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EFECTOS DE LOS CAMBIOS EN EL PATRÓN DE PRECIPITACIONES SOBRE LOS MICROORGANISMOS Y LOS PROCESOS DEL SUELO

TESIS DOCTORAL

Lourdes Morillas Viñuales

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EFECTOS DE LOS CAMBIOS EN EL PATRÓN DE PRECIPITACIONES SOBRE LOS MICROORGANISMOS Y LOS PROCESOS DEL SUELO

Memoria que la Licenciada Lourdes Morillas Viñuales presenta para aspirar al grado de doctora por la Universidad Plablo de Olavide de Sevilla

Esta memoria ha sido realizada bajo la dirección de:

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CERTIFICA

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Efectos de los cambios en el patrón de precipitaciones sobre los microorganismos y los procesos del suelo"**, son aptos para ser presentados por la Lda. Lourdes Morillas Viñuales ante el Tribunal que en su día se designe, para aspirar al Grado de Doctora por la Universidad Pablo de Olavide.**

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extiendo el presente certificado a 21 de abril de 2014

Dr. Antonio Gallardo Correa

Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca de Postgrado para la Formación de Profesorado Universitario (AP2008-02985) del Ministerio de Educación.

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Los trabajos de investigación presentados en esta Tesis Doctoral se ha realizado en el Departamento de Sistemas Físicos, Químicos y Naturales de la Universidad Pablo de Olavide de Sevilla.

A mis padres,

Gracias por ayudarme a llegar tan lejos.

If you want to build a ship, don't
drum up the people to gather wood,
divide the work, and give orders.
Instead, teach them to yearn for the
vast and endless sea”

Antoine de Saint Exupéry

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ÍNDICE

1. RESUMEN

El objetivo de esta tesis doctoral es evaluar los efectos de los cambios en el patrón de precipitación sobre los microbios y sobre una amplia variedad de procesos del suelo en distintos ecosistemas. Para ello, hemos estudiado cuatro zonas diferentes: dos ecosistemas mediterráneos (un pinar y un matorral), en los que se ha llevado a cabo un estudio de campo observacional no manipulativo y un ecosistema semiárido y otro templado en los que se han realizado experimentos manipulativos. En el capítulo 1 evaluamos los efectos de los ciclos naturales de humedecido y secado del suelo sobre el pool de nitrógeno (N) en los dos ecosistemas mediterráneos anteriormente mencionados muestreando semanalmente durante un año. Nuestros resultados mostraron que el pool de N tiene una alta variabilidad temporal que es independiente del contenido en materia orgánica y del carbono (C) y N lábil del suelo. De este modo, es esperable que los cambios en los pulsos de agua producidos por el cambio climático tengan un impacto significativo sobre la disponibilidad y reciclaje de las formas orgánicas e inorgánicas de N.

En el capítulo 2 estudiamos las tasas de respiración de estos dos mismos ecosistemas utilizando el mismo diseño muestral que en el capítulo 1. Nuestros resultados evidenciaron que mientras que la tasa de respiración en el pinar aumentaba durante los ciclos de humedecido y disminuía durante los de secado, la tasa de respiración en el matorral no respondía a la humedad del suelo. Nuestros resultados apuntaron a que las bajas concentraciones de fósforo en el matorral estaban limitando la respuesta de la respiración del suelo a los pulsos de agua. En el capítulo 3 evaluamos la respuesta estacional de los microbios del suelo al consumo de diferentes fuentes de C en relación a las concentraciones de C lábil del suelo y calculamos la diversidad microbiana en los dos ecosistemas mediterráneos de estudio. Nuestros resultados evidenciaron que las concentraciones de C lábil del suelo no son un predictor fiable de la respuesta microbiana a distintas fuentes de C. También encontramos un patrón estacional diferente de la respuesta microbiana a las distintas fuentes de C en los dos ecosistemas, probablemente debido a la distinta fertilidad de sus suelos.

En el capítulo 4 analizamos cómo la longitud y la intensidad de los ciclos de humedecido y la presencia de la costra biológica del suelo (CBS) determinan las variables relacionadas con el ciclo del C y N en un ecosistema semiárido. Nuestros resultados indicaron que los eventos de humedecido más largos e intensos pueden estar

relacionados con un aumento en las tasas de descomposición que compensaría las pérdidas de nutrientes asociadas con los eventos de humedecido cortos. Esta tendencia es mucho más evidente en CBS que en suelo desnudo. De esta forma, los cambios en la longitud e intensidad de los eventos de humedecido y la presencia de la CBS podrían alterar la estructura y la función de la comunidad del suelo. Por último, en el capítulo 5 evaluamos cómo el efecto combinado de la adición de N en el suelo y más frecuentes ciclos de humedecido y secado afectan al flujo de los gases de efecto invernadero (GEIs), la diversidad funcional microbiana y los ciclos del N y C. Nuestros resultados no sólo indicaron que la intensificación de la frecuencia de los ciclos de humedecido y secado afecta a los flujos de los GEIs y a la capacidad del suelo de ciclar N y C, si no también demostraron que la adición de N cambia las respuestas de estas variables a los pulsos de humedad del suelo. La población microbiana vio aumentada su diversidad en respuesta a la adición de N, pero no se vio afectada por la frecuencia de los ciclos de humedecido y secado del suelo. Estos resultados confirman que cambios tanto en la frecuencia de los ciclos de humedecido y secado como en la adición de N en el suelo pueden inducir alteraciones significativas en la población microbiana y los procesos del suelo.

Los resultados obtenidos en esta tesis doctoral no sólo han mejorado nuestro conocimiento sobre la importancia del efecto que los pulsos de agua tienen sobre los microorganismos y los procesos del suelo, sino que además han explorado las consecuencias de futuros cambios en los pulsos de agua provocados por el cambio climático, tales como cambios en la longitud de los eventos de humedecido o en la frecuencia de los ciclos de humedecido y secado del suelo. También se ha explorado el papel de moduladores de la respuesta a los pulsos de agua como la CBS o la adición de N. Estos resultados pueden ser de ayuda para realizar previsiones más aproximadas de las consecuencias del cambio global sobre los ciclos biogeoquímicos y los microorganismos del suelo.

2. INTRODUCCIÓN GENERAL

2.1. Cambio climático y consecuencias sobre la humedad del suelo

Durante las últimas décadas se han invertido grandes esfuerzos en determinar cuáles serán las consecuencias del cambio global, que se ha visto intensificado desde 1950. Uno de los aspectos más importantes de este fenómeno desde el punto de vista de los ciclos biogeoquímicos es el cambio climático (Gallardo et al., 2009). Según las previsiones, el cambio climático producirá un aumento de las temperaturas, un cambio en el tamaño y la frecuencia de las precipitaciones y un incremento de los periodos de sequía (Huntington, 2006; IPCC, 2013). Muchos han sido los estudios que han relacionado estos cambios ambientales con modificaciones en los procesos del suelo en ecosistemas naturales (Skopp et al., 1990; Voroney, 2007; Evans & Wallenstein, 2011). Actualmente, está ampliamente aceptado que la naturaleza episódica de la disponibilidad de agua determina de forma directa e indirecta un gran número de procesos biogeoquímicos a través de los ciclos de secado y rehumedecido del suelo en ecosistemas limitados por la disponibilidad de agua (Cui & Caldwell, 1997; Collins et al., 2008). Por ejemplo, las precipitaciones puntuales afectan de forma directa a la frecuencia y a la duración de los ciclos de secado y rehumedecido del suelo, pero estos ciclos pueden controlar indirectamente la actividad de los microorganismos del suelo, lo cual determina en última instancia el reciclaje de C y N. Incluso se ha afirmado que los cambios en la naturaleza de estas precipitaciones pueden llegar a ser más importantes que los cambios en la temperatura o en la cantidad total de precipitación (Austin et al., 2004).

Aunque han pasado 40 años desde que Noy-Meir (1973, 1974) describió cómo las precipitaciones puntuales afectan a la mayoría de los procesos del suelo en ecosistemas áridos, aún quedan muchas cuestiones por resolver relacionadas con cómo los ciclos de secado y rehumedecido del suelo controlan los ciclos biogeoquímicos en ecosistemas limitados por la disponibilidad de agua. La naturaleza efímera del efecto de los pulsos de agua en el flujo de nutrientes, como por ejemplo pérdidas gaseosas tras una precipitación (Schlesinger & Peterjohn, 1991), o la respuesta instantánea de los microorganismos del suelo a los cambios de humedad del suelo (Freckman et al., 1987;

Schwinning & Sala, 2004) contribuye a la dificultad de obtener resultados fiables de los efectos de los pulsos de agua sobre el funcionamiento de los ecosistemas.

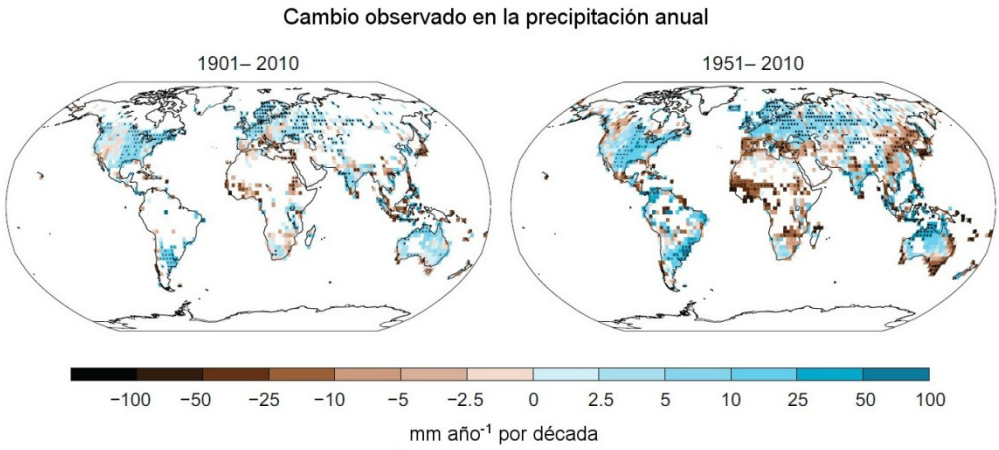

Figura 1. El aumento en la temperatura del aire proyectado, conducirá a una mayor capacidad de la atmósfera para retener agua, acelerando el ciclo hidrológico y alterando las características de las precipitaciones (cantidad, frecuencia, intensidad, duración…). La frecuencia y la intensidad de las precipitaciones torrenciales es probable que aumenten en norte América y Europa. Imagen modificada de IPCC, Climate Change (2013).

2.2. Efectos de los pulsos de agua sobre los ciclos biogeoquímicos

Los efectos del de secado y rehumedecido del suelo en los procesos biogeoquímicos han sido estudiados tanto en sistemas agrícolas como naturales. Estos ciclos de secado y rehumedecido del suelo afectan a todos los aspectos del reciclaje de nutrientes, incluyendo la mineralización de C y N (Birch, 1964; Agarwal et al., 1979; Seneviratne & Wild, 1985; Degens & Sparling, 1995), la biomasa microbiana (Bottner, 1985; Kieft et al., 1987; Van Gestel et al., 1993) y flujo de gases de efecto invernadero (Groffman & Tiedje, 1988; Mummey et al., 1994; Ruser et al., 2006; Norton et al., 2008).

- **Ciclo de nutrientes**

Tanto la fase de humedecido como la de secado afectan a los procesos del suelo, pero es la dinámica cíclica la que determina la diferente respuesta del suelo comparada con condiciones de humedad constante (Austin et al., 2004). Por ejemplo, la acumulación de N inorgánico suele ocurrir durante los periodos secos debido a que la difusión de los iones se encuentra muy restringida en finas películas de agua del suelo seco y a que los

sumideros de N inorgánico están limitados por el reducido crecimiento microbiano y la disminuida toma de nutrientes por parte de las plantas en condiciones de sequía (Barber, 1995; Stark & Firestone, 1995). Los ciclos de secado y rehumedecido del suelo generalmente estimulan la mineralización de C y N (frecuentemente durante las primeras horas tras el rehumedecido para el caso del C, Mikha et al., 2005). Muchos menos estudios se han centrado en el efecto de estos pulsos de humedad sobre el ciclo del P. La mayoría de éstos encontraron un aumento de P inorgánico en respuesta a repetidos ciclos de secado y rehumedecido del suelo (Grierson et al., 1998; Turner & Haygarth, 2001).

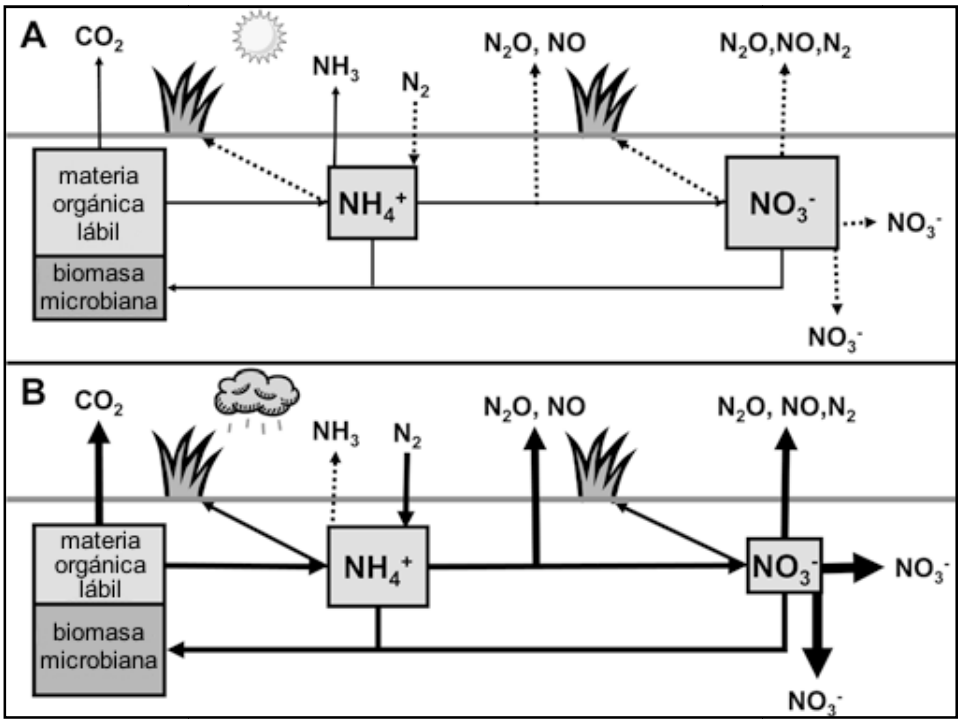

Figura 2. Esquema de los ciclos biogeoquímicos del C y del N en ecosistemas limitados por agua bajo condiciones de secado (A) y de rehumedecido (B) del suelo. El grosor de las flechas indica la importancia relativa de estos procesos en las dos situaciones. Las líneas punteadas indican un flujo muy bajo o indetectable. Durante el periodo en el que el suelo está seco, el reciclaje de C y N disminuye, aumenta la muerte microbiana y la toma de nutrientes por parte de las plantas está restringida, produciendo un aumento en el nitrato y la materia orgánica lábil del suelo. Cuando el suelo se rehumedece, se estimula la mineralización de C y N y se producen grandes cambios en la relación de la biomasa microbiana del suelo y la materia orgánica lábil, aumentando las pérdidas potenciales de N debido al aumento de la nitrificación, desnitrificación y lavado. Imagen modificada de Austin et al., 2004.

- **Microorganismos**

El secado y rehumedecido de los suelos supone un gran estrés fisiológico para las comunidades microbianas que habitan en la superficie de los suelos. El rehumedecido de un suelo seco puede inducir la muerte de una proporción significativa de la biomasa microbiana debido al cambio repentino en potencial hídrico del suelo que puede llegar a causar un choque osmótico induciendo a la lisis de las células microbianas (Bottner, 1985; Van Gestel et al., 1992). Este fenómeno puede directa o indirectamente afectar a la composición de la comunidad microbiana del suelo (Fierer et al., 2003). Alternativamente, los microbios podrían ajustar su potencial hídrico liberando solutos intracelulares (Halverson et al., 2000). Estos sustratos lábiles de C y N pueden ser rápidamente mineralizados por los microbios que hayan sobrevivido al choque osmótico, produciendo un pulso de mineralización de C y de N (Birch, 1959; Kieft et al., 1987). Por otro lado, los ciclos de secado y rehumedecido puede causar la rotura de agregados del suelo, poniendo al alcance de los descomponedores materia orgánica previamente protegida (Adu and Oades, 1978; Appel, 1998; Lundquist et al., 1999a). Fierer y Schimel (2002) encontraron que los suelos sometidos a frecuentes ciclos de secado y rehumedecido emitieron una menor cantidad de CO_2 y también aumentaron la actividad de las poblaciones de nitrificantes autótrofas. Seis semanas después del último ciclo de secado y rehumedecido, las tasas de respiración en los suelos fueron significativamente más bajas que las observadas en los suelos no estresados, sugiriendo que los ciclos de secado y rehumedecidos pueden producir cambios significativos en la dinámica microbiana del C y el N, y que estos efectos pueden durar más de un mes después del último ciclo de secado.

Los microorganismos del suelo juegan un papel clave en la retención y liberación de nutrientes en ecosistemas naturales, siendo al mismo tiempo una fuente y sumidero de éstos. La cantidad de biomasa microbiana varía a lo largo del año, siendo esta dinámica temporal muy importante en el grado de liberación o inmovilización de nutrientes en el medio (Bauthus & Barthel, 1995; Diaz-Ravina et al, 1995). El crecimiento de la biomasa microbiana se ve influenciado por factores tales como la humedad del suelo, la temperatura, la aireación, la cantidad y naturaleza del C orgánico en el medio o el pH del suelo, existiendo distintos óptimos en función del tipo de microorganismos (Vitousek, 1982; Cochran et al., 1989; Tietema & Wessel, 1992; Wardle, 1998).

- **Flujo de gases de efecto invernadero**

Los principales gases implicados en el efecto invernadero son el dióxido de C (CO_2), el óxido nitroso (N_2O) y el metano (CH_4). Éstos dos últimos tienen potencial de calentamiento global 298 y 25 veces mayores que el del CO_2, respectivamente (Kaye et al., 2005; IPCC, 2013). Ha sido ampliamente probado que los ciclos de secado y rehumedecido del suelo tienen consecuencias evidentes para la mineralización de C (Fierer & Schimel, 2002; Mikha et al., 2005; Xiang et al., 2008). Al frecuentemente observado incremento en la respiración del suelo en respuesta a un pulso de humedad, se le conoce como el "Efecto Birch" (Birch, 1958). Muchos estudios se han concentrado en evaluar el efecto de los ciclos de secado y rehumedecido sobre la respiración del suelo (Franzluebbers et al., 2000; Mamilov & Dilly, 2002; McCulley et al., 2007), sin embargo aún permanecen sin resolver muchas cuestiones referentes a la dependencia de otras variables, el origen y la magnitud de la este pico de respiración (Xiang et al., 2008). Recientes estudios de laboratorio (Miller et al., 2005), modelos matemáticos (Yuste et al., 2005), y studios conceptuales (Schimel et al., 2007) sugieren que los ciclos de secado y rehumedecido del suelo pueden acelerar la pérdida de C en comparación con un suelo en condiciones de humedad constante, aunque hay excepciones (Mikha et al., 2005).

El N_2O que se emite en los suelos es un intermediario de la nitrificación y de la desnitrificación (Sahrawat & Keeney, 1986; Granli & Bøckman, 1994; Bremner, 1997). Tanto estudios de laboratorio como de campo han encontrado un aumento de las tasas de emisión de N_2O como consecuencia del aumento del contenido hídrico del suelo (Cates & Keeney, 1987; Rudaz et al., 1991), éstos resultados son atribuidos a un aumento en la desnitrificación inducida por una disminución de la difusión del O_2 en el suelo (Mosier et al., 1986; Corre et al., 1996; Ruser et al., 2001). Sin embargo, se ha encontrado una alta variabilidad en la magnitud de este pico de emisión y la contribución de la nitrificación y desnitrificación (Firestone & Tiedje, 1979). Por otro lado, el flujo de CH_4 depende de las condiciones físicas que determinan las tasas de difusión del suelo. Por este motivo, la absorción este gas por parte del suelo se ve frecuentemente aumentada durante los periodos secos de los suelos debido al aumento de la difusión (Ridgwell et al., 1999). Sin embargo, existe un gran desconocimiento sobre el efecto que tiene la dinámica cíclica de la humedad sobre el flujo de este gas.

2.3. Moduladores de las respuestas del suelo a los pulsos de agua

Hay algunos factores que pueden tener un efecto modulador de los procesos del suelo a los pulsos de agua. Nosotros hemos querido evaluar la influencia que la costra biológica del suelo y la deposición atmosférica de N tienen sobre las respuestas de las variables que estudiamos a los pulsos de agua. Hemos elegido testar el efecto de estos moduladores debido a su creciente interés sobre los ciclos biogeoquímicos a nivel mundial (Sala et al., 2000; Maestre et al., 2011).

- **Costra biológica del suelo**

La costra biológica del suelo, que está compuesta por algas eucariotas, cianobacterias, hongos, musgos y líquenes, cubre los primeros milímetros de la superficie en la mayoría de ecosistemas áridos y semiáridos a través de todo el mundo, y son uno de los componentes bióticos más importantes de estas áreas (Belnap & Lange, 2003). Todos estos componentes, constituyen una comunidad biótica especializada que ejerce una fuerte influencia sobre los procesos claves de los ecosistemas, tales como la escorrentía e infiltración de las precipitaciones (Alexander & Calvo, 1990; Belnap, 2006), la respiración del suelo (Maestre & Cortina, 2003), la fijación y transformación de N (Belnap, 2002; Castillo-Monroy et al., 2010), y mejora las condiciones para la proliferación del microorganismos del suelo (Belnap & Lange, 2003). Durante los últimos 20 años ha habido un creciente interés por la costra biológica del suelo a través de todo el mundo, lo cual ha mejorado nuestro conocimiento sobre la estructura, composición fisiología y biogeografía de éstos organismos (Belnap & Lange, 2003). Sin embargo, el efecto de la humedad sobre la costra biológica del suelo ha sido identificado como un tema clave para el futuro, ya que ha sido escasamente investigado (Maestre et al., 2011).

Figura 3. Fotografías de la costra biológica del suelo.

- **Deposición atmosférica de N**

La deposición atmosférica de N es, además del cambio climático, uno de los mayores impulsores del cambio global (Schlesinger, 2013). Los altos niveles de deposición atmosférica de N provenientes tanto del consumo de combustibles fósiles como de actividades agrarias, está llegando a ser un problema a nivel mundial (Vitousek et al., 1997, Galloway & Cowling, 2002). Este exceso de deposición de N puede tener serias consecuencias sobre los ecosistemas, tales como imbalances de nutrientes, acidificación del suelo, eutroficación de aguas, cambios en los ciclos de C y N, y aumentos en las emisiones de N_2O del suelo, las cuales a su vez, contribuirían al calentamiento global (Balota et al., 2004, Lal, 2004, Fenn et al., 1998). Recientemente se ha demostrado que el enriquecimiento de N está también asociado al descenso de la biomasa microbiana del suelo (Treseder, 2008), y teniendo en cuenta la importancia de este reservorio de nutrientes, es previsible que afecte a las tasas de transformación de la materia orgánica y la disponibilidad de nutrientes para las plantas (Sardans et al., 2008). La interacción de varios impulsores del cambio global como la deposición atmosférica de N y el cambio climático, pueden interactuar de formas que son difíciles de predecir en base a la respuesta de un solo impulsor (Shaw et al., 2002), por eso es necesario estudiar el efecto combinado de éstos para poder hacer previsiones más aproximadas.

2.4. Áreas de estudio

Aunque la mayor parte de los estudios realizados en esta tesis se han desarrollado en dos ecosistemas mediterráneos, también hemos querido evaluar el efecto de los ciclos de secado y rehumedecido del suelo en un ecosistema semiárido y en uno templado. El motivo por el que nos hemos centrado en ecosistemas limitados por la disponibilidad de agua, es porque la asincronía entre la estación de crecimiento y la disponibilidad de agua aumenta la importancia de este recurso en el ecosistema. Los ecosistemas en los que se ha llevado a cabo esta tesis se describen a continuación.

- **Ecosistemas mediterráneos**

Las zonas de estudio en las que se han desarrollado la mayoría de los experimentos de esta tesis han sido un pinar y un matorral situados en el suroeste de España (37° 21'N; 5° 56' O). El año en el que se realizaron los estudios fue más húmedo de lo habitual, habiendo recibido unas precipitaciones anuales de 853 mm el pinar y 846 mm el matorral. Los suelos de estas zonas tienen un perfil típico A(B)C. El pinar está compuesto por *Pinus pinea* L. y algunas herbáceas anuales, mientras que el matorral está dominado por *Quercus coccifera* L., *Cistus albidus* L., *Genista hirsuta* Vahl. y *Arbutus unedo* L.

En la cuenca mediterránea, la actividad biológica se restringe a los pocos periodos húmedos que coinciden con altas temperaturas, siendo de esta forma las características climáticas de la región mediterránea las que más determinan los recursos nutricionales de estos suelos. La relación inversa que frecuentemente se encuentra entre la temperatura y la humedad óptimas para el crecimiento microbiano hacen que las tasas de descomposición y mineralización de la materia orgánica sólo sean elevadas en periodos cortos e impredecibles a lo largo del año (kruger et al., 1983), produciendo una baja disponibilidad de nutrientes. Los ciclos de secado y rehumedecido del suelo durante la estación de crecimiento son los que probablemente más determinen las tasas de actividad microbiana, y su efecto no ha sido suficientemente estudiado en ecosistemas mediterráneos (Li et al., 2006). La actividad biológica está inhibida en condiciones de suelo seco, y los períodos de sequía producen una capa superior seca del suelo que disminuye el N disponible (Garwood & Tyson, 1973). Sin embargo, los

microorganismos pueden sobrevivir en forma inactiva en los suelos secos, volviendo a reactivarse al aumentar el contenido hídrico del suelo.

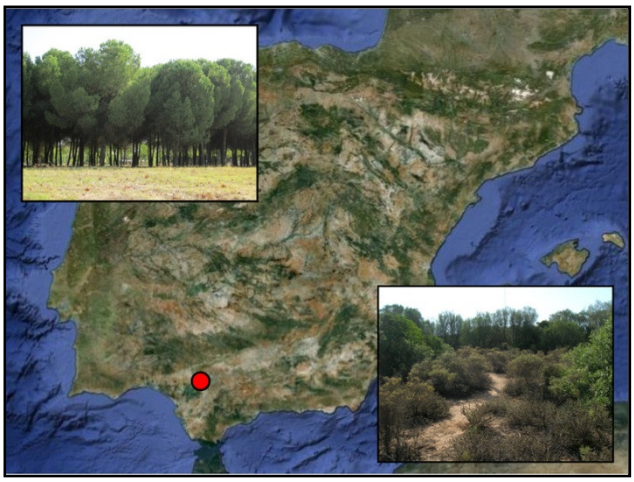

Figura 4. Localización e imágenes de los ecosistemas mediterráneos que han sido estudiados en esta tesis. La pimera imagen se corresponde con el pinar y la segunda con el matorral.

- **Ecosistema semiárido**

El cuarto capítulo de esta tesis se ha llevado a cabo con un suelo procedente de la Estación Experimental de Aranjuez, situado en el centro de España (40° 02′ N; 3° 37′ O). El clima en esta zona es Mediterráneo semiárido, con una media anual de 388 mm de precipitación y 14ºC de temperatura. El suelo tiene una importante proporción de yeso y presenta una cobertura de plantas perennes por debajo del 40%, principalmente *Stipa tenacissima* L. y *Retama sphaerocarpa* L. El suelo desprovisto de plantas aloja costra biológica del suelo bien desarrollada dominada por líquenes (Castillo-Monroy et al., 2010).

Figura 5. Localización e imagen del ecosistema semiárido que ha sido estudiado en esta tesis.

- **Ecosistema templado**

A pesar de que los ciclos de secado y rehumedecido de los suelos tienen un papel muy relevante en ecosistemas con una fuerte estacionalidad en las precipitaciones como los mencionados anteriormente, estos ciclos también ocurren frecuentemente en una gran variedad de ecosistemas y tienen importantes consecuencias sobre los ciclos biogeoquímicos (Kieft et al., 1987; Groffman & Tiedje, 1988; García-Méndez et al., 1991; Mummey et al., 1994; Cui & Caldwell, 1997; Ryan et al., 1998; Pulleman & Tietema, 1999). El quinto capítulo de esta tesis se ha realizado en el Cary Institute of Ecosystem Studies, en el suroeste del estado de Nueva York, USA (41.797°N, 73.734°W). El bosque del que se recogieron las muestras de suelo está dominado por *Quercus rubra* L., *Quercus prinus* L. y *Carya* sp. El clima en esta zona es continental húmedo, con unas medias anuales de 1110 mm de precipitación y 9.6 ^{o}C de temperatura. El suelo es franco limoso y tiene buen drenaje. Desde 1996, la mitad de las parcelas muestreadas fueron tratadas periódicamente con NH_4NO_3 y la otra mitad permanecieron sin tratar como control.

Figura 6. Localización e imagen del ecosistema templado que ha sido estudiado en esta tesis.

2.5. Objetivos de la tesis y estructura en capítulos

El objetivo general de esta tesis es evaluar como los cambios en el patrón de precipitación afectarán a los ciclos biogeoquímicos, los microorganismos del suelo y la emisión de gases de efecto invernadero en distintos ecosistemas y evaluar la importancia de los ciclos de secado y rehumedecido del suelo como controladores de los procesos del suelo. Además, analizaremos cómo la costra biológica del suelo y la deposición atmosférica de N modulan la respuestas de las variables de estudio a los ciclos de secado y rehumedecido del suelo.

De forma más concreta, abordaremos los siguientes objetivos específicos a través de los cinco capítulos de esta tesis. En el **capítulo 1** se estudia la importancia de los cambios en el contenido hídrico del suelo sobre el pool del N en dos ecosistemas mediterráneos, un pinar y un matorral. En este estudio evaluamos N orgánico y N inorgánico, así como la disponibilidad de N mediante el uso de resinas de intercambio iónico. En el **capítulo 2** se evalúa la respuesta de las tasas de respiración de estos dos mismos ecosistemas a los ciclos de secado y rehumedecido del suelo en relación a su estado nutricional, analizando también la variación temporal intra e inter estacional de las variables estudiadas. En el **capítulo 3** se determina si los cambios estacionales en la respuesta microbiana a la adición de diferentes fuentes de C produce un patrón similar en el pinar y el matorral nombrados anteriormente, y se evalúa su posible relación con algunas variables orgánicas del ciclo del C en el suelo. En el **capítulo 4** se estudia el

papel modulador de la costra biológica sobre el efecto de la longitud y la intensidad de los ciclos de secado y rehumedecido del suelo en una serie de variables relacionadas con el ciclo del N y del C. Finalmente, en el **capítulo 5** se evalúa el potencial modulador de la deposición atmosférica de N sobre el efecto de la frecuencia de los ciclos de secado y rehumedecido del suelo en la comunidad microbiana, los ciclos biogeoquímicos y la emisión de los gases de efecto invernadero.

Los resultados obtenidos de este trabajo permitirán una mejor comprensión de los mecanismos implicados en la respuesta de los procesos del suelo y la comunidad microbiana a los pulsos de agua sobre diferentes ecosistemas. Estos conocimientos serán de gran valor para realizar previsiones sobre las consecuencias del cambio climático esperado para las próximas décadas, pudiendo llegar a ser una importante herramienta a la hora de prever futuros escenarios.

