC microsites (Fig.6).

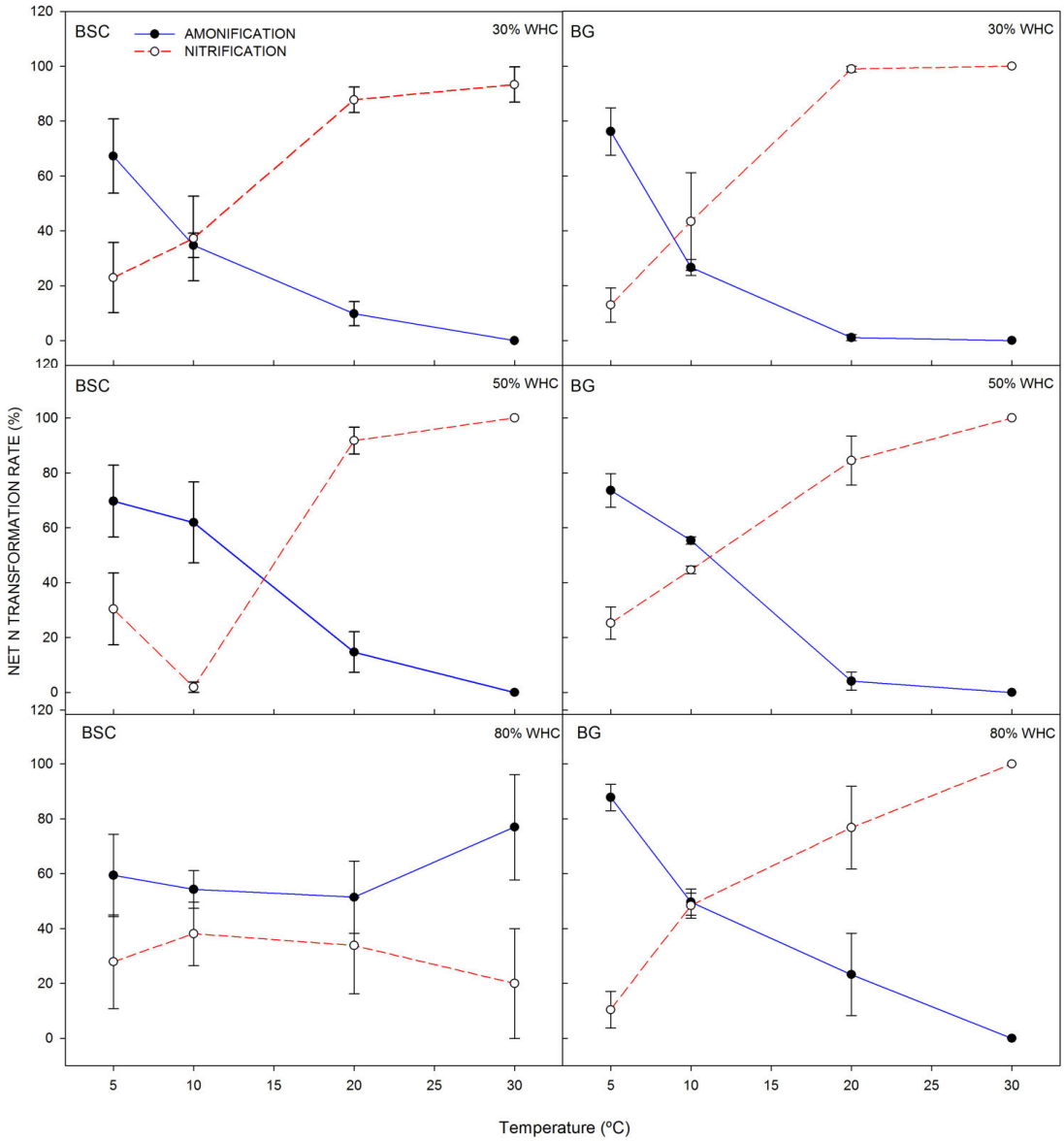

Figure 4. *Changes in the relative dominance of nitrogen transformation processes (net ammonification and nitrification rates) under the different combinations of soil water content and temperature in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

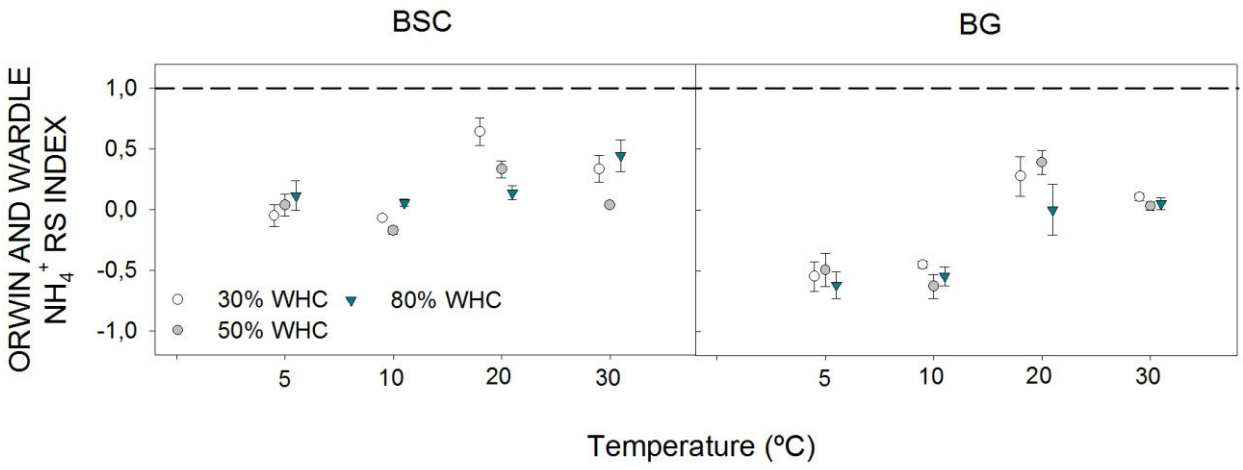

Figure 5. *Changes in the Orwin and Wardle Resistance index for NH_4^+-N under different combinations of soil water content and temperature in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

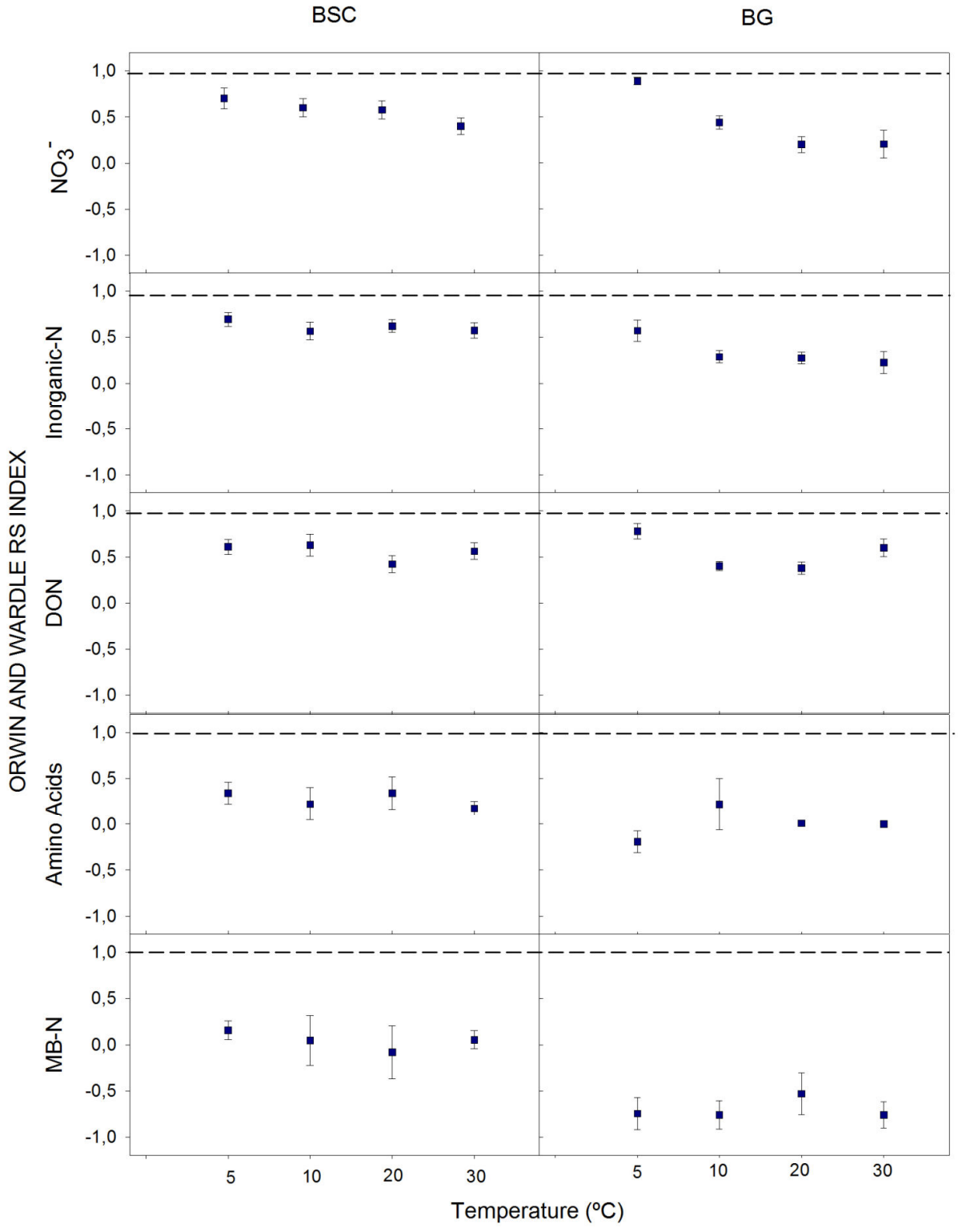

Figure 6. *Changes in the Orwin and Wardle Resistance index for NO_3^--N, inorganic-N, DON and amino acids (after incubation) under the different temperature levels evaluated in biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 5).*

Discussion

N concentrations

According to our hypothesis, we found that virtually all of the N variables studied had a different response to changes in temperature when BSCs were present, as indicated by the significant temperature × microsite interactions found. However, similar interactions were not observed for soil water content. The different variables evaluated were highly sensitive to changes in temperature, but not to the later variable within the range evaluated in this study (30-80% of water holding capacity). Thus, the observed increase of microbial biomass-N and NO_3^- and the decrease of NH_4^+ and amino acids with the increase in temperature would suggest an increased use by microorganisms of these reduced substrates, increasing nitrification and microbial immobilization. We expected a higher influence of soil water content on N transformations and concentrations, given the influence of this variable on most soil processes in drylands, such as soil respiration (e.g., Fernández et al. 2006; Almagro et al. 2009; Castillo-Monroy et al. 2011a), N mineralization (Kladivko and Keeney 1987; Gallardo and Merino 1998) and microbial biomass (Gallardo and Schlesinger 1993; 1995). The low influence of soil water content in our study may reflect the adaptation of the soil microbial community to the dry conditions typically maintained during long periods of time in the study area (Castillo-Monroy et al. 2011a). Thus, these microorganisms may be more responsive to the more continuously changing temperature than to changes in soil water content. However, we cannot discard that our minimum soil water content (30% of WHC) was not low enough to produce metabolic stress in our microbial populations. While 30% of WHC has been found to be the wilting point in soils such as those studied (Marqués et al 2008), surface soils (0-5 cm) at BS and BSC microsites at the study area commonly experience very low soil moisture values (below 20% of WHC during dry periods; Castillo-Monroy et al. 2010; 2011a). Thus, significant microbial functioning exists at 30% of WHC, with no apparent response to increasing soil moisture, emphasizing the idea that levels of soil humidity limiting plant production are not necessary coupled with limitation of microbial functioning, the latter being extended during longer periods of time in semi-arid ecosystems. The lack of response to 80% WHC as compared to lower WHC levels observed in our studied variables may be the consequence of the hierarchy of responses to water pulses size suggested for arid and semi-arid ecosystems (Schwinning and Sala 2004). Soil microbes involved in processes such as N mineralization and decomposition can be physiologically active in small amounts of water, while other microbes driving processes not measured in this study, such as microbialN fixation and predation, may require higher amount of water to be active (Cui and Caldwell 1997, Austin et al. 2004, Schwinning and Sala 2004).