Capítulo 1

Wetting and drying events determine soil N pools in two Mediterranean ecosystems

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To improve our knowledge of how nutrient cycling in Mediterranean environments responds to climate change, we evaluated the effects of the continuous changes in soil nitrogen (N) pools during natural wetting and drying events. We measured soil N pools (microbial biomass [MB-N], dissolved organic nitrogen [DON], NH_4^+ and NO_3^-) and N ion exchange resins at weekly intervals for one year in two contrasting Mediterranean ecosystems. All soil N fractions in both ecosystems showed high intraseasonal and interseasonal variability that was greater in inorganic soil fractions than in organic N soil fractions. MB-N, DON and resin-NH_4^+ showed increased concentrations during wetting events. Only the soil NO_3^- and resin-NO_3^- showed the opposite trend, suggesting a different response to water pulses compared to the other soil variables. Our results show that N pools are continuously changing, and that this high variability is not associated with the total amount of organic matter and labile soil carbon (C) and N soil fractions found in each ecosystem. The highest variability was found for inorganic N forms, which suggests that organic N forms are more buffered in soils exposed to wetting-drying cycles. Our results suggest that the changes in wetting-drying cycles expected with global climate change may have a significant impact on the availability and turnover of organic and inorganic N.

Keywords: Ion exchange resins N; nutrient cycling; precipitation; Dissolved organic N; microbialbiomass-N.

Introduction

Mediterranean ecosystems are predicted to experience important changes in the rainfall dynamics due to climate change, including a higher frequency of intense precipitation and drought events (Bates et al. 2008). These ecosystems are characterized by humid and cold temperature winters and dry and high temperature summers, and they are particularly susceptible to wetting and drying episodes due to the infrequency of rainfall events and the often warm and dry climate that favors rapid soil drying (Schröter, et al.2005). These changes may affect the turnover of carbon (C) and N in soils, but the direction of such changes is still unclear (Bortner and Matzner, 2009). A large number of laboratory studies during recent decades have assessed the changes in C and N turnover during wetting-drying cycles. However, the results of these studies are not conclusive (see Table 1 in Bortner and Matzner, 2009), most likely due to the application of different experimental conditions (Bortner and Matzner, 2009). Few field studies have been conducted, and most of those simulated reductions in rainfall (Davidson et al., 2004; Sotta et al., 2007; Yahdjian et al., 2006) or employed artificial wetting (Borken et al., 2006; Emmett et al., 2004) and reached contradictory conclusions. Consequently more detailed field observations are demanded to clarify the role of wetting-drying cycles in N pool changes.

Wetting-drying cycles influence microbial biomass and its activity (Bottner, 1985; Orchard and Cook, 1983; Skopp et al., 1990; Voroney, 2007). Increased amounts of N are mineralized after wetting-drying cycles compared with the amount that is mineralized in soils that are kept moist (Sorensen, 1974; Soulides and Allison, 1961). The mechanisms that explain this increased turnover are well known. During soil drying, microbes may accumulate solutes such as amino acids, carbohydrates, polyols and inorganic solutes to decrease osmotic potential in the cell and equilibrate with their environment (Halverson et al., 2000; Harris, 1981). The rapid changes in the soil water potential associated with rewetting cause microbes to undergo osmotic shock, which induces microbial cell lysis (Bottner, 1985; Van Gestel et al., 1992, 1993) and the release of the previously accumulated intracellular solutes (Halverson et al., 2000). A significant fraction of decomposable organic substrates are derived in part from the death of a portion of soil microorganisms (Jenkinson, 1966; Shields et al., 1974; Sorensen, 1983).

Additionally, the wetting-drying of soil may break soil aggregates and expose physically protected organic matter (Denef et al., 2001a, b; Lundquist et al., 1999a; Wu and Brookes, 2005). This previously unavailable organic matter can be rapidly mineralized by the microbial community (Appel, 1998). Thus, wetting-drying cycles may indirectly control the activity of soil organisms (Evans and Wallenstein, 2011) and ultimately determine nutrient turnover (Fierer and Schimel, 2002).

Improving our knowledge of the dynamics of wetting-drying cycles is crucial to predicting how nutrient cycling in Mediterranean environments will respond to ongoing global environmental change. However, it is difficult to predict the response to wetting or drying events because of the complexity of the processes involved. For example, in semi-arid ecosystems Schwinning and Sala (2004) have developed the idea that there is a hierarchy of soil moisture pulse events with a corresponding hierarchy of ecological responses, in which small pulses only trigger a small number of relatively minor ecological events and larger pulses trigger a more inclusive set and some larger ecological events. A higher level of complexity arises from the inverse metabolic activity hypothesis (Huxman et al., 2004), which suggests that ecosystems functioning at rates higher than their maximum will exhibit relatively low pulse responses compared to ecosystems functioning at lower rates. We think that these mechanisms might be operating in Mediterranean ecosystems as well, since small and large pulses of water inputs are also frequent.

In order to test the hypothesis that pulses of water will make noticeable changes in soil N pools, we evaluated the effects of the continuous changes on these pools during wetting-drying cycles in two Mediterranean ecosystems: a pine forest and a shrubland. These two plant communities were chosen as representative of two of the most common terrestrial ecosystems in SW Spain. We focused on the analysis of field observations to avoid the problems associated with manipulative experimental techniques. Our novel approach consisted of weekly soil sampling over the course of one year. This intensive sampling permitted consideration of all intraseasonal variability in the studied variables, identified different wetting or drying events in different seasons and explored the existence of an emerging and common pattern in the response of nutrient dynamics to these wetting-drying cycles.

In this study, we explored changes in N pools, including in dissolved organic N (DON) and microbial biomass N (MB-N), the most important labile organic pools in soils. Because N pools do not reflect the soil ion diffusion rates, which can be

dramatically affected during wetting-drying cycles (Li et al., 1993; Qian and Schoenau, 2001), we utilized ion resin membranes to estimate the effect of natural wetting and drying events on N availability. This technique is a tool which estimates plant uptake in simulating a plant root surface (Duran et al., 2013), and thus to be a better indicator of net N mineralization, avoiding N mineral leaching or crop capture. We first attempted to quantify the intraseasonal and interseasonal temporal N variation in soils and discern the consistency of temporal variability in the two Mediterranean ecosystems. Owing to the magnitude of the biogeochemical pulses during wetting-drying events may depend on substrate availability (Austin, 2011; Austin et al., 2004; Collins et al., 2008; Ma et al., 2012), we expected a higher variability of soil N pools in ecosystems rich in soil organic matter and nutrients compared to poorer ecosystems.

We also analyzed whether the soil variability was higher in organic or inorganic forms of N because resilience to wetting and drying events may be related to the different turnover rates of soil N fractions. We searched for directional changes in the soil N pools during wetting and drying events and examined the consistency of these changes in the two different ecosystems and contrasting climatic seasons. We also tested the hypothesis that the response of N pools to wetting-drying cycles may differ from N availability because diffusion rates are not accounted for.

Materials and methods

Study area

This study was conducted in a pine forest and a shrubland ecosystem in southwest Spain (37° 21'N; 5° 56' W). The distance between these study sites is 14.5 km. The Mediterranean climate has a 30-year average rainfall and temperature of 565.7 mm and 19.0°C, respectively. The study year was wetter than normal (852.6 mm in the pine forest and 845.7 mm in the shrubland). The soils in these areas have a typical A(B)C profile with a sandy clay loam and loamy sand texture in the pine forest and the shrubland respectively. Table 1 presents the main soil properties of the study sites. The pine forest includes *Pinus pinea* L. and some annual herbs. The shrubland is dominated by *Quercus coccifera* L., *Cistus albidus* L., *Genista hirsuta* Vahl. and *Arbutus unedo* L. Seasonally, the pine forest is subjected to fewer rapid changes in the soil water content than the shrubland soil due to its thicker litter layer and canopy shading. Net primary

production in these types of pine forest is significantly higher than in the shrublands (Merino et al. 1988).

Table 1. Soil physical and chemical properties of the top 10 cm for the pine forest and shrubland sites. Soil sampling was done weekly during 2009-2010 for MB-N, DON, NH_4^+-N, NO_3^-- N, PO_4^{3-}-P, IEMs, phenols, hexoses and aromatic compounds (n=312) and twice a year (summer and winter) for the remaining variables (n=24)

	Pine Forest		Shrubland	
	Mean	SE	Mean	SE
Clay (%)*	23.6	1.71	6.63	0.80
Silt (%)	12.8	3.64	12.5	0.69
Sand (%)*	63.6	5.26	81.0	0.36
Bulk density (g cm^{-3})*	1.16	0.07	1.41	0.09
Water content (%)*	12.4	0.53	7.98	0.33
Water holding capacity (%)*	45.87	0.58	27.73	0.76
pH*	7.2	0.03	5.49	0.06
Organic matter (%)*	2.84	0.21	1.91	0.17
Phenols (mg kg^{-1} soil)*	10.42	1.56	6.61	0.43
Hexoses (mg kg^{-1} soil)*	39.33	2.35	12.03	0.34
Aromatic compounds (mg kg^{-1} soil)*	127.74	9.29	32.8	3.06
Total N (%)*	0.15	0.02	0.10	0.01
C/N	10.9	0.49	13.09	1.91
MB-N (mg kg^{-1} soil)*	62.7	1.88	35.6	1.46
DON (mg kg^{-1} soil)*	12.0	0.39	8.82	0.48
NH_4^+-N (mg kg^{-1} soil)*	0.32	0.04	0.35	0.08
NO_3^-- N (mg kg^{-1} soil)*	3.22	0.23	1.38	0.16
NH_4^+-N IEMs (µg cm^{-2} day^{-1})	0.24	0.01	0.31	0.02
NO_3^-- N IEMs (µg cm^{-2} day^{-1})	0.48	0.03	0.52	0.03
Sodium bicarbonate PO_4^{3-}-P (mg kg^{-1} soil)*	2.39	0.09	0.53	0.03
Mg (meq $100g^{-1}$)*	1.16	0.08	0.51	0.03
K (meq $100g^{-1}$)*	0.62	0.03	0.16	0.01
Ca (meq $100g^{-1}$)*	12.2	0.82	8.08	0.52
Na (meq $100g^{-1}$)*	0.31	0.01	0.18	0.02

Sampling design

The soil sampling was conducted at weekly intervals for one year (from October 2009 to October 2010) to explore the dynamics of the N pools in the soils in response to periods of increasing or decreasing moisture availability. Six soil samples were collected randomly from the top 10 cm of the soil profile at each study site using a circular soil corer (5 cm diameter × 10 cm height). The samples were taken from the top 10 cm of the soil profile because most of soil nutrients in a Mediterranean ecosystem accumulate in the first few cm of the soil profile (Lugo et al., 1990). The soil samples were transported in polyethylene bags to the laboratory, stored at 3 °C in laboratory refrigerators and processed as soon as possible. All samples were processed in less than three days. This procedure ensured that the soil samples did not experience relevant changes (Gonzalez-Quiñones et al., 2009).

Laboratory analysis

The soil texture was estimated using the hydrometer method proposed by Kroetsch and Wang (2008). The gravimetric soil water content was calculated in fresh 5 g subsamples after drying in an 80 ^{o}C oven for 48 h. The water holding capacity was determined for each soil type as the gravimetric water content of soil that was saturated and allowed to drain freely over 48 h in a filter funnel. The soil pH was measured in 1:5 soil-water solutions. The soil organic matter was analyzed via the wet oxidation techniques of Skjemstad and Baldock (2006). Soil phenols, hexoses and aromatic compounds were determined following Chantigny et al. (2006). Soil subsamples were extracted with 0.5 *M* K_2SO_4 at a ratio of 1:5, followed by shaking for 1 h at 200 rpm at 20^{o}C. The extracts were filtered through a 0.45 µm Millipore filter, and they were measured colorimetrically by using a microplate reader. The total soil N was measured by standard Kjeldahl procedures (Rutherford et al., 2007). The soil PO_4^{3-}-P content was extracted with 100 ml of 0.5 M $NaHCO_3$ at a ratio of 1:20, and the concentration in the extract was determined by the molybdenum blue colorimetric method (Allen et al., 1986). Mg, K, Ca and Na were determined by atomic absorption spectrophotometry.

To measure ammonium (NH_4^+-N), nitrate (NO_3^--N) and DON, the soil subsamples were extracted with 0.5 *M* K_2SO_4 at a ratio of 1:5, followed by shaking for 1

h at 200 rpm at 20°C. The extract was filtered through a 0.45 µm Millipore filter (Jones and Willett, 2006). The NH_4^+-N concentration was estimated directly via the indophenol blue method using a microplate reader (Sims et al., 1995). The NO_3^--N was first reduced to NH_4^+-N with Devarda alloy, and the concentration was determined as the difference between the Devarda-incubated and unincubated samples. The DON in the extracts was first oxidized to NO_3^--N with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 °C for 55 min and then reduced to NH_4^+-N with Devarda alloy (Sollins et al., 1999). The DON contents were calculated as total dissolved N minus inorganic N.

MB-N was determined using the fumigation-extraction method proposed by Brookes et al. (1985). Twenty grams of fresh soil subsamples were fumigated with chloroform for 5 days. The non-fumigated replicates were used as controls. The fumigated and non-fumigated samples were extracted with 100 ml of K_2SO_4 0.5 *M* and filtered through a 0.45-µm Millipore filter. The extracts were digested as described above. The total N content in the digested extracts was determined by colorimetry (indophenol blue method) with a microplate reader (Sims et al., 1995). The MB-N concentration was estimated as the difference between the total N in fumigated and unfumigated digested extracts divided by a Kn (fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 (Brookes et al., 1985).

The availability of NH_4^+-N and NO_3^--N was measured in situ with ion-exchange membranes (resins; Subler et al., 1995). We selected this technique to generate minimal disturbances in the superficial soil communities and permit intensive sampling over multiple time periods at the same spatial location. Six anion and cation resins (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) were installed per site each week during the one-year sampling period. These resins were first subjected to expansion treatment by submersion in distilled water at 82–90°C for 48 h. Next, the resins were cut into 2.5 × 2.5 cm squares, attached to a plastic rod with acrylic glue and inserted into the soil at a 0.5–3 cm depth. The difference between this depth and the top 10 cm of soil from which the soil cores were collected makes them not directly comparable in terms of soil depth. The resins likely reflect the most organic horizon compared with the top 0 to 10 cm of the soil profile. During each sampling period, the resins were incubated in the field for 7 days. Following collection, the resins were taken to the laboratory and dried at ambient temperature. The resins were carefully separated from the plastic rod, brushed to remove soil particles, and placed into 125 ml

flasks for extraction with 25 ml of 2 *M* KCl via orbital spinning (1 h at 200 rpm). The extracts were analyzed to measure NH_4^+-N and NO_3^--N, as explained above.

Statistical and numerical analyses

During the study year, we identified eight major intervals of increasing soil water content (wetting events) and eight periods of decreasing soil water content (drying events) at each study site (Figure 1 a and b). We identified the intervals in all seasons, and the interval length ranged from 1 and 9 weeks. To calculate the impact of further wetting events on an already wet soil or a relatively dry soil on N dynamics, we classified these intervals based on occurrence on dry or wet soil. We defined dry or wet soil based on the initial water content, with the wet soil found approximately between November and April. A wetting interval was arbitrarily considered to occur within a wet soil if the water content starting point (the driest point) was above 10% in the pine forest or 5% in the shrubland. A drying interval was considered to occur within a dry soil if the water content starting point (the wettest point) was below 15% for both sites.

The increases or decreases in soil variables were calculated as the difference between the final and initial concentration in each wetting or drying interval, except for the NO_3^- and resin-NO_3^-, with a lag time of one week with respect to the wetting and drying events. We performed a Spearman correlation between changes in soil variables during wetting and drying events, mean temperature, mean soil water content (SWC), length of the wetting and drying event (LE) and length of the previous wetting or drying event before the next event (LPE).

To assess the effect of the study sites and seasons on the analyzed soil variables, we used a linear mixed model that treated the study site as a fixed effect and the seasons as random effects. We used linear mixed models that are particularly useful in settings with repeated measurements, such as our sampling design. The effect of each independent variable on the model was analyzed using a permutation test (1000 permutations of raw data). The linear mixed model and permutation tests were run using the libraries "nlme" and "pgirmess", respectively, in the R statistical package, version 2.15 (R Development Core Team 2012). The effects of the wetting and drying events on the changes in the analyzed variables for each site were determined using a linear model and permutation tests as described above. We adopted a

significance level of $p<0.05$. We used coefficients of variation (CV) as a metric for soil variability.

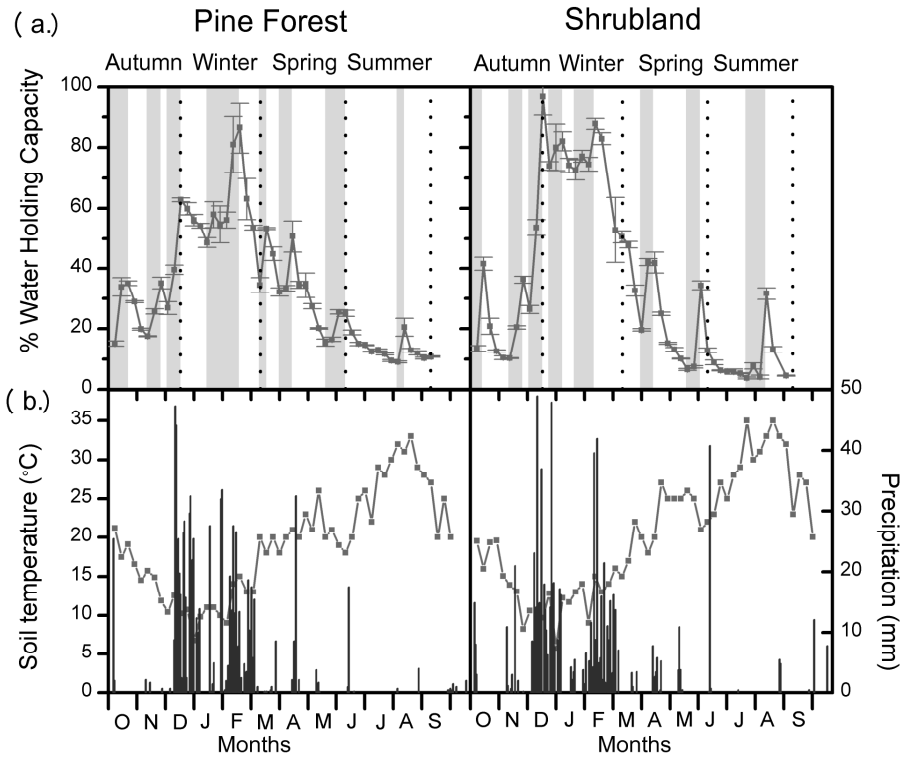

Figure 1. Soil water holding capacity (a) measured at weekly intervals in the top 10 cm of the soil profile during the study year in the pine forest and shrubland sites. Shaded bands indicate the selected wetting phases. Error bars are ±2SE. Soil temperature and precipitations (b) in the pine forest and shrubland plant communities during the study year

Results

Soil differences between ecosystems

Significant differences for soil texture and for all other physical properties were found between the two sites (Table 1). The pine forest showed both the highest water holding capacity and water content, and a neutral pH as opposed to the more acidic shrubland soil. All soil chemical variables were also significantly different between ecosystems, with the exception of the C:N ratio, and the resin-NH_4^+ and -NO_3^-.

Intraseasonal and annual variability

The maximum MB-N values were found in autumn and winter at the pine forest and in winter at the shrubland, while the minimum values were found in

summer at the pine forest and in autumn and summer at the shrubland (Figure 2a). The MB-N contents ranged between 10.7 and 110.8 mg kg^{-1} soil in the pine forest and 0.09 and 64.5 mg kg^{-1} soil in the shrubland. The highest CV for this variable were found in summer (CV=0.65 and 0.79 in the pine forest and the shrubland, respectively) at both study sites (Table 2). We detected significant differences in the MB-N contents between the two study sites and the seasons (Table 3). Differences between seasons were also observed when we analyzed each site separately ($P_{Shrubland}<0.0001$, $P_{pine\ Forest}<0.0001$).

Table 2. Coefficients of variation for the soil variables due to seasons and sites (annual)

Pine Forest						Shrubland				
Variables	Autumn	Winter	Spring	Summer	Annual	Autumn	Winter	Spring	Summer	Annual
MB-N	0.35	0.38	0.41	0.65	0.50	0.71	0.50	0.56	0.79	0.65
DON	0.55	0.37	0.51	0.35	0.53	0.59	0.39	0.53	0.57	0.84
NO_3^--N	0.66	1.09	0.95	1.29	1.21	1.24	1.82	2.16	1.56	1.95
Resin-NH_4^+-N	0.32	0.79	0.45	0.35	0.91	0.35	0.64	0.53	0.45	0.97
Resin-NO_3^--N	0.78	0.86	0.81	1.15	0.99	0.94	0.52	0.77	1.12	1.04

The maximum DON contents were found in summer at both study sites, while the minimum DON values were similar in the remainder seasons (but with minimum peaks in spring, Figure 2b). The DON values ranged between 2.81 and 26.4 mg kg^{-1} soil in the pine forest and 0.84 and 21.73 mg kg^{-1} soil in the shrubland. The highest CV values for this variable were found in autumn (CV=0.55 and 0.59 in the pine forest and the shrubland, respectively) at both study sites (Table 2). We detected significant differences in this variable between study sites and seasons (Table 3).

Table 3. Permutation test evaluating the effect of the site and season on soil variables

Variables	P site	P season	P Site x Season
MB-N	<0.0001	<0.0001	<0.0001
DON	<0.0001	<0.0001	0.071
NH_4^+-N	0.752	0.028	0.054
NO_3^--N	<0.0001	0.004	0.019
Resin-NH_4^+-N	<0.0001	<0.0001	<0.0001
Resin-NO_3^--N	0.163	0.921	0.205

Very low (or undetectable) NH_4^+-N values were found throughout the whole year at both study sites. The maximum values were recorded in winter, summer and autumn, and the minimum values were found in spring at both study sites (Figure 3a). The NH_4^+- N contents ranged from 0 to 2.53 mg kg^{-1} soil in the pine forest and from 0 to 3.87 mg kg^{-1} soil in the shrubland. The highest coefficients of variation (CV=4.03 and 4.55 in the pine forest and the shrubland, respectively) in this variable were found in autumn at both study sites (Table 2). We detected significant differences between the seasons but not between sites (Table 3).

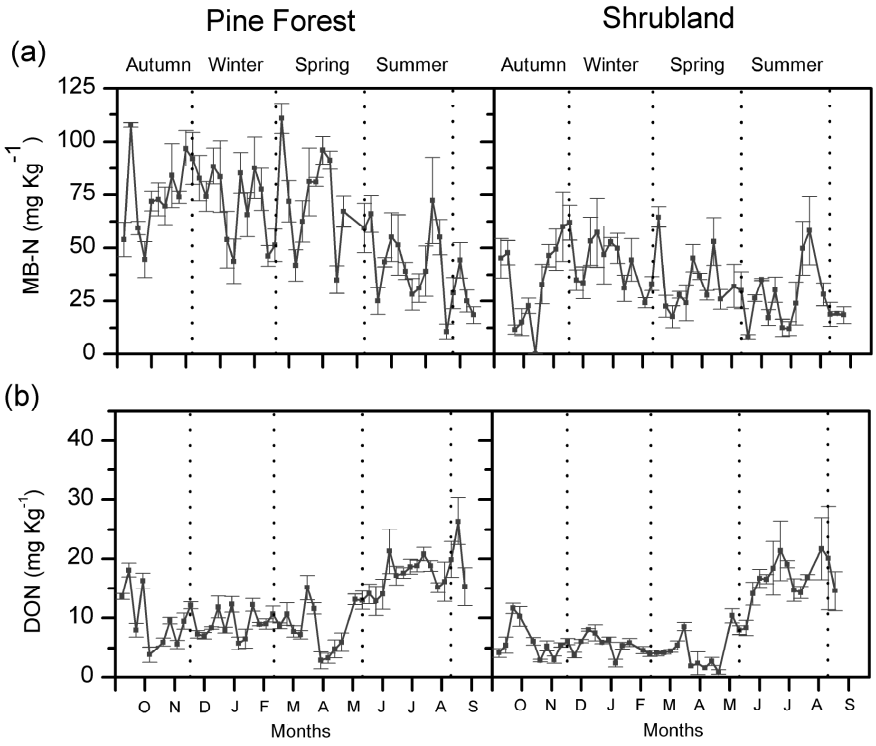

Figure 2. MB-N (a) and DON (b) measured at weekly intervals throughout the year in the pine forest and shrubland plant communities.

The maximum NO_3^--N contents were observed in autumn at both study sites, while the minimum NO_3^--N values were observed in the rest of the seasons (Figure 3b). The NO_3^--N contents ranged from 0 to 12.3 mg kg^{-1} soil in the pine forest and from undetectable to 11.13 mg kg^{-1} soil in the shrubland. The highest CV values were found in summer (1.29) and spring (2.16) in the pine forest and the shrubland, respectively (Table 2). We detected significant differences in the contents of NO_3^--N between the study sites and seasons (Table 3).

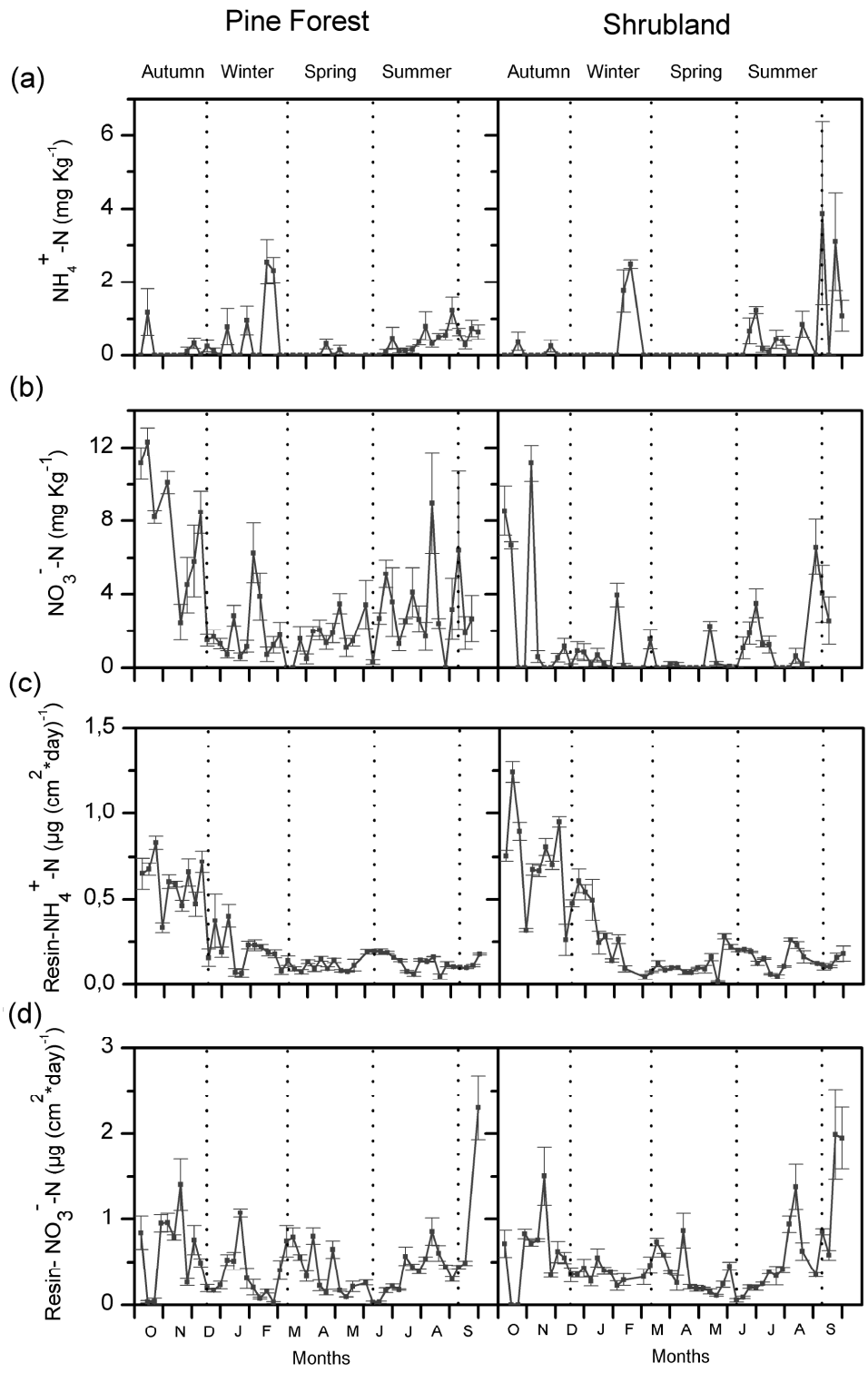

Figure 3. NH_4^+-N (a), NO_3^--N (b), resin-NH_4^+-N (c) and resin-NO_3^--N (d) measured at weekly intervals during the study period in the pine forest and shrubland plant communities

For resin-NH_4^+-N, the highest CV values was found in winter (CV=0.79 and 0.64 in the pine forest and the shrubland, respectively) at both study sites (Table 2). Significant differences were observed among seasons for this variable (Table 3), with

the maximum and minimum peaks occurring in autumn and spring, respectively, for both study sites (Figure 3c). Significant differences were detected in the mean values between the two sites (Table 3), with values ranging between 0.047 and 0.83 μg cm^{-2} day^{-1} in the pine forest and 0.014 and 1.24 μg cm^{-2} day^{-1} in the shrubland. We observed a significant site x season interaction (Table 3) because differences between sites were only found in autumn.

For resin-NO_3^--N, the highest CV values were found in summer (CV=1.15 and 1.12 in the pine forest and the shrubland, respectively) at both study sites (Table 2). Significant differences were not found between seasons for this variable (Table 3) or sites (Table 3; Figure 3d). The values ranged between 0.027 and 2.3 μg cm^{-2} day^{-1} in the pine forest and between 0.09 and 2.16 μg cm^{-2} day^{-1} in the shrubland.

Changes during wetting-drying events

Positive and negative changes were observed during the wetting and drying events, respectively, for MB-N ($p<0.0001$), DON ($p=0.015$) and resin-NH_4^+-N ($p=0.002$) in the pine forest soils (Figure 4 and 5). However, the opposite trend (increases during the drying phases and decreases during the wetting phases) was found for NO_3^--N ($p=0.104$) and resin-NO_3^--N ($p<0.0001$, Figure 5). In the shrubland soils, these differences between wetting and drying events were of a lower magnitude but still significant for MB-N ($p<0.0001$), DON ($p=0.05$), NO_3^--N ($p=0.045$) and resin-NO_3^--N ($p=0.006$, Figure 4 and 5). The pattern noted for the whole year was also observed for the dry and wet soils for most variables (Table 4). However, some differences emerged for soil NO_3^--N, as the variations between the wetting and drying events were apparent only for the dry season at both sites ($p=0.085$ and $p=0.008$ in the pine forest and the shrubland, respectively). Similarly, the differences for resin-NH_4^+-N were apparent only in the dry season for both sites ($p=0.032$ and $p=0.07$ in the pine forest and the shrubland, respectively).

Table 4. Permutation test evaluating the effect of the wetting-drying events for all conditions (whole year) or within the wet and dry soils on soil variables

	Pine Forest			Shrubland		
Variables	Whole year	Dry soil	Wet soil	Whole year	Dry soil	Wet soil
MB-N	< 0.0001	0.09	0.029	< 0.0001	0.016	0.305
DON	0.015	0.141	0.071	0.05	0.381	0.049
NH_4^+-N	0.971	0.121	0.719	0.262	0.271	Undetectable
NO_3^--N	0.05	0.085	0.916	0.045	0.008	0.897
Resin-NH_4^+-N	0.002	0.032	0.315	0.565	0.07	0.296
Resin-NO_3^--N	< 0.0001	0.205	0.026	0.006	0.03	0.398

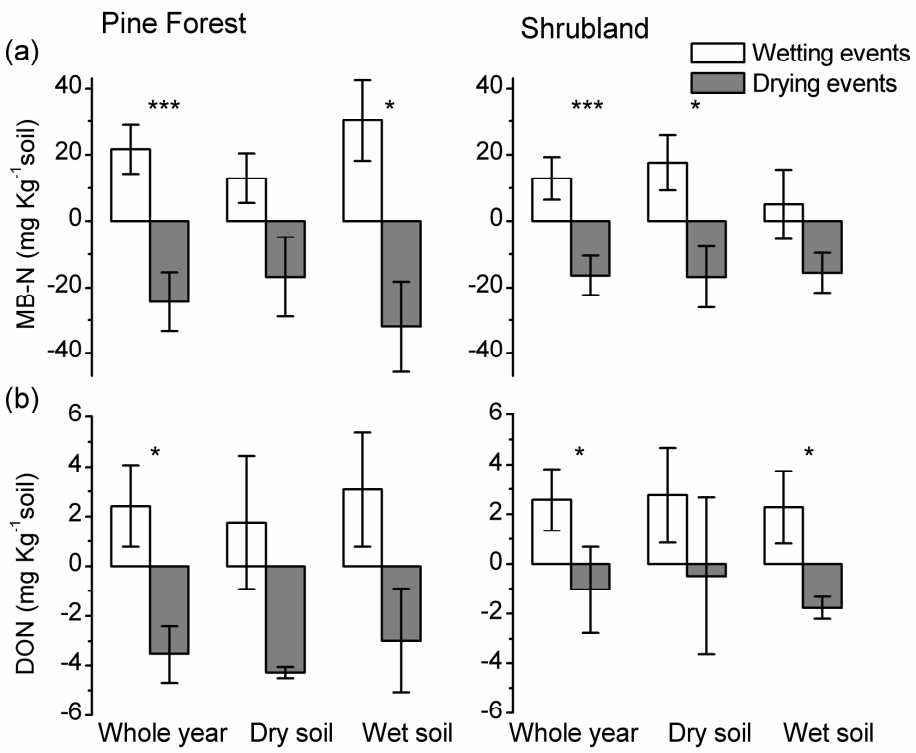

Figure 4. Increments of MB-N (a) and DON (b) during eight wetting and drying events for each study site. Error bars are ±2SE. n=8 for "Whole year" and n=4 for "Dry soil" and "Wet soil"

The changes in soil variables during wetting and drying events were not correlated with the mean temperature of each time period. In the pine forest during the drying events, the changes in the amount of NH_4^+-N and NO_3^--N were positively correlated with the mean SWC (Table 5). We also found negative correlations between the changes in the MB-N during wet events and the LE and LPE in the pine forest and the shrubland, respectively (Table 5). We identified a positive correlation between

changes in NO3 and resin- NO_3^--N and LE in the shrubland during wet and dry events, respectively.

Table 5. Spearman correlation matrix (rho) between changes in soil variables during wetting and drying events, and the mean temperature, mean soil water content (SWC), the length of the wetting and drying event (LE) and the length of the previous wetting or drying event before the next event (LPE). Significant correlations (P≤0.05) are indicated in bold numbers. N=8.

		MB-N (µg/g)	DON (µg/g)	NH_4^+-N (µg/g)	NO_3^--N (µg/g)	NH_4^+-N IEMs (µg/(cm²*day))	NO_3^-- N IEMs (µg/(cm²*day))
Pine forest Wet event	T	0.2143	0.1905	-0.4059	-0.1905	-0.3214	0.3214
	SWC	0.1667	-0.2619	0.4312	0.2619	-0.0357	-0.4643
	LE	**-0.8301**	0.2554	0.6191	0.0255	0.6736	-0.3930
	LPE	-0.3604	-0.3063	-0.0374	-0.0180	0.4638	0.3424
Dry event	T	-0.5476	-0.7143	0.7413	0.3571	0.4048	-0.2619
	SWC	-0.0878	0.6786	**-0.8901**	**-0.714**	0.3333	0.3810
	LE	0.1708	-0.2594	-0.0374	-0.5124	0.0732	0.5855
	LPE	-0.7093	0.3947	0.2464	0.4335	0.2167	-0.0985
Shrubland Wet event	T	-0.1429	0.0952	-0.535	-0.3214	0.5238	-0.1191
	SWC	-0.1905	0.0238	0.1336	0.6429	-0.6667	0.4048
	LE	-0.2887	-0.0138	0.5916	**0.8964**	-0.4949	-0.1237
	LPE	**-0.8001**	0.3273	-0.429	0.4058	0.2546	0.2364
Dry event	T	-0.3095	0.4643	0.6547	0.3095	-0.5238	0.5714
	SWC	0.0238	-0.2500	-0.131	-0.5000	0.3333	-0.5476
	LE	-0.0603	0.2342	0.5314	0.1566	-0.3615	**0.7711**
	LPE	-0.1336	-0.1690	0.7906	-0.2673	-0.4009	0.4009

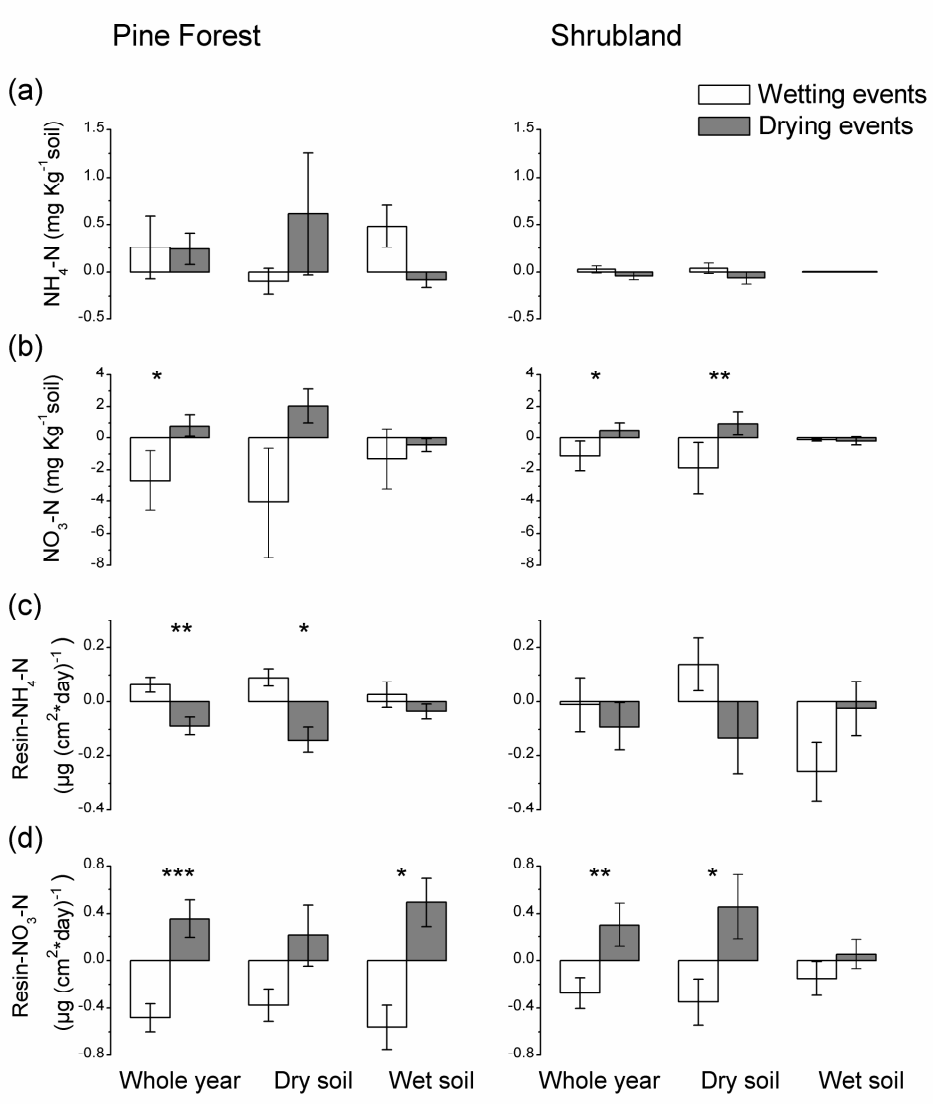

Figure 5. Increments of NH_4^+-N (a), NO_3^--N (b), resin-NH_4^+-N (c) and resin-NO_3^--N (d) during eight wetting and drying events for each study site. Error bars are ±2SE. n=8 for "Whole year" and n=4 for "Dry soil" and "Wet soil".

Discussion

Our results showed a high intraseasonal variability for most soil variables together with a clear pattern of increase and decrease for these variables during the wetting and drying periods respectively. This intraseasonal variability was as high as the annual variability, suggesting that variability of our soil variables may be more dependent on wetting-drying events than seasonal differences. If microbial responses to re-wetting are triggered by water content but limited by substrate availability, such as labile C and nutrient pools (Austin, 2011; Austin et al., 2004; Collins et al., 2008; Ma et al., 2012), we would expect to find stronger responses and more variability in the

pine forest (where there is a higher soil organic matter content and lower C-to-N ratio) than the shrubland. However, the variability in the soil N variables was similar in both ecosystems; this result provides little support for the substrate limitation hypothesis in these ecosystems (Jenerette and Chatterjee, 2012).

The inorganic forms of N were more variable than the organic forms, suggesting that the turnover of microbial biomass and organic polymers is more resilient to wetting-drying cycles than inorganic forms of N due to the many processes involved in transformation, inputs (atmospheric N deposition) and outputs (leaching and denitrification), in addition to internal ammonification and nitrification processes. Other authors have reported similar results. For example, the wetting of dry soil from a semi-arid ecosystem was found to initially stimulate gross N mineralization to a greater extent than N immobilization and cause a short-lived wetting pulse of inorganic N (Saetre and Stark, 2005). Less variability in the organic N forms may agree with the observation that the C cycle is less sensitive than the N cycle to wetting-drying cycles (Borken and Matzner, 2009).

The values of most of the analyzed variables increased during wetting events, as expected due to the increasing microbial activity observed by numerous authors (e.g., Borken et al., 2003; Lee et al., 2004; Sponseller, 2007). Several mechanisms have been proposed to explain the directional changes in microbial biomass and N pools detected during the wetting-drying cycles, which were also observed in the present study. For example, dry intervals can result in high microbial mortality due to desiccation and radiation damage (Castenholz and Garcia-Pichel, 2000). These dead microorganisms are readily decomposed by surviving organisms when the soil is rewetted. Surviving microorganisms may experience greater stress than during the drying interval and possibly die following wetting (Schimel et al., 2007).

Sudden changes in soil moisture are stressful to microbes because they must expend energy to regulate osmotic pressure in relation to their microenvironment (Bottner, 1985; Van Gestel et al., 1993). To achieve osmotic regulation as the soil dries, many microbes synthesize solutes such as aminated sugars and amino acids (Csonka, 1989). Because the soil water potential increases rapidly after precipitation events, the microbes must release solutes before osmotic pressure bursts their cells (Halverson et al. 2000; Wood et al., 2001). These mechanisms are complex, and the dominance of one process over another may explain the contrasting directional changes that several authors observed during wetting-drying cycles (Fierer et al., 2003; Halverson et al.,

2000; Schimel et al., 2007). Borken and Matzner (2009) argue that the contradictory results of these studies may depend on experimental and local conditions, such as the intensity, frequency and duration of drying and wetting cycles. In the present study, we observed an increase and decrease in most variables during the wetting and drying phases, respectively. We did not perform a manipulative experiment (i.e., reduce or increase precipitation). These changes were observed under natural conditions in the two different ecosystems for both dry and wet seasons during the year and provide strong support for the directional changes in these variables reported in Mediterranean ecosystems.

The soil NO_3^- pools and resin-NO_3^- showed a trend opposite to that of MB-N and DON, increasing during drying events and decreasing during wetting events. Unsurprisingly, the soil NH_4^+ pools showed no detectable directional changes during the wetting and drying events because the NH_4^+-N concentration was very low in both ecosystems during the year. However, the resin-NH_4^+ showed an inverse tendency to resin-NO_3^-, suggesting rapid nitrification during the dry season. The mineralization process is known to be less affected by drought than by other microbial-driven soil processes (Reynolds et al., 1999; Smolander et al., 2005). Schwinning and Sala (2004) suggested that small pulses may occur during drying events due to the higher water content in the soil during early morning hours, which can be sufficient to trigger nitrification but not to trigger plant nutrient uptake (Gelfand and Yakir, 2008).

Inorganic N may also accumulate during drying events because ion diffusion is severely restricted in thin water films in dry soils and because inorganic N sinks are limited by reduced microbial growth and limited plant uptake (Barber, 1995; Stark and Firestone, 1995). The length of the wetting or drying events also influenced the response of some soil variables. In the pine forest, we detected a negative correlation between changes in MB-N during a wetting event and their length, indicating the short-lived nature of the microbial biomass pulses after rewetting (Landesman and Dighton, 2011). This correlation was not found in the shrubland site, in which the only correlation was the length of the previous drying event. This different pattern showed that these changes in microbial biomass after rewetting in different ecosystems cannot be explained by a single mechanism. We also found a positive correlation between the changes in soil NO_3^- during wetting events and their length, which suggests that large decreases in soil NO_3^- were produced at the beginning of the wetting event (by leaching or by plant and microbial uptake) and that NO_3^- levels were later recovered by

soil nitrification. Unsurprisingly, the length of the drying event affected only to the resin- NO_3^-, emphasizing the importance of diffusion in dry soils.

We expected to observe different patterns of change during the wetting and drying events based on whether they occurred on wet or dry soils. However, our observations of most of the analyzed variables revealed very similar responses to wetting and drying events, suggesting that the initial soil water content and temperature are not as determinant as abrupt changes in soil humidity for triggering a microbial response to these events. These results do not support the inverse metabolic activity hypothesis (Huxman et al., 2004), which predicts larger pulse responses outside of the primary growing season (wet soil). However, we only studied the top 10 cm of the soil profile, and we would expect a reduced effect of wetting-drying cycles in deeper soils. Because most fine roots are found in this horizon, we argue that these results are relevant for soil biogeochemistry and plant nutrition.

Based on diffusion limitations, we expected to find clear differences between inorganic N pools and resin-N availability. We focused on NO_3^--N because of the low (or even undetectable) NH_4^+-N concentration in the soils. Both soil NO_3^- pools and resin-NO_3^- showed increases during soil drying and decreases during soil wetting. However, these changes in resin-NO_3^- were patent during both wet and dry soils. Besides, the relative magnitude of the changes was greater for resin-NO_3^- than for NO_3^- in all cases. Thus, the measurement of N availability, including diffusion rates, provides more information than traditional soil N pool measurements in ecosystems under frequent wetting-drying cycles and emphasizes the importance of soil diffusion for nutrient availability during these cycles.

Conclusions

Soil variables showed a high intraseasonal variability and a clear pattern of increase and decrease during the wetting and drying periods. This high variability was not associated with the amount of labile C and N pools available for microbial processes. Because we found that inorganic N forms have the highest variability, we suggest that organic N forms are more buffered in the soils exposed to wetting-drying cycles. A future with more intense and frequent cycles may produce greater losses of inorganic N. Increases in the concentrations of N variables clearly occurred during wetting events, independent of the time of the year or the ecosystem type, except for

soil NO_3^-, which showed the opposite trend. Soil N measurements taking in account diffusion rates improve the understanding of the responses of N pools to wetting and dry cycles. These trends may be sufficiently robust to extend to other Mediterranean ecosystems because these results are based on an analysis of field observations without experimental manipulation. Further research is necessary to determine how these increases and decreases in N availability synchronize with plant demand and uptake.

Acknowledgments

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Capítulo 2

Unpredictable soil respiration responses to wetting and drying cycles in Mediterranean ecosystems

Lourdes Morillas and Antonio Gallardo

A better knowledge of changes in soil respiration during natural wetting and drying events in highly susceptible regions such as Mediterranean ecosystems is essential, as expected climate changes lead to increased precipitation variability. We analyzed soil respiration rates, mineral nitrogen, ion-exchange resin mineral nitrogen and phosphate at weekly intervals over one year in two Mediterranean ecosystems: a pine forest and a shrubland. Higher soil respiration rates were detected in the pine forest than the shrubland. The pine forest showed an increased soil respiration rate during the wetting events and a decreased rate during the drying events, while the soil respiration of the shrubland did not show any relationship to wetting-drying cycles. Soil and resin mineral nitrogen showed increased concentrations during the drying events in both ecosystems, but we found no trend for the phosphate at any site. The lack of a soil respiration response to water pulses at the shrubland was unexpected because the nearby pine forest showed a well-defined pattern. The two ecosystems are separated by few kilometres, yet their soil respiration responses to water pulses ranged from predictable to unpredictable. Although we cannot identify the process underlying these differences, our results suggested that phosphorous limitation may restrict the soil respiration response to wetting-drying events.

Keywords: Soil moisture; Precipitation; Mineral nitrogen; phosphate; resin mineral-N; Carbon mineralization.

Introduction

Climate models predict an intensification of the hydrologic cycle, which will result in longer dry periods and more intense rainfall events (Huntington, 2006). These climatic changes may have implications for biogeochemical processes including changes in the long term of carbon (C) and nitrogen (N) pools in soils (Lindner et al., 2010). Many laboratory studies evaluated the soil biochemical response to intermittent water availability (Birch, 1964; Fierer and Schimel, 2002; Evans and Wallenstein, 2011), but these results do not readily transfer to field conditions due to the combination of different mechanisms (Borken and Matzner, 2009). The influence of plants (water and nutrient uptake, root production, exudation), heterogeneous patterns of soil moisture, soil aggregation, and different temperature regimes may affect C and N mineralization, and fluxes may differ under field conditions compared to laboratory studies. Few field studies assess the effects of wetting-drying cycles on soil biochemical response, and most of these studies manipulated natural rainfall (Yahdjian et al., 2006). Borken and Matzner (2009) highlighted the contradictory conclusions that these researches reached, since some of them found increase, some others decrease, and some others no change in C mineralization during drying and wetting relative to moist control or moist condition before drying, most likely resulting from the application of different experimental conditions. The results of these studies are inconsistent due to the wide variety of processes involved in N and C cycling. Non-manipulative field experiments would provide valuable information on this topic.

The changes in soil moisture and temperature are considered to be the main drivers of mineralization processes in Mediterranean ecosystems (Noy-Meir, 1973). Both C and N mineralization rates generally increase following the rewetting of dry soil (Cui and Caldwell, 1997; Franzluebbers et al., 2000) since soil microbial activity is no longer limited by water, but the source of this burst of C and N is still unclear. Few studies have considered the effect of wetting-drying events on phosphorous (P) cycling (Austin et al., 2004), although P might be highly limiting in terrestrial ecosystems as a consequence of increased atmospheric N deposition (Elser et al., 2007). We evaluated the effects of the continuous changes in soil respiration rates, mineral N, resin-mineral N and PO_4^{3-} during natural wetting-drying cycles in two Mediterranean ecosystems: a pine forest and a shrubland. The relevance of water pulses on N cycle has been previously proved in these study sites (Morillas et al., 2013). We conducted our study with a non-manipulative field study to overcome the problems associated with

experimental techniques and other types of manipulations that provide inconsistent results, most likely due to varying experimental designs, incubation temperatures, soil properties and treatments. We conducted an intensive weekly sampling over a year to capture the entire intra-seasonal variability in the studied variables and to identify different wetting or drying events in each season. This survey allows exploration of any emergent and common patterns in the C mineralization response to these wetting-drying cycles, a topic scarcely studied in natural conditions.

Enhanced knowledge of the implications of wetting-drying events is crucial to understand these effects on soil respiration rates and nutrient cycling in Mediterranean environments. This valuable information will permit prediction of how these variables respond to ongoing global environmental change. This study responds to the need for more research in Mediterranean ecosystems and recognizes that current climate models indicate these regions are highly susceptible to global change (Giorgi, 2006).

Our specific goals were (i) to study whether the response to wetting-induced pulses on soil respiration rates produced a similar pattern in our two study ecosystems, (ii) to identify the intraseasonal or interseasonal temporal variation in the analyzed soil variables and determine the consistency of this temporal variability in the two studied Mediterranean ecosystems, and (iii) to test whether the directional changes in soil respiration and nutrient contents during wetting and drying events are dependent on the soil water content or the temperature and length of the wet or dry period.

Material and methods

Study area

This study was conducted in a pine forest and a shrubland ecosystem in southwest Spain (37° 21'N; 5° 56' W) with a typical Mediterranean-type climate. The distance between these study sites is 14.5 km. In these Mediterranean sites 30-year average rainfall and temperature was of 565.7 mm and 19.0 ◦C, respectively. The study year was wetter than normal (852.6 mm in the pine forest and 845.7 mm in the shrubland).The soils in these areas show a typical A(B)C profile. Table 1of chapter 1 presents the main properties of these soils. The pine forest contains the species *Pinus pinea* L., with scarce annual herbs and forbs under their canopies. The shrubland is dominated by *Quercus coccifera* L., *Cistus albidus* L., *Genista hirsuta* Vahl. and *Arbutus unedo* L.

Field sampling

To explore the temporal dynamics of soil respiration and nutrient availability, we conducted soil sampling at weekly intervals for one year, from October 2009 to October 2010. Six soil samples from each study site were collected randomly from the top 10 cm of the soil profile with a circular soil corer (5 cm diameter × 10 cm height). Soil samples were transported in refrigerated plastic bags to the laboratory and stored at 3 °C. Litter and stones were removed from soils prior to analysis and they were processed as soon as possible (less than three days in all cases). On each sampling date, the soil respiration rates were determined as the surface CO_2 efflux utilizing a portable soil respiration system (EGM-4 PP SYSTEMS) with a chamber of 10 cm ∅ and 15.5 cm depth. The soil respiration and soil temperature were measured between 10:00 am and 11 am for each sampling date. On six randomly chosen spots these variables were monitored in each sampling week by using a digital soil thermometer and a portable chamber which were placed on a different location on each sampling date.

Laboratory analysis

The soil texture was estimated with the hydrometer method suggested by Kroetsch and Wang (2008). The soil conductivity and pH were measured in 1:2.5 and 1:5 soil-water solutions, respectively. The soil organic matter was analyzed via the wet oxidation techniques utilized by Skjemstad and Baldock (2006). The carbonate levels were analyzed according to Boon Goh and Mermut (2007). The total soil N was measured utilizing standard Kjeldahl procedures (Rutherford et al., 2007). The dissolved organic nitrogen (DON) was analyzed following the Sollins et al. (1999) methodology. The DON contents were calculated as total dissolved N minus mineral N. The microbial biomass-N (MB-N) was determined with the fumigation-extraction method proposed by Brookes et al. (1985). The MB-N concentration was estimated as the difference between the total N in fumigated and unfumigated digested extracts divided by a Kn (fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 (Brookes et al., 1985). All these variables were analysed for the general description of the two study sites.

The following analyses were done for monitoring purposes. The gravimetric soil moisture was calculated in fresh 5 g subsamples after drying at 80 ^oC for 48 h until

constant weight. The water holding capacity (WHC, %) was determined for each soil type as the gravimetric water content of soil that was saturated and allowed to drain freely over 48 h in a filter funnel. To measure the mineral N, the soil subsamples were extracted utilizing 0.5 M K_2SO_4 at a ratio of 1:5. Soil samples were shaken with the extractant in an orbital shaker at 200 rpm for 1 h at 20^oC and filtered through 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 seven days following the extraction. The NH_4^+-N concentration was directly estimated with the indophenol blue method utilizing a microplate reader (Sims et al., 1995). The NO_3^--N was first reduced to NH_4^+ with Devarda alloy, and its concentration was determined as described above. The NO_3^--N concentration in the extracts was calculated as the difference between the Devarda-incubated and unincubated samples. The mineral N is expressed as the sum of NH_4^+ and NO_3^-.

The availability of NH_4^+-N, NO_3^--N and mineral N was measured in situ utilizing ion-exchange membranes (resins; Subler et al., 1995). We selected this technique because it generates minimal disturbances to soil surface communities and allows intensive sampling over multiple time periods at the same spatial location. Six anion and cation resins (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) were installed per site and week over the one year sampling period. These resins were first subjected to expansion treatment by submersion in distilled water at 82–90°C for 48 h. Then, the resins were cut into 2.5 × 2.5 cm squares, attached to a plastic rod with acrylic glue and inserted into the soil at a 0.5–3 cm depth. The resins were incubated in the field for seven days during each sampling period. Following collection, the resins were taken to the laboratory and dried at ambient temperature. The resins were carefully separated from the plastic rod, brushed to remove soil particles and placed into 125 ml flasks for extraction with 25 ml of 2 M KCl via orbital spinning (1 h at 200 rpm). The extracts were analyzed to measure the NH_4^+-N and NO_3^--N utilizing the above method. To measure PO_4^{3-}-P, the soil subsamples were extracted with 100 ml of 0.5 M $NaHCO_3$ at a ratio of 1:20, and the concentration in the extract was determined with the molybdenum blue colorimetric method (Allen et al., 1986).

Statistical and numerical analyses

During the study year, we identified eight major intervals of increasing soil water content (wetting events) and eight periods of decreasing soil water content (drying events) at each study site (Figure 1 a and b of chapter 1). We found these intervals in all seasons and identified interval lengths ranging from 1 and 9 weeks. We classified these intervals into dry or wet soil occurrences to calculate how further wetting events on an already wet soil or a relatively dry soil might affect the dynamics of the studied variables. A wetting interval was arbitrarily considered to occur within a wet soil if the water content starting point (the driest point) was above 10% in the pine forest or 5% in the shrubland. A drying interval was considered to occur within a dry soil if the water content starting point (the wettest point) was below 15% in both sites. The increases or decreases in soil variables were calculated as the difference between the final and initial concentration in each wetting or drying interval. We calculated the Spearman correlation between these changes in soil variables during wetting and drying events and the mean soil temperature, mean soil water content, the length of the wetting and drying event and the length of the previous wetting or drying event before the next event. The daily precipitation values were obtained from the AEMET weather station network in close proximity to the study sites.

To assess the effect of the study sites and soils on the analyzed soil variables, we utilized a linear mixed model in which the study site was treated as a fixed effect and the soil variables were treated as random effects. We utilized linear mixed models that are particularly useful in settings (such as our sampling design) that require repeated measurements. The effect of each independent variable on the model was analyzed with a permutation test (1000 permutations of raw data). The linear mixed model and permutation tests were conducted utilizing the libraries "nlme" and "pgirmess", respectively, in the R statistical package, version 2.15 (R Development Core Team 2012). The effects of the wetting and drying events on the changes in the analyzed variables for each site were determined with a linear model and permutation tests as described above. We adopted a significance level of $p<0.05$. We used coefficients of variation (CV) as a metric for soil variability.

Results

Intraseasonal and annual variability

Higher soil respiration rates were detected in the pine forest compared to the shrubland (Table 1), ranging between 0.12 and 0.76 g CO_2 m^{-2} $hour^{-1}$ for the first and between 0.04 and 0.67 gCO_2 m^{-2} $hour^{-1}$ for the second. We detected significant differences among seasons (Figure 2, Table 2), with a significant interaction between site and season. Differences between seasons were found during a separate site analyses ($P_{Shrubland}$=0.001, $P_{pine\ Forest}$<0.0001). The highest coefficients of variation (CV) values for this variable were found in winter and autumn for the pine forest and the shrubland, respectively (Table 3).

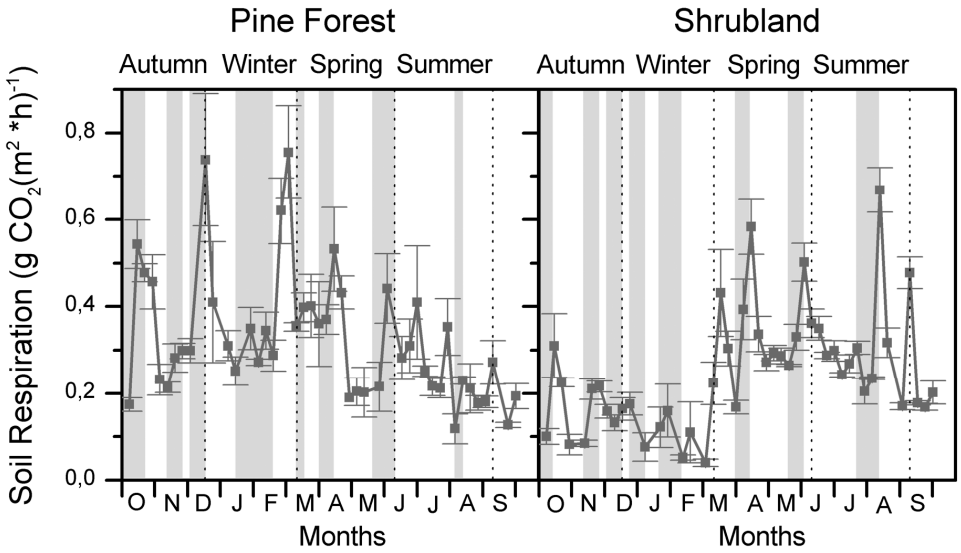

Figure 2. Soil respiration rates measured at weekly intervals during the study period in the pine forest and shrubland plant communities

Significantly higher values of mineral N were found in the pine forest compared to the shrubland (Table 1 and 2), with a significant interaction between site and season. Differences between the seasons were found during a separate site analyses ($P_{Shrubland}$=0.009, $P_{pine\ Forest}$=0.009). The maximum and minimum mineral N values were found in winter and spring for both study sites (Figure 3a). The highest CV for this variable was found in summer and spring in the pine forest and the shrubland, respectively (Table 3).

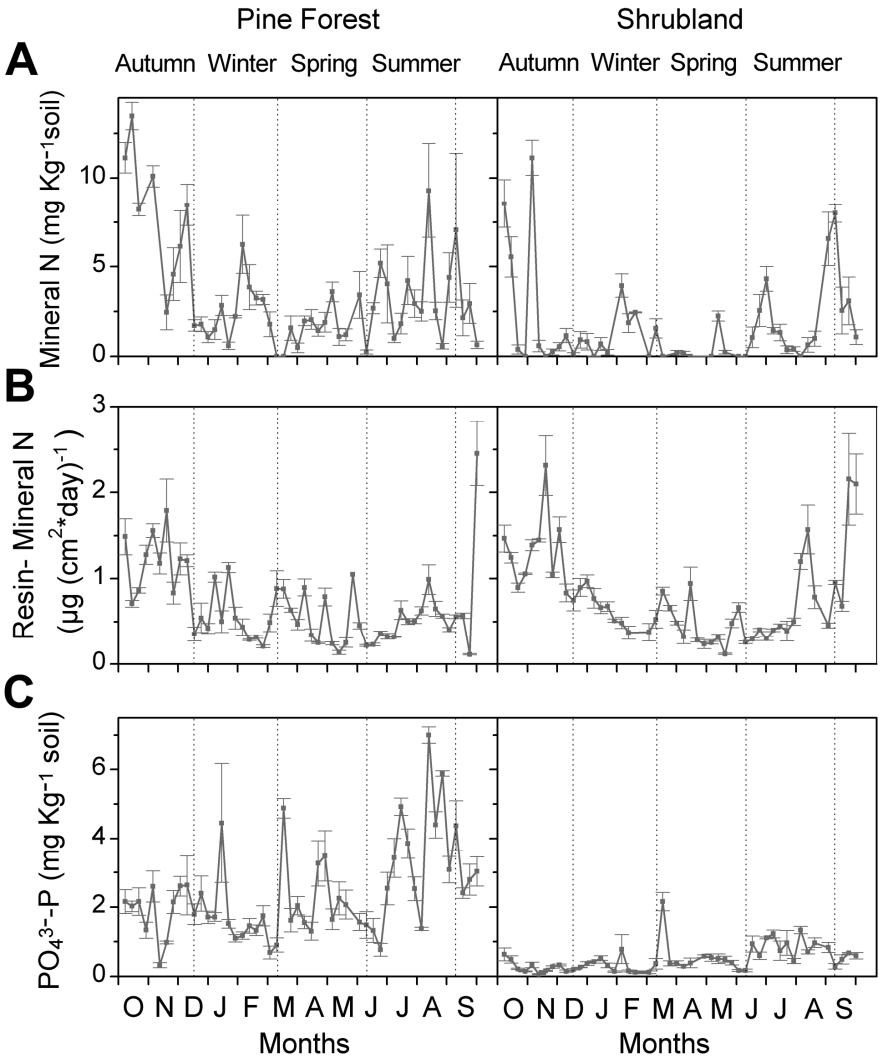

Figure 3. Mineral N (a), Resin Mineral N (b) and PO_4^{3-} -P (c) measured at weekly intervals throughout the study year in the pine forest and shrubland plant communities.