It is interesting to note that there were not significant differences between BSC and BG soils in the initial conditions of C and N sources such as hexoses, pentoses, DON, and MB-N. We also did not find differences between nitrification inhibitors such as phenols, and other soil variables such as pH or the C:N ratio. We would expect that these initial differences between BSC and BG microsites may have resulted in divergent microbial community compositions (e.g. Zaady et al. 2011). The lack of differences in these initial soil variables between these microsites suggests that the different responses to changes in temperature and soil water content of the variables evaluated can only be explained by the different microorganism communities under BSCs with regard to those existing in BG areas. While particular differences in microbial communities between BSC and BG microsites in our study area have not explored yet, these are likely to occur, as recent studies that have shown important effects of BSC composition, richness and degree of development on the abundance and composition of soil microorganisms (Yeager et al. 2004, Housman et al. 2007, Soule et al. 2009, Castillo-Monroy et al. 2011b).

N transformation rates

Ammonification was the dominant N transformation process at both BSC and BG microsites under low temperatures and 30-50% WHC, whereas nitrification was the dominant process at high temperatures. An increase in nitrification parallel to augments in temperature up to moderate water content (30-60% WHC) has also been observed by other authors (Szukics et al. 2010; Bregliani et al. 2010). However, at higher water content levels, the prevalence of nitrification was turned into a dominance of ammonification in BSC soils at the highest temperatures, whereas in BG soils nitrification continued to be the dominant process at this temperature (Fig 3). These results suggest a differential response of microorganisms in BSC and BG microsites under these conditions. Previous studies have observed an accumulation of ammonium in cyanobacteria-covered soils, which was not observed under well-developed and lichen-dominated BSC soils (Housman et al. 2007). While denitrification can substantially differ depending on the degree of development of the BSC community (Barger et al. 2005), we assume it was not an important process in our experiment. As we applied the same treatments to both BSC and BG soils, and our soils were maintained under their water holding capacity, we do not expect anaerobic conditions to be prevalent in either treatment, even if they occur in some soil micropores. The abundance of nitrate in both treatments also indicated that denitrification was not important in our experiment. Our results suggest that, in semi-arid soils like those studied, small-scale spatial differences in factors like soil temperature, water content and the microbial community, which are strongly modified by BSCs (Castillo-Monroy et al. 2011; Maestre et al. 2011; Gundlapally and García-Pichel 2006), may have more

influence on the relative dominance of N transformation processes than differences in the overall availability of N (Schimel and Bennet 2004).

Orwin and Wardle Resistance index

The RS index obtained with variables such as DON, ammonium, nitrate and inorganic N showed a high sensitivity to changes in temperature at both BSC and BG microsites. However, changes in soil water content did not have any impact on the RS index for these variables. Thus, temperature, rather than water content, seems to be the main factor affecting the resistance of the N variables in the studied ecosystem.

Our results showed that soils under well-developed BSCs had a higher RS index than BG soils for most of the variables evaluated (ammonium, nitrate, inorganic-N, amino acids and MB-N), although a significant interaction with T appeared for ammonium and nitrate. These significant interactions can also be seen for DON, concluding that the presence of BSCs influenced alone, or in combination with temperature, all the studied variables. This effect may be a consequence of the more stable environment provided by BSCs versus the BG microsite. Because the RS index was predominantly higher for most N variables under BSCs, well-developed BSC communities may contribute to protect the N cycle in arid and semi-arid ecosystems by making their soils more resistant to the expected changes in climatic variability expected under the ongoing climatic change, helping to maintain soil functioning and fertility under future climatic conditions. These results highlight the importance of BSCs as a key player of N cycling in drylands, and complement and expand the findings of previous studies showing the influence of these organisms over the N cycle (Belnap 2002; Housman et al. 2007; Delgado-Baquerizo et al. 2010; Castillo-Monroy et al. 2010)

Concluding remarks

Our results showed that changes in temperature (between 5ºC and 30ºC) were more important than variation in soil water content (between 30% and 80% of field capacity) to determine the relative dominance of N transformation rates in a semi-arid grassland. Biological soil crusts significantly increased the resistance of the N variables studied to changes in temperature. Our results complement those of previous studies highlighting the key role of BSCs as modulators of N dynamics in dryland ecosystems, and indicate that the maintenance of well-developed BSC communities can minimize the impacts of expected increase in temperature variability with climate change on important variables of the N cycle.

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Supplementary material

Appendix 1. *Summary results of the PERMANOVA analyses carried out with all the studied variables. NH_4^+-N = N as ammonium; $N0_3$-N = N as nitrate; DON = Dissolved organic nitrogen; MB-N= Microbial biomass-N; PO_4^{3-}P = P as phosphate. MI = microsite, RES = residuals, SWC= soil water content, T = temperature.*

Variable	Source	df	MS	Pseudo-F	P(perm)
N and P concentrations (before incubation)					
Phenols	MI	1	7.2	<0.01	0.8
	RES	8	106.18		
Hexoses	MI	1	8265.6	1.51	0.24
	RES	8	5474.9		
Pentoses	MI	1	1288.2	0.19	0.63
	RES	8	6698.9		
NH_4^+-N	MI	1	191.67	27.43	0.02
	RES	6	6.99		
$N0_3^-$-N	MI	1	2866.2	32.7	0.01
	RES	8	87.65		
Inorganic-N	MI	1	3721	51.86	0.02
	RES	6	71.75		
DON	MI	1	88.89	3.53	0.13
	RES	7	25.21		
MB-N	MI	1	320.58	3.31	0.13
	RES	8	96.76		
Amino Acids	MI	1	491.82	16.603	< 0.01
	RES	7	236.98		
Hexose:Pentose ratio	MI	1	0.53	0.93	0.37
	RES	7	0.56		
N and P concentrations (after incubation)					
NH_4^+-N	T	3	15177	14.17	< 0.001
	SWC	2	3018	2.82	< 0.01
	MI	1	35075	32.76	< 0.001
	T × SWC	6	2002.9	1.87	< 0.001
	T × MI	3	9126.6	8.52	< 0.001
	SWC × MI	2	3005.7	2.81	< 0.001
	T × SWC × MI	6	2034.1	1.9	< 0.01
	RES	80	1070.9		
$N0_3^-$-N	T	3	1286.1	5.24	< 0.001
	SWC	2	364.2	1.48	0.21
	MI	1	35917	146.36	< 0.001
	T × SWC	6	450.23	1.83	0.06
	T × MI	3	852.81	3.48	< 0.001
	SWC × MI	2	718.36	2.93	0.03
	T × SWC × MI	6	447.58	1.82	0.05
	RES	85	245.4		
Inorganic-N	T	3	141.59	0.55	0.65

	SWC	2	331.01	1.29	0.28
	MI	1	65163	254.65	< 0.001
	T × SWC	6	462.39	1.81	0.11
	T × MI	3	112.19	0.44	0.73
	SWC × MI	2	440.44	1.72	0.19
	T × SWC × MI	6	424.79	1.66	0.14
	RES	71	255.9		
DON	T	3	1187	3.8	< 0.01
	SWC	2	207.69	0.67	0.63
	MI	1	10530	33.74	< 0.001
	T × SWC	6	529.32	1.7	0.07
	T × MI	3	617.45	1.98	0.07
	SWC × MI	2	129.71	0.42	0.84
	T × SWC × MI	6	327.79	1.05	0.3944
	RES	76	312.16		
Amino Acids	T	3	118000	29.78	< 0.001
	SWC	2	2508.7	0.63	0.54
	MI	1	437000	109.91	< 0.001
	T × SWC	6	4975.8	1.25	0.29
	T × MI	3	27008	6.8	< 0.001
	SWC × MI	2	14640	3.69	0.03
	T × SWC × MI	6	3168	0.8	0.58
	RES	85	3972		
MB-N	T	3	4106.2	2.33	< 0.001
	SWC	2	3283.2	1.86	0.04
	MI	1	12320	6.99	< 0.001
	T × SWC	6	1367.2	0.78	0.86
	T × MI	3	3585.8	2.04	0.01
	SWC × MI	2	2983.5	1.69	0.06
	T × SWC × MI	6	2098.6	1.19	0.19
	RES	84	1761.7		
N transformation rates					
N Transformation rate	T	3	4198.1	1.9	0.01
	SWC	2	3787.8	1.72	0.04
	MI	1	22805	10.34	< 0.001
	T × SWC	6	3051.7	1.38	0.04
	T × MI	3	5219.4	2.37	< 0.001
	SWC × MI	2	4597.8	2.08	0.01
	T × SWC × MI	6	3950.9	1.79	< 0.001
	RES	81	2205.7		
Mineralization rate	T	3	3043.9	2.48	< 0.001
	SWC	2	1159.3	0.94	0.48
	MI	1	9896.9	8.05	< 0.001
	T × SWC	6	2086.8	1.7	0.01
	T × MI	3	1884.8	1.53	0.07
	SWC × MI	2	2323.6	1.89	0.04
	T × SWC × MI	6	1836.7	1.49	0.04
	RES	75	1229.3		
Ammonification rate	T	3	27799	12.96	< 0.001
	SWC	2	5431.5	2.53	< 0.001
	MI	1	6081.3	2.84	< 0.001
	T × SWC	6	3162.4	1.47	< 0.001
	T × MI	3	3738.1	1.74	< 0.001

	SWC × MI	2	2950.2	1.38	0.04
	T × SWC × MI	6	2525.7	1.18	0.06
	RES	89	2144.4		
Nitrification rate	T	3	12038	6.26	< 0.001
	SWC	2	2629.9	1.37	0.13
	MI	1	6387.7	3.32	< 0.001
	T × SWC	6	3078.3	1.6	0.01
	T × MI	3	3136.6	1.63	0.03
	SWC × MI	2	2911.7	1.51	0.08
	T × SWC × MI	6	2422.4	1.26	0.11
	RES	86	1923.2		
Depolymerization rate	T	3	4826.7	0.97	0.67
	SWC	2	4869.1	0.98	0.58
	MI	1	5633.2	1.14	0.09
	T × SWC	6	5126.5	1.03	0.21
	T × MI	3	4981.3	1	0.45
	SWC × MI	2	4753	0.96	0.71
	T × SWC × MI	6	5024.4	1.01	0.36
	RES	80	4962.5		
Orwin and Wardle Resistance index					
NH_4^+-N	T	3	2.1	49.55	< 0.001
	SWC	2	< 0.01	1.61	0.21
	MI	1	2.92	68.83	< 0.001
	T × SWC	6	0.15	3.47	< 0.01
	T × MI	3	0.26	6.14	< 0.001
	SWC × MI	2	0.11	2.56	0.08
	T × SWC × MI	6	< 0.01	0.89	0.5
	RES	73	< 0.01		
$N0_3^-$-N	T	3	1.09	19.56	< 0.001
	SWC	2	< 0.01	1.16	0.32
	MI	1	0.44	7.85	< 0.01
	T × SWC	6	< 0.01	0.5	0.81
	T × MI	3	0.33	5.98	< 0.001
	SWC × MI	2	< 0.01	0.48	0.62
	T × SWC × MI	6	< 0.01	0.49	0.81
	RES	74	< 0.01		
Inorganic-N	T	3	0.25	5.95	< 0.001
	SWC	2	0.14	3.23	0.05
	MI	1	1.67	39.03	< 0.001
	T × SWC	6	< 0.01	1.14	0.35
	T × MI	3	< 0.01	1.5	0.22
	SWC × MI	2	< 0.001	< 0.01	0.91
	T × SWC × MI	6	< 0.01	0.82	0.56
	RES	67	< 0.01		
DON	T	3	0.32	8.97	< 0.001
	SWC	2	< 0.01	2.58	0.08
	MI	1	< 0.001	0.15	0.7
	T × SWC	6	< 0.01	1.28	0.28
	T × MI	3	0.15	4.1	0.01
	SWC × MI	2	< 0.01	1.54	0.22
	T × SWC × MI	6	< 0.01	1.09	0.38

	RES	65	< 0.01		
Amino acids	T	3	0.13	1.03	0.39
	SWC	2	0.14	1.12	0.34
	MI	1	1.67	13.67	< 0.001
	T × SWC	6	0.18	1.51	0.18
	T × MI	3	0.31	2.57	0.06
	SWC × MI	2	< 0.01	0.19	0.83
	T × SWC × MI	6	< 0.01	0.66	0.68
	RES	76	0.12		
MB-N	T	3	< 0.01	0.11	0.95
	SWC	2	0.34	1.72	0.19
	MI	1	12.15	61.58	< 0.001
	T × SWC	6	0.13	0.67	0.67
	T × MI	3	0.23	1.15	0.34
	SWC × MI	2	0.13	0.65	0.52
	T × SWC × MI	6	< 0.01	0.38	0.89
	RES	69	0.2		

Appendix 2. *Summary results of the PERMANOVA analyses conducted when interactions between the main factors were found. NH_4^+-N = N as ammonium; $N0_3$–N = N as nitrate; DON = Dissolved organic nitrogen; MB-N= Microbial biomass-N; PO_4^{3-}P = P as phosphate. MI = microsite, RES = residuals, SWC= soil water content, T = temperature.*

Variable	Factor	Source	df	SS	Pseudo-F	P(perm)
N and P concentrations (after incubation)						
NH_4^+-N	T: 5ºC	SW	2	14.45	0.3	0.75
		MI	1	1394.4	58.01	< 0.001
		SW × MI	2	23.87	0.5	0.62
		RES	17	408.6		
	T: 10ºC	SW	2	45.28	0.51	0.61
		MI	1	2365.4	53.08	< 0.001
		SW × MI	2	21.21	0.24	0.79
		RES	19	846.76		
	T: 20ºC	SW	2	159.12	2.11	0.14
		MI	1	1187.5	31.53	< 0.001
		SW × MI	2	210.33	2.79	0.08
		RES	22	828.65		
	T: 30ºC	SW	2	291.12	12.31	< 0.001
		MI	1	395.54	33.45	< 0.001
		SW × MI	2	327.96	13.87	< 0.001
		RES	22	260.16		
	T: 30ºC; BSC	SW	2	673.51	16.74	< 0.001
		RES	10	201.14		
	T: 30ºC; BARE SOIL	SW	2	0.43	0.55	0.7
		RES	5	1.95		
	SWC: 30%	T	3	1278.5	17.61	< 0.001
		MI	1	1533.4	63.38	< 0.001
		T × MI	3	382.08	5.26	0.01
		RES	27	653.27		
	SWC: 50%	T	3	1530.2	13.94	< 0.001
		MI	1	1114.9	30.47	< 0.001

		T × MI	3	486.92	4.44	0.01
		RES	26	951.27		
	SWC: 80%	T	3	279.53	3.4	0.03
		MI	1	2512.6	91.72	< 0.001
		T × MI	3	57.28	0.7	0.56
		RES	27	739.63		
MB-N	T; BSC	T	3	1045.5	0.79	0.5
		RES	51	22247		
	T; BARE SOIL	T	3	1670.9	9	<0.001
		RES	49	2925.7		
N transformation rates						
Mineralization rate	BSC; T x SWC	T	3	2,65	0,58	0,63
		SWC	2	3,99	1,32	0,27
		T × SWC	6	10,29	1,13	0,37
		RES	34	51,2		
	BG; T x SWC	T	3	1,81	4	0,01
		SWC	2	0,49	1,64	0,2
		T × SWC	6	0,3	0,33	0,92
		RES	41	6,2		
	SWC: 30%	MI	1	3,28	9,56	<0.001
		RES	29	9,96		
	SWC: 50%	MI	1	0,81	0,71	0,42
		RES	35	39,89		
	SWC: 80%	MI	1	0,32	0,41	0,55
		RES	29	22,97		
N transformation rate	BSC; T x SWC	T	3	1,95	1	0,4
		SWC	2	8,76	6,73	<0.01
		T × SWC	6	11,87	3,04	0.01
		RES	39	25,37		
	BG; T x SWC	T	3	1,93	3,97	0,01
		SWC	2	0,03	0,09	0,9
		T × SWC	6	1,11	1,14	0,35
		RES	42	6,8		
	T: 5ºC	SW	2	0.23	0.14	0.88
		MI	1	0.51	0.62	0.46
		SW × MI	2	0.59	0.36	0.72
		RES	19	15.5		
	T: 10ºC	SW	2	0.4	5.53	0.01
		MI	1	5.75	159.5	< 0.001
		SW × MI	2	0.71	9.8	< 0.001
		RES	19	0.68		
	T: 20ºC	SW	2	< 0.01	< 0.01	0.91
		MI	1	3.63	12.38	< 0.001
		SW × MI	2	0.35	0.6	0.55
		RES	22	6.44		
	T: 30ºC	SW	2	11.37	12.48	< 0.001
		MI	1	< 0.01	< 0.01	0.84
		SW × MI	2	9.05	9.94	< 0.001
		RES	21	9.57		
	T: 30ºC; BSC	SW	2	17.61	12.8	< 0.001
		RES	9	6.19		
	T: 30ºC; BARE SOIL	SW	2	< 0.01	0.14	0.87

		RES	12	3.38		
	SWC: 30%	T	3	3.99	1.76	0.18
		MI	1	7.89	10.45	< 0.001
		T × MI	3	1.41	0.62	0.62
		RES	29	21.9		
	SWC: 50%	T	3	0.8	1.34	0.29
		MI	1	3.03	15.22	< 0.001
		T × MI	3	0.55	0.92	0.46
		RES	25	4.97		
	SWC: 80%	T	3	3.32	5.62	< 0.001
		MI	1	< 0.001	< 0.01	0.89
		T × MI	3	7.6	12.87	< 0.001
		RES	27	5.32		
	SWC: 80%; BSC	T	3	8.66	14.9	< 0.001
		RES	12	2.33		
	SWC: 80%; BARE SOIL	T	3	0.85	1.43	0.27
		RES	15	2.99		
Ammonification rate	SWC: 30%	T	3	31497	51.69	< 0.001
		MI	1	35.56	0.18	0.68
		T × MI	3	481.76	0.79	0.51
		RES	29	5890.5		
	SWC: 50%	T	3	36447	37.13	< 0.001
		MI	1	101.94	0.31	0.58
		T × MI	3	299.83	0.31	0.82
		RES	30	9815.2		
	SWC: 80%	T	3	8226.7	4.4	0.01
		MI	1	3898.4	6.25	0.02
		T × MI	3	13035	6.97	< 0.001
		RES	30	18701		
	SWC: 80%; BSC	T	3	1685.3	0.63	0.59
		RES	15	13279		
	SWC: 80%; BARE SOIL	T	3	19576	18.05	< 0.001
		RES	15	5422.1		
Nitrification rate	T; BSC	T	3	26139	7,16	< 0.001
		RES	51	61995		
	T; BARE SOIL	T	3	19771	60,34	< 0.001
		RES	51	16710		
	SWC: 30%	T	3	41097	24,11	< 0.001
		MI	1	110,33	0,19	0,66
		T × MI	3	606,31	0,36	0,79
		RES	28	15908		
	SWC: 50%	T	3	44132	70,13	< 0.001
		MI	1	528,39	2,52	0.12
		T × MI	3	3493	5,55	< 0.001
		RES	29	6083		
	BSC; SWC: 50%	T	3	29762	36,9	< 0.001
		RES	14	3759		
	BG; SWC: 50%	T	3	17567	37,8	< 0.001
		RES	15	2323		
	SWC: 80%	T	3	8812	3,41	0.03
		MI	1	4665	8,9	< 0.001
		T × MI	3	11339	4,39	0.01

		RES	29	24962		
	BSC; SWC: 80%	T	3	819	0,21	0.88
		RES	15	19496		
	BG; SWC: 80%	T	3	18299	15,6	< 0.001
		RES	14			
Orwin and Wardle Resistance index						
NH_4^+-N	T: 5ºC	SW	2	< 0.01	0.19	0.82
		MI	1	1.88	37.95	< 0.001
		SW × MI	2	< 0.01	0.61	0.55
		RES	17	0.84		
	T: 10ºC	SW	2	0.11	5.33	0.02
		MI	1	1.16	112.93	< 0.001
		SW × MI	2	< 0.01	2.1	0.15
		RES	16	0.16		
	T: 20ºC	SW	2	0.68	4.68	0.02
		MI	1	0.14	1.92	0.18
		SW × MI	2	0.2	1.38	0.27
		RES	20	1.45		
	T: 30ºC	SW	2	0.23	3.57	0.05
		MI	1	0.28	8.72	0.01
		SW × MI	2	0.16	2.53	0.11
		RES	20	0.64		
	SWC: 30%	T	3	3.15	21.12	< 0.001
		MI	1	0.99	19.87	< 0.001
		T × MI	3	< 0.01	0.5	0.69
		RES	24	1.19		
	SWC: 50%	T	3	2.7	41.82	< 0.001
		MI	1	0.42	19.48	< 0.001
		T × MI	3	0.54	8.37	< 0.001
		RES	24	0.52		
	SWC: 50%; BSC	T	3	0.64	14.21	< 0.001
		RES	14	0.21		
	SWC: 50%; BARE SOIL	T	3	2.34	25.58	< 0.001
		RES	10	0.31		
	SWC: 80%	T	3	1.5	9.05	< 0.001
		MI	1	1.76	31.67	< 0.001
		T × MI	3	0.4	2.39	0.09
		RES	25	1.39		
$N0_3$_N	T; BSC	T	3	0.47	2.28	0.09
		RES	43	2.98		
	T; BARE SOIL	T	3	3.9	34.99	< 0.001
		RES	47	1.75		
DON	T; BSC	T	3	0,27	1,95	0.13
		RES	40	1,86		
	T; BARE SOIL	T	3	1,2	12,57	<0.001
		RES	41	1,31		

CAPÍTULO 6

Biological soil crusts promote N accumulation in response to dew events in dryland soils

Manuel Delgado-Baquerizo, Fernando T. Maestre, Jesús G.P. Rodríguez, Antonio Gallardo

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Abstract

Dew is an important source of water in drylands, particularly for biological soil crusts (BSCs), a soil community dominated by lichens, mosses and cyanobacteria that is prevalent in these environments and play important roles in nutrient cycling. While BSCs can retain and use water from dew, the effects of dew events on the cycling of nitrogen (N) and carbon (C) in BSC-dominated ecosystems are largely unknown. We conducted an experiment to evaluate the effects of BSCs and dew on N and C cycling; intact soil cores from either bare ground or BSC-dominated microsites were incubated during 14 days under control and artificial dew addition treatments. A positive increment in the amount of total available N and phenols was observed in response to dew events under BSCs. We also found an increase in the concentration of dissolved organic N, as well as in the pentoses:hexoses ratio, under BSCs, suggesting that dew promoted an increase in the decomposition of organic matter at this microsite. The increase in the amount of available N commonly observed under BSCs has been traditionally associated with the fixation of atmospheric N_2 by BSC-forming cyanobacteria and cyanolichens. Our results provide a complementary explanation for such an increase: the stimulation of microbial activity of the microorganisms associated with BSCs by dew inputs. These effects of dew may have important implications for nutrient cycling in drylands, where dew events are common and BSCs cover large areas.

Key words: DON; drylands; carbohydrates; phenols; pentose: hexose ratio

Introduction

Drylands (arid, semi-arid and dry-subhumid ecosystems) are a key terrestrial biome, covering 41% of the Earth's land surface and supporting over 38% of the total global population (Reynolds et al., 2007). In these ecosystems, where water availability is the most important factor limiting biological processes (Whitford, 2002), dew events are a common source of water for plants, biological soil crusts dominated by mosses, lichens and cyanobacteria, invertebrates, and small vertebrates (Kidron et al., 2002; Zhang et al., 2009). Information about dew events and their biological implications is still scarce as they have been traditionally considered a minor component of the water balance (Wallin, 1967). However, studies conducted during the last decades indicate that dew plays a significant role in the local water balance of semiarid vegetation in drylands as they may provide up to 40% of the water inputs received every year (Kidron, 2000; Jacobs et al., 2002; Moro et al., 2007; Lekouch et al., 2011). For instance, up to 195 days of dew and foggy mornings were recorded in the Negev Desert of Israel, providing an annual mean of 33 mm of dew and fog precipitation (Evenari, 1981). Water pulses from dew differ from those of rainfall events in their size and

frequency; while dew events are common throughout the year, and typically range from 0.15 mm to 0.30 mm per day (Kidron 2000; Jacobs et al., 2000; Moro et al., 2007), the smallest rainfall events in arid/semiarid ecosystems are less than 5 mm (Loik et al., 2004), and show irregular patterns of distribution both intra- and inter-annually (Westoby, 1972; Noy-Meir, 1973). Water inputs from rainfall events play a critical role on biogeochemical processes in drylands as even small pulses can activate processes such as nitrification, soil respiration and denitrification (Cui and Caldwell, 1997; Smart et al., 1999; Austin et al., 2004). However, not much is known about the influence that very small water pulses, such as those from dew events, have on biogeochemical cycles in drylands.