We detected significant differences in the resin mineral N values among seasons but not between sites (Table 1 and 2). The maximum and minimum resin mineral N contents at both study sites were found in autumn and spring (Figure 3b). We found a significant site by season interaction. Differences between seasons were found during a separate site analyses ($P_{Shrubland}<0.0001$, $P_{pine\ Forest}<0.0001$). The highest CV values for this variable were found in the summer at both study sites (Table 3).

Table 2. Permutation test performed on the linear mixed models evaluating the effect of Site and Season on soil respiration and soil nutrient variables during the whole year

	P site	P season	P site x season
Respiration	< 0.0001	< 0.0001	< 0.0001
Mineral N	< 0.0001	0.012	0.015
Resin-Mineral N	0.068	< 0.0001	0.005
PO_4^{3-}-P	< 0.0001	0.471	< 0.0001

The PO_4^{3-} content was significantly higher in the pine forest (ranging between 0.33 and 7.00 mg kg^{-1} soil, mean of 2.37 mg kg^{-1} soil) than the shrubland (values between 0.07 and 2.16 mg kg^{-1} soil, mean of 0.53 mg kg^{-1} soil, Table 1). We did not observe significant differences between seasons, with the maximum and minimum peaks in both study sites occurring in summer and autumn, respectively (Figure 3c). However, the significant interaction that we have found during the statistical analyses in each site indicates that the seasons influence the differences between sites (Table 2). Differences between seasons were found during a separate site analyses for both study sites ($P_{Shrubland}$=0.007, $P_{pine\ Forest}$=0.007). The highest CV values for this variable were found in winter at both study sites (Table 3).

Table 3. Coefficients of variation for the soil respiration and soil nutrients variables at the two study sites during the different seasons

Pine Forest						Shrubland				
Variables	Autumn	Winter	Spring	Summer	Annual	Autumn	Winter	Spring	Summer	Annual
Respiration	0.48	0.60	0.51	0.54	0.59	0.70	0.62	0.56	0.46	0.62
Mineral N	0.84	0.94	0.92	1.25	1.17	1.26	1.41	2.23	1.36	1.74
Resin-Mineral N	0.42	0.62	0.66	0.97	0.77	0.40	0.46	0.67	0.92	0.77
PO_4^{3-}-P	0.59	0.79	0.67	0.58	0.69	0.91	1.09	1.06	0.65	0.92

Changes during wetting-drying events

The soil respiration in the pine forest increased during wetting events and decreased during drying events, but we did not find any significant response to wetting and drying events in the shrubland soils (Table 4a, b and c, Figure 4). The inverse pattern of increases during the drying phases and decreases during the wetting

phases was found for mineral N and resin mineral N (Figure 5), although these differences only were significant for the last variable. In the shrubland soils, the resin mineral N differences between wetting and drying events were of a lower magnitude yet still significant (Figure 4, Table 4). The pattern noted above can be observed during separate analysis of the dry and wet soils (Table 4). However, some differences emerged for soil resin mineral N, where the variations between the wetting and drying events were only apparent for the wet soil at both sites. No trend was observed for the PO_4^{3-}-P at any site.

Table 4. Probabilities that the differences of mean soil respiration and soil variables between wetting and drying events were found by random. Data were also analyzed separately for the wet and dry soils. For simplicity, P > 0.1 is arbitrary showed as non-significant (NS)

	Pine Forest			Shrubland		
Variables	Whole year	Wet soil	Dry soil	Whole year	Wet soil	Dry soil
Respiration	0.001	0.063	0.006	NS	0.073	NS
Mineral N	0.080	NS	0.093	0.096	NS	NS
Resin-Mineral N	0.005	0.009	NS	0.017	0.062	NS
PO_4^{3-}-P	NS	NS	NS	NS	NS	NS

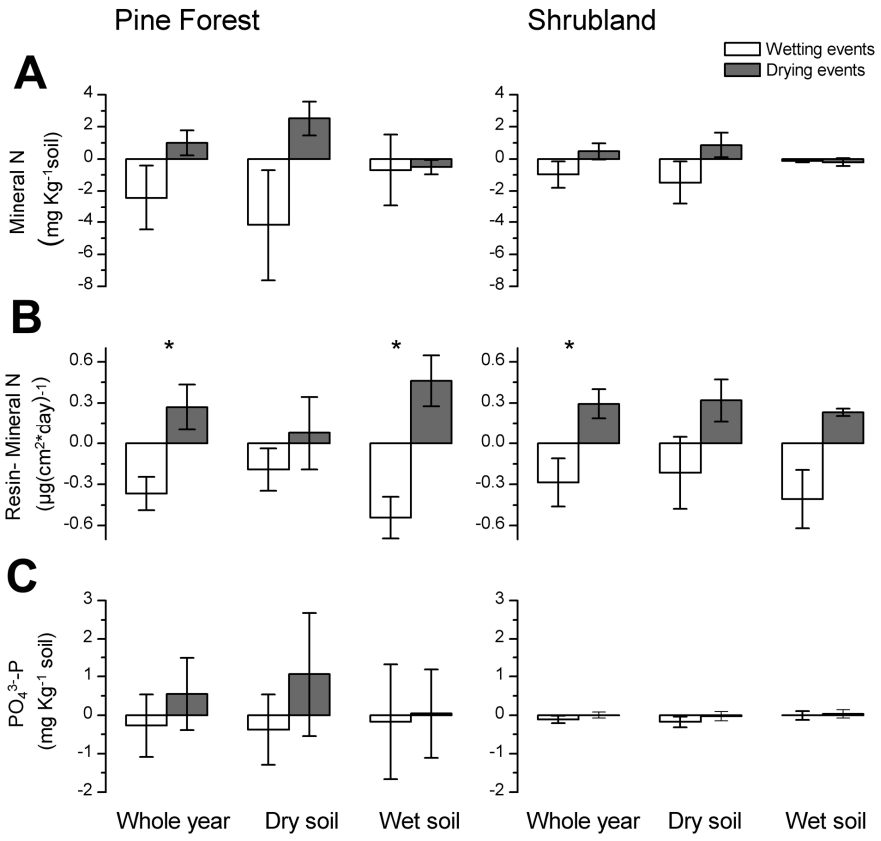

Figure 4. Increments of Mineral N (a), Resin Mineral N (b) and PO_4^{3-} -P (c) during eight wetting and drying events for each study site. Error bars are ±2SE. Asterisks indicate significant differences.

The changes in soil respiration during wetting and drying events did not show any significant correlations with the mean soil temperature, the mean soil water content, the length of the wetting and drying event and the length of the previous wetting or drying event before the next event (Table 5).

Table 5. Spearman correlation matrix (rho) between changes in soil respiration during wetting and drying events, and the mean soil temperature, mean soil water content (SWC), the length of the wetting and drying event (LE) and the length of the previous wetting or drying event before the next event (LPE). None of the correlations were statistically significant ($p < 0.05$). N=8.

	Pine forest		Shrubland	
	Wet event	Dry event	Wet event	Dry event
T	-0.0714	0.0476	-0.0857	-0.0857
SWC	-0.1905	0.0149	-0.3143	-0.3143
LE	0.1916	-0.6099	0.3928	0.3928
LPE	-0.5225	0.2955	-0.2060	-0.2060

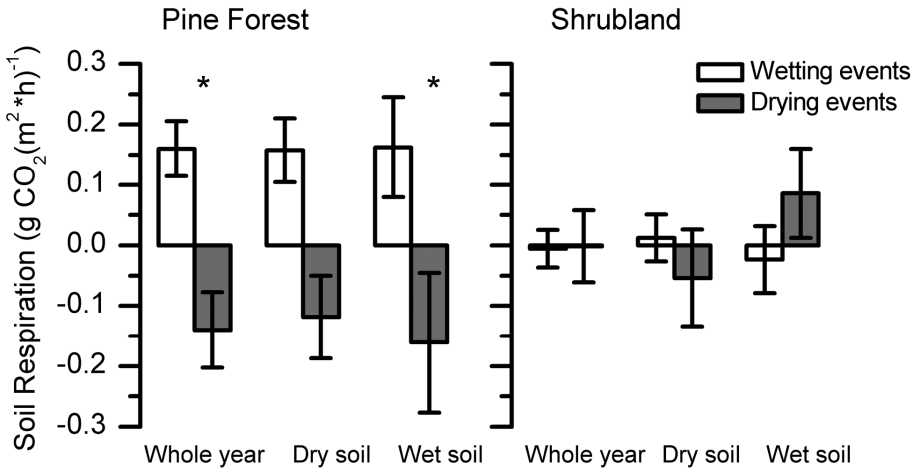

Figure 5. Increments of soil respiration rate during eight wetting and drying events in each study site. Error bars are ±2SE. Asterisks indicate significant differences.

Discussion

While we found a clear soil respiration response to wetting and drying pulses in the pine forest, this response did not exist in the shrubland. We cannot easily explain the reason for this difference. Although the two ecosystems present different soil organic matter and soil texture (Table 1), these differences might not be large enough to predict a completely different microbial response to wetting and drying events. We did not intend to compare both ecosystems, since they are unreplicated, indeed, we chose these two ecosystems to serve as site pseudoreplicates and expected to describe the same pattern of soil respiration with modulation of local soil conditions. But the lack of response to wetting and drying events in the grassland and the striking differences in the inorganic P between the two sites may shed light on the mechanisms contributing to this different site behavior. Recent studies have supported the idea that the response to a drying-wetting event is triggered by soil wetting but regulated by resource limitation (Jenerette and Chatterjee, 2012). The lack of a common pattern in response to drying and wetting events in both sites is even more striking since the variability of analyzed variables was similar in both ecosystems. The intra-seasonal variability at our study sites was as high as the inter-seasonal variability in all analyzed variables,

suggesting that the magnitude of changes is more dependent on short-term events than seasonal differences.

Soil respiration pulses are generally derived from the microbial consumption of soil organic matter, which is highly responsive to precipitation pulses (Austin et al., 2004). Several mechanisms may explain the soil respiration rate changes detected during wetting-drying cycles. The decomposable organic substrates are derived partially from the death of a portion of soil microorganisms and partially from the non-living soil organic matter (Jenkinson, 1966). The portion of the microbial biomass that dies under dry conditions (Bottner, 1985) is readily decomposed by the surviving organisms when the soil is rewetted. The rapid changes in soil water potential associated with rewetting may cause microbes to undergo osmotic shock, including microbial cell lysis (Van Gestel et al., 1993) or release of intracellular solutes (Halverson et al., 2000). The drying and rewetting process makes the non-living soil organic matter available for decomposition by the physical disruption of the soil structure, the substrate desorption from surfaces (Adu and Oades, 1978), and by the increased microbial mobility and diffusion of soluble organic compounds (Lund and Gokssyr, 1980). On the other hand, wetting events of a dry soil also produces a CO_2 burst in the short term, due to the physical displacement of air present in the soil pores.

However, none of these mechanisms help us to understand the lack of relationship between the soil respiration and wetting-drying events at the shrubland site. Its lower amount of soil organic matter may explain a lower response as outlined above, but this potential explanation fails to account for the absence of response. Other studies indicate no systematic difference in C mineralization after wetting-drying events (Borken and Matzner, 2009). Approximately 50% of these studies revealed an increase in soil respiration following wetting-drying events, whereas the other studies showed either no change or a decrease in soil respiration relative to a moist control soil (van Veen et al., 1985; Degens and Sparling, 1995; Fierer and Schimel, 2002, 2003). However, our study is not directly comparable with the above research because we did not compare soils under wetting-drying cycles with soils under constant moisture. Our novel approach aimed to identify differences between wetting and drying phases in natural conditions throughout one year.

Besides, it is important to consider the role of plant root respiration, which makes a large contribution to soil respiration (Schwinning et al., 2002). We should take into account that measured variable is an integrated measure of heterotrophic and

autotrophic respiration (Hanson et al., 2000). Autotrophic respiration could be masking any pattern related to heterotrophic respiration. In fact, soil respiration is relatively high in dry months of the year in the shrubland, and it could be due to higher autotrophic respiration in this ecosystem. It is interesting to note that in the shrubland respiration rates were much lower in autumn and winter compared to the pine forest, while in spring and summer respiration rates were higher in the shrubland. We attribute this fact to site differences in soil water availability, productivity and plant community phenology (Carbone et al., 2008). Plant (as well as microbial) uptake will influence mineral N content and the uptake will in turn depend on soil moisture. In the short-term, the activity of soil microbial biomass may be less sensitive to low soil water content than is water uptake by plants (Singh et al., 1989), and small rain events might be of insufficient magnitude to produce a plant response, nevertheless it may generate a rapid rise in activity of soil microbes (Schwinning and Sala, 2004). Thus, soil microorganism may become active in moments when plant roots are not, diminishing competition for mineral N and raising the immobilization in microbial biomass (Bolton et al., 1993).

Soil organic matter availability to microorganisms is related to its position within the soil matrix (Elliot and Coleman, 1986). Taking into account the higher clay plus silt content of the pine forest soil compared to that of the shrubland, we would expect a higher degree of soil aggregation, and therefore a higher proportion of the organic carbon chemically protected and thus unavailable to microorganisms decomposition. However, the higher labile organic C content (phenols, hexoses and aromatic compounds) found in the pine forest than in the shrubland suggested higher C availability in the pine forest site. On the other hand, we have found a declining linear response of CO_2 soil effluxes to increases in soil moisture in the shrubland community during winter, when WHC was above 80%. The high bulk density shown by the soil shrubland may cause anaerobic conditions, stopping of gas diffusion. Thus, differences between soil textures may help to explain this distinct behavior of soil respiration across ecosystems. Although the soil organic matter and soil texture may somehow affect the lack of a soil respiration response to wetting-drying cycles in the shrubland, the low PO_4^{3-} values found in this soil (Table 1) may limit the response of soil microbial activity to water pulses. The seasonality of soil PO_4^{3-} may offer additional clues. During the growing season, we observed an increase of the soil PO_4^{3-} coupled with a soil respiration rise, which suggests that these two variables are dependent.

Thus, the soil microbial activity may be limited by the shrubland PO_4^{3-} availability, which minimizes the triggered effect of water pulses. However, our descriptive experimental approach cannot demonstrate that the PO_4^{3-} availability governs soil respiration.

Other mechanisms may explain the strikingly different soil respiration responses to the wetting and drying events in both ecosystems. For example, the differing soil hydrophobicity is largely dependent on the amount and quality of the organic matter (Franco et al., 2000), and different microbial communities have different resilience to water stress (Landesman and Dighton, 2010). We explored the additional possibility that soil respiration relates to the mean soil temperature, mean soil water content, length of the wetting and drying event and length of the previous wetting or drying event before the next event, but we failed to find an effect in either ecosystem.

The effect of wetting and drying events on soil respiration seems to be ecosystem-specific, although we cannot clearly identify the ecosystem process underlying these differences. Our initial goal was to obtain a "mean response" to wetting and drying events in two ecosystems under the same climate and similar soil characteristics. Instead, we demonstrated that two ecosystems located in the same climatic regime and separated by a few kilometres can present both predictable and unpredictable responses to water pulses. More research is needed to understand the effect of wetting-drying cycles on soil respiration in a changing world where these episodes are predicted to intensify.

Acknowledgments

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Capítulo 3

Temporal pattern of microbial functional diversity in two Mediterranean ecosystems

Lourdes Morillas and Antonio Gallardo

Under Revision in The Scientific World Journal

Soil microbial communities drive crucial ecosystem processes, and the way they respond to changes in seasonal climatic conditions can be a critical factor for predicting shifts in ecosystem processes. We assessed the seasonal response of soil microbial substrate induced respiration (SIR) to different amino acids, carbohydrates and carboxylic acids by using MicroResp technique; following this method we have also calculated the Shannon Index diversity of microbial consumption of C sources. We also measured in situ soil hexoses, aromatic compounds and phenols as a proxy of soil C availability. Soils of two Mediterranean ecosystems located in southwest Spain: a pine forest and a shrubland community were sampled at weekly intervals for soil C variables and 10 times along all seasons for SIR measurements during one year. Both plant communities showed clear differences in carbon source preferences, with higher respiration rates in the pine forest than in the shrubland. Soil hexoses, aromatic compounds and phenols showed a clear seasonality in both ecosystems, with minimum during the wet period and maximum during the dry season. Our results showed that the soil labile C concentration was a poor predictor of microbial response to different C sources addition in our two sites. We have also found that the seasonal changes on soil microbial response to different C sources addition produce a different pattern in our two ecosystems, most likely due to the contrasting fertility. Lastly, we conclude that the microbial consumption of the studied C sources may be partially determined by their physico-chemical properties.

Keywords: Soil moisture; Hexoses; Aromatic compounds; Phenols; MicroResp; Carbon source consumption.

Introduction

Seasonal precipitation pattern is a key limiting factor regulating primary productivity, soil microbial activity, and soil biochemical dynamics in Mediterranean ecosystems (Bell et al., 2008). As decomposers, soil microbial communities mediate critical ecosystem processes and the activity of these microbial communities is primarily regulated by moisture availability (Fierer et al, 2003). The soils of Mediterranean ecosystems are particularly susceptible to seasonal dynamics due to the infrequency of rainfall events and the often warm and dry climate that favors rapid soil drying (Fierer and Schimel, 2002). Thus, soil water content may play a critical role for the soil biological responses in Mediterranean ecosystems (Jarvis et al., 2007). Soil moisture can also strongly affect carbon transformations (Fierer and Schimel, 2002) throughout soil aeration creating anaerobic and aerobic conditions that promote a diversity of microorganisms (Fierer et al., 2003) and nutrient cycling through microbial activity (Austin et al., 2004). Previous researches established that soil temperature and water content affect decomposition rates of soil organic carbon (Hogg et al., 1992; Howard and Howard, 1993; Fierer and Schimel, 2002), and could also affect the quantity as well as the chemical characteristics of dissolved organic carbon (Lundquist et al., 1999a; Chow et al., 2003; Blodau et al., 2004). Hexoses, phenols and aromatic compounds are part of soil carbon pool. Aromatic compounds are positively correlated to the amount of XAD-8 adsorbable dissolved organic C (DOC) (Dilling and Kaiser, 2002; Kalbitz et al., 2003), therefore we measured aromatic compounds as surrogate of DOC. Phenols mainly derive from decomposition of polyphenolic plant metabolites, such as tannins and lignine (Guggenberger and Kaiser, 2003). Soil hexoses are labile energy sources to soil microbes. Labile organic carbon is a precursor of microbial growth and activity, thus driving decomposition processes in the soil (Boyer and Groffman, 1996). Soil labile organic carbon have been proposed as an indicator of the carbon availability to soil microorganisms (DeLuca and Keeney, 1993), so hexoses, phenols and aromatic compounds contents in soil may be related to microbial response to different C sources addition.

Fluctuations in soil water content are expected to be even greater in the future due to more frequent extreme weather events resulting from climate change (Bernstein, 2007; IPCC, 2007). Soil biogeochemical responses to moisture pulses driven by dynamic precipitation patterns are especially complex and difficult to predict (Collins et al., 2008). Under these conditions, the role of seasons regulating ecosystem function may

become critical (Knapp et al., 2002), since seasonal changes may modify soil microbial community functionality that could alter rates of nutrient cycling and soil biological responses. More detailed descriptions of the microbial seasonal dynamics could improve predictions for how microbially-mediated processes will respond to global changes (Treseder et al., 2012).

Since soil microbes are key drivers of biogeochemical cycles, the way they respond to seasonal precipitation changes can be a critical factor for predicting shifts in ecosystem processes. Sudden changes in soil water content are stressful to microorganism because they must expend energy to regulate osmotic pressure (Bottner, 1985; Van Gestel et al., 1993). These physiological adaptations to soil water content require a great investment of resources (Schimel et al., 2007), thus, the capability of soil microbes to perform these processes may be limited by the soil nutritional status. For this reason, we expect that soil with distinct fertilities lead soil microbes to respond in different ways to changes in soil water content. One means of assessing the heterotrophic function of a soil microbial community is to measure the ability of the active microbial community to utilize a range of organic carbon sources. We have analyzed our samples using MicroResp method, which has been reported to have greater sensitivity in resolving soil differences compared to Biolog (Chapman et al., 2007) or the Degens and Harris method (Lalor et al., 2007). The carbon sources utilized have different characteristics that affect to microbial degradation due to their distinct molecular weight, solubility, oxidation state and heat of combustion. We hypothesized these physico-chemical properties of the different C source, being all other conditions equal, should determine the preferences of soil microorganism for the C sources.

We studied two Mediterranean ecosystems: a pine forest with a higher level of organic matter content and nutrients, and a shrubland with lower C and nutrient availability. Few studies have explored the soil hexoses, aromatic compounds and phenols levels throughout the year, even less is known about how the seasonal changes in these soil variables affect to microbial response to different C sources addition in Mediterranean ecosystems. On the other hand, the biochemical reasons of the different microbial consumption of different carbon sources have been scarcely analyzed. This represents a significant gap in understanding the role of these carbon substrates of great ecological importance on soil microbial consumption and diversity. An improved

knowledge of how seasonal changes affect these soil variables is of critical importance as these shifts could alter carbon substrate consumed by microbial biomass.

The objectives of this research were (i) to assess whether the seasonal changes on soil microbial response to different C sources addition produce a similar pattern in two contrasting ecosystems, (ii) to determine whether hexoses, phenols and aromatic compounds contents in soil are related to microbial response to different C sources addition throughout the year, (iii) to evaluate the importance of physico-chemical properties of the studied C sources for the microbial consumption.

Material and methods

Study area

This research was carried out in a pine forest and a shrubland community ecosystem located in southwest Spain (37° 21'N; 5° 56' W) with a Mediterranean-type climate. The Mediterranean climate has a 30-year average rainfall and temperature of 565.7 mm and 19.0°C, respectively. The study year was wetter than normal (852.6 mm in the pine forest and 845.7 mm in the shrubland). The soils in these areas show a typical A (B) C profile. Table 1 of chapter 1 presents the main properties of these soils. The pine forest is comprised of the species *Pinus pinea* L., with scarce annual herbs under their canopies. The shrubland is dominated by *Quercus coccifera* L., *Cistus albidus* L., *Genista hirsuta* Vahl. and *Arbutus unedo* L. The pine forest is seasonally subjected to fewer rapid changes in the soil water content than the shrubland soil due to a thicker litter layer and canopy shading.

Field sampling

We conducted soil sampling at weekly intervals for one year, from October 2009 to October 2010. Six soil samples from each study site were collected randomly from the top 10 cm of the soil profile with a circular soil corer (5 cm diameter × 10 cm height). The soil samples were transported in polyethylene bags to the laboratory, sieved (2 mm mesh) and separated into two fractions. One fraction was immediately frozen for microbial analysis, the other was stored at 3 °C in refrigerators and processed as soon as possible (less than three days in all cases) for biogeochemical

analyses. This procedure ensured that the soil microbial populations did not experience relevant changes (Gonzalez-Quiñones et al., 2009). Two sampling of each season were selected randomly for microbial analysis. On each sampling date, the soil respiration rates were determined as the surface CO_2 efflux utilizing a portable soil respiration system (EGM-4 PP SYSTEMS) with a chamber of 10 cm ∅ and 15.5 cm depth. The soil respiration and soil temperature were measured at approximately 10:00 am on each sampling day. The daily precipitation values were obtained from the AEMET weather station network in close proximity to the study sites.

Laboratory analysis

The gravimetric soil moisture was calculated in fresh 5 g subsamples after drying in an 80 ^{o}C oven for 48 h. The water holding capacity (WHC, %) was determined for each soil type as the gravimetric water content of soil that was saturated and allowed to drain freely over 48 h in a filter funnel. The soil texture was estimated with the hydrometer method suggested by Kroetsch and Wang (2008). The soil conductivity and pH were measured in 1:2.5 and 1:5 soil-water solutions, respectively. The soil organic matter was analyzed via the wet oxidation techniques utilized by Skjemstad and Baldock (2006). The carbonate levels were analyzed according to Boon Goh and Mermut (2007). The soil PO_4^{3-}-P content was extracted with 100 ml of 0.5 M $NaHCO_3$ at a ratio of 1:20, and the concentration in the extract was determined with the molybdenum blue colorimetric method (Allen et al., 1986). The total soil N was measured utilizing standard Kjeldahl procedures (Rutherford et al., 2007). The NH_4^+-N concentration was estimated directly via the indophenol blue method using a microplate reader (Sims et al., 1995). The NO_3^--N was first reduced to NH_4^+-N with Devarda alloy, and the concentration was determined as the difference between the Devarda-incubated and unincubated samples. The DON in the extracts was first oxidized to NO_3^--N with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 ^{o}C for 55 min and then reduced to NH_4^+-N with Devarda alloy (Sollins et al., 1999). The DON contents were calculated as total dissolved N minus inorganic N. MB-N was determined using the fumigation-extraction method proposed by Brookes et al., (1985). The total N content in the digested extracts was determined by colorimetry (indophenol blue method) with a microplate reader (Sims et al., 1995). The MB-N concentration was estimated as the difference between the total N in fumigated and

unfumigated digested extracts divided by a Kn (fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 (Brookes et al., 1985).

The availability of NH_4^+-N and NO_3^--N was measured in situ utilizing ion-exchange membranes (resins, Subler et al., 1995). Six anion and cation resins (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) were installed per site and week over the one year sampling period. The resins were incubated in the field for seven days during each sampling period. Following collection, the resins were extracted with 25 ml of 2 M KCl via orbital spinning. The extracts were analyzed to measure the NH_4^+-N and NO_3^--N utilizing the above method. All these variables were analysed for the general description of the two study sites.

The following analyses were done for monitoring purposes. To measure hexoses, aromatic compounds and phenols, the soil subsamples were extracted with 0.5 *M* K_2SO_4 at a ratio of 1:5, followed by shaking for 1 h at 200 rpm at 20^{o}C. The extract was filtered through a 0.45 µm Millipore filter. All concentration were estimated following (Chantigny and Angers, 2008) using a microplate reader.

We analyzed soil heterotrophic microbial communities with the MicroResp system (Campbell et al., 2003). This method tests 15 carbon sources that vary in structural complexity (Oren and Steinberger, 2008). Carbon sources were selected depending on their ecological importance to soil and their solubility in water. In particular rhizospheric C substrates were chosen taking into account the relevance of root inputs for microbial metabolism. We utilized amino acids (L-alanine, L-lysine, arginine, L-cysteine and N-acetyl-glucosamine [NAGA]), carbohydrates (D-fructose, D-galactose, D-glucose, L-arabinose and D-trehalose) and carboxylic acids (citric acid, L-malic acid, oxalic acid, oxoglutaric acid and amino butyric acid [GABA]). In functional terms, the substrate utilization rates of the carbon sources correspond to the catabolic attributes of different soil microbial functional groups (Zak et al., 1994). Even if we cannot evaluate microbial communities in relation to taxonomic or phylogenetic diversity (Øvreås, 2000), we still can use MicroResp results to explain functional diversity shifts. Before performing the MicroResp method, defrosted soils were introduced into the flasks and pre-incubated for five days at 25 ^{o}C. The moisture within the flaks was corrected to 40% WHC in order to condition the soils and reestablish active microbial populations. Although potential changes in microbial communities may have occurred due to freeze-thaw cycles, samples are still comparable because all the soils had the same treatments. To avoid changes in soil moisture content during

incubation, flasks were covered with plastic wrap. Each C source was dissolved in deionised water and added to soils to deliver 30 mg C g soil water^{-1}. Approximately 0.4 g of soil was placed in the 96 deep-well plates volumetrically. To estimate the evolved CO_2, a colorimetric method relying on the change in the pH of a gel-based solution of bicarbonate was used. After that, the plates were incubated for 6 h and read at 570 nm. The results were calculated on the basis of water, which represents the basal respiration.

Statistical analysis

To assess the effect of sampling date and study site on soil C variables, and the effect of sampling date, study site, incubation time and carbon source types on the microbial respiration to different C sources (microbial functional diversity) we used the semi-parametric PERMANOVA approach (Anderson, 2001).We chose this approach because our data did not follow ANOVA assumptions (normality and homogeneity of variances). This analysis does not make distributional assumptions and is compatible with any distance measure. Sampling date, study site, incubation time and carbon source types were treated as fixed factors. When significant interactions between two fixed factors were found, we conducted separate PERMANOVA analyses for the different factors. PERMANOVA analyses were carried out using 9999 permutations (permutation of raw data) in each analysis. Principal component analysis (PCA) was conducted using the package Vegan of R 3.0 (R Core Team, 2013).

Results

Water and temperature seasonality

Soil water availability showed a strong seasonality in both ecosystems, with a central period of highest values from early winter to mid-spring and lowest values in summer (Figure 1a of chapter 1). Temperature increased from winter minimum to summer maximum, with intermediate values for spring and autumn (Figure 1b of chapter 1).

Seasonal changes in soil sources of labile carbon

Soil hexoses, aromatic compounds and phenols showed a clear seasonality in both ecosystems, with minimum during the wet period and maximum during the dry season (Figure 2a, 2b and 2c). We found significantly higher values in the pine forest compared to the shrubland for all these variables (Table 2). We also detected significant differences among sampling dates and significant interactions between site and sampling date. Differences among the sampling date were found during a separate site analyses for soil hexoses, aromatic compounds and phenols (Table 2).

Table 2. PERMANOVA analyses evaluating the effect of the study sites and sampling dates on the soil concentration of hexoses, aromatic compounds (AC) and phenols. Individual analysis for each study site is also shown.

		Hexoses		AC		Phenols	
	df	Pseudo-F	P	Pseudo-F	P	Pseudo-F	P
Site	1	264.5	<0.0001	189.7	<0.0001	14.7	<0.0001
Sampling date	3	15.9	<0.0001	14.6	<0.0001	22.8	<0.0001
Site x Date	3	12.1	<0.0001	12.5	<0.0001	15.5	<0.0001
Pine forest	3	14.9	<0.0001	16.1	<0.0001	22.8	<0.0001
Shrubland	3	3.4	0.026	3.7	0.021	4.2	0.008

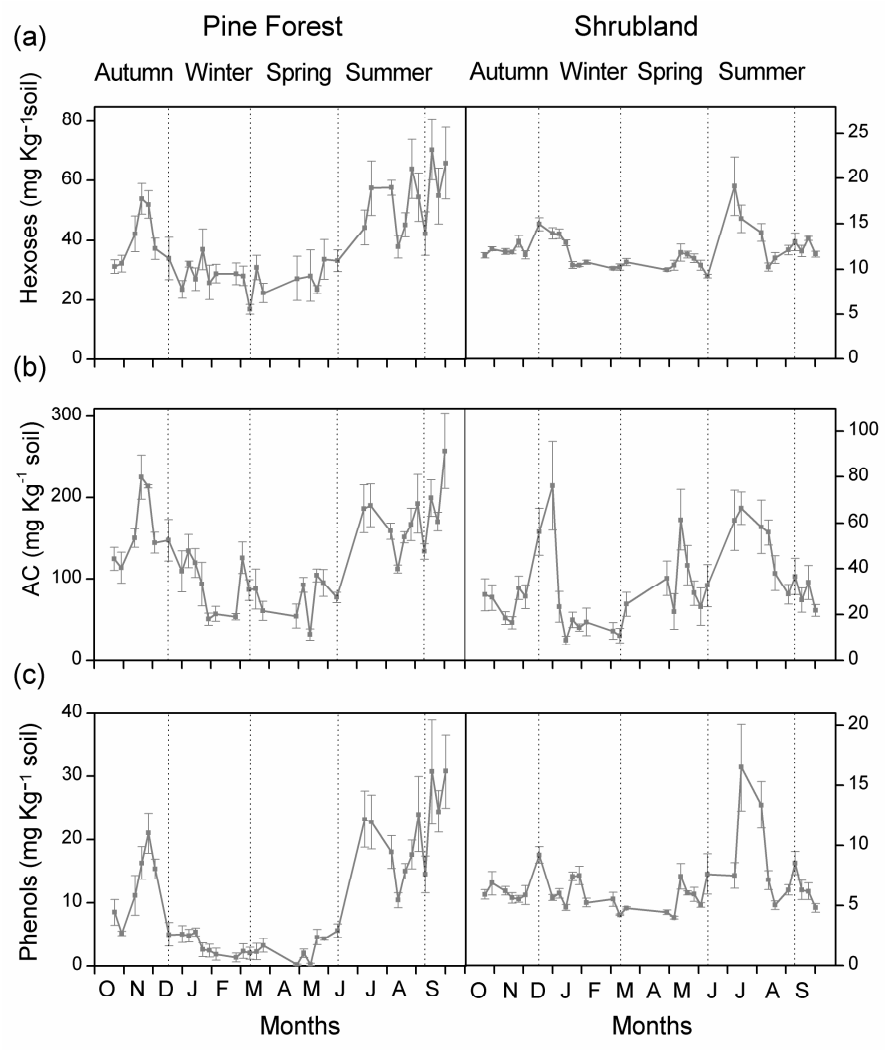

Figure 2. Hexoses (a), aromatic compounds (AC, b) and phenols (c) concentrations in the top 10 cm of soil profile measured at weekly intervals along the study period at the pine forest and shrubland plant community.