Biological soil crusts (BSCs) play key functional roles in drylands worldwide, where they are a prevalent biotic component (Eldridge and Green, 1994; Belnap and Lange, 2003; Maestre et al., 2011). These crusts control the carbon (C) cycle in drylands by fixing atmospheric C through photosynthesis (Belnap et al., 2004), by contributing to soil CO_2 efflux (Castillo-Monroy et al., 2011a), and by affecting the activity of β-glucosidase (Bowker et al., 2011). Key processes of the nitrogen (N) cycle, such as N fixation (Belnap, 2002), mineralization-depolymerization (Castillo-Monroy et al., 2010), and gaseous N losses (Barger et al., 2005), are also driven by BSCs. The influence of BSCs on C (i.e. photosynthesis) and N (i.e. mineralization) processes in response to small rainfall events (around 2 mm) has been previously observed (e.g., Belnap et al., 2004; Delgado-Baquerizo et al., 2012). By increasing the roughness and microtopography of the soil surface, BSCs can increase its area, and augment the amount of dewfall reaching the soil (Rao et al., 2009). Therefore, BSCs are likely to determine potential effects of dew events on soil biogeochemical processes.

The effects of BSCs on soil N and C dynamics in response to water inputs from dew have not been studied before. We aimed to do so by evaluating how simulated dew conditions affect multiple variables related to N (ammonium, nitrate, dissolved organic nitrogen [DON], amino acids, total available N) and C (carbohydrates, phenols, pentoses:hexoses ratio and carbohydrates: available N ratio) cycling in soils from microsites differing in the degree of BSC development (bare ground and well-developed BSCs areas). These cryptogams have been observed to be physiologically active during dew events (Veste et al., 2008**;** Wilske et al., 2008; Rao et al., 2009; Pintado et al., 2010), and maintain abundant and rich fungal communities underneath them (Bates et al., 2010). Thus, we hypothesized that the microbial communities associated with BSCs may carry out microbial decomposition and N mineralization in response to dew events, uptaking C labile sources, and producing N available to plants and microorganisms (Schimel and Bennett, 2004) at a faster rate than those present in bare ground areas.

Methods

Sampling design and laboratory analyses

Soils and BSCs for this study were collected at the Aranjuez experimental station, located in central Spain (40º02' N – 3º 37'W; 590 m a.s.l.; 8º slope Facing SE). The climate is Mediterranean semi-arid, with an average annual rainfall and temperature of 349 mm and 14.5 ºC, respectively (1986-2012 period). Perennial plant cover is below 40%, and is dominated by the tussock grass *Stipa tenacissima* L. (18% of plant cover) and the N-fixing shrub *Retama sphaerocarpa* (L.) Boiss (6% of plant cover). Open areas between plant patches host well developed BSCs 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 Castillo-Monroy et al., 2010 for a full checklist). Bare ground and BSC-dominated areas cover 28% and 32% of the total area of the study site, respectively. The soil is classified as Xeric Haplogypsid (Marqués et al., 2008), and has a fine texture dominated by the presence of gypsum. See Appendix A for further information on the soils from the study site.

Soil sampling was carried out during the spring of 2010. Twelve intact soil cores (5 cm depth, 7.5 cm diameter) were collected under each of two microsites: well-developed BSCs (cover of lichens and mosses > 75%; see Appendix B of Castillo-Monroy et al., 2010) and bare ground areas (BG hereafter) devoid of vascular vegetation and visible BSC components (cover of mosses and lichens < 15%; see Appendix B of Castillo-Monroy et al., 2010). This depth was chosen to keep the influence of BSCs in its entirety (usually top 0-4 cm of soil, Castillo-Monroy et al., 2010; Delgado-Baquerizo et al., 2010). After sampling, soil cores were transported to the laboratory and air-dried at room temperature for four weeks.

Air-dried soil cores with and without BSCs (with six replicates each) were incubated in a plant growth chamber for 14 days under two treatments: with and without (control) dew. Day (9 hours of light, 20% relative humidity, 20ºC) and night (15 hours darkness, 80% relative humidity, 10ºC) conditions simulated in this chamber follow climatic conditions typically found in early spring in the study area, when BSCs and soil microbial communities are most active (Castillo-Monroy et al., 2011a). In the dew treatment, soils were watered automatically three times during the darkness period to keep soil humidity constant at a rainfall equivalent of 0.15 mm per pot (1% of soil water content in our soils). The amount and duration of dew events were selected according to the number and duration of dew events observed in ecosystems similar to that studied here (Jacobs et al., 2002; Kidron, 2000; Moro et al., 2007). The amount of dew added to the soil was monitored

by weighting all the cores every 12 hours (Appendix B). In the control treatment, soils were not watered at any time during the experiment.

Before and after the experiment, we collected 2.5 g of soil (0-2 cm depth) from each replicated core. This depth was chosen because the effects of BSCs on soil properties are mainly noticeable in the first two cm of the soil profile (e.g., Bowker et al., 2011; Castillo-Monroy et al., 2011b; Maestre et al., 2012). Soil samples were extracted with K_2SO_4 0.5 M in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 hour at 20ºC and filtered to pass a 0.45-µm Millipore filter (Jones and Willett 2006). The filtered extract was kept at 2 ºC until colorimetric analyses, which were conducted within 24 hours following the extraction. Sub-samples of each non-incubated (air-dried) extract were taken for measurements of amino acids, phenols and carbohydrates (sum of pentoses and hexoses) according to Chantigny et al. (2006). The pentoses:hexoses ratio was calculated from these variables. Ammonium, nitrate, DON were measured by following Delgado-Baquerizo et al. (2011). Potentially available N (total available N hereafter) was calculated as the sum of ammonium, nitrate and DON as described in Delgado-Baquerizo and Gallardo (2011). We used the ratio carbohydrates:total available N as an indicator of the relative C and N availability for soil microorganisms in drylands, as this ratio can provide additional insights compared to the classic total C-to-N ratio (Gallardo and Schlesinger, 1992; 1995).

We also collected samples of BSC-forming lichens to measure their respiration in response to simulated dew events. We weighed 12 samples (~5 g) of BSCs (without soil) in petri dishes. Six of these samples were subjected to each treatment (control and dew, as described above), and were placed in the plant growth chamber. Lichen respiration was measured multiple times during a 12 hour period, including measurements of night and light conditions, by using an Infra-Red Gas Analyzer (SBA-4, PP-Systems, Hitchin, U.K).

Numerical and statistical analyses

We calculated the absolute increment in the values of soil C variables (hexoses, pentoses, phenols, and hexose: pentose ratio) and N variables (ammonium, nitrate, DON, amino acids, total available N, C-phenols: available N and carbohydrates: available N ratio) after 14 days of incubation (regarding initial concentrations). One-way ANOVAs were used to evaluate differences between microsites (BSC and BG) in the initial values (before incubations) of C and N variables. Differences in the increment of these variables were evaluated using two-way ANOVAs, with microsites (BSC and BG) and treatments (control and dew) as fixed factors. Separate ANOVAs

were conducted for each variable. To investigate interactions, data were divided into subsets based on one of the factors of interaction, and then were subjected to ANOVA. Differences in the respiration of BSC-forming-lichens between control and dew treatments were evaluated by using repeated-measures ANOVA. All statistical analyses were carried out using IBM SPSS 15.0 (SPSS Inc, Chicago, IL, USA). The experiment-wide error rate was not adjusted for multiple testing, as this approach is considered excessively conservative (Gotelli and Ellison, 2004).

Results

Before incubations, the concentration of ammonium, amino acids, total available N and phenols was higher in BG than in BSC microsites ($p<0.05$; Table 1). At this time, differences in the concentration of nitrate, DON, carbohydrates, and in the hexoses:pentoses, carbohydrates:total available N and phenols:carbohydrates ratios, were not found between microsites ($p>0.05$; Table 1).

Table 1. *Initial concentration of carbon and nitrogen variables in both biological soil crust (BSC) and bare ground (BG) microsites. Data represent means (SE), n = 12. Significance levels between microsites are as follows: *p < 0.05; **p<0.01; ***p< 0.001.*

Variable	BG	BSC
NH_4^+ (mg N kg^{-1} soil)**	5.23 (0.50)	3.22 (0.18)
NO_3^- (mg N kg^{-1} soil)	5.98 (0.70)	4.78 (0.80)
DON (mg N kg^{-1} soil)	26.31 (1.96)	21.28 (1.56)
Amino acids (mg N kg^{-1} soil)**	0.76 (0.09)	0.35 (0.07)
Total available N (mg N kg^{-1} soil)*	37.52 (2.62)	29.07 (2.01)
Carbohydrates (mg C kg^{-1} soil)	110.22 (9.83)	95.60 (12.64)
Phenols (mg C kg^{-1} soil)**	23.31 (2.59)	11.07 (1.06)
Ratio pentoses: hexoses	0.66 (0.20)	0.45 (0.13)
Carbohydrates: total available N	3.00 (0.28)	3.53 (0.59)
Phenols: carbohydrates	0.22 (0.03)	0.15 (0.03)

A significant Microsite x Treatment interaction was found for the increment in DON ($p<0.05$; Appendix C, Fig. 1A). The addition of dew increased DON (regarding control) in the BSC microsite ($p<0.05$; Fig.1A; appendix C). Soils under BSCs showed a greater decrease in DON than BG soils in the control treatment ($p=0.05$; Appendix C), but the inverse result was found under dew conditions ($p<0.01$; Fig.1A; Appendix C). A Microsite x Treatment interaction was also found

when analyzing the increment in ammonium ($p<0.01$; Appendix C). In the dew treatment, soils from bare ground areas showed a greater decrease in ammonium than those from the BSC microsite ($p<0.05$), which was not observed in the control treatment ($p>0.05$; Fig.1B; Appendix C). A Microsite x Treatment interaction was observed for the increment in total available N ($p<0.01$; Appendix C). Dew additions increased total available N with respect to control in the BSC microsite ($p<0.01$), a response that was not observed in the BG microsite ($p>0.05$; Fig.1C; Appendix C). Significant differences between microsites were not found in the control treatment ($p>0.05$), albeit BSC soils had a higher increase in the total available N than BG soils with dew ($p<0.01$; Fig.1C; appendix C). Significant differences were not observed between the microsites or treatments when analyzing the increment in nitrate and amino acids ($p>0.05$; Fig.1D and E; appendix C). A Microsite x Treatment interaction was found for the increment of phenols ($p<0.01$; Appendix C). The addition of dew promoted a significant increase in phenols for the BSC and a decrease in phenols for the BG microsites ($p<0.05$; Fig.2A; appendix C). Dew decreased the amount of carbohydrates found ($p<0.01$) regardless of the microsite considered ($p>0.05$; Fig 2B; Appendix C). The increment in the pentoses:hexoses ratio showed a marginally significant tendency ($p=0.08$) to increase in response to dew additions at both BSC and BG microsites (Fig.2C; Appendix C). Dew decreased the carbohydrates:total available N ratio ($p<0.01$), regardless of the microsites considered ($p>0.05$; Fig 2B; Appendix C).

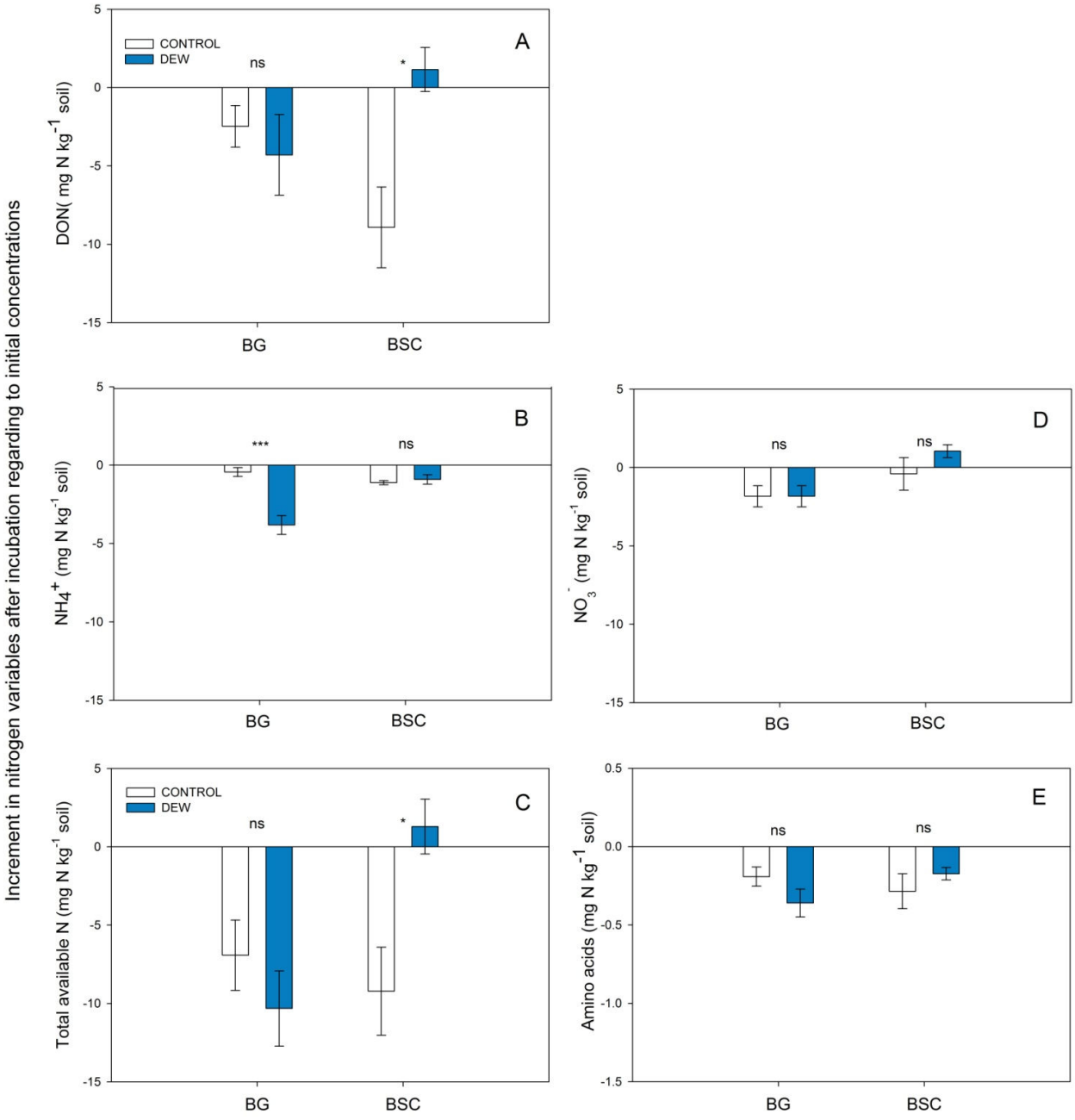

Figure 1. *Increment in the studied nitrogen variables after 14 days of incubation under control and simulated dew conditions for biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 6). Significance levels between treatments (control and dew) are as follows: [a]p<0.1 (marginally significant); *p < 0.05; **p<0.01; ***p< 0.001.*

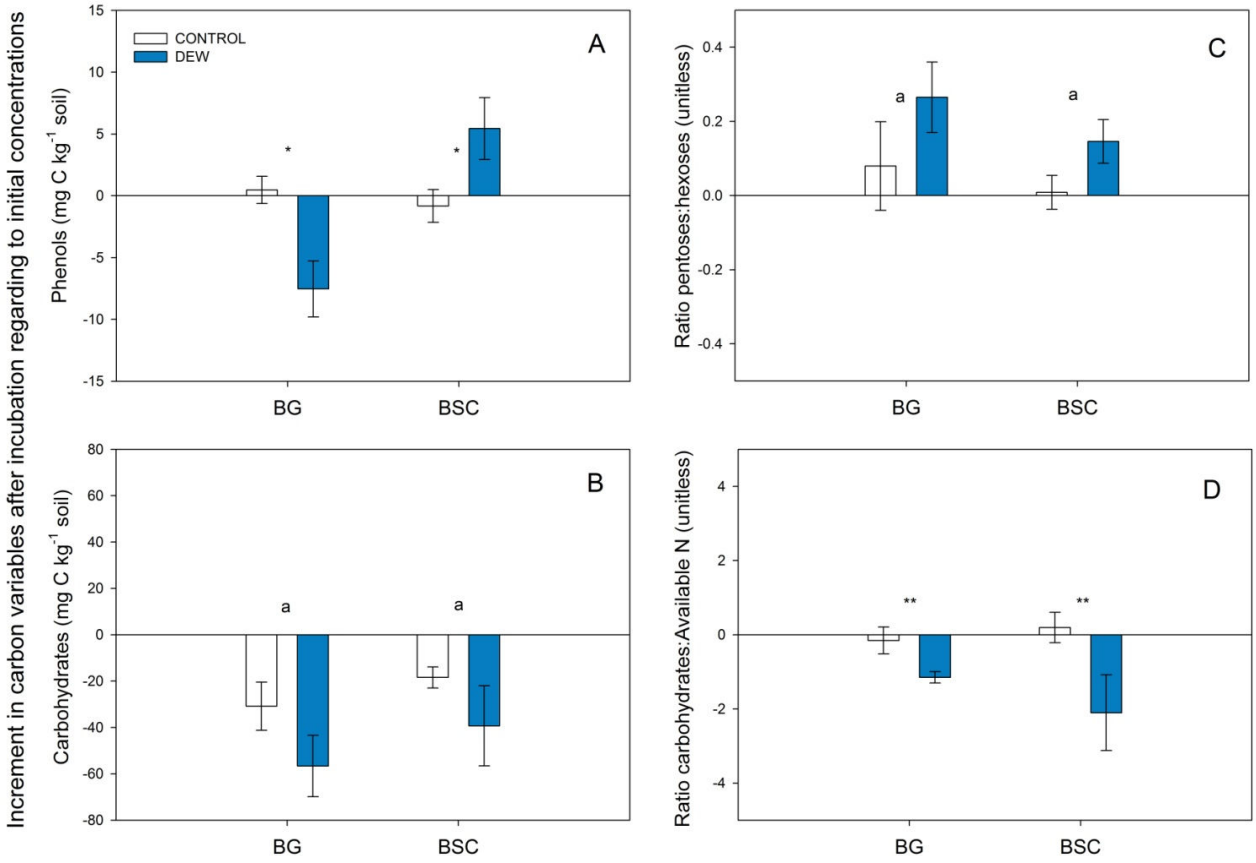

Figure 2. *Increment in the studied carbon variables after 14 days of incubation under control and simulated dew conditions for biological soil crust (BSC) and bare ground (BG) soils. Data are means ± SE (n = 6). Significance levels between treatments (control and dew) are as follows: [a]p<0.1 (marginally significant); *p < 0.05; **p<0.01; ***p< 0.001.*

The respiration of BSC-forming-lichens was always higher in the dew treatment than in the control, where respiration was not detected (p<0.01; Fig.3; Appendix C), with the highest peak of crust respiration found after the first dew pulse (Fig. 3).

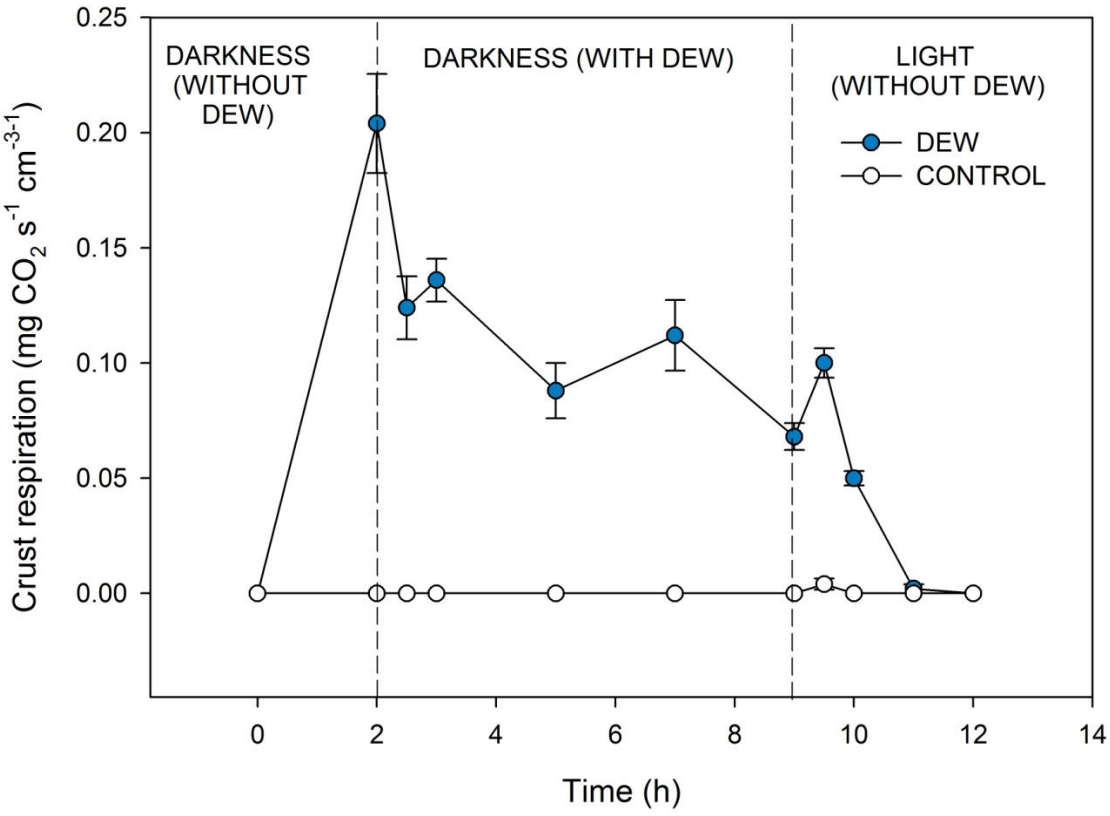

Figure 3. *Monitoring of the respiration of biological soil crust-forming lichens over a 12 hours period for both control and dew treatments. Data are means ± SE (n = 6).*

Discussion

Our results show that water pulses similar to those provided by dew events under field conditions can activate the microbial communities associated with BSCs, promoting microbial mineralization and decomposition of soil-organic matter (Schwinning and Sala, 2004). They also indicate that well developed BSCs change the response of C and N variables to dew pulses, which could be mediated by the activity of bacteria and fungi. The observed positive increment in the total available N under BSCs in response to dew events may be promoted by higher fungal:bacteria ratios observed at this microsite (compared to bare ground areas) in our study area (Appendix A). Similarly, well-developed fungal communities under BSCs have been reported in other dryland ecosystems (Bates et al., 2010). Fungi-dominated communities have a higher microbial N-use efficiency than bacterial-dominated communities, producing biomass with a higher C:N ratio (Paul and Clark, 1996), and immobilizing less N per unit of assimilated C (Austin et al., 2004). In addition, cyanobacteria and green algae communities, which are usually linked to BSCs (Belnap and Lange, 2003; Belnap et al., 2004), could also be responsible for the higher N availability observed under BSCs due to their high capacity in fixing atmospheric N (Belnap and Lange, 2003).