Seasonal changes in carbon microbial consumption

In the pine forest, microbial consumption of amino acids and carbohydrates showed maximum values in summer, with the lowest values in autumn and winter (Figure 3a and 3b). The increase in microbial responses to carboxylic acids from winter to summer was less pronounced than amino acids and carbohydrates, with a 6-h response peak in autumn (Figure 3c). In the shrubland, a different pattern emerged, with maximum in autumn for amino acids, carbohydrates and carboxylic acids and no clear trend afterwards. In both ecosystems the 6-h and 24-h responses to carbon sources showed parallel lines, with significantly lower rates and less marked seasonal differences for the 24-h responses for all the carbon sources types (Figure 3). We also

found significant differences among sampling dates for all carbon sources types and sites (Table 3). Interactions between incubation time and sampling dates were found for all these variables, excepting amino acids and carboxylic acids consumption in the pine forest and shrubland respectively (Table 3). Differences among sampling dates for all carbon sources types were found during a separate analysis for each incubation time (Table 3). We also found a greater SIR response to amino acids, carbohydrates and carboxylic acids in the pine forest than in the shrubland site (df=1, Pseudo-F=29.8, 89.1 and 69.4, respectively, P<0.0001 for all the cases).

Table 3. PERMANOVA analyses evaluating the effect of incubation times and sampling dates on the mean SIR response to three types of low molecular weight carbon sources (amino acids, carbohydrates and carboxylic acids). Separate analyses for the two incubation times are also showed.

	Pine forest			Shrubland	
	df	Pseudo-F	P	Pseudo-F	P
Amino acids					
Incubation time	1	85.2	<0.0001	65.9	<0.0001
Sampling date	9	37.7	<0.0001	14.3	<0.0001
Incubation time x Date	9	1.8	0.058	5.5	<0.0001
6-h response	9	14.2	<0.0001	11.5	<0.0001
24-h response	9	53.1	<0.0001	6.3	<0.0001
Carbohydrates					
Incubation time	1	489.7	<0.0001	540.1	<0.0001
Sampling date	9	58.4	<0.0001	24.4	<0.0001
Incubation time x Date	9	5.7	<0.0001	8.1	<0.0001
6-h response	9	28.1	<0.0001	19.1	<0.0001
24-h response	9	53.7	<0.0001	28.4	<0.0001
Carboxylic acids					
Incubation time	1	781.9	<0.0001	339.5	<0.0001
Sampling date	9	18.2	<0.0001	4.1	<0.0001
Incubation time x Date	9	3.1	0.002	1.5	0.152
6-h response	9	9.2	<0.0001	2.5	0.007
24-h response	9	27.6	<0.0001	6.4	<0.0001

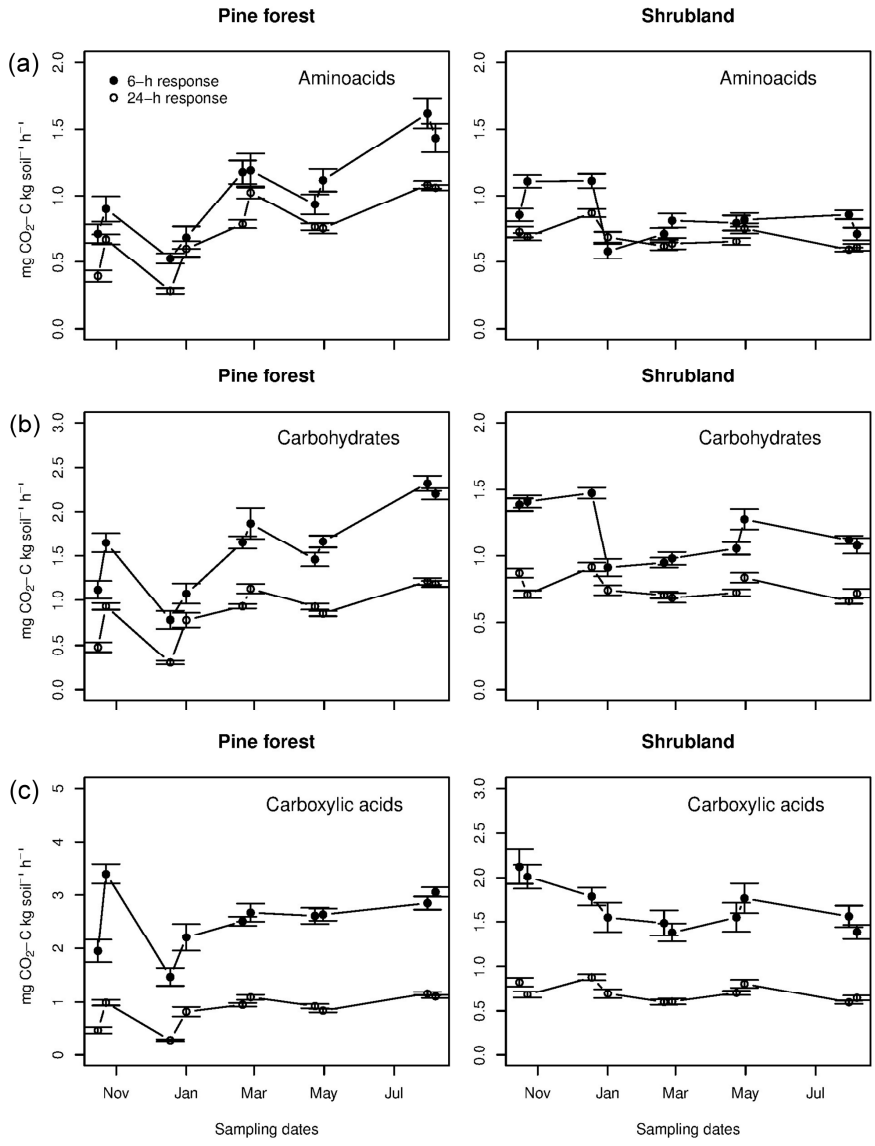

Figure 3. SIR due to amino acid (a), carbohydrates (b) and carboxylic acid (c) microbial consumption at 6 and 24 hours response at the pine forest and shrubland plant community soils through the different sampling dates.

Carbon source preferences

Amino acids were the most reduced C sources, showing the highest heat of combustion per gram of substance or per gram of C, while carboxylic acids were the most oxidized compounds (Table 4). Carboxylic acids were the carbon sources that showed the highest 6-h response as compared with carbohydrates and amino acids, the latest showing the minimum values in both ecosystems (Figure 3a, 3b and 3c). Both plant communities were clearly different, with higher respiration rates and C sources

preferences in the pine forest than in the shrubland (Figure 3, Table 3), being the responses to C sources much more plastic in the pine forest than in the shrubland (Figure 4).

Table 4. Physico-chemical properties of the C sources added to the soils. Heat of combustion data were taken from Kienzle et al., (2002) and Domalsky (1972).

C source	Formula	MW	C:O	Heat of combustion (Hc; kcal mol-1)	Hc:MW	Hc:C	Solubility (25ºC) (g/L)
Amino acids							
Alanine	$C_3H_7NO_2$	89.09	1.5	-386.7	-4.3	-128.9	166.5
Arginine	$C_6H_{14}N_4O_2$	174.2	3	-893.9	-5.1	-149.0	148.7
Aminobutyric acid	$C_4H_9NO_2$	103.12	2	-545.5	-5.3	-136.4	1300
Cysteine	$C_3H_7NO_2S$	121.16	1.5	-540.5	-4.5	-180.2	280
Lisine	$C_6H_{14}N_2O_2$	146.19	3	-880.3	-6.0	-146.7	1500
Carboxylic acids							
Citric acid	$C_6H_8O_7$	192.12	0.86	-468.6	-2.4	-78.1	730
Malic acid	$C_4H_6O_5$	134.09	0.80	-317.4	-2.4	-79.3	558
Oxalic acid	$C_2H_2O_4$	90.03	0.50	-58.7	-0.7	-29.4	143
Oxoglutaric acid	$C_5H_6O_5$	146.11	1.00	-429.9	-2.9	-86.0	100
Carbohydrates							
Arabinose	$C_5H_{10}O_5$	150.13	1.00	-559.0	-3.7	-111.8	1000
N-Acetilglucosamine	$C_8H_{15}NO_6$	221.21	1.33	-973.3	-4.4	-121.7	50
Fructose	$C_6H_{12}O_6$	180.16	1.00	-672.0	-3.7	-112.0	3750
Galactose	$C_6H_{12}O_6$	180.16	1.00	-670.1	-3.7	-111.7	683
Glucose	$C_6H_{12}O_6$	180.16	1.00	-671.4	-3.7	-111.9	910
Tetrahalose	$C_{12}H_{26}O_{13}$	342.30	0.92	-1348.8	-3.9	-112.4	689

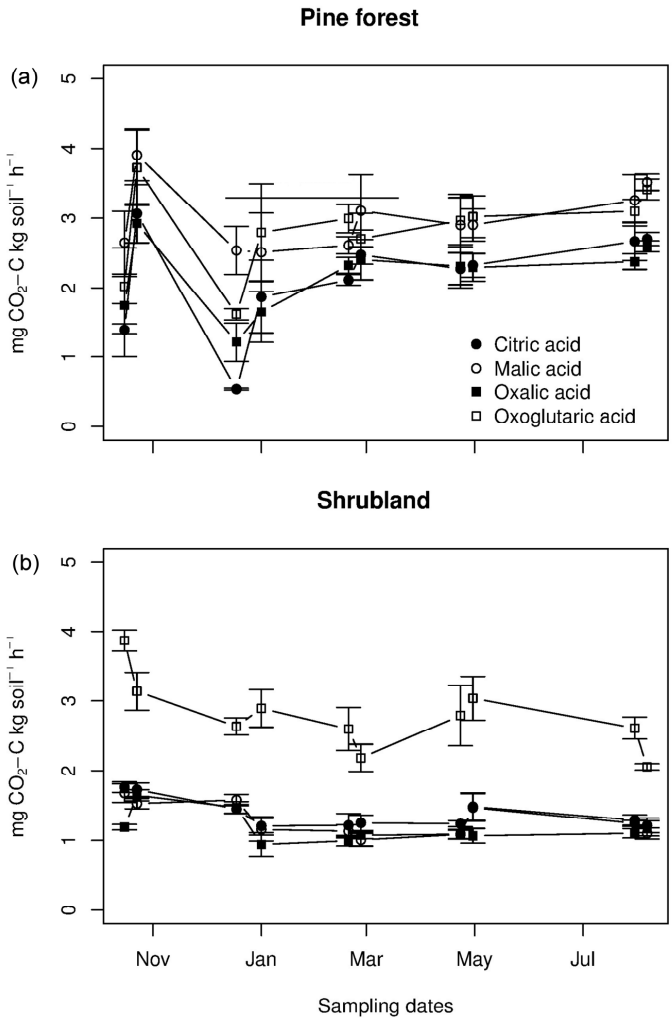

Figure 4. Principal-component analyses of carbon microbial consumption data of both studied soils.

We found differences among sampling dates and amino acids, carbohydrates and carboxylic acids types in both ecosystems (Table 5). In the pine forest, malic acid and oxoglutaric acid showed the highest respiration rates, while citric acid and oxalic acid showed the minimum (Figure 5a). However in the shrubland, oxoglutaric acid was the highest consumed C source, with lowest values for the other three carboxylic acids (Figure 5b). Glucose and fructose were the most consumed labile carbohydrates in the pine forest (Figure 6a), but no differences were found in the shrubland (Figure 6b). Among the amino acids C sources, the arginine was the less consumed in both ecosystems, with no significant differences between the other amino acids (Figure 7a and 7b).

Table 5. (a) PERMANOVA analyses evaluating the effect of sampling dates on the mean SIR response to the different amino acids, carbohydrates and carboxylic acids. (b) PERMANOVA analyses evaluating the effect of incubation times and sampling dates on the Shannon index of diversity of SIR response to the different low molecular weight carbon sources. Separate analyses for the two incubation times are also shown.

a

		Pine forest		Shrubland	
	df	Pseudo-F	P	Pseudo-F	P
Aminoacids					
Sampling date	9	39.7	<0.0001	12.8	<0.0001
Amino Acid type	4	36.8	<0.0001	11.7	<0.0001
Date x Amino Acid type	36	0.6	0.968	0.6	0.969
Carbohydrates					
Sampling date	9	34.6	<0.0001	12.9	<0.0001
Carbohydrates type	5	12.7	<0.0001	3.2	0.012
Date x Carbohydrates type	45	0.3	0.999	0.5	0.998
Carboxylic acids					
Date	9	6.6	<0.0001	2.9	0.005
Carboxylic acids type	3	6.2	<0.0001	38.7	<0.0001
Date x Carboxylic acids type	27	0.2	0.999	0.5	0.986

b

		Pine forest		Shrubland	
	df	Pseudo-F	P	Pseudo-F	P
Shannon Index					
Incubation time	1	335.7	<0.0001	111.4	0.002
Sampling date	9	12.6	<0.0001	3.3	<0.0001
Incubation time x Date	9	7.9	<0.0001	2.6	0.006
6-h response	9	11.4	<0.0001	2.9	0.007
24-h response	9	2.3	0.033	1.9	0.074

Microbial functional diversity

Microbial functional diversity tended to increase from minimum winter values to the highest summer values in both ecosystems for the 6-h responses (Figure 8a and 8b). However, whereas in the pine forest the maximum values were found in summer, in the shrubland soil, these maximum were found in the autumn. We found significant differences among sampling dates and incubation times for both ecosystems. No significant differences were found in a separate analysis between sampling dates of the 24-h responses to C sources in the shrubland ecosystem (Table 5).

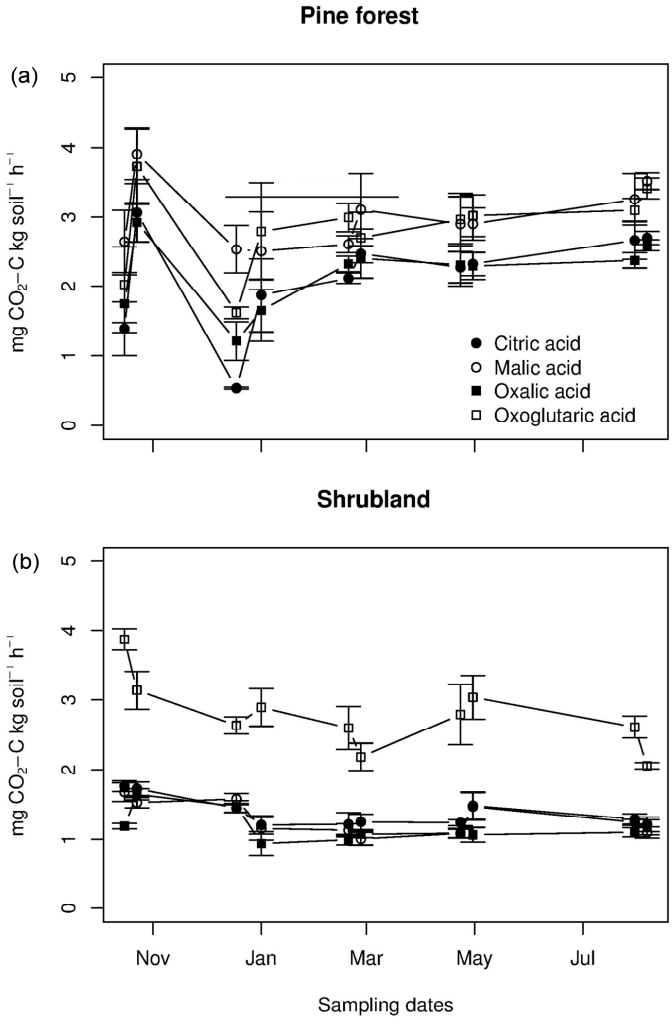

Figure 5. SIR due to microbial consumption of different carboxylic acids at the pine forest (a) and shrubland plant community (b) soils through the different sampling dates.

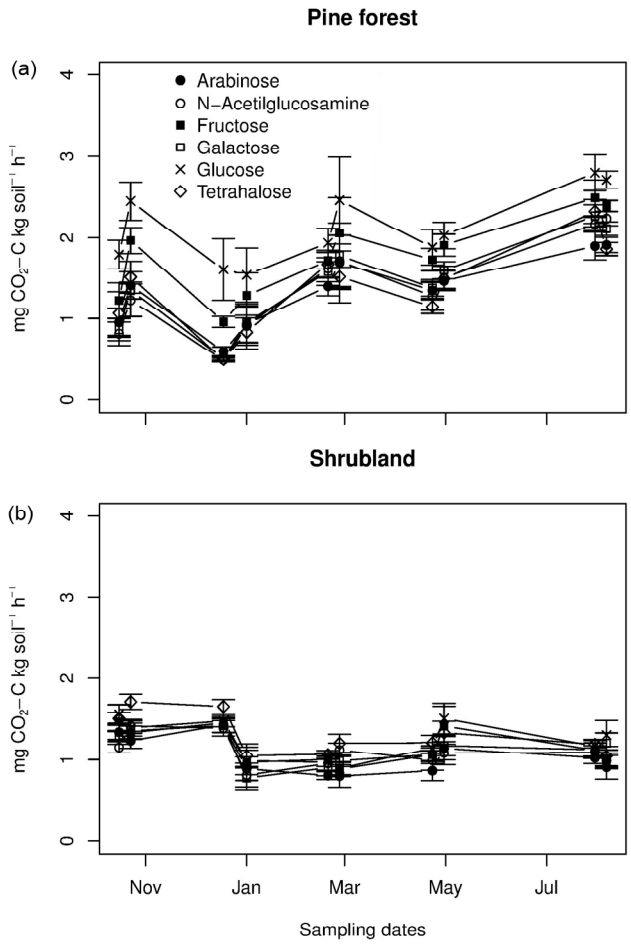

Figure 6. SIR due to microbial consumption of different carbohydrates at the pine forest (a) and shrubland plant community (b) soils through the different sampling dates.

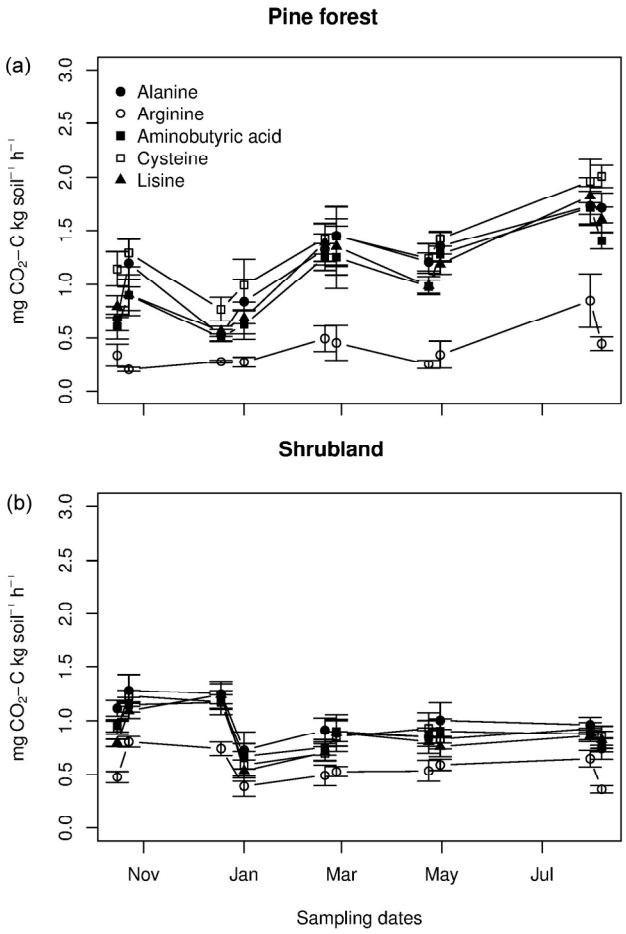

Figure 7. SIR due to microbial consumption of different amino acids at the pine forest (a) and shrubland plant community (b) soils through different sampling dates.

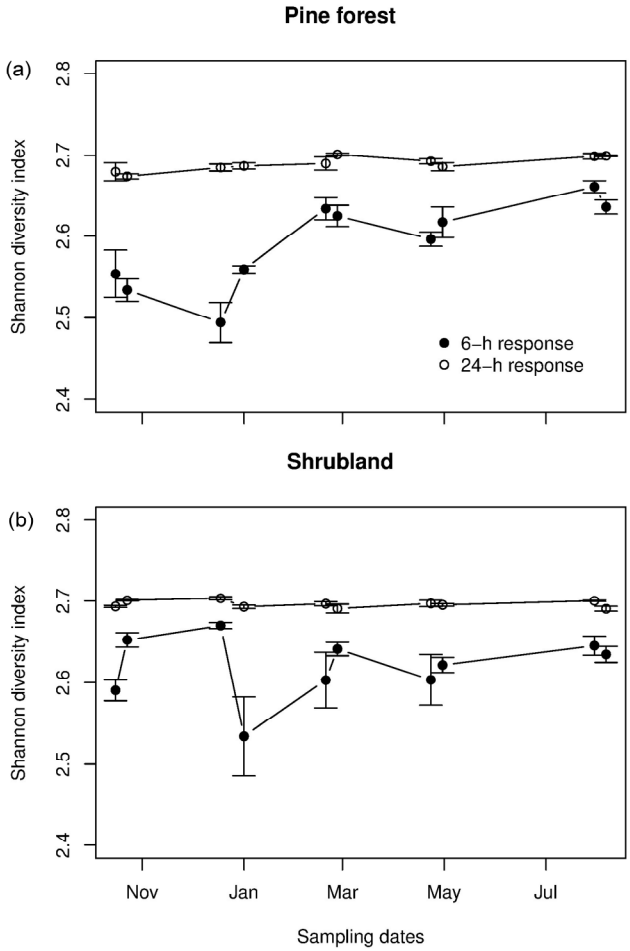

Figure 8. Shannon diversity index at 6 and 24 hours response at the pine forest (a) and shrubland plant community (b) soils through the different sampling dates.

Discussion

Soil labile C concentration is the result of the balance between inputs such as litter and root decomposition or root exudates and outputs such as microbial consumption and soil leaching (Cheshire, 1979, Strobel, 2001). Thus, the seasonal pattern of soil labile C that we can see in the pine forest is likely reflecting the fast consumption by soil microbes and/or rapid leaching from soil during winter, and the higher litter inputs mediated by plants in spring and summer. The less clear pattern in the shrubland might be due to the lower primary production and labile C concentration in this soil, however soil solution concentration is also buffered by sorption to the solid phase, which is soil specific and may help to explain the differences found between the two ecosystems (Jones et al. 2003). Because it has been proposed that the capacity of a microbial community to mineralize specific organic C

substrates likely reflects differences in the type, abundance and bioavailability of C substrates present in the in situ organic matter pool (Hamer and Marschner, 2005; Orwin et al., 2006; Banning et al., 2012), we expected that this seasonal pattern observed in the soil labile C was also reflected in changes in the respiration rates induced by the addition of labile C sources. However, although some common patterns were observed, such as the summer maximum in the pine forest, and the winter and spring minimum in the shrubland, we can conclude that the soil labile C concentration is a poor predictor of microbial response to different C sources addition in our two sites.

The Microresp method, such as another SIR method, is based in the rapid response of the soil microbial community to different C sources, with the essential premise that no changes in the original microbial community are induced by the method (Pennaten et al., 2004; Lagomarsino et al. 2007). Short incubations are therefore preferred, and although we found the same temporal pattern in the 6-h and 24-h incubations, differences between sampling dates and between the different C sources tended to decreases in the 24-h incubation, suggesting a late consume of non-preferred C sources, and explaining the observed increase in microbial functional diversity in the 24-h incubations for all sampling dates and sites.

A higher soil respiration of soil microbial communities in response to carboxylic acids addition than to carbohydrates or amino acids have been found for several authors (Bell et al., 2009, García-Palacios et al., 2011, Banning et al., 2012), but the reasoning of this differential response has not been clearly elucidated. Indeed, carboxylic acids are more oxidized compounds, yielding lower heat of combustion than amino acids or carbohydrates. However van Hees et al. (2005) calculated that 60-90% of organic acid substrate C evolved as CO_2 in the short term, as opposed to carbohydrates (20-60%) and amino acids (10-30%). We propose that carboxylic acids are preferred C sources over carbohydrates and amino acids because these simple organic acids are not directly involved in synthesis of more complex compounds inside the cell. Thus, the low response of SIR to amino acids found in this study and others (García-Palacios et al., 2011) does not mean a lower consumption by soil microbes, but only that amino acids or carbohydrates are not preferentially used as a primary source of energy as compared with carboxylic acids. Accordingly, van Hees et al. (2005) explained the highest respiration percentages for labile organic acids based on the large ATP requirement for uptake into cells and synthesis of metabolic monomers from these

compounds (Stouthamer, 1976). However, if enough time is allowed, such as in a 24-h incubation period, preferences for C sources disappeared, suggesting that new components of the microbial community may emerge specialized in the most abundant substrate. The relative low preference of amino acids as a carbon source for metabolism may also be enhanced in poor nutrient sites. Our two ecosystems showed low soil nitrogen availability which may explain that amino acids could be preferentially used for protein synthesis.

Differences within use of carboxylic acids C sources are even more difficult to explain. While in the pine forest microbes response was highest for malic acid and oxoglutaric acid, in the shrubland the highest response was for the oxoglutaric acid. A preference for this compound is expected based in the lowest oxidation state and highest heat of combustion as compared with the other carboxylic acids, but no physico-chemical properties can explain the differences between carboxylic acids found in the pine forest. Different responses to carboxylic acids have been explained based on their availability in the soil profile. One controlling factor may be the extent of exposure of microorganisms to specific organic acids in soils (Banning et al. 2012). Different carboxylic acids have been found to have different depth distribution patterns in forest soil horizons, which have been suggested to relate to organic matter content, pH and complexation with metal cations (Tani and Highashi, 1999). Differences in microbial community structure have also been proposed to influence the availability of specific carboxylic acid. For example, van Hees et al. (2002) found higher soil capacity to mineralize the organic acids in the presence of ectomycorrhizal fungi.

Within amino acids the only clear pattern was that arginine was the less consumed, which was observed for both ecosystems. Arginine has the highest molecular weight and lowest solubility of all other amino acids, which may difficult the movement through cell membranes. Besides, arginine differs from the other amino acids in that it has two amine groups and four N atoms in its structure. Before entering in the Krebs cycle to be catabolized, amino acids have to be deaminized, which is an energy-consuming process and other possible reason why arginine is negatively discriminated as a C source. Less clear differences emerged among the carbohydrates C sources in both ecosystems. Only in the pine forest glucose and fructose were more respired that the other C sources. N-Acetilglucosamine should be preferred by soil microorganism based on its lowest oxidation state and highest heat of combustion, however its solubility is very low as compared with the other carbohydrates. N-

Acetilglucosamine is part of a biopolymer in the bacterial cell wall and the main component of the cell walls of fungi, and part of the N-Acetilglucosamine can be directed to anabolism more than to catabolism.

Based on the C source consumption, the soil samples in the pine forest showed more diversity of responses than the shrubland. The difference may be based on the lower fertility of the shrubland soils compared with the pine forest soil, which precludes soil microbes in the shrubland site to rapid response to C additions. There are also differences in terms of microbial functional diversity as measured by the Shannon index between sites, since in the shrubland site the maximum diversity was reached in the autumn samples, while in the pine forest the maximum was reached in the summer samples, although microbial functional diversity increased in both sites from winter to summer. These increases were due to the relatively higher consumption of amino acids in summer, which may be related to the known mechanism of solutes incorporation in microbial cells (particularly dissolved organic nitrogen substances) to overcome water stress in summer (Csonka, 1989).

In conclusion, our results showed that the soil labile C concentration is not a reliable predictor of microbial response to different C sources addition in our two study sites. We have also found that the seasonal changes on soil microbial response to different C sources addition produce a different pattern in our two ecosystems, most likely due to the contrasting fertility. Finally, we suggest that the common pattern showed in microbial consumption of the studied C sources may be partially determined by their physico-chemical properties.

Acknowledgments

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Capítulo 4

Biological soil crusts and wetting events: Effects on soil N and C cycles

Lourdes Morillas and Antonio Gallardo

Biological soil crusts (BSC) communities control many functional processes in arid and semiarid ecosystems, where biological activity is closely influenced by soil wetting. Our goal was to analyze how the length of wetting events and the presence of BSC determine soil variables related to C and N cycling in a semiarid ecosystem. We applied three watering treatments (1, 6 and 10 days) on soils from two microsites (BSC and bare ground) in a microcosm experiment. We analyzed N in microbial biomass [MB-N], dissolved organic nitrogen [DON], NH_4^+-N, NO_3^--N, Resin-NH_4^+-N and -NO_3^--N, phenols and carbohydrates. Our results showed that longer lasting wetting events kept higher mineral and organic N as well as labile organic C in soils under BSC, which suggest that longer wetting events may be related to an enhancement in the decomposition rate that compensate for nutrient losses associated to short lasting wetting events. This trend is much less obvious in bare ground than under BSC. Our data suggest that changes in the length of wetting events and the presence of BSC with climate changes could alter future soil community structure and function.

Keywords: Drylands; DON; Microbial Biomass -N; Mineralization rate, Nitrogen cycle, Carbon cycle.

Introduction

Arid and semiarid ecosystems (also known as drylands) cover approximately 40% of Earth's terrestrial surface (Reynolds et al. 2007). Soils in dryland areas are commonly covered with biological soil crust (BSC), which is variably composed of cyanobacteria, bacteria, mosses, lichens, algae, and fungi. BSC are globally widespread and constitute a key biotic component of these areas (Belnap and Lange 2003), since they can completely cover plant interspaces in undisturbed areas and represent up to 70% of the living cover in these sparsely-vegetated areas (Belnap 1995). These crusts exert a strong effect on key ecosystem processes including water retention and infiltration (Eldridge et al. 2010), soil respiration (Maestre and Cortina 2003), nitrogen and carbon fixation and transformations (Belnap 2002; Evans and Lange 2003; Castillo-Monroy et al. 2010).

Since BSC are such an important part of soil drylands, there has been concern related to the effect of projected climate change on their functioning (Belnap et al. 2008; Grote et al. 2010; Maestre et al. 2013) and the ecosystem processes that are affected by them (Maestre et al. 2010). As their activity is directly tied to periods following rain events, any change in the timing and duration of precipitation will likely affect their physiological functioning (e.g., C and N fixation) and their response to stress. Future climate scenarios predict important changes in the temperature and rainfall dynamics of drylands, including larger and less frequent rain events (Knapp et al. 2008) and more extreme events (Easterling et al. 2000; Solomon et al. 2007). Nevertheless, how much precipitation will change is still a matter of debate (Weltzin and McPherson 2003). These climatic changes could have important implications, because previous research on vascular plants suggested that shifts in timing and size of individual precipitation events might be as or more relevant for ecosystem function than shifts in the absolute amount of annual precipitation (Cable and Huxman 2004; Schwinning et al. 2004). All this suggests that soil nutrients may be affected not only by the amount of rainfall, but by the length of precipitation event. Nevertheless, there have been few studies on the effects of the length of wetting event on soil nutrients, and no studies to our knowledge, on the combined effects of the length of wetting event and biological soil crusts on soil nutrients.