The immobilization of total available N, ammonium and amino acids observed in the control treatment under BSCs, as well as the decrease of carbohydrates and the carbohydrates:available N ratio observed in both BG and BSC microsites, suggests that even the 80% relative humidity present in the plant growth chamber during the incubation period in the control treatments may be enough to activate N cycling processes under BSCs. Both green algae and the lichens containing them as photobionts have been shown to use atmospheric moisture to carry out photosynthesis and growth (Thomson and Iltis, 1968; Belnap and Lange, 2003). Thus, bacterial populations with rapid expansion may be active under these conditions, promoting an initial phase of immobilization followed by a mild phase of mineralization under dew conditions, as suggested by Austin et al. (2004). The observed trend towards a positive increase in the pentoses:hexoses ratio in both microsites, and the increase of DON under BSCs in response to dew events, suggests a higher plant-derived decomposition of organic matter in this microsite (Chantigny et al., 2006; Schimel and Bennet, 2004). Thus, fungal-dominated microbial communities, which are more desiccation-tolerant than those dominated by bacteria (Adebayo and Harris, 1971; Austin et al., 2004), may be more efficient promoting decomposition under dew events (Austin et al., 2004; Butterbach-Bahl and Per Gundersen 2011).

Furthermore, the wilting point in soils of the area studied is reached near 30% of water holding capacity (Marqués et al., 2008), whereas surface soils (0-5 cm) at BS and BSC microsites in the study area commonly experience low soil moisture values (below 15%) during a significant part of the year (Castillo-Monroy et al., 2011a). This fact emphasizes the idea that soil moisture levels limiting plant production are not limiting microbial function. This allows soil microorganisms associated with BSCs operating without nutrient plant competition to accumulate N, which can be used by plants during their main activity periods (Singh et al., 1989).

Moreover, the increment in phenols observed under BSCs may be the result of either organic matter decomposition or active microbial synthesis. In either case, such increment may suggest an allelopathic effect that may prevent other groups of microorganisms from occupying a microsite with high total available N as a result of the positive impacts of BSCs on this variable (Chantigny et al., 2006; Zhang et al., 2009). For example, catechol and hydroxamate siderophores, produced by some groups of microbes have been implicated in microbial competition through the chelation of Fe m (Martinez et al., 1990; Diarra et al., 1996).

Finally, the respiration detected in the BSC-forming lichens when dew was added, together with the observed changes in C and N variables, supported the idea that even small water pulses can

activate soil microbial communities associated to BSCs in drylands and the metabolism of C and N in soils (Schwinning and Sala, 2004). In addition, the highest peak in crust respiration observed immediately after a dew event agree with previous studies showing rapid physiological responses of BSC-forming lichens to small water pulses (Veste et al., 2008**;** Wilske et al., 2008; Rao et al., 2009). Our results also showed a decrease in soil carbohydrates with dew, suggesting that the crust respiration responses to dew events may be modulated by the reserve of labile soil carbohydrates.

To conclude, we found that dew-like water inputs can promote the activation of microorganisms involved in the C and N cycles in dryland soils, and that this response is modulated by well developed BSC communities. To our knowledge, such effects of dew and BSCs on nutrient cycling have not been reported before. Increases in the availability of N under BSCs have been commonly observed in many drylands worldwide (Belnap, 2002; Zaady, 2005; Delgado-Baquerizo et al., 2010; Su et al., 2011). Such an increase is often associated with the fixation of atmospheric N_2 by BSC-forming cyanobacteria and cyanolichens (Belnap, 2002). Our results provide an alternative explanation for the increase of N typically observed under BSCs: the stimulation of microbial activity of the microorganisms associated with BSCs by dew inputs. Given the degree of development of BSCs in drylands worldwide, and the importance of water inputs from dew, the production of N under dew conditions can make an important contribution to the total N available for plants and microorganisms in these regions. The different response found in BSC and BG microsites suggest that different microbial communities may modulate the hierarchical response to soil moisture pulse events commonly observed in drylands (Schwinning and Sala, 2004). Our results complement those of previous studies highlighting the key role of BSCs as modulators of C and N dynamics in dryland ecosystems, and indicate that the conservation of well-developed BSC communities is crucial to maintain and increase N availability in drylands.

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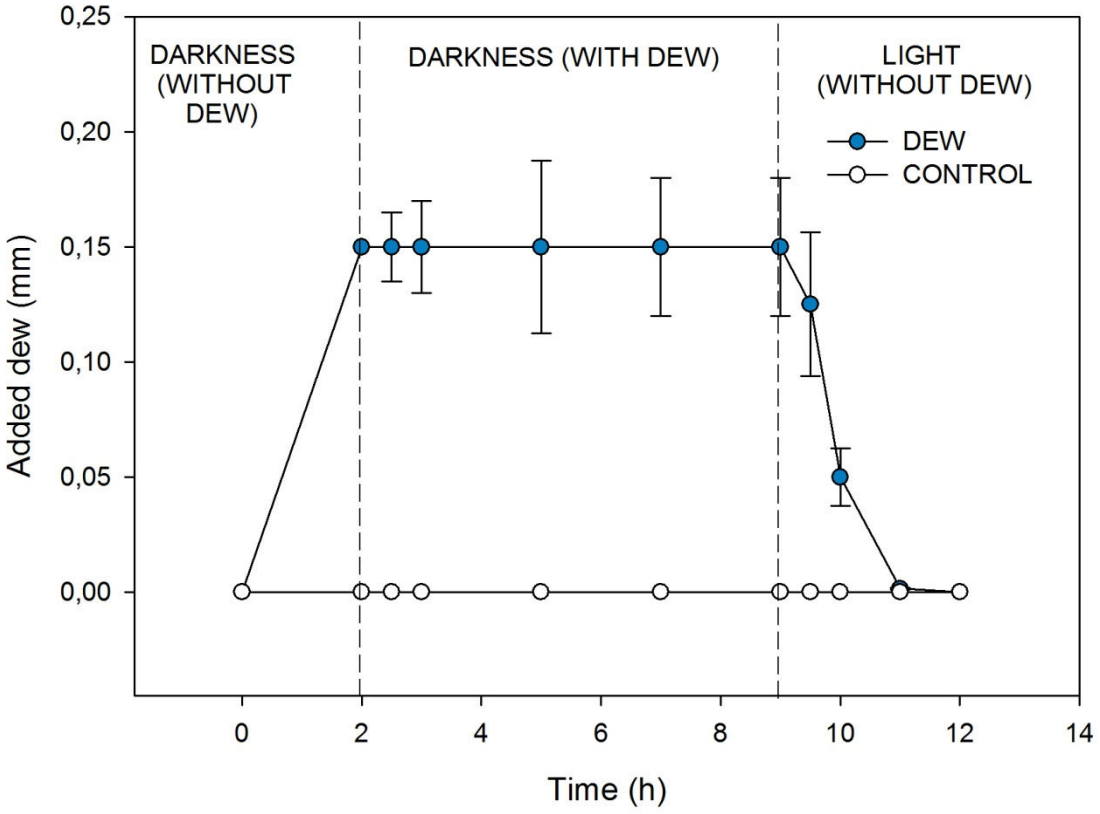
Supplementary material

Appendix A. *Texture, pH, C:N ratio and fungal:bacterial ratio from the top 2 cm of the mineral soil profile for the Bare soil and BSC microsites in the study site. Texture and pH data represent means ± SE (n = 5). Fungi:Bacterial and C:N ratio represent means ± SE (n = 10).*

	Bare soil	**BSC**
Silt (%)[1]	38.0 ± 1.09	30.0±2.75
Clay (%)[1]	6.3 ± 0	6.3 ± 0
Sand (%)[1]	55.7 ± 1.09	63.7 ± 2.75
pH[1]	7.2 ± 0.06	7.4 ± 0.02
Total C (%)[2]	2.16 ± 0.21	4.14 ± 0.30
Total N (%)[2]	0.13 ± 0.01	0.22 ± 0.02
C:N ratio	14.4 ± 0.71	17.32 ± 1.03
Fungal:bacterial ratio[3]	0.003 ± 0.001	0.02 ± 0.01

[1]*Data from Castillo-Monroy et al. (2010).*
[2] *Total N and C were obtained using a CN analyzer (Leco CHN628 Series, Leco Corporation, St. Joseph, MI, USA)*
[3] *Bacterial 16S and fungal 18s rRNA genes were measured using quantitative PCR as described in Evans and Wallenstein (2011).*



Appendix B. *Monitoring during a period of 12 hours of the mm of dew added on the experimental pots in both control and dew treatments. Data are means ± SE (n = 6).*

***Appendix C**. Summary results of the ANOVA analyses carried out with all the studied variables. NH_4^+-N = N as ammonium; $N0_3^-$-N = N as nitrate; DON = Dissolved organic nitrogen; mi = microsite, tr= treatment, ti= period of incubation, res = residuals.*

Variable	Factor	Source	DF	SS	F	p
DON		mi	1	1.247	0.045	0.834
		res	1	85.673	3.102	0.096
		mi	1	178.66	6.469	0.021
		res	17	27.617		
mi x tr interaction	BG	tr	1	9.768	0.312	0.589
		res	10	31.289		
	BSC	tr	1	225.423	10.076	0.016
		res	7	22.371		
	CONTROL	mi	1	103.942	4.947	0.057
		res	8	21.01		
	DEW	mi	1	75.71	2.261	0.167
		res	9	33.491		
NH_4^+		mi	1	7.062	7.025	0.015
		tr	1	14.306	14.232	<0.01
		mi x tr	1	18.335	18.239	<0.001
		res	20	1.005		
mi x tr interaction	BG	tr	1	39.969	25.95	<0.001
		res	12	1.54		
	BSC	tr	1	0.105	0.519	0.492
		res	8	0.203		
	CONTROL	mi	1	1.497	4.206	0.065
		res	11	0.356		
	DEW	mi	1	21.523	11.965	<0.01
		res	9	1.799		
Total available N		mi	1	114.177	3.494	0.078
		tr	1	66.545	2.036	0.171
		mi x tr	1	254.689	7.794	0.012
		res	18	32.678		
	BG	tr	1	37.337	1.044	0.329
		res	11	35.771		
	BSC	tr	1	245.412	8.822	0.021
		res	7	27.818		
	CONTROL	mi	1	14.402	0.419	0.534

		res	9	34.392		
	DEW	mi	1	343.128	11.081	<0.01
		res	9	30.964		
NO_3^-		mi	1	14.15	3.45	0.078
		tr	1	9.721	2.37	0.139
		mi x tr	1	0.115	0.028	0.869
		res	20	4.102		
Amino acids		mi	1	0.751	0.305	0.587
		tr	1	0.279	0.114	0.74
		mi x tr	1	6.743	2.741	0.113
		res	20	2.46		
Phenols		mi	1	220.123	8.539	<0.01
		tr	1	4.948	0.192	0.666
		mi x tr	1	328.572	12.746	<0.01
		res	22	25.778		
	BG	tr	1	207.079	9.039	0.012
		res	11	22.908		
	BSC	tr	1	126.44	4.414	0.04
		res	11	28.648		
	CONTROL	mi	1	5.025	0.562	0.471
		res	10	8.938		
	DEW	mi	1	588.557	14.783	<0.01
		res	12	39.812		
Carbohydrates		mi	1	1399.609	1.241	0.277
		tr	1	3472.851	3.079	0.093
		mi x tr	1	38.784	0.034	0.855
		res	22	1128.012		
Ratio pentoses:hexoses		mi	1	0.051	1.156	0.296
		tr	1	0.149	3.339	0.083
		mi x tr	1	0.003	0.074	0.788
		res	19	0.045		
Carbohydrates: N available ratio		mi	1	0.485	0.395	0.538
		tr	1	14.448	11.754	0.003
		mi x tr	1	2.266	1.843	0.191
		res	18	1.229		
BSC respiration		tr	1	0.16	821.02	<0.001
		ti	10	0.09	26.53	<0.001