Despite some recent studies have proved that carbon mineralization rates in deserts are highly affected by photodegradation (Austin, 2011), water availability plays a central part in affecting many biotic processes in aridland ecosystems (Noy-Meir,

1973). In arid and semi-arid regions, biological activity is closely related to the frequency, size, and timing of rainfall events (Noy-Meir 1973), since low soil water potentials can limit microbial contact with available substrate (Orchard and Cook 1983). Hence, most of these ecosystems show a pulse-dynamic response to precipitation, in which individual rainfall events allow short pulses of resource availability to microbes (Huxman et al. 2004). Soil surface properties, such as the existence of a biological soil crust, determine the location, amount, and timing of water infiltration into drylands soils (Eldridge and Greene 1994; Belnap and Lange 2003), which affects the type and size of microbial response.

Rapid rewetting of dry soils may kill up to one-third of the soil microbes due to osmolysis, since microbial biomass accumulate solutes during dry intervals in order to rise desiccation tolerance (Kieft et al. 1987). Thus, microbes must rapidly acclimate through physiological mechanisms to survive. Nevertheless, acclimation strategies have physiological costs that may change the composition of the soil microbial community, which may involve shifts in ecosystem-level C and nutrient flows (Schimel et al. 2007). Excessive stress will force microbes into dormancy (Farrar and Reboli 1999; Suzina et al. 2004) or will even kill them, which will result in organic carbon and nutrients release. This resource and soil moister pulse may stimulate an increase of microbial biomass (Kieft et al. 1987) and microbial metabolic rates such as decomposition and mineralization of soil organic matter (Schwinning and Sala 2004; Fisher and Whitford 1995) or, alternatively, these resources might be lost to deeper soils or to the atmosphere (Miller et al. 2005). Some studies suggest dryland microorganisms will be relatively resilient to changes in precipitation regime, since they are adapted to extreme environments (Kleidon et al. 2000; Ward 2009). In contrast, others researches suggest these microbes are already living near the physiological limits of tolerance, and hence they are likely to be particularly sensitive to climatic changes (Sala and Lauenroth 1982; Weltzin et al. 2003; Schwinning et al. 2004).

Improving our knowledge on the effect of length of wetting event on soil nutrients in BSC areas is critical to improve our understanding of nutrient cycling in arid and semiarid environments, in order to accurately predict how they will respond to the ongoing global environmental change. To advance in this direction, we evaluated how different length of wetting events affect multiple variables related to N (N in microbial biomass [MB-N], dissolved organic nitrogen [DON], NH_4^+-N, NO_3^--N,

Resin-NH_4^+-N and Resin-NO_3^--N) and C (phenols and carbohydrates) cycling in BSC and bare ground in a microcosm experiment.

We hypothesize that physical processes leading to nutrient losses such as leaching and runoff are faster than biological processes related to decomposition and mineralization rates. Therefore, longer wetting events should be conservative for soil nutrients. We also hypothesize that soils under BSC will show a different response of the soil variables to different lengths of wetting events than bare ground samples, due to the interferences of BSC in the water infiltration and to the different composition of microbial communities found under BSC (Delgado-Baquerizo et al., 2013a). Therefore, an enhancement in the microbial metabolic rates may be expected in longer wetting events, altering inorganic and organic N as well as labile C sources as a result of increasing both decomposition and mineralization rates.

Material and methods

Study area

This study was conducted in the Aranjuez Experimental Station, located in central Spain (40° 02′ N; 3° 37′ W; 590 m; 8 ° slope facing SE). The climate is Mediterranean semiarid, with a 30-year average rainfall and temperature of 388 mm and 14°C. There is a pronounced dry season from June to September, with only small amounts of rain. The soil in this area is derived from gypsum outcrops, and it is classified as Xeric Haplogypsid (Marqués et al. 2008). It is characterized by a fine texture, dominated by the presence of gypsum. Table 1 presents the relevant physical and chemical properties of this soil. Perennial plant cover is below 40% and is dominated by *Stipa tenacissima* L. (18% of plant cover) and the N-fixing shrub *Retama sphaerocarpa* L. (6% of plant cover). The open areas between plant patches host well-developed BSC community dominated by lichens such as *Diploschistes diacapsis* Ach., *Squamarina lentigera* Weber., *Fulgensia subbracteata* Nyl., *Toninia sedifolia* Scop., and *Psora decipiens* Hedw. (see Castillo-Monroy et al. 2010 for a full species checklist). Bare ground and BSC-dominated areas cover 28 and 32% of the study area, respectively.

Experimental Design

Soil sampling and laboratory analyses were performed in spring of 2011, the most biologically active season at the study site (Castillo-Monroy et al. 2011). In May 2011, we randomly selected 30 intact soil cores (4 cm depth, 7 cm diameter) under each of two studied soils: well-developed BSC communities (cover of mosses and lichens > 75%; see Appendix B of Castillo-Monroy et al. 2010) and bare ground areas lacking in vascular vegetation and BSC components (cover of lichens and mosses < 15%; see Appendix B of Castillo-Monroy et al. 2010). This soil depth was selected to keep the whole effect of BSC (top 0-4 cm of soil, Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010). Soil cores were transported to the laboratory and air-dried at room temperature for three weeks. Soil cores with and without BSC were incubated in the laboratory for 17 days and watered once a day until saturation for one, six and ten days (1-d, 6-d and 10-d, hereafter), conforming three treatments of one wetting and drying cycle with different length of watering. The incubation was performed in a sunlight location in the laboratory. The soil cores were allowed to drain after each watering in order to avoid anoxic conditions. Ten replicates were used for each combination of treatments. From each sample, we collected 3 g of soil from each replicated core at different times: immediately before the first watering (T 0), 1 day after T 0 (T 1), 2 days after T 0 (T 2), 6 days after T 0 (T 6), 10 days after T 0 (T 10) and 17 days after T0 (T 17).

We also measured mineral N availability in situ by using ion-exchange membranes (resins; Subler et al. 1995; Durán et al., 2013a). Resins were the technique of choice to produce minimal disturbances in the soil communities and allow intensive sampling over multiple time periods at the same spatial location. Five pairs of anionic and cationic resins (types AMI-7001S and CMI-7000S, Membranes International INC, New Jersey) were installed per each replicated core during the experiment. Each pair of resins was incubated in soil during different periods of time throughout the experiment (from T 0 to T 1, from T 1 to T 2, from T 2 to T 6, from T 6 to T 10 and from T 10 to T17).

Laboratory analysis

To measure ammonium (NH_4^+-N), nitrate (NO_3^--N) and DON, the soil subsamples were extracted with 0.5 *M* K_2SO_4 at a ratio of 1:5, followed by shaking for 1 h at 200 rpm at 20°C. The extract was filtered through a 0.45 µm Millipore filter (Jones and Willett 2006). The NH_4^+-N concentration was estimated directly via the indophenol blue method using a microplate reader (Sims et al. 1995). The NO_3^--N was first reduced to NH_4^+-N with Devarda alloy, and the concentration was determined as the difference between the Devarda-incubated and unincubated samples. The DON in the extracts was first oxidized to NO_3^--N with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 °C for 55 min and then reduced to NH_4^+-N with Devarda alloy (Sollins et al. 1999). The DON contents were calculated as total dissolved N minus inorganic N.

MB-N was determined using the fumigation-extraction method proposed by Brookes et al. (1985). Fresh soil subsamples were fumigated with chloroform for 5 days. The non-fumigated replicates were used as controls. The fumigated and non-fumigated samples were extracted with K_2SO_4 0.5 *M* at a ratio of 1:5 and filtered through a 0.45-µm Millipore filter. The extracts were digested as described above. The total N content in the digested extracts was determined by colorimetry (indophenol blue method) with a microplate reader (Sims et al. 1995). The MB-N concentration was estimated as the difference between the total N in fumigated and unfumigated digested extracts divided by a Kn (fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 (Brookes et al. 1985). Potentially available N (total available N hereafter) was calculated as the sum of ammonium, nitrate and DON.

The ion-exchange resins were first subjected to expansion treatment by submersion in distilled water at 82–90°C for 48 h. Next, the resins were cut into 2.5 × 2.5 cm squares and inserted into the soil at a 0.5–3 cm depth. After soil incubation time, the resins were collected and dried at ambient temperature. The resins were carefully brushed to remove soil particles, and placed into 125 ml flasks for extraction with 25 ml of 2 *M* KCl via orbital spinning (1 h at 200 rpm). The extracts were analyzed to measure NH_4^+-N and NO_3^--N by following Morillas et al. (2013).

To measure carbohydrates (sum of pentoses and hexoses) and phenols, the soil subsamples were extracted with 0.5 *M* K_2SO_4 at a ratio of 1:5, followed by shaking for 1 h at 200 rpm at 20°C. The extract was filtered through a 0.45 µm Millipore filter. All

concentrations were estimated following Chantigny et al. (2006). All results were expressed on a dry soil basis.

Statistical and numerical analyses

Differences in soil variables concentration between watering treatments and microsites were evaluated via repeated-measures ANOVA by using IBM SPSS 15.0 (SPSS Inc, Chicago, IL, USA). Net changes of soil variables during the experiment for each watering treatment was calculated as the difference between T 17 and T 0 concentrations. Since these data did not follow ANOVA assumptions (normality and homogeneity of variances), the effects of watering treatments (1-d, 6-d and 10-d) and microsites (BSC and bare ground) on net changes were tested using the semi-parametric PERMANOVA approach (Anderson 2001). BSC presence or absence and watering treatments were treated as fixed factors. PERMANOVA does not make distributional assumptions and is compatible with any distance measure. To investigate interactions, data were divided into subsets based on one of the factors of interaction, and then were subjected to PERMANOVA analyses. PERMANOVA analyses were performed by 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).

Results

Soil differences between microsites

Before any treatment the concentration of resin-NH_4^+, resin-NO_3^- and carbohydrates was significantly higher in bare ground than in BSC, meanwhile NH_4^+-N showed the opposite trend (Table 1). Initial differences in the concentrations of MB-N, DON, NO_3^--N, mineral N, total available N and phenols were not found between microsites (Table 1).

Table 1. Physical and chemical properties for the top 4 cm of the soil profile for the bare ground and biological soil crust (BSC) microsites (n=30).

	Bare ground		BSC	
	Mean	SE	Mean	SE
MB-N (mg kg^{-1} soil)	58.67	8.68	64.95	8.38
DON (mg kg^{-1} soil)	27.06	1.84	26.08	1.51
NH_4^+-N (mg kg^{-1} soil)***	0.85	0.21	2.25	0.41
NO_3^-- N (mg kg^{-1} soil)	7.75	0.70	6.22	0.51
Min N (mg kg^{-1} soil)	8.56	0.80	8.32	0.69
Total available N (mg kg^{-1} soil)	35.34	2.68	34.93	1.95
Resins-NH_4^+ (µg cm^{-2} day^{-1})*	0.16	0.01	0.14	0.01
Resins-NO_3^- (µg cm^{-2} day^{-1})*	0.67	0.06	0.51	0.05
Phenols (mg kg^{-1} soil)	22.44	2.52	22.81	3.43
Carbohydrates (mg kg^{-1} soil) ***	67.71	6.68	34.61	4.30

*Significant differences between microsites in the initial soil variables concentrations are as follows: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ (n=5). Total available N is the sum of mineral N plus DON.

Soil variables concentration

We found a significant effect of the different watering treatments on the C variables (phenols and carbohydrares) and on NO_3^- (both NO_3^- concentration and NO_3^--Resin), but not on soil MB-N, DON and NH_4^+. (Online Resource 1, Figure 1, 2, 3 and 4). The presence of BSC affected significantly all variables, excepting MB-N and NH_4^+-Resins (Online Resource 1). For all watering treatments, DON concentrations reached their minimum values earlier in BSC than in bare ground, while maximum NO_3^- concentrations appeared earlier in BSC than in bare ground (Figure 1b and 2b). Soils under BSC showed lower values of DON than in bare ground for all watering treatments (Figure 1b). Increases of NH_4^+ were only perceptible under BSC (Figure 2a). We found a significant effect of time for all analyzed variables, excepting soil NH_4^+ (Online Resource 1). There was a significant time x microsite interaction for all variables, except for phenols (Online Resource 1). We also found a significant time x treatment and treatment x microsite interaction for NO_3^--Resin and NH_4^+-Resin, respectively (Online Resource 1).

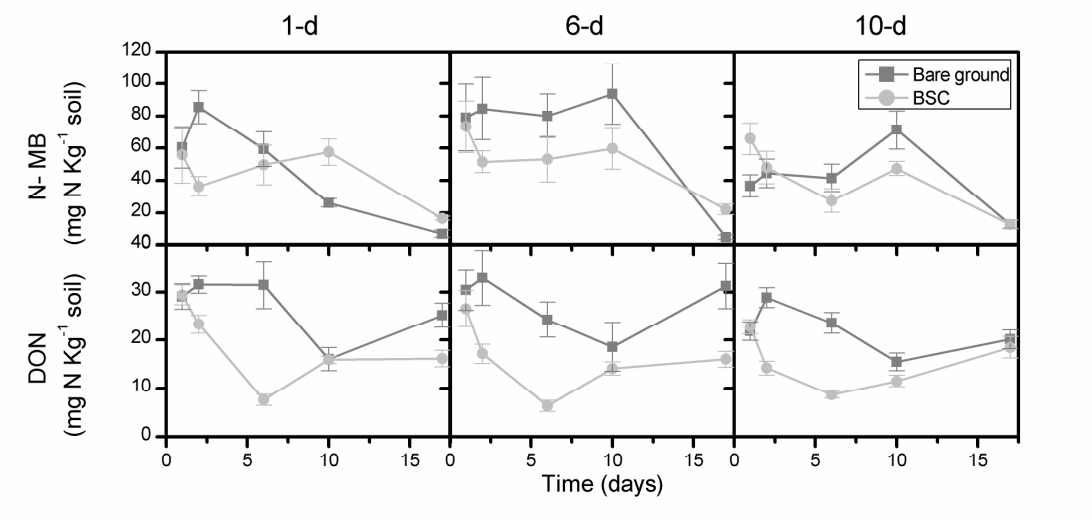

Figure 1. MB-N (a) and DON (b) measured at T 0, T 1, T 6, T 10 and T 17 in the top 4 cm of the soil profile throughout the study in the bare ground and BSC microsites. Error bars are ±2SE (n=10)

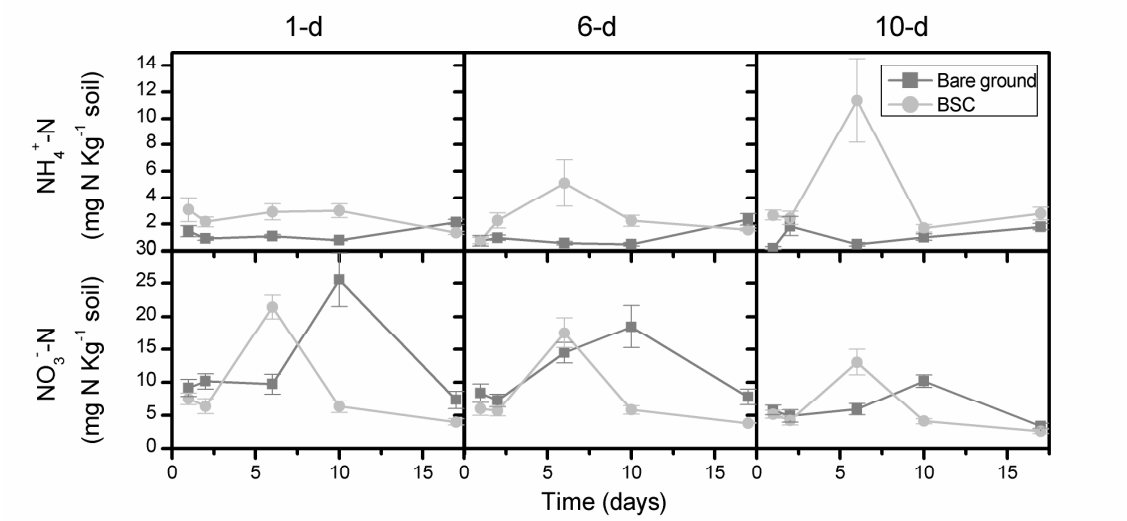

Figure 2. NH_4^+-N (a) and NO_3^--N (b) measured at T 0, T 1, T 6, T 10 and T 17 in the top 4 cm of the soil profile throughout the study in the bare ground and BSC microsites. Error bars are ±2SE (n=10)

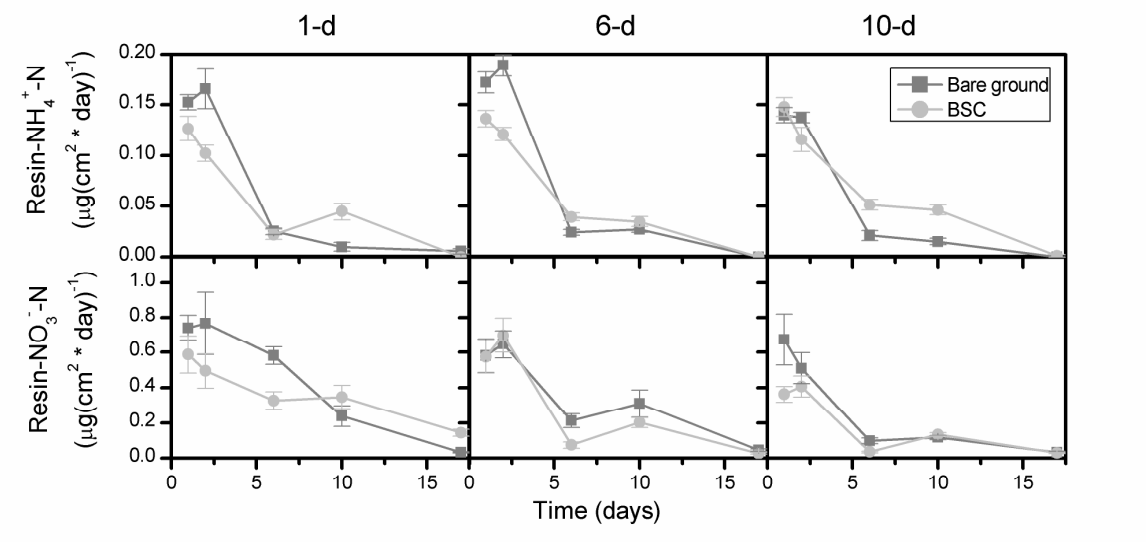

Figure 3. Resin-NH_4^+-N (a) and Resin-NO_3^--N (b) measured at T 0, T 1, T 6, T 10 and T 17 in the top 4 cm of the soil profile throughout the study in the bare ground and BSC microsites. Error bars are ±2SE (n=10).

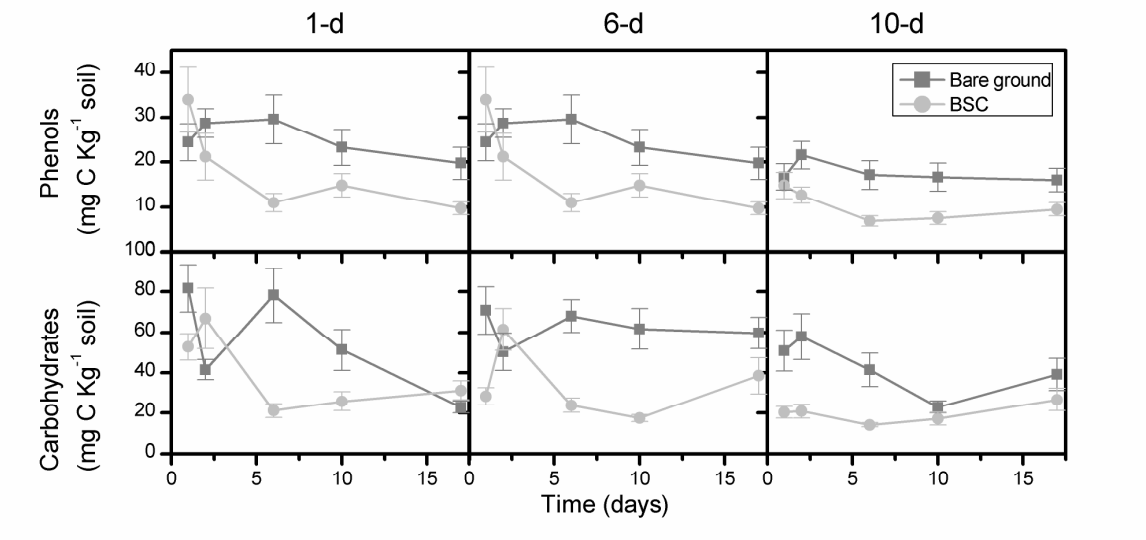

Figure 4. Phenols (a) and Carbohydrates (b) measured at T 0, T 1, T 6, T 10 and T 17 in the top 4 cm of the soil profile throughout the study in the bare ground and BSC microsites. Error bars are ±2SE (n=10)

Soil variables net changes

There was a significant effect of watering treatment on the net change of all analyzed variables, except for NO_3^- (Table 2, Figure 5 and 6). Microsite significantly affected all soil variables net changes, excepting MB-N and NO_3^- (Table 2, Figure 5 and 6). There was a significant treatment x microsite interaction for all the soil variables net changes, excepting NO_3^-, phenols and carbohydrates (Table 2). For the 1-d watering the

net changes were negative for all soil variables under the BSC. For the 6-d and 10-d treatments these values tended to be less negative or even positive for all variables excepting MB-N and resin-N. In the bare ground only DON and NH_4^+ showed positive values for the 1-d watering treatment, and for the 6-d and 10-d watering treatments only NH4 and carbohydrates showed a significant trend toward less negative or more positive values. Net changes of DON showed an inverse pattern in bare ground than under BSC.

Table 2. PERMANOVA test evaluating the effect of the microsite and length of wetting event (treatment) on the net changes (differences between final and initial values) of soil variables

Soil variables	$P_{Microsite}$	$P_{Treatment}$	$P_{Microsite \times Treatment}$
MB-N	0.152	< 0.001	< 0.001
DON	< 0.001	0.006	< 0.001
NH_4^+-N	< 0.001	< 0.001	0.016
NO_3^--N	0.068	0.194	0.566
Resin-NH_4^+-N	< 0.001	< 0.001	< 0.001
Resin-NO_3^--N	< 0.001	< 0.001	< 0.001
Phenols	< 0.001	< 0.001	0.074
Carbohydrates	< 0.001	< 0.001	0.259

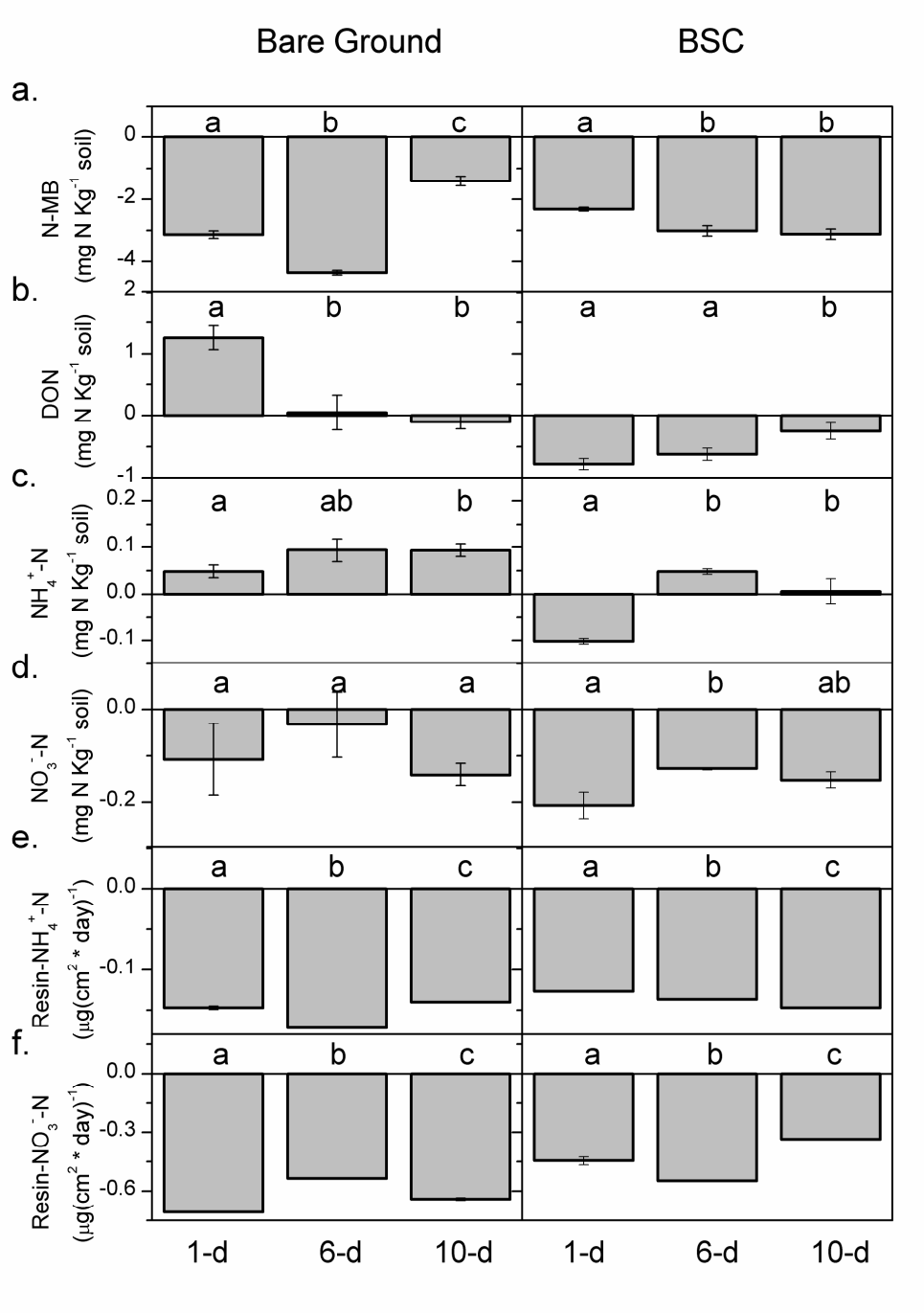

Figure 5. MB-N (a), DON (b), NH_4^+-N (c), NO_3^--N (d), Resin-NH_4^+-N (e) and Resin-NO_3^--N (f) net changes for each watering treatment measured as the difference between T 17 and T 0 concentrations in the bare ground and BSC microsites. Error bars are ±2SE (n=10)

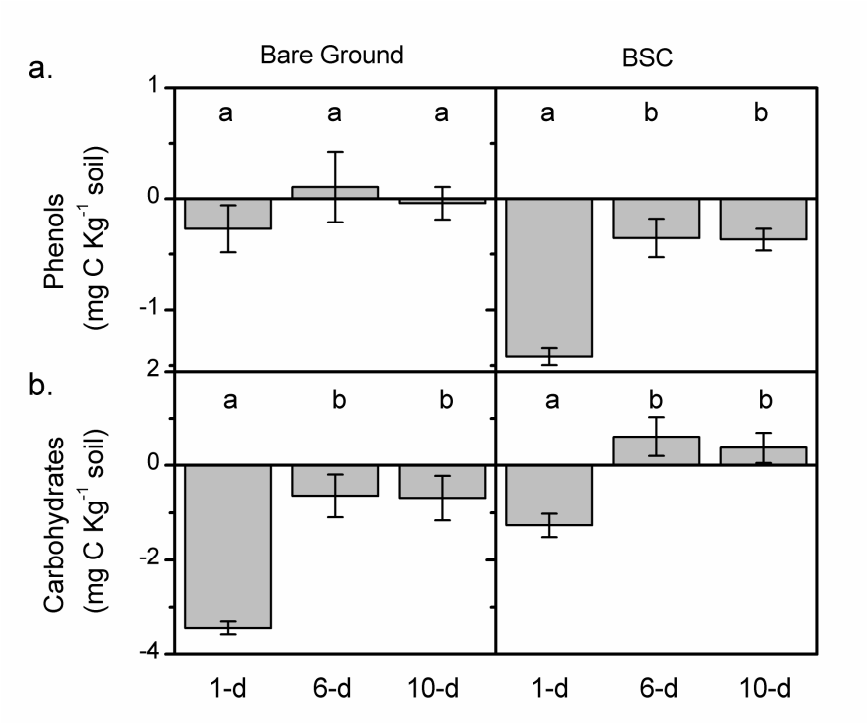

Figure 6. Phenols (a) and Carbohydrates (b) net changes for each watering treatment measured as the difference between T 17 and T 0 concentrations in the bare ground and BSC microsites. Error bars are ±2SE (n=10)

Discussion

As we expected, increasing length of wetting event had a noticeable effect on bare ground and biological soil crust microsites, though it was larger on the latest. Our results showed that longer wetting events produced higher mineral N and organic C production, which suggests that longer wetting events may be related to an enhancement in microbial metabolic rates such as both decomposition and mineralization rates. Our results are consistent with those of Schwinning and Sala (2004), who suggested that there is a hierarchy of soil moisture pulse events with a corresponding hierarchy of ecological responses in semi-arid ecosystems. Based on this idea, small watering events trigger only a few relatively minor ecological responses, whereas larger events trigger more complex and larger ecological responses. However, we like to point out a complementary idea; the results observed under small watering events may be a consequence of the dominance of physical processes, such as leaching

or runoff versus biological processes, such as those derived from the microbial metabolism.

Mineral N production increased with the length of wetting event supporting that microbial activity is mainly limited by soil moisture. High levels of soil moisture reduce the transport rate of oxygen through soil and decrease oxygen availability to the microbes whereas too low levels of soil moisture inhibit cellular activity (Grote et al. 2010). Since dryland soils are moist and metabolically active <10% of the time (Lange et al. 1994), long wetting events are unlikely to occur frequently. Nevertheless, these findings prove the potential for C and N cycles in dryland soils to respond to a broad range of length of wetting events. Our findings suggest that longer wetting events can be related to an increase in the microbial metabolic rates such as both decomposition and mineralization rates, but this increase was observed earlier under BSC, suggesting better conditions for microbial activity. However, the presence of BSC may slow down water infiltration (Maestre et al. 2011), avoiding rapid leaching of labile solutes, which may contribute to the earlier increase in mineral and organic compound in soils. We found net changes of NH_4^+, NO_3^-, DON, phenols and carbohydrates increased for the two longest wetting events in BSC, while only net changes of NH_4^+ and carbohydrates showed the same trend in bare ground. Since this trend was more obvious in BSC than in bare ground, our results suggest that BSC communities may benefit these microbial activities throughout the larger amount of heterotrophic bacteria and fungi compared to bare ground (Belnap and Lange 2003), and that BSC communities plays a key role in nutrient conservation in semiarid ecosystems. Thus, the decrease of BSC cover predicted under climatic change scenarios (Maestre et al. 2013) may have consequences on the soil balance of soil labile C and N cycling, even without considering changes in the length of wetting events. Besides, we found that all the soil studied variables, excepting MB-N and NH_4^+, had a different response throughout the studied period to distinct length of wetting event when BSC were present. For all treatments, DON concentration decreased faster in BSC than in bare ground, while NO_3^- showed the inverse response, increasing faster in BSC than in bare ground. Increases of NH_4^+ were only perceptible under BSC. These findings suggest a differential response of microbes in BSC and bare ground microsites under different length of wetting events. Other researchers have observed rapid physiological responses of BSC to water pulses (Wilske et al. 2008).