	tr x ti	10	0.09	26.53	<0.001
	res	40			

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6. DISCUSION GENERAL

En el **capítulo 1** se presentan los resultados del primer estudio empírico global sobre biogeoquímica de zonas áridas, donde detectamos un posible desequilibrio entre los ciclos del C, N y P derivado del incremento de aridez pronosticado para finales del siglo XXI (Gao y Giorgi, 2008; Dai 2013). Los resultados obtenidos indican que este aumento de la aridez conllevará una disminución del control biótico (p. ej. menor cobertura vegetal) y un incremento del abiótico (p. ej. mayor dominio de la meteorización mecánica) sobre los ciclos biogeoquímicos en las zonas áridas. Nuestro estudio sugiere que los nutrientes asociados a procesos biológicos como el C y N (p. ej. fotosíntesis, descomposición de materia orgánica, fijación de N atmosférico) disminuirán con el incremento de aridez, mientras aquellos nutrientes asociados con procesos geoquímicos (p. ej. meteorización de la roca) como el P se verán favorecidos, generando desbalances entre los ciclos biogeoquimicos del C, N y P (Figura 6.1). Este previsible desacople de los ciclos del C, N y P en las zonas áridas puede derivar en importantes impactos a nivel mundial sobre reacciones biogeoquímicas determinantes de la producción primaria y otros procesos a nivel de ecosistema (Finzi et al. 2009; Peñuelas et al. 2012). Por ejemplo, una disminución del N disponible derivado del incremento de aridez podría limitar la capacidad de las plantas para amortiguar el incremento de CO_2 derivado de la actividades humanas a nivel global, ya que las tasas fotosintéticas dependen de la actividad de la enzima RuBisCo (ribulose bisphosphate carboxylase oxigenase) que comprende más de un 50% del N en la hoja (Thornton et al. 2007; Finzi et al. 2009; Peñuelas et al. 2012). Por otro lado, una disminución del N en relación al P disponible con el incremento de aridez podría generar efectos ecosistémicos a corto plazo, afectando por ejemplo al crecimiento de grupos concretos de plantas en función de su estequiometria (Peñuelas & Sardans 2009). También podría tener efectos evolutivos a más largo plazo seleccionando plantas y microorganismos en función de sus niveles de N en nucleótidos y aminoácidos, lo que podría afectar a la estructura del ecosistema y a los rasgos funcionales de los seres vivos en zonas áridas (Acquisti et al. 2009). Además, la disminución de N ligada a la disminución de materia orgánica, en ecosistemas ya típicamente pobres en este nutriente (Cookson et al. 2006; Delgado-Baquerizo et al. 2011), limitará la mineralización de este elemento en el suelo y su disponibilidad para plantas y microorganismos. En ecosistemas pobres en N, donde la producción de hojarasca y consiguiente descomposición son lentas, las plantas y microorganismos tienden a inmovilizar este nutriente en lugar de mineralizarlo, debido a alta escasez de este nutriente en el ecosistema (Schimel & Bennet 2004). Esta retención del N por parte de los microorganismos se ha observado también durante periodos de sequía en zonas áridas (Schimel et al. 2007). Todo ello sugiere que la disminución del N derivada del incremento de aridez podría resultar en una retroalimentación negativa sobre la inmovilización de este nutriente, incrementando las tasas de retención de N y haciendo a este nutriente incluso más limitante en las zonas áridas.

La relación entre la aridez y los contenidos de C y N no fue lineal y siguió una regresión cuadrática negativa. De este modo, el desbalance observado en los ciclos del C, N y P puede ser particularmente frágil en la transición entre ecosistemas semiáridos a áridos, donde se observó la disminución más abrupta del C y N. Este tipo de información altamente extrapolable es difícil de obtener a escala regional o local, donde las distintas zonas bioclimáticas (ecosistemas seco-subhumedos, semiaridos y áridos) suelen estar poco representados. Sin embargo no podemos obviar la cantidad de varianza no explicada observada en la regresión entre la aridez y N total en el suelo (65.2% de la varianza total), que podría estar generada por los distintos tipos de suelo, especies de plantas e historias de uso por parte de las poblaciones locales (Maestre et al. 2012a).

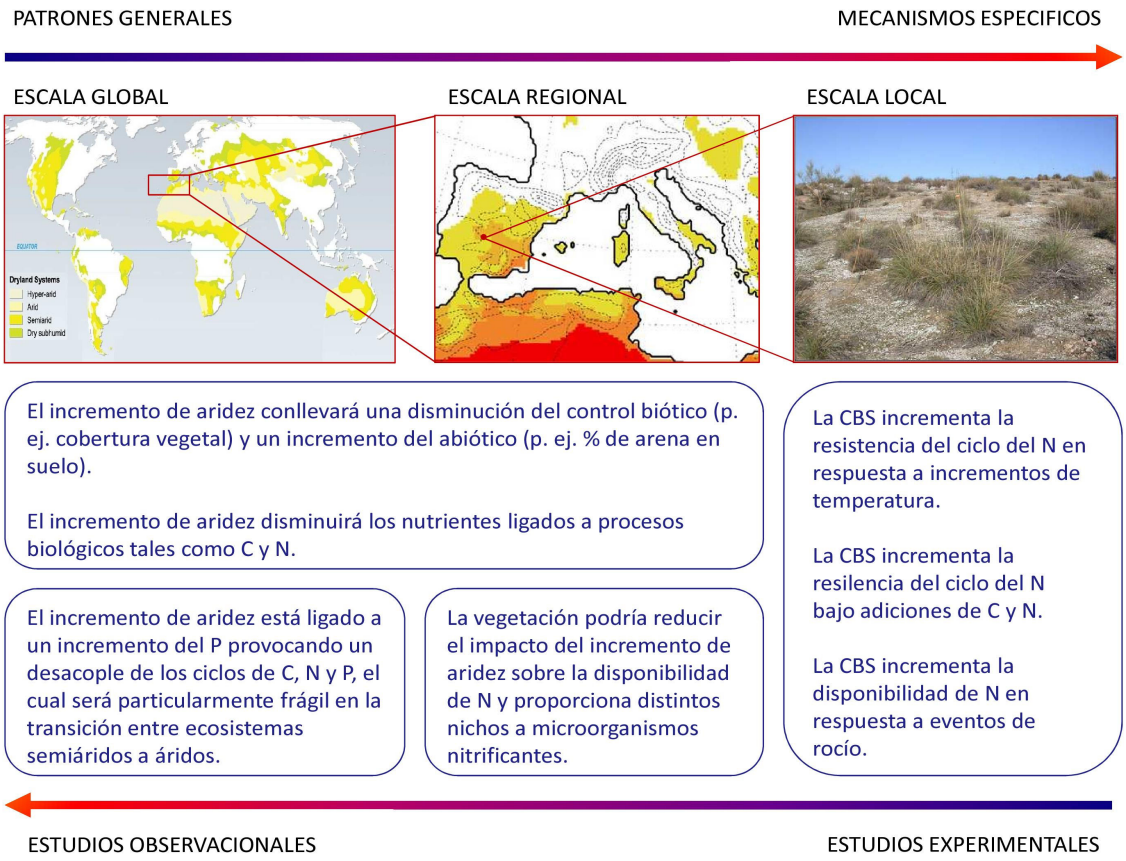

Figura 6.1. *Resumen de los resultados más importantes sobre el impacto del cambio global sobre el ciclo del N en las distintas escalas espaciales consideradas en la presente tesis doctoral.*

Una forma de eliminar parte del "ruido" causado por distintos tipos de suelo y especies de plantas observadas en el **capítulo 1**, es analizar el ciclo del N a una escala menor. Para ello realizamos un estudio observacional sobre un gradiente regional de espartales (*Stipa tenacissima*) en la cuenca Mediterránea, seleccionando siempre parcelas sobre suelos calizos y bajo el mismo tipo de vegetación. El incremento de aridez mostró el mismo patrón observado a escala global,

disminuyendo el efecto biológico (p. ej. menor cobertura vegetal) e incrementando el efecto abiótico (p. ej. mayor % de arena). Del mismo modo, el incremento de la aridez estuvo ligado a una disminución del N disponible en suelo para plantas y microorganismos (incluyendo formas orgánicas e inorgánicas), manteniéndose el patrón encontrado a escala global. Estudios regionales anteriores habían mostrado patrones contradictorios del efecto de la precipitación sobre la disponibilidad de N (Austin 1998; Schuur and Matson 2001; Aranibar 2004; McCulley 2009). Factores tales como predominancia de distintos micrositios (p. ej. vegetación vs. suelo desnudo), así como la historia de una determinada región, podrían determinar la respuesta del ciclo del N a impactos derivados del cambio climático. Así pues, a escala regional (**capítulo 2**) emergen nuevos mecanismos, como el papel fundamental que la presencia de distintos micrositios (p. ej. suelo desnudo vs. microambientes vegetados) juega en la disponibilidad de N en respuesta a un incremento de aridez. Ésta fue el factor más importante que determinó la disponibilidad de N en el suelo desnudo, mientras que el C orgánico se postuló como otra variable importante a la hora de determinar la disponibilidad de N bajo *S. tenacissima*. El mayor contenido de C orgánico en el suelo bajo *S. tenacissima* (y por tanto de materia orgánica), podría favorecer la despolimerización de dicha materia (producción de NOD), incrementando la disponibilidad de N en suelo (Cookson et al., 2006). Estudios anteriores han mostrado que *S. tenacissima* atenúa el exceso de radiación y temperatura (Maestre et al. 2001), aumentando la infiltración bajo su cubierta, actuando como un sumidero de agua procedente de escorrentía (Cerdà 1997; Maestre et al. 2002; Puigdefábregas et al. 1999), e incrementando el contenido de nurientes y materia orgánica (Martínez-Sánchez et al. 1994; Maestre et al. 2001; Goberna et al. 2007) en el suelo en comparación con zonas abiertas desprovistas de vegetación, lo que hace que esta especie forme verdaderas "islas de fertilidad" (*sensu* Reynolds et al. 1999). De este modo, la perdida de cobertura vegetal observada a escala regional y global asociadas al incremento de aridez tendría un impacto negativo indirecto sobre la disponibilidad de N para plantas y microorganismos del suelo mediante la disminución de su materia orgánica (Figura 6.1).

La aridez también fue el factor más importante que determinó el cociente entre arqueas (AOA) y bacterias (AOB) nitrificantes en suelo a lo largo del gradiente regional estudiado (**capítulo 3**). Sin embargo, la presencia de *S. tenacissima* afectó la respuesta de dicho cociente a la aridez, modulando además, otros factores como el contenido de nutrientes o el pH del suelo, altamente relacionados con la abundancia de AOB y AOA edáficas (Nicol et al. 2008; Verhamme et al. 2011). Así pues, AOB fue siempre el grupo de microorganismos nitrificantes más abundante bajo esparto, mientras que AOA, altamente resistente al estrés hídrico y nutricional (Adair y Schwartz 2008; Verhamme et al. 2011), lo fue en las zonas de suelo desnudo, en las regiones con mayor aridez. La

menor dominancia de AOA bajo esparto podría venir dada por las mejores condiciones microclimaticas y nutricionales tipicamente observadas bajo su copa (Maestre et al. 2001; 2003; Goberna et al. 2007). De hecho, una disminución de la abundancia absoluta de AOA fue observada en este estudio con el incremento de fertilidad, mientras que AOB mostró una tendencia opuesta. Los resultados obtenidos en los **capítulos 2 y 3** indican que en regiones como la estudiada, la vegetación podría reducir los impactos del incremento de aridez derivado del cambio climático sobre la disponibilidad de N en zonas áridas, proporcionando distintos nichos a los diferentes grupos de nitrificantes presentes en el suelo. Sin embargo, otros ecosistemas con cubiertas contínuas tales como bosques templados y boreales, podrían generar un menor gradiente de micrositios y por tanto poseer una menor capacidad para generar nichos específicos para distintos grupos de microorganismos del suelo.

Las escalas regionales y globales abordadas en este estudio proporcionaron información única acerca del efecto modulador de las plantas vasculares sobre la disponibilidad de N y la abundancia de orgánismos nitrificantes. Este efecto sería más difícil de observar a escalas inferiores debido a la menor variabilidad en el grado de cobertura, la diversidad de especies o el concepto de aridez, dificilmente aplicable a pequeña escala. Sin embargo, la existencia de micrositios concretos (ej. costra biológica del suelo) y factores microclimáticos específicos (ej. eventos de rocío) que ocurren a escala local podrían determinar la respuesta del ciclo de N al cambio global y son ignorados a escalas superiores (Figura 6.1).