It is interesting to note the strong decrease of net change of MB-N found for all watering treatments and microsites. Several previous studies (Henry et al. 2005; Chung et al. 2007; Allison and Martiny 2008; Zelikova et al. 2012) found that more frequent wetting and drying cycles regarding to the control may modify microbial community composition. Zelikova et al. (2012) found a decrease in both bacterial and fungal biomass in frequently watered soils in a desert ecosystem with presence of BSC. They suggested that a direct response to watering or an indirect response to the decrease of moss cover could be potential explanations for this microbial biomass reduction. Fierer et al. (2003) found shifts in microbial processes 6 weeks after the end of several wetting and drying cycles, and these changes were related to shifts in the composition of the microbial population. Pesaro et al. (2004) found that respiration rates recovered rapidly after drought, meanwhile microbial biomass remained depressed for at least one month. Even tolerable stresses suppose C and N costs on microbes that they must overcome in order to survive and remain active, these costs may have relevant influences on ecosystem functioning (Shimel et al. 2007).

In drylands, dry soil and the stress imposed by sudden rewetting can select against gram negative bacteria and favor gram positive bacteria and fungi (Shimel et al. 2007), this selection gain importance due to different groups of microbes perform distinct biogeochemical functions. Most of microbes responsible for soil organic matter breakdown are gram positives and fungi, and therefore they are drought tolerant, for this reason soil respiration may be only slightly sensitive to climate-induced community changes (Schimel 1995a). Nevertheless, most of the microbes performing specialized functions in soil, such as nitrifiers (Schimel 1995a), are gram negatives and therefore can be more sensitive. However, our results showed both decomposition and mineralization rates increased in response to longer wetting events, suggesting that both decomposer and nitrifier microbes are equally sensitive to the length of wetting events.

Physicochemical decomposition might also be relevant in this ecosystem, since the slightly alkaline pH shown in these soils favors high oxidative enzyme potentials (Stursova et al. 2006). These conditions allow the breakdown of recalcitrant organic compounds such as phenols. Net changes of these compounds increased in response to longer wetting events only for BSC, highlighting the role of BSC microsites as a factor modulating physicochemical decomposition of recalcitrant organic compounds.

Alternatively, the increment of this variable observed under BSC can be the result of active microbial synthesis.

It is noticeable that most of the net changes in soil variables are negative, our experimental design cannot provide a certain understanding for these findings, but it might be related to the leakage of C and N with wetting (Kieft et al. 1987). Thus, soils that are wetted for a longer period are likely to lose more nutrients than those receiving less watering. Because soil water content determines soil microbial activity (Morillas et al. 2013), a shift in precipitation patterns as predicted by climate change models, can change microbial responses in a pulse driven ecosystem (Rustad et al. 2000; Easterling et al. 2000). Our data suggest that changes in the length of wetting events and the presence of BSC with climate changes could alter the C and N balance in semiarid soils.

Conclusions

Results from this study support the hypothesis that soil N and C cycling is markedly affected by the length of wetting events and the presence of BSC, and such changes could alter future soil community structure and function. Our findings show that longer wetting events may activate the microbial communities, mainly those related to BSC, stimulating microbial decomposition and mineralization of soil organic matter. They also indicate that BSC change the response of DON, NH_4^+ and NO_3^- to wetting events, producing a faster or higher microbial response compared to bare ground. The different response found in BSC and bare ground suggests that distinct microbial communities may modulate the response to wetting events in these microsites. These findings highlight the relevance of BSC as a key factor of C and N cycling in drylands, and complement the results of previous researches.

Acknowledgments

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also appreciate the help of Cristina Allely and Ana Prado during the field sampling and the lab analysis.

Online Resource 1. Repeated measurements ANOVA analyses for all the studied variables. Mi = Microsite, Ti = Time, Tr = Treatment (watering length), BG= Bare ground.

	Factor	Source	df	F	p
MB-N					
		Ti	4	27.13	0.0005
		Mi	1	1.54	0.238
		Tr	2	1.96	0.208
		Ti x Mi	4	4.90	0.042
		Ti x Tr	8	13.75	0.069
		Tr x Mi	2	0.78	0.490
		Tr x Mi x Ti	8	2.31	0.336
Interaction Ti x Mi	Mi (BG)	Ti	4	21.44	0.001
		Tr	2	2.01	0.195
		Ti x Tr	8	11.86	0.080
	Mi (BCS)	Ti	4	8.75	0.011
		Tr	2	0.76	0.497
		Ti x Tr	8	1.32	0.498
DON					
		Ti	4	44.12	< 0.0001
		Mi	1	41.25	< 0.0001
		Tr	2	3.41	0.085
		Ti x Mi	4	30.03	< 0.0001
		Ti x Tr	8	9.77	0.096
		Tr x Mi	2	0.63	0.554
		Tr x Mi x Ti	8	10.75	0.087
Interaction Ti x Mi	Mi (BG)	Ti	4	11.64	0.005
		Tr	2	1.70	0.242
		Ti x Tr	8	15.23	0.063
	Mi (BCS)	Ti	4	85.71	< 0.0001

		Tr	2	4.13	0.058
		Ti x Tr	8	3.00	0.273
NH_4^+					
		Ti	4	3.26	0.095
		Mi	1	40.07	< 0.0001
		Tr	2	2.35	0.156
		Ti x Mi	4	32.31	< 0.0001
		Ti x Tr	8	1.22	0.525
		Tr x Mi	2	3.06	0.103
		Tr x Mi x Ti	8	2.01	0.374
Interaction Ti x Mi	Mi (BG)	Ti	4	14.43	0.003
		Tr	2	2.13	0.180
		Ti x Tr	8	1.81	0.403
	Mi (BCS)	Ti	4	6.24	0.025
		Tr	2	2.36	0.156
		Ti x Tr	8	3.17	0.261
NO_3^-					
		Ti	4	34.33	< 0.0001
		Mi	1	12.33	0.006
		Tr	2	53.27	< 0.0001
		Ti x Mi	4	19.09	0.001
		Ti x Tr	8	6.86	0.133
		Tr x Mi	2	2.35	0.157
		Tr x Mi x Ti	8	67.59	0.015
Interaction Ti x Mi	Mi (BG)	Ti	4	15.02	0.003
		Tr	2	13.95	0.002
		Ti x Tr	8	14.21	0.067
	Mi (BCS)	Ti	4	37.46	< 0.0001

		Tr	2	7.73	0.014
		Ti x Tr	8	0.84	0.644
NH_4^+- Resin					
		Ti	4	1635.59	< 0.0001
		Mi	1	3.40	0.097
		Tr	2	4.32	0.053
		Ti x Mi	4	13.65	0.003
		Ti x Tr	8	0.67	0.717
		Tr x Mi	2	12.92	0.003
		Tr x Mi x Ti	8	7.10	0.129
Interaction Ti x Mi	Mi (BG)	Ti	4	677.83	< 0.0001
		Tr	2	11.26	0.004
		Ti x Tr	8	31.07	0.031
	Mi (BCS)	Ti	4	313.70	< 0.0001
		Tr	2	3.93	0.064
		Ti x Tr	8	0.71	0.699
Interaction Tr x Mi	Tr (A)	Ti	4	144.77	< 0.0001
	Tr (B)	Ti	4	107.41	< 0.0001
	Tr (C)	Ti	4	313.73	< 0.0001
NO_3^-- Resin					
		Ti	4	43.02	< 0.0001
		Mi	1	11.95	0.007
		Tr	2	11.64	0.004
		Ti x Mi	4	28.67	0.0004
		Ti x Tr	8	84.78	0.011
		Tr x Mi	2	0.29	0.751
		Tr x Mi x Ti	8	4.08	0.211
Interaction Ti x Mi	Ti (T1)	Mi	1	4.99	0.052

		Tr	2	0.86	0.457
		Mi x Tr	2	2.75	0.123
	Ti (T2)	Mi	1	3.11	0.111
		Tr	2	6.78	0.018
		Mi x Tr	2	1.15	0.361
	Ti (T3)	Mi	1	32.84	0.0002
		Tr	2	104.97	< 0.0001
		Mi x Tr	2	7.91	0.012
	Ti (T4)	Mi	1	0.02	0.882
		Tr	2	17.46	0.001
		Mi x Tr	2	1.26	0.331
	Ti (T5)	Mi	1	17.32	0.002
		Tr	2	13.49	0.002
		Mi x Tr	2	41.86	< 0.0001
Interaction Mi x Tr (T3)	Mi (BG)	Tr	2	71.30	< 0.0001
	Mi (BCS)	Tr	2	52.35	< 0.0001
Interaction Mi x Tr (T5)	Mi (BG)	Tr	2	1.99	0.198
	Mi (BCS)	Tr	2	13.55	0.002
Phenols					
		Ti	4	15.46	0.002
		Mi	1	41.48	0.0001
		Tr	2	5.73	0.028
		Ti x Mi	4	4.47	0.051
		Ti x Tr	8	3.55	0.238
		Tr x Mi	2	2.08	0.187
		Tr x Mi x Ti	8	0.77	0.674
Carbohydrates					
		Ti	4	9.47	0.009

		Mi	1	39.49	0.0001
		Tr	2	16.36	0.001
		Ti x Mi	4	14.37	0.003
		Ti x Tr	8	12.36	0.076
		Tr x Mi	2	2.49	0.143
		Tr x Mi x Ti	8	16.59	0.058
Interaction Ti x Mi	Mi (BG)	Ti	4	11.86	0.005
		Tr	2	5.70	0.028
		Ti x Tr	8	6.77	0.135
	Mi (BCS)	Ti	4	10.52	0.007
		Tr	2	47.05	< 0.0001
		Ti x Tr	8	4.38	0.198

Capítulo 5

Increased N supply modulates the effect of drying-rewetting frequency on soil C and N cyclingand soil-atmosphere trace gases exchange

Lourdes Morillas, Jorge Durán, Alexandra Rodríguez, Javier Roales, Antonio Gallardo, Gary M. Lovett and Peter M. Groffman

Despite climate change and atmospheric nitrogen (N) deposition are two of the most important and concomitant global change drivers, the non additive effects that they can generate on soil response remain to be tested. We aimed to assess how the combined effect of soil N additions and more frequent drying-rewetting events affects carbon (C) and N cycling, functional microbial diversity and greenhouse gases (GHGs) soil-atmosphere exchange. To do so, we manipulated the frequency of soil drying-rewetting events on ambient and N-treated soils from a template forest. To evaluate how N-addition modulates the resistance of the soil to changes in the frequency of drying-rewetting events, we calculated the Orwin and Wardle Resistance index. Our results indicate that the intensification of the frequency of drying-rewetting cycles can affect the ability of soil to cycle C and N and the soil:atmosphere GHGs exchange, and that soil N input change the response of these variables to drying-rewetting cycles. We found a buffer effect of N-treated soil to changes in the frequency of the stresses, making soils more resistant to the predicted climate change. Overall, repeated drying-rewetting cycles led to reduction in NO_3^-, potential nitrification rate and GHGs emissions, and to increase in NH_4^+ and mineral N. The addition of N affected the functionality of the microbial population and increased the microbial functional diversity of the soil, but we found no effect of the intensification of the drying-rewetting cycles on these variables. Our results suggest that the combination of increased drying-rewetting cycles and N input can induce significant changes in C and N cycles, soil:atmosphere GHGs exchange and soil microbial community, therefore, they should be incorporated into models of the global change on soil C and N cycling.

Keywords: global change, nutrient cycling, precipitation pattern, microbial functional diversity, nitrogen addition.

Introduction

Human activities are altering the global climate. As a result, an intensification of the hydrological cycle manifested as raised evapotranspiration and soil drought coupled to more frequent and intense storms (Wetherald and Manabe, 2002), and an increase in the frequency of soil drying-rewetting cycles are expected (Huntington, 2006; IPCC, 2007). Soil moisture is one of the most critical controls of soil biogeochemical processes (Parton et al., 1987; Paul and Clark, 1996), and many studies have shown that changes in hydrological dynamics (i.e. drying-rewetting cycles) due to climate change can deeply alter soil carbon(C) and nitrogen (N) cycling (Cui and Caldwell, 1997), microbial biomass and activity (Stark and Firestone, 1995; Schimel et al., 1999), and the emission of greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4; Matson et al., 1991).

Atmospheric N deposition is one of the major drivers of global environmental change (Schlesinger, 2013). High levels of atmospheric N deposition from both fossil fuel combustion and agricultural activities are increasingly becoming a problem worldwide (Vitousek et al., 1997; Galloway and Cowling, 2002). This excess in N deposition can have serious consequences on ecosystems, such as nutrient imbalances, soil acidification, waters eutrophication, changes in C and N cycles, and increases in N_2Osoil emissions (Balota et al., 2004; Lal, 2004; Fenn et al., 1998; Driscoll et al., 2003). Recent reviews about the effect of N addition on soil C cycling in forest soils also show that increased N inputs can influence C storage through its effect on various aspects of soil C cycling such as changes in organic matter decomposition and soil respiration, among others (see Nave et al., 2009; Janssens et al., 2010; Liu and Greaver, 2010). However, other studies show that this effect can be transient (Hagedorn et al., 2012), highly heterogeneous (Pregitzer et al., 2008), modulated by the tree species composition (Rodríguez et al. under review) or insignificant (Lovett and Goodale, 2011).

While soil N additions reportedly affects soil C and N cycling and GHGs soil:atmosphere exchange, fewer studies have examined how the frequency of drying-rewetting cycles influences these processes (Fierer and Schimel, 2002). Further, to our knowledge, how increased N supply modulates soil response and resistance to changes in the frequency of drying-rewetting stresses has never been thoroughly tested before, even when climate change and atmospheric N deposition are two of the most important and concomitant global change drivers in the Northern Hemisphere (Sala et

al., 2000; Gruber and Galloway, 2008). It is well known that ecosystems frequently face multiple human-driven problems that can generate non-additive effects unpredictable from one single-factor studies. However most of the studies about global change effects on ecosystems have focused on the effects of one single driver (Sala et al., 2000; Matesanz et al., 2009).There is therefore a major challenge in developing a better understanding of how the combined effect of soil N additions and more frequent drying-rewetting events affects soil processes.

As soil microbes are mediators of biogeochemical cycles, understanding their responses to environmental changes will be critical to comprehend and project the effects of different drivers of global change on biogeochemical cycles and feedbacks, and to preserve ecosystem services (Nie et al., 2013). Thus, improving our knowledge of the combined effects of changes in N supply and changes in the frequency of drying-rewetting cycles on the soil microbial community is crucial to our understanding of future ecosystem C and N cycling in a global change scenario. Rapid changes in soil moisture are stressful to microbes mainly because they have to invest energy to regulate osmotic pressure (Bottner, 1985; Van Gestel et al., 1993). Since these physiological adaptations to soil moisture need for a large investment of resources, the ability of soil microorganisms to carry out these processes can be restricted by the nutritional status of the soil and other soil conditions (Schimel et al., 2007). Thus, N-supply could modulate, through its own effect on soil C and N cycling and on the microbial community, the response of soil microorganisms to different frequency of drying-rewetting cycles.

The goal of this study was to assess the effect of the frequency of drying-rewetting events on the microbial community and microbial processes of soil forests with different N-supply. To do so, we subjected N-treated and non-treated (ambient) soils from a 15 years N addition experiment (Lovett and Goodale, 2001) to different frequency of drying-rewetting stresses, and measured different variables related to C and N cycling, and to the soil:atmosphere GHGs exchange. To address the role of microbial populations on the observed response, we calculated the soil functional microbial diversity. To better understand how soil N supply modulates the response of microbial biomass and activity to changes in drying-rewetting frequency, we calculated the Orwin and Wardel Resistance Index (2004) for all variables. We hypothesized that (i) the ability to cycle C and N and the soil:atmosphere GHGs

exchange will be influenced by the intensification of the frequency of drying-rewetting cycles, but that (ii) this effect will be modulated by previous soil N-supply conditions.

Materials and methods

Research site and soil sampling

This study wascarried out at the Cary Institute of Ecosystem Studies, Millbrook, in southeastern New York State, USA (41.797°N, 73.734°W). The forest is comprised of upland mixed-oak woods dominated by red oak (*Quercus rubra* L.), chestnut oak (*Quercus prinus* L.) and several species of hickory (*Carya* sp.). The site has a humid continental climate, with an annual average temperature of 9.6 °C and precipitation averages 111 cm y^{-1}. Soils in this area are well-drained silt loams of the Nassau and Woodlawn series (Glitzenstein et al., 1990) classified as lithic dystrudepts. Ambient inorganic N deposition (wet + dry) at this site average approximately 9 kg N ha^{-1} y^{-1} (Lovett and Goodale, 2011). In 1996, six pairs of plots of 20 m in diameter each were selected. The two plots of each pair were within 40 m of each other. One plot of each pair was continuously N-treated adding granular NH_4NO_3 to the forest floor at a rate equivalent of 100 kg N ha^{-1} y^{-1} from 1996 to 1999, and then the N addition rate was diminished to 50 kg N ha^{-1} y^{-1} from 2000 to 2012. This annual application was distributed into four equal doses each year. The other plot of each pair was left untreated as an ambient plot [see Lovett and Goodale (2011) for more information about the research site]. In May 2012, three sampling points were randomly selected at each of the six pairs of plots, and cleared form fresh litter plus some Oi horizon to facilitate soil sample collection. We took three samples of the first 10 cm of the soil profile from each plot using a 5 cmdiameter corer. All samples from the ambient and from the N-treated plots were combined separately into two composite samples, hereafter referred to as 'ambient' and 'N-treated', respectively.

Soil incubation

The two composite samples were homogenized by hand to remove roots and rocks and oven dried at 38° C to constant weight. Soil water holding capacity (WHC) was determined as the gravimetric water content of soil that was saturated and

allowed to drain freely over 48 h in a filter funnel. Soils were then placed in tins (150 g of soil per tin) and conditioned for 4 days at 35% WHC (21% of soil water content) at 20-22 °C. Soils from 5 randomly selected tins of each type of soil were used for chemical and trace gases analysis (see below) at time zero (T_0). Then, 5 replicates (tins) of each soil type (N-treated and ambient) where selected for each drying-rewetting treatment. Soils were incubated during 31 days in darkness at 30 °C, and initially at 35% WHC. To avoid soil drying and allowing gases exchange, tins were closed with plastic wrap secured with a rubber band, and tins were daily weighed to make sure that the soil WHC was the appropriate. We applied 4 different drying-rewetting stress treatments. In the 'D_0' treatment soils were constantly kept at 35% of WHC, while in 'D_1', 'D_2' and 'D_4' treatments, soils were subjected to one, two and four drying-rewetting events, respectively: D_1 received a drying-rewetting event at the end of the experiment; D_2 received one drying-rewetting event in the middle of the experiment (during the second week) and one drying-rewetting event at the end of the experiment; D_4 received four drying-rewetting events, one each week of the incubation.

Drying-rewetting events consisted of a 2-day drying period followed by a rewetting. We dried soils by removing the plastic wrap and incubating at 30 °C under forced air flow so soil moister reached~5% of soil water content. To rewet the soils we homogeneously added deionized water using a syringe until the tins gained the weight corresponding to the soil 35% WHC. At the end of the last drying-rewetting event, all soils were rewetted at 35% WHC and incubated for three more days. After that, soils were harvested and one fraction was immediately frozen for microbial analysis, the other was stored at 3 °C for chemical analysis. This procedure ensured that the soil microbial populations did not experience relevant changes (Gonzalez-Quiñones et al., 2009).

Soil, trace gases and microbial analyses

Soil and trace gases analyses were carried out at the beginning (T_0) and at the end of the incubation. Soil samples were analyzed for water content by drying a subsample at 60 °C for 48 h (McInnes and Weaver, 1994). We measured soil NH_4^+, NO_3^-, inorganic N, microbial biomass C and N, potential microbial respiration and potential mineralization and nitrification rates using the chloroform fumigation -incubation method (Jenkinson and Powlson, 1976) as described by Durán et al. (2013b).

Soil-atmosphere CO_2, N_2O and CH_4 fluxes were determined for all soil samples by placing each tin inside a Mason jar and incubating for 150 minutes at 30 °C. Then, the jar headspace was sampled through rubber septa on Mason jar lids with a fine needle polypropylene syringe. Samples were then transferred to evacuated glass vials and stored at room temperature before analysis by gas chromatography using electron capture, thermal conductivity, and flame ionization detection for N_2O, CO_2 and CH_4, respectively.We calculated fluxes from the linear rate of change in gas concentration, the jar internal volume, and soil volume.

We estimated soil microbial functional diversity by analyzing soil heterotrophic microbial communities with the MicroResp system (Campbell et al., 2003). This method tests 15 carbon sources that vary in structural complexity (Oren and Steinberger, 2008). Carbon sources were selected depending on their ecological importance to soil and their solubility in water. Specifically, 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, malic acid, oxalic acid and amino butyric acid) (Delgado-Baquerizo et al., 2013b). Before performing the MicroResp method, defrosted soils were introduced into the flasks and pre-incubated for five days at 25 ^{o}C. The moisture within the flaks was corrected to 40% WHC in order to condition the soils and reestablish active microbial populations. To avoid changes in soil moisture content during incubation, flasks were covered with plastic wrap. Each C source was dissolved in deionized water and added to soils to deliver 30 mg C g soil water^{-1}. Approximately 0.4 g of soil was placed in the 96 deep-well plates volumetrically. To estimate the evolved CO_2, a colorimetric method relying on the change in the pH of a gel-based solution of bicarbonate was used. After that, the plates were incubated for 6 h and read at 570 nm. The results were calculated on the basis of water, which represents the basal respiration.

Data and statistical analyses

Percent change of all analyzed variables was calculated as follows:

$$\frac{\left(D_x - \overline{D_0}\right) \times 100}{\overline{T_0}}$$

where D_X is the studied variable valueat the end of the disturbance, $\overline{D_0}$ is the variable mean value for the samples that were kept at constant moisture and $\overline{T_0}$ is the variable mean value before the incubation.

To test how soil N treatment affected the resistance of the soil to different drying-rewetting treatments, we calculated the Orwin and Wardle Resistance Index (RS, 2004) for all the studied variables by using the following equation:

$$RS = 1 - \frac{2\left|X_0\right|}{\left(D_0 + \left|X_0\right|\right)}$$

where X_0 is the difference between the control (D_0) and the disturbed soil at the end of the disturbance. Disturbance is used as the period of time that the soils were exposed to the drying-rewetting treatments (31 days). This index is standardized by the control and it is bounded by −1 (less resistance) and +1 (maximal resistance).

We calculated the Shannon-Weaver Diversity Index (H') to assess microbial functional diversity using the soil respiration responding to the different C sources as (Shannon and Weaver, 1963):

$$H' = -\sum_{i=1}^{s} pi.\ln pi$$

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).

We assessed how the studied variables were influenced by N treatment (ambient vs. N-treated) and the different drying-rewetting treatments by using the semiparametric PERMANOVA approach developed by Anderson (2001). We established the presence/absence of soil N treatment and the different drying-rewetting treatments as fixed factors. Significant differences among drying-rewetting treatments were evaluated for each N treatment by using a posteriori pairwise comparison with the PERMANOVA t-statistic. PERMANOVA analyses were performed using 9999 permutations and the Euclidean distance. We used Primer 6 and

Permanova+ (PRIMER-E Ltd, Plymouth, UK) to carry out all statistical analyses. Principal component analysis (PCA) was conducted using SPSS 17.

Results

Soil variables

All studied soil variables, except potential mineralization rate, were significantly affected by drying-rewetting treatment ($P<0.001$). Soil NH_4^+-N and inorganic N significantly increased from treatment D_0 to treatment D_4 while NO_3^--N, microbial biomass C and potential nitrification rate showed the opposite trend (Table 1). Soil N treatment significantly altered all soil variables, except potential N mineralization rate (Table 1). Soil NO_3^--N, inorganic N and potential nitrification rate, were positively and significantly affected by the N additions, while the rest of variables showed the opposite trend (Table 1). We also found a significant N x drying-rewetting treatment interaction for all soil variables, except for microbial biomass N and potential N mineralization.

Table 1. Absolute valuesof the studied variables for the different N and drying-rewetting treatments.Data represent means ± SE (n=5). Different superscripts indicate significant differences among drying-rewetting treatments after permutational-repeated measures ANOVA (9999 permutations, $P < 0.05$). Asterisks indicate significant differences between N treatments (** means $p<0.01$, *** means $p<0.001$).A= ambient soils;N= N-treated soils.

		T_0	D_0	D_1	D_2	D_4
NH_4-N ***	A	78.2 ± 0.97^{A}	124.1 ± 1.05^{B}	125.8 ± 1.26^{BC}	130.0 ± 1.40^{CD}	133.1 ± 1.14^{D}
	N	84.6 ± 0.34^{a}	102.1 ± 1.83^{b}	110.1 ± 2.62^{c}	113.4 ± 1.56^{c}	121.3 ± 2.38^{d}
NO_3-N ***	A	0.27 ± 0.00^{A}	1.85 ± 0.04^{B}	1.81 ± 0.04^{B}	1.74 ± 0.04^{B}	1.41 ± 0.04^{C}
	N	1.64 ± 0.01^{a}	21.5 ± 0.18^{b}	20.8 ± 0.50^{bc}	19.1 ± 0.59^{c}	15.1 ± 0.34^{d}
Inorganic N **	A	78.4 ± 0.97^{A}	126.1 ± 1.09^{B}	127.6 ± 1.27^{BC}	131.7 ± 1.39^{CD}	134.6 ± 1.11^{D}
	N	86.3 ± 0.31^{a}	124.1 ± 2.21^{b}	133.6 ± 1.48^{c}	132.5 ± 1.86^{c}	137.0 ± 3.06^{c}
Microbial	A	169.1 ± 0.78^{A}	214.4 ± 3.30^{B}	219.7 ± 4.57^{B}	219.5 ± 1.03^{B}	214.8 ± 3.91^{B}
Biomass N ***	N	158.5 ± 2.02^{a}	199.8 ± 5.09^{bc}	198.9 ± 0.89^{b}	196.1 ± 0.41^{c}	199.3 ± 1.66^{bc}
Microbial	A	1676.5 ± 73.6^{A}	1563.2 ± 32.9^{A}	1509.6 ± 30.1^{A}	1415.7 ± 14.4^{B}	1320.5 ± 8.26^{C}
Biomass C ***	N	1632.9 ± 97.1^{a}	1258.5 ± 27.6^{b}	1212.5 ± 39.7^{bd}	1056.8 ± 45.9^{cd}	1066.1 ± 64.1^{d}
Pot Nitrification	A	0.065 ± 0.002^{A}	0.113 ± 0.001^{AB}	0.100 ± 0.004^{AB}	0.098 ± 0.007^{B}	0.092 ± 0.005^{C}
***	N	0.260 ± 0.010^{a}	1.42 ± 0.078^{b}	1.29 ± 0.031^{bc}	1.43 ± 0.099^{b}	1.05 ± 0.095^{c}

Pot Mineralization	A	0.92±0.19^{A}	1.68±0.18^{B}	1.89±0.30^{B}	1.45±0.11^{AB}	1.77±0.15^{B}
	N	1.06±0.02^{a}	1.44±0.21^{ab}	1.74±0.33^{b}	1.69±0.28^{ab}	1.35±0.74^{ab}
Pot microbial respiration ***	A	41.7±1.79^{A}	23.2±0.73^{B}	23.0±0.23^{B}	23.6±0.74^{B}	21.0±0.19^{C}
	N	43.6±1.79^{a}	17.7±0.29^{b}	19.2±0.10^{c}	17.8±0.15^{b}	18.4±0.45^{bc}
CO_2-C ***	A	73.8±1.42^{A}	30.4±0.02^{B}	42.2±2.70^{C}	35.8±0.79^{D}	30.6±1.10^{B}
	N	93.4±2.16^{a}	23.2±1.68^{b}	32.6±0.76^{c}	29.5±1.98^{cd}	28.5±0.41^{d}
N_2O-N ***	A	-0.037±0.11^{A}	6.74±0.32^{B}	5.06±0.17^{C}	3.36±0.20^{D}	1.52±0.22^{E}
	N	-0.258±0.33^{a}	28.6±0.53^{b}	35.9±4.34^{b}	26.5±2.71^{b}	16.1±0.61^{c}
CH_4-C ***	A	-64.6±1.59^{A}	-49.9±1.06^{B}	-48.1±3.44^{BC}	-44.3±1.79^{C}	-38.4±1.97^{C}
	N	-50.2±1.47^{a}	-2.02±0.79^{b}	-0.70±1.21^{b}	-3.33±2.28^{bc}	-5.95±1.22^{c}

NH_4-N, NO_3-N, inorganic N and microbial biomass N are expressed in ug N gr dry soil^{-1}; microbial biomass C is expressed in ug C gr dry soil^{-1}; potential N mineralization and nitrification are expressed in ug N gr dry soil day^{-1}; potential microbial respiration is expressed in ug C gr dry soil day^{-1}; CO_2-C and CH_4-C are expressed in mg C Kg soil^{-1} day^{-1}; and N_2O-N is expressed in mg N Kg soil^{-1} day^{-1}.

Except for soil microbial biomass N and potential N mineralization rate, there was a significant effect of drying-rewetting treatment on the percent change of all analyzed variables (Table 2). The percent change of soil NH_4^+-N in both N treatments and of inorganic N in ambient soils increased concurrently and significantly with the number of drying-rewetting cycles (Figure 1a and c). The percent change of soil NO_3^--N in both N treatments, of microbial biomass C and potential microbial respiration in ambient soils, and of potential nitrification in N-treated soils were negatively and significantly affected by the number of drying-rewetting cycles (Figure 1b, e, f and h). Soil N treatment significantly affected the percent change of all variables, except for microbial biomass N and C (Table 2, Figure 1). Nitrogen addition positively affected the percent change of all variables, excepting that of NO_3^--N, potential nitrification and potential mineralization. There was a significant N x drying-rewetting treatments interaction for the percent change of soil NO_3^--N and potential nitrification and microbial respiration rates (Table 2).

Table 2. Summary of permutational-repeated measures ANOVA exploring the effect of N-and drying-rewetting (D-R) treatments on the percent change of the studied variables. Ambient (A) and N-treated (N) soils were also analyzed separately for all variables in order to assess the effect of the drying-rewetting treatment in both of them (9999 permutations, $P < 0.05$)

	All soils combined				Ambient and N-treated separated			
		df	Pseudo-F	P	Soil	df	Pseudo-F	P
NH_4^+-N	N-treat	1	17.612	<0.001				
	D-R treat	2	10.895	<0.001	A	2	5.678	0.029
	N xD-R	2	0.657	0.526	N	2	6.571	<0.001
NO_3^--N	N-treat	1	27.923	<0.001				
	D-R treat	2	45.604	<0.001	A	2	15.201	0.001
	N x D-R	2	7.011	0.006	N	2	28.946	<0.001
Inorganic N	N-treat	1	7.067	0.015				
	D-R treat	2	3.455	0.046	A	2	5.118	0.038
	N x D-R	2	0.895	0.419	N	2	0.979	0.423
Microbial	N-treat	1	4.444	0.052				
biomass N	D-R treat	2	0.301	0.739	A	2	0.558	0.592
	N x D-R	2	0.878	0.435	N	2	1.236	0.337
Microbial	N-treat	1	2.668	0.117				
biomass C	D-R treat	2	6.613	0.007	A	2	11.735	0.001
	N x D-R	2	0.662	0.525	N	2	2.309	0.145
Potential	N-treat	1	5.626	0.026				
nitrification	D-R treat	2	5.529	0.009	A	2	0.530	0.602
	N x D-R	2	3.852	0.040	N	2	4.917	0.035
Potential	N-treat	1	4.601	0.039				
mineralization	D-R treat	2	0.316	0.736	A	2	1.062	0.394
	N x D-R	2	0.409	0.679	N	2	0.186	0.834
Potential	N-treat	1	436.25	<0.001				
microbial	D-R treat	2	4.229	0.031	A	2	6.241	0.023
respiration	N x D-R	2	5.615	0.013	N	2	3.017	0.109
CO_2-C	N-treat	1	0.004	0.971				
	D-R treat	2	12.201	<0.001	A	2	11.222	<0.001
	N x D-R	2	3.899	0.031	N	2	2.247	0.149
N_2O-N	N-treat	1	0.871	0.371				
	D-R treat	2	13.941	<0.001	A	2	70.287	<0.001
	N x D-R	2	3.437	0.049	N	2	8.981	0.004

CH_4-C	N-treat	1	17.318	<0.001				
	D-R treat	2	0.204	0.813	A	2	3.775	0.047
	N x D-R	2	6.275	0.005	N	2	2.525	0.118

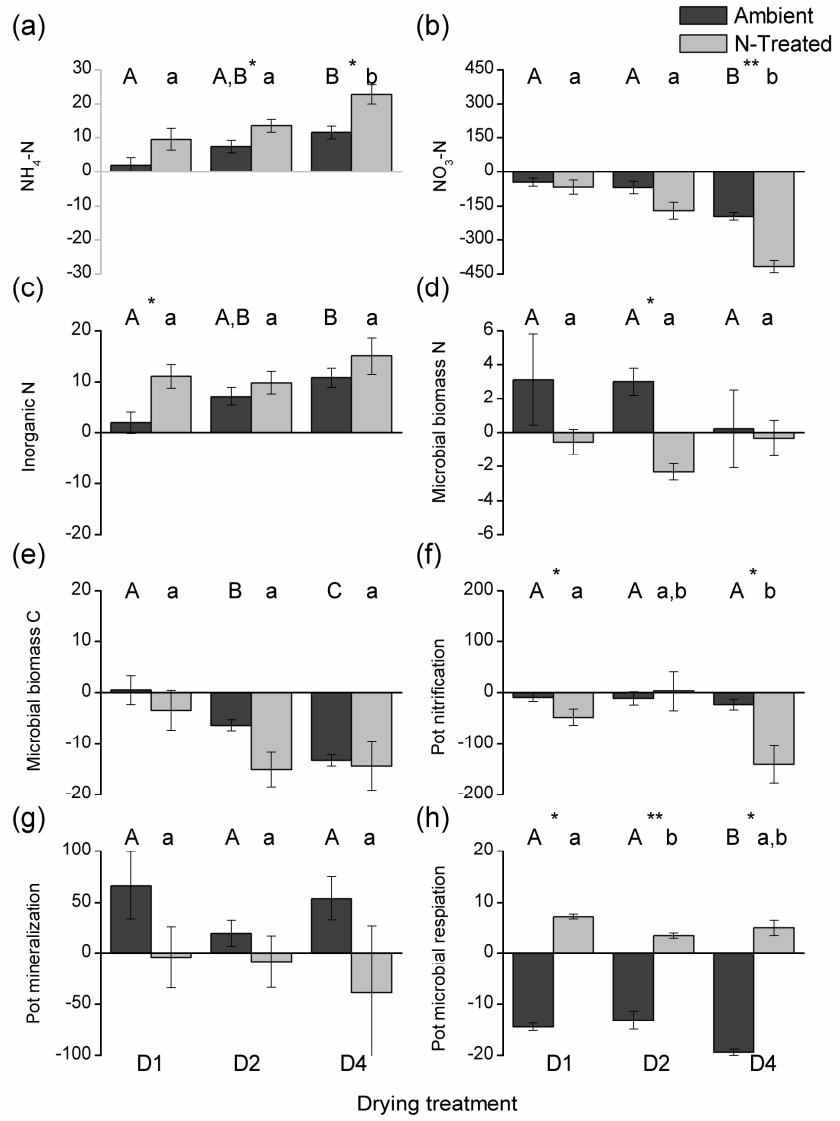

Figure 1. Percent changes of soil NH_4^+ (a), NO_3^- (b), inorganic N (c), microbial biomass N (d), microbial biomass C (e), potential nitrification (f), potential mineralization (g) and potential microbial respiration (h) measured following incubation in ambient and N-treated soils. Values are means ± standard error. Different letters show statistically significant differences at $P < 0.05$. Asterisks indicate significant differences between N treatments (* means $p<0.05$, ** means $p<0.01$)

Drying-rewetting treatment significantly altered resistance of soil NH_4^+-N, NO_3^--N and microbial biomass C (Table 3). Resistance of NH_4^+-N and NO_3^--N in both N treatments, and of inorganic N, microbial biomass C, and microbial respiration in ambient soils decreased with the number of drying-rewetting cycles (Table 3, Figure 2).

Resistances of soil NH_4^+-N, inorganic N, and potential mineralization rate were significantly and negatively affected by N-fertilization, whereas the effect on the microbial biomass N resistance was significant and positive (Table 3, Figure 2). A significant N x drying-rewetting treatments interaction was also observed for resistance in potential microbial respiration (Table 3).

Table 3. Summary of permutational-repeated measures ANOVA exploring the effect of N and drying-rewetting (D-R) treatments on the Orwin-Wardle resistance index (RS) of the studied variables. Ambient (A) and N-treated (N) soils have been analyzed separately for all variables in order to assess the effect of the drying-rewetting treatment in both of them (9999 permutations, $P < 0.05$).

	All soils combined				Ambient and N-treated separated			
		df	Pseudo-F	P	Soil	df	Pseudo-F	P
NH_4^+-N	N treat	1	32.896	<0.001				
	D-R treat	2	10.143	<0.001	A	2	5.315	0.033
	N xD-R	2	0.972	0.392	N	2	6.485	0.007
NO_3^--N	N treat	1	1.900	0.181				
	D-R treat	2	41.587	<0.001	A	2	18.138	<0.001
	N x D-R	2	0.665	0.522	N	2	23.697	<0.001
Inorganic N	N treat	1	8.711	0.007				
	D-R treat	2	2.959	0.069	A	2	4.553	0.043
	N x D-R	2	0.565	0.572	N	2	0.936	0.423
Microbial	N treat	1	9.200	0.009				
biomass N	D-R treat	2	0.295	0.745	A	2	1.224	0.325
	N x D-R	2	1.584	0.240	N	2	0.604	0.578
Microbial	N treat	1	0.918	0.341				
biomass C	D-R treat	2	4.724	0.022	A	2	16.355	<0.001
	N x D-R	2	0.640	0.537	N	2	1.462	0.271
Potential	N treat	1	0.002	0.878				
nitrification	D-R treat	2	3.137	0.063	A	2	0.500	0.602
	N x D-R	2	0.619	0.549	N	2	3.743	0.052
Potential	N treat	1	7.165	0.014				
mineralization	D-R treat	2	0.946	0.397	A	2	0.651	0.533
	N x D-R	2	1.386	0.263	N	2	1.565	0.248
Potential	N treat	1	0.542	0.482				
microbial	D-R treat	2	2.994	0.059	A	2	11.217	0.004
respiration	N x D-R	2	9.211	0.002	N	2	3.600	0.072

CO_2-C	N treat	1	7.543	0.014				
	D-R treat	2	13.646	<0.001	A	2	13.383	<0.001
	N x D-R	2	1.441	0.257	N	2	2.789	0.105
N_2O-N	N treat	1	5.311	0.034				
	D-R treat	2	9.413	0.002	A	2	63.434	<0.001
	N x D-R	2	2.600	0.097	N	2	2.211	0.151
CH_4-C	N treat	1	53.99	<0.001				
	D-R treat	2	2.797	0.079	A	2	2.708	0.109
	N x D-R	2	2.368	0.116	N	2	1.149	0.344

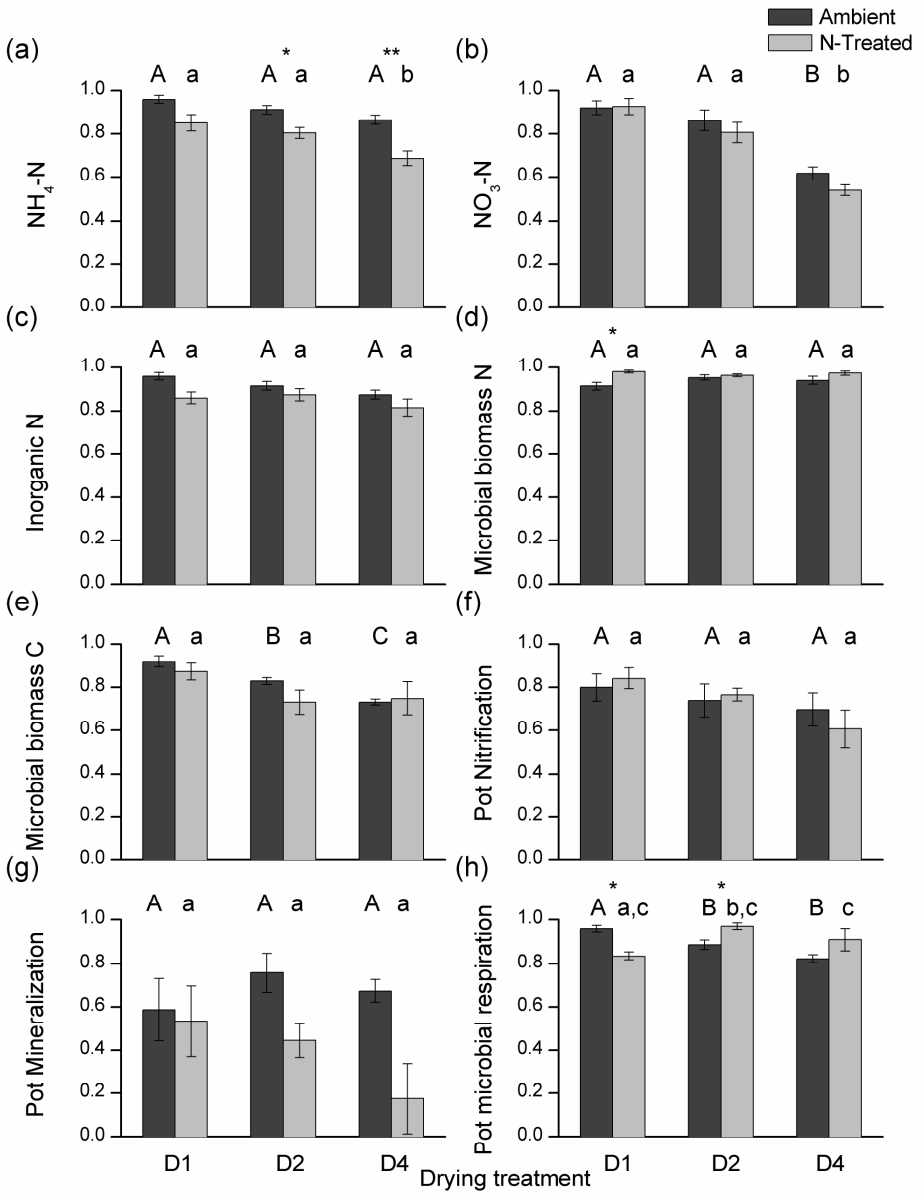

Figure 2. Orwin and Wardle Resistance index for soil NH_4^+ (a), NO_3^- (b), inorganic N (c), microbial biomass N (d), microbial biomass C (e), potential nitrification (f) potential mineralization (g) and potential microbial respiration (h) measured following incubation in

ambient and N-treated soils. Values are means ± standard error. Different letters show statistically significant differences at P < 0.05. Asterisks indicate significant differences between N treatments (* means p<0.05, ** means p<0.01)

Trace gases

Soil CO_2 (F=19.87, P<0.0001) and N_2O (F=14.63, P<0.0001) emissions were significantly affected by drying-rewetting treatment. Emissions of CO_2 and N_2O decreased from D_1 to D_4 in both N treatments whereas CH_4 uptake decreased from D_0 to D_4 in ambient soils and increased from D_1 to D_4 in N-treated soils (Table 1). Soil N treatment significantly affected all trace gases variables, positively to N_2O and CH_4 and negatively to CO_2 (Table 1). We also found a significant N x drying-rewetting treatments interaction for N_2O and CH_4 (F=6.51, P<0.001; F=7.16, P=0.001, respectively).

The percent change of CO_2 and N_2O emissions were significantly affected by drying-rewetting treatment (Table 2). Percent change of CO_2 emission in both N treatments and CH_4 uptake in ambient soils decreased significantly with the number of drying-rewetting cycles (Figure 3a and c), whereas the opposite trend was found for that of N_2O emission in both N treatments and for that of CH_4 uptake in N-treated soils (Figure 3b). Percent change of CH_4 uptake, but not of CO_2 or N_2O emissions, was significantly affected by soil N treatment (Table 2, Figure 3). This percent change of CH_4 was increasing as the number of drying-rewetting treatments increased, but in the opposite direction for each N treatment (positively in the case of N-treated soils and negatively in the ambient ones). We also found a significant N x drying-rewetting treatments interaction for the percent change of all trace gases variables (Table 2).

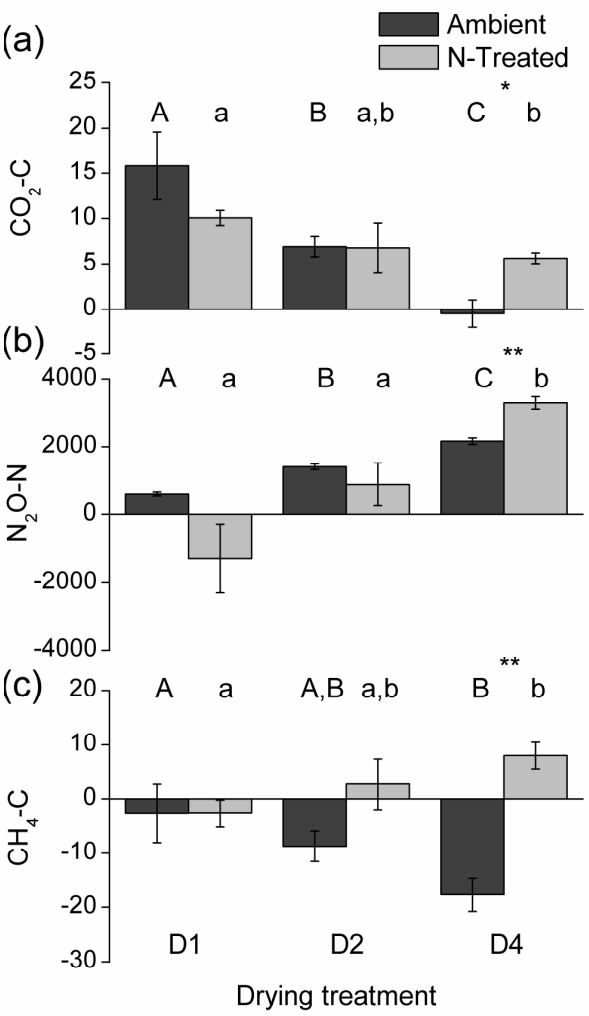

Figure 3. Percent changes of soil: atmosphere CO_2-C (a), N_2O-N (b) and CH_4-C (c) exchange measured following incubation in ambient and N-treated soils. Values are means ± standard error. Different letters show statistically significant differences at $P < 0.05$. Asterisks indicate significant differences between N treatments (* means $p<0.05$, ** means $p<0.01$)

The resistances of CO_2 and N_2O emissions were also significantly altered by the drying-rewetting treatment (Table 3). In both N treatments, the resistance of CO_2 emissions was higher in D_4 than in D_1, whereas that of N_2O emission showed the opposite trend (Figure 4a and b). We also observed a significant N treatment effect on the resistances of trace gases soil:atmosphere exchange with lower values of resistance of CO_2 emissions and higher values of that of N_2O emissions and CH_4 uptake in N-treated than in ambient soils (Table 3, Figure 4).

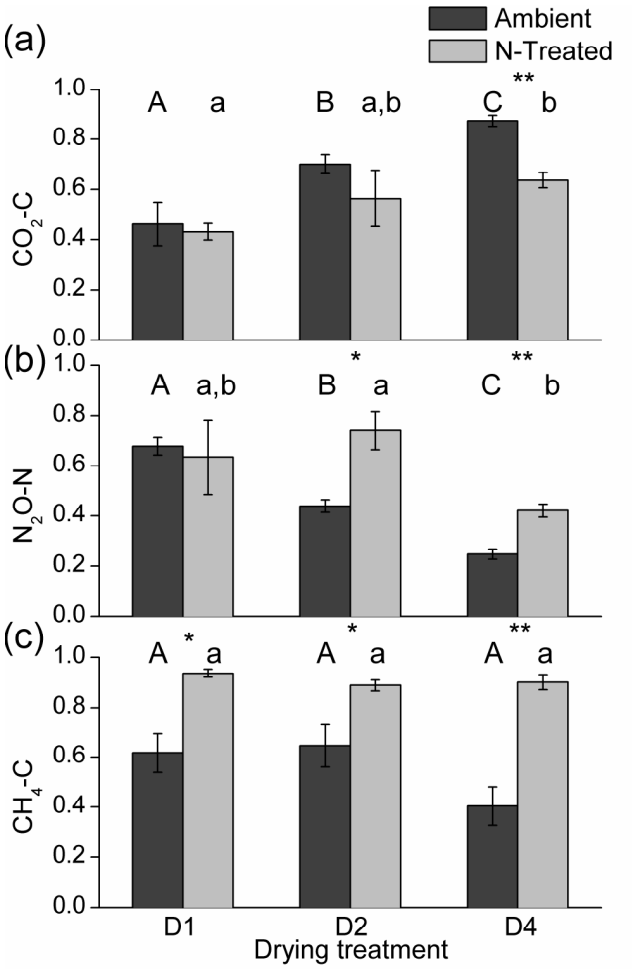

Figure 4. Orwin and Wardle Resistance index for soil: atmosphere CO_2-C (a), N_2O-N (b) and CH_4-C (c) exchange measured following incubation in ambient and N-treated soils. Values are means ± standard error. Different letters show statistically significant differences at $P < 0.05$. Asterisks indicate significant differences between N treatments (* means $p<0.05$, ** means $p<0.01$)

Microbial functional diversity

Soil N treatment significantly and positively affected the microbial functional diversity ($F=6.44$, $P=0.015$), whereas we did not find any effect of drying-rewetting treatment on this variable (Figure 5). The PCA did show an evident separation between N treatments, but it was not clear the discrimination of the drying-rewetting treatment (Figure 6).

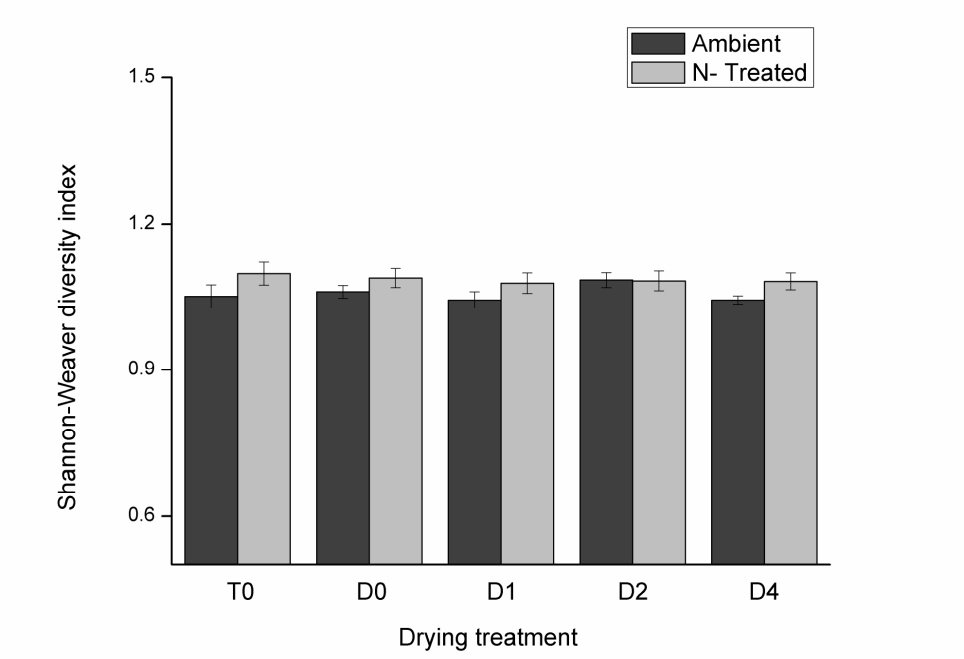

Figure 5. Shannon-Weaver soil microbial functional diversity index following incubation in ambient and N-treated soils. Values are means ± standard error.

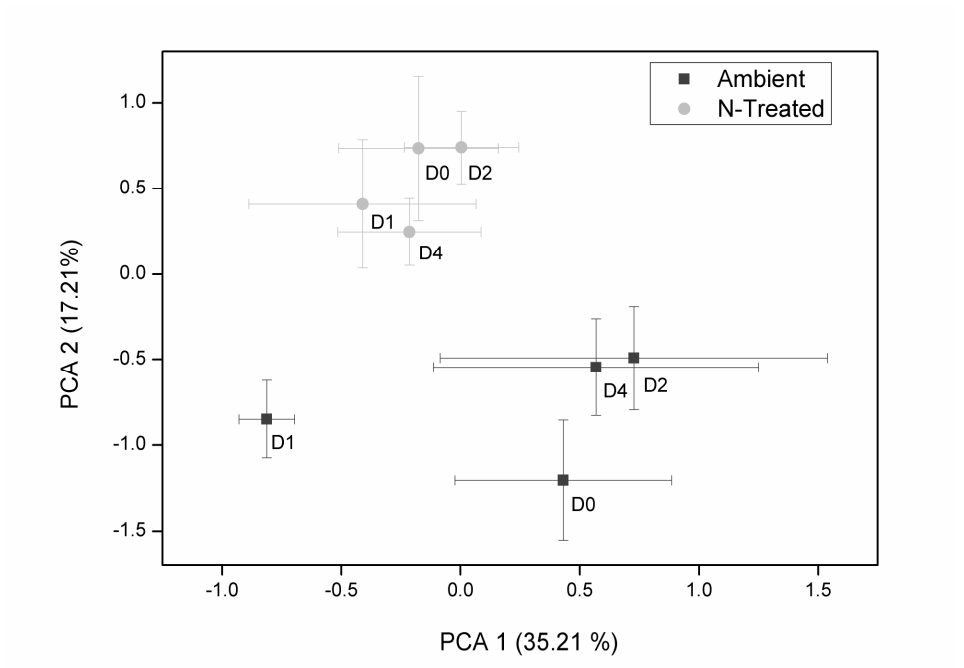

Figure 6. Principal-component analyses of soil microbial functional diversity data following incubation in ambient and N-treated soils.

Discussion

Our results support our first hypothesis, demonstrating that the projected climate change-driven increase in the frequency of drying-rewetting cycles affects the ability of soil to cycle C and N and the soil:atmosphere GHGs exchange. Our study also shows that not all studied variables are equally affected by different drying-rewetting frequency scenarios, with some variables such as microbial biomass N, N mineralization rate and microbial functional diversity appearing to be more resistant to changes, independently of the previous N supply conditions, than mineral N, microbial biomass C or trace gasses fluxes. Further, the drying-rewetting treatment affected more to the percent change and resistance of ambient than N-treated soils, which gives support to our second hypotheses about the modulator effect of the N supply conditions.

Different mechanisms could explain the observed increases in NH_4^+ and mineral N in both N treated and ambient soils in response to the increase in the number of drying-rewetting cycles. Frequent drying-rewetting can cause soil aggregate disruption and release of physically protected organic matter (Adu and Oades, 1978; Lundquist et al., 1999a). Through this mechanism, previously protected soil organic matter can be exposed to microbial attack and mineralization (Appel, 1998; Miller et al., 2005). Alternatively, frequent changes in soil moisture related to rewetting can induce microbes to undergo osmotic shock and cell lyses due to the rapid change in water potential (Harris 1981; Kieft et al., 1987). This decrease in the amount of microbes reduces the mineral N that is consumed, which facilitates its accumulation. Also, microbes surviving osmotic shock must release intracellular solutes (Halverson et al., 2000; Schimel et al., 2007) which involve labile N and C solutes that can be easily mineralized by the extant microbes, thus allowing a pulse of inorganic N (Birch, 1959; Kieft et al., 1987). It is interesting to note that the inorganic N pool consisted almost entirely of NH_4^+, and a flush of NH_4^+ related to soil rewetting has been earlier reported by others researches (Birch, 1959; Cabrera, 1993).

These results are consistent with the observed significant decrease in soil CO_2 emissions and microbial biomass C (in both N treatments), and in soil microbial respiration (in ambient soil) with the increased amount of drying-rewetting cycles. Microbes might have been suffering from the stress of frequent drying-rewetting treatments, which likely led to the above described substrate release and a decrease of

soil microbial respiration. This assumption is consistent with a number of studies that have found a decrease in microbial biomass after drying-rewetting events (Sorensen, 1983; Van Gestel et al., 1996), and support the idea that increased drying-rewetting cycles due to projected climate change will negatively affect the development of soil microbial populations (Xiang et al., 2008). Further, we show that, concurrently to the increase of microbial death, surviving microbes under stress may have modified its metabolism by accelerating nutrient (i.e. N) turnover (Mamilov and Dilly, 2002), which is consistent with other studies that exposed soil microbes to different types of stresses (Fließbach et al., 1994; Moreno et al., 1999; Pratt and Barreiro, 1998) and supports the idea that enhanced metabolism rates is likely a microbial unspecific response to the stress (Selye, 1950).

The contrasted difference between the slightly declining trend in microbial biomassin N-treated soils and the abrupt microbial decline in ambient soils responding to increased drying-rewetting cycles supports the hypothesized modulator effect of the previous N supply. This modulation might be due to the greater microbial functional diversity found in N-treated soils (compared to ambient soils), which has been shown to allow microbes to cope better with disturbances such as drastic osmotic shock upon soil rewetting (Allison and Martiny, 2008). Alternatively, the higher mineral N content found in the N-treated soils might have helped microbes to overcome the drying-rewetting stress better than in the ambient plots as the ability of soil microorganisms to regulate the osmotic pressure in presence of rapid changes in soil moisture relies in part in their capability to make a large investment of resources and, therefore, in the nutritional status of the soil (Bottner, 1985; Van Gestel et al., 1993; Schimel et al., 2007).

It has been proved that the frequency of drying-rewetting cycles has consequences for soil C mineralization rates (Fierer and Schimel, 2002; Mikha et al., 2005; Xiang et al., 2008). In our study, one drying-rewetting stress (D_1) consistently produced a significant increase in C mineralization (i.e. CO_2 emissions) respect to samples that not experience any stress (D_0). Further, C mineralization decreased as we increased the amount of drying-rewetting cycles from 1 to 4. These findings are consistent with a number studies that show decreases in C mineralization rates when increasing the frequency of drying-rewetting cycles (Magid et al., 1999; Fierer and Schimel, 2002; Mikha et al., 2005). The pattern found in the response of CO_2 emissions in our study could be explained by the combination of two different mechanisms. First, the disruption of the soil structure and physically protected organic matter with the

first drying-rewetting cycle, which could lead to an increase in the material available for microbes and, therefore, the mineralization of C. Then, after a series of drying-rewetting events, this labile organic matter is reduced by the microbial uptake, diminishing the size of the CO_2 pulse. Second, an increase in microbial mortality as we enhance the frequency of drying-rewetting cycles, supported by the observed decrease in C microbial biomass, could also be behind the C mineralization response (Fierer and Schimel, 2003).

The effect of drying-rewetting cycles on NO_3^- was roughly parallel to that on potential nitrification rate, C mineralization and C microbial biomass, suggesting a notable decrease in the nitrifier population in frequently stressed soils. Other studies found similar results following repeated drying-rewetting cycles (Franzluebbers et al., 1994; Xiang et al., 2008). This finding is also supported by the observations of Stark and Firestone (1995), who concluded that nitrifiers are highly sensitive to moisture stress. Since soil NO_3^- and/or its production have been demonstrated to largely determine denitrification rates, the observed reductions in the NO_3^- content and in the nitrification rate with the frequency of drying-rewetting cycles could be linked to the observed decrease of N_2O emissions in our soils with the drying-rewetting cycles increase. Although the soil moisture in our incubations should not have led to extended anaerobic conditions (a traditionally assumed requirement for denitrification processes), it has been recently proved that small areas suffering brief periods of low O_2 availability can be of great relevance emitting significant amounts of N_2O, especially in N-rich soils (McClain et al., 2003; Groffman et al., 2009). In addition, Groffman (2012) demonstrated that soil texture and N and C mineralization may produce large spatial and temporal variation in N_2O emissions; and Robertson et al. (1995) showed that heterotrophic nitrifiers bacteria such as *Alcaligenes faecalis* and *Thiosphaera pantotrophacan* denitrify under aerobic conditions. Regarding the comparison between soils, N treatment significantly increased the N_2O emissions compared to ambient soils, adding more evidence to the dependence of denitrification on the availability soil NO_3^- (Flessa and Beese, 2000).

Long-term N enrichment diminished soil CH_4 uptake, whereas the direction of the effect of drying-rewetting cycles on this gas fluxes varied depending on the soil N previous conditions. The main driver of soil CH_4 uptake is accepted to be the soil physical conditions that determine soil diffusion rates (Ridgwell et al., 1999), but inorganic N availability have also been recognized to affect this flux (Steudler et al.,

1989; Mosier et al., 1991). Consistent with our results, previous studies have also reported reduced CH_4 uptake in N fertilized soils due to additional C substrates compared to unfertilized soils (Lu et al., 2000; Zheng et al., 2007). We observed an increase in the CH_4 uptake in frequently stressed N-treated soils, but the opposite trend in ambient soils. These findings highlight the importance of the increased soil N inputs on the climate change through its effect on greenhouse gasses emissions, but also suggest previous soil N conditions as a significant modulator of the drying-rewetting stress effect on something so important such as the soil feedback to the climate change.

The percent change of most of the variables was affected by the drying-rewetting cycles. In addition, when analyzing N treatments separately, both the percent change and the RS index show that the ambient soils were consistently more sensitive to the drying-rewetting treatment. These findings add further evidence to the idea that while the intensification of drying-rewetting cycles will trigger changes in ecosystem processes (Fenn et al., 1998; Driscoll et al., 2003; Borken and Matzner, 2009), higher N supply may contribute to stabilize crucial ecosystem processes, hence making soils more resistant to the projected climate change. Interestingly, while in ambient soils, the percent change and the resistance of both C- and N-related variables were equally affected by the drying-rewetting treatment, in N-treated soils none of the C-related variables were affected. This unexpected finding seems to add a new dimension to the widespread idea that N addition can have a stabilization effect on soil C (Janssens et al. 2010; Rodríguez et al., under review).

The long-term N addition not only had an effect in the size and functionality of the microbial population, but also in the soil microbial functional diversity. The increased microbial functional diversity observed in N-treated soils compared with ambient soils contrasts with previous studies that reported a decrease in microbial diversity with N additions (Campbell et al., 2010) or no significant effects of this treatment on bacterial diversity (Ramirez et al., 2010; Fierer et al., 2011). These different findings among studies suggest that the effects of N amendments on bacterial diversity levels are variable and likely site-dependent (Fierer et al., 2011). On the other hand, we did not find a change in the functional diversity of soil microbial community due to the increased frequency of drying-rewetting cycles. Three mechanisms found in literature may explain this lack of response. First, microbial communities are metabolically very flexible and physiologically tolerable to environmental changes resulting in microbial populations surprisingly resistant to change (Meyer et al., 2004). Second, soil microbial

adaptation to drying-rewetting stress can occur without change in its diversity by developing strategies such as the building of thicker polysaccharide layers (Roberson and Firestone, 1992) or the accumulation of higher levels of osmoprotective solutes (Kempf and Bremer, 1998). And third, microbial communities can maintain their functionality despite stresses due to functional redundancy (Allison and Martiny, 2008). It is important to note that while our soils were subjected to 16 years of N addition, they just experienced the different drying-rewetting stresses for a short-term period. Thus, how these soils microbial diversity and functionality would response to long-term drying-rewetting and N addition interactive stresses is yet to be explored.

We conclude that the ability of temperate forests soils to cycle C and N will be significantly altered by the changes in precipitation patterns projected by future climate change scenarios. Our results demonstrate that repeated drying-rewetting cycles are likely to modify C and N cycling through changes in soil inorganic N pools, C and N mineralization rates, and GHG emissions, with likely implications in the ecosystem