Los diversos estudios a escala local realizados en esta tesis resaltan la importancia de la costra biológica del suelo (CBS) como moduladores de la respuesta del ciclo del N a distintos agentes de cambio global. Por ejemplo, en el **capítulo 4** mostramos como la CBS incrementó la resiliencia de procesos básicos del ciclo del N frente a distintas bajo adiciones de C y N (Figura 6.1). La presencia de CBS podría jugar un papel fundamental a nivel local en respuesta a los desequilibrios de C, N y P ligados al incremento de aridez mostrados en el **capítulo 1** de esta tesis a nivel global. En este sentido, se ha visto que la CBS incrementa procesos tales como la fijación de C y N (Belnap 2002; Belnap & Lange 2003; Castillo-Monroy et al. 2011a;b; Elbert et al. 2012) y la actividad de la enzima fosfatasa en zonas áridas respecto a micrositios de suelo desnudo (Castillo-Monroy et al. 2011; Bowker et al. 2011, Maestre et al. 2012b). Este hecho podría permitir a los microorganismos asociados a la CBS adquirir los nutrientes limitantes derivados de los desequilibrios causados por el cambio global, de una manera más eficiente que a los microorganismos que se desarrollan en suelo desnudo. Así pues, la limitación relativa de P en los sitios más fértiles observados en el **capítulo 1** podría ser equilibrada con la producción de enzimas fosfatasas siempre que el N estuviera disponible (Vitousek 2004; Naninnpieri et al. 2011; Bowker et

al. 2011), mientras que la limitación de N y C en los sitios más aridos podría ser compensada por la mayor fijación de N y C de los microorganismos asociados a la CBS con respecto al suelo desnudo (Belnap 2002; Vitousek 2004; Castillo-Monroy et al. 2011b).

Tal como muestran los resultados del **capítulo 5**, la CBS juega también un importante papel controlando los efectos de distintos agentes de cambio climático (incremento de temperatura y contenido de agua en suelo) en variables clave del ciclo del N. Estas variables fueron altamente sensibles a cambios en temperatura, pero no a cambios en la humedad del suelo. Un estudio previo en zonas templadas-frías había mostrado una disminución del nitrato e incremento del amonio bajo condiciones del 70% de la capacidad de campo (Szukics et al. 2010). Sin embargo, la falta general de respuesta de las variables medidas del ciclo del N a cambios en el estado hídrico del suelo encontrada en el **capítulo 5**, sugiere que procesos tales como la mineralización en ecosistemas, áridos pueden ser llevados a cabo de igual manera entre el 30 y el 80% de la capacidad de campo. En este sentido, Schwinning y Sala (2004) hipotetizaron que en ecosistemas áridos existe una respuesta proporcional de procesos y funciones ecosistemicas en función de la duración y tamaño de los pulsos de agua. De este modo, procesos tales como mineralizacion e inmobilización del N pueden estar activos incluso desde pequeños pulsos de agua, mientras que otros procesos no evaluados en esta tesis, tales como la fijacion de N o la predación entre organismos del suelo, podrían requerir contenidos de agua más elevados (Austin et al. 2004; Schwinning y Sala 2004).

Otro factor a tener en cuenta a nivel local son los eventos de rocío, que aunque comunes en zonas áridas (Kidron 2000; Jacobs et al. 2002; Moro et al. 2007), son obviados en los índices de aridez actuales, como el empleado en los capítulos **1-3** (UNEP 1992). En el **capítulo 6** observamos como la CBS favorece la acumulación de N total disponible y NOD para plantas y microorganismos en respuesta a pequeños pulsos de agua (1% de la capacidad de campo) similares a los proporcionados por los eventos de roció en zonas áridas (Kidron 2000; Jacobs et al. 2002; Moro et al. 2007). El incremento de N disponible ligado al descenso de carbohidratos bajo CBS en respuesta a los eventos de rocio, disminuyó la relación C:N en el suelo, lo que podría favorecer la nitrificación local del N. De hecho, una tendencia no significativa a incrementar el nitrato en suelo bajo CBS fue observada en respuesta a estos pequeños pulsos de agua. La mayor abundancia de hongos encontrados bajo CBS en nuestra zona de estudio podría estar ligada a la mayor disponibilidad de NOD observada en estos microambientes. No en vano, las comunidades de hongos del suelo juegan un papel fundamental en la descomposición de la materia orgánica (Austin et al. 2004; Robertson & Groffman 2007), y son más resistentes que las bacterias a los periodos de desecación, pudiendo estar activos en respuesta a pequeños pulsos de agua (Austin et al. 2004). Esta producción de N en respuesta a pequeños pulsos de agua puede incrementar la disponibilidad de N

para las plantas durante sus períodos de actividad, pudiendo explicar también las mayores concentraciones de N orgánico observadas bajo CBS con respecto al suelo desnudo (Delgado-Baquerizo et al. 2010; Castillo-Monroy et al. 2010; Bowker et al. 2011).

La disminución del control biológico sobre zonas áridas observados a escala global y regional, junto con la capacidad de mantener activos los procesos asociados al ciclo del N con pequeños volúmenes de agua, nos sugiere que aspectos del cambio global, tales como el incremento de temperatura y la deposición de N, pueden resultar en una mayor predominancia de formas de N inorgánicas en los suelos de las zonas áridas (Schlesinger et al. 1990). En este sentido, la CBS favorecería la presencia de amonio (Delgado-Baquerizo et al. 2010; Castillo-Monroy et al. 2010), manteniendo la diversidad de formas de N disponibles para plantas y microorganismos. Sin embargo, la CBS no está exenta de los impactos causados por el cambio climatico. Por ejemplo, Reed et al. (2012) mostraron que cambios en la frecuencia y tamaño de los pulsos de agua en suelos desérticos impacta negativamente sobre la cobertura de musgos, incrementando la nitrificación en suelo. Además, Escolar et al. (2012) mostraron que un incremento de temperatura media de unos 2.6ºC conlleva una disminución de casi el 40% de la cobertura de líquenes asociados a la CBS en la misma zona de estudio donde se basaron los **capítulos 4-6**. El efecto de la disminución de la CBS sobre el ciclo del N no ha sido medido en esta tesis, pero Delgado-Baquerizo et al. (2010) mostraron que un incremento en la dominancia de formas inorganicas de N está asociado a una menor cobertura de CBS, mientras que una mayor dominancia de NOD está asociado a CBS más desarrolladas. La falta de información previa a la presente tesis doctoral nos impedía sugerir el impacto indirecto de la perdida de CBS derivada del cambio climatico sobre el ciclo del N. Sin embargo, ahora podemos afirmar que la disminución de la CBS derivada del cambio climatico, podría limitar su impacto positivo sobre el ciclo del N en zonas áridas (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010), disminuyendo su capacidad para mantener la dominancia de formas de N en suelo, a la hora de afrontar desequilibrios en los ciclos biogeoquímicos del C, N y P en suelo o incrementos de temperatura derivados del cambio global. En este sentido, los impactos derivados del cambio global sobre la cobertura de CBS podrían generar una sinergía negativa sobre el ciclo del N al incrementar la nitrificación del N órganico y amonio previamente acumulado bajo la CBS (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010).

Los resultados obtenidos en esta tesis muestran como la CBS incrementa la resiliencia del ciclo del N a cambios en los cocientes de C, N y P y una mayor resistencia del ciclo del N a cambios en temperatura (Orwin & Wardle 2004), manteniendo la dominancia de formas disponibles de N en el suelo, cuyos cambios podrían afectar a la toma preferencial de N por parte de las distintas especies presentes en estos ecosistemas (Warren 2009). Además, la CBS fomenta el

incremento de formas orgánicas de N en suelo en respuesta a pequeños pulsos de agua similares a eventos de rocío, incrementando el N disponible para plantas y microorganismos del suelo. La existencia de micrositios y de factores microclimáticos específicos que ocurren a escala local, como la presencia de CBS, son obviados en los modelos actuales de cambio climático (Parton et al. 1994; Parton et al. 1998; Shen et al. 2008), aunque podrían determinar la respuesta del ciclo de N a dicho cambio. En este sentido, factores tales como el número de días de rocío o la cobertura de CBS en suelo podrían ser incluidos como variables dentro de modelos ecosistemicos CENTURY y PALS (Parton et al. 1994; Parton et al. 1998; Reynolds et al. 2004; 2007; Shen et al. 2008), en donde la costra biológica podría ser considerada como un nuevo grupo funcional.

El conjunto de estudios realizados en esta tesis ha ayudado a profundizar nuestro conocimiento sobre los papeles que juegan la CBS y la vegetación como moduladores del impacto del cambio global sobre el ciclo del N en las zonas áridas en las escalas locales y regionales, respectivamente. Del mismo modo, concluimos que un incremento de aridez a nivel mundial podría llevar a un desacople de los ciclos del C, N y P en suelo en los ecosistemas más áridos, lo que posiblemente afectará a los procesos y servicios ecosistémicos que prestan estos ambientes. Sin embargo, mucho queda aún por descubrir acerca de cómo diferentes factores climaticos y micrositios modulan otros aspectos del ciclo del N no tratados en esta tesis doctoral, tales como los intercambios de N entre el suelo y la atmósfera (Barger et al. 2005; Robertson & Groffman 2007). También se necesita más información sobre los efectos del cambio global sobre las comunidades microbianas, que en última instancia, son las que determinan los ciclos biogeoquímicos de elementos esenciales para el desarrollo de la vida (Fierer et al. 2012). Los distintos resultados obtenidos en esta tesis demuestran que el estudio de las causas e impactos derivados del cambio global requiere del entendimiento de fenómenos y características ecosistémicas ligados a distintas escalas espaciales (Levin 1992), que nos permitan ir desde patrones generales ligados a escala global a los mecanismos y factores concretos que actúan a escalas locales y regionales.

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7. CONCLUSIONES GENERALES

1. El incremento de aridez pronosticado para finales de siglo disminuirá los nutrientes ligados a procesos biológicos (como el C y el N) y aumentara aquellos bajo un mayor control geoquímico (como el P) en las zonas áridas del planeta, desacoplando los ciclos del C, N y P a escala global.

2. El desacople observado en los ciclos del C, N y P puede ser particularmente frágil en la transición entre ecosistemas semiáridos a áridos, donde se observó una disminución abrupta del C y N.

3. La aridez mostró una fuerte relación negativa con la disponibilidad de N (amonio, nitrato y DON) en formaciones de *Stipa tenacissima* situadas a lo largo de un gradiente regional desde España a Túnez, siendo su importancia a la hora de determinar esta disponibilidad mayor que la de factores como la textura del suelo o la cobertura vegetal.

4. Un incremento en la aridez estuvo ligado a un incremento en el cociente AOA:AOB a lo largo del gradiente regional estudiado. El gen AOB fue más abundante en los sitios más fértiles y debajo de esparto, mientras que AOA, más resistente al estrés hídrico y nutricional fue más abundante en los micrositios con menor disponibilidad hídrica (suelo desnudo).

5. La vegetación podría reducir los impactos negativos del incremento de aridez derivado del cambio climático sobre la disponibilidad de N y los microorganismos nitrificantes en zonas áridas. La concentración de recursos bajo la vegetación, que permite la creación dedistintos nichos a diferentes grupos de nitrificantes autótrofos presentes en el suelo, podría ser el mecanismo responsable de dicha reducción.

6. La costra biológica del suelo (CBS) aumentó la resiliencia del ciclo del N a desbalances del C y N con respecto a P.

7. La CBS incremento la resistencia de las variables asociadas al ciclo del N en respuesta a cambios de temperatura.

8. La CBS incrementó la concentración del DON y de la disponibilidad de N para plantas y microorganismos en respuesta a pequeños pulsos de agua similares a los esperados por eventos de rocío en ecosistemas áridos.

9. El incremento de temperatura, así como de deposición de N derivados de las actividades humanas pueden resultar en una mayor predominancia inorgánica del ciclo del N en zonas áridas, disminuyendo el N orgánico disponible para plantas y microorganismos.

10. Nuestros resultados demuestran que para la comprensión del impacto derivado del cambio global sobre zonas áridas es necesario estudiar distintas escalas espaciales que nos permitan abarcar desde patrones generales ligados a la escala global a factores concretos que actúan a escala local.

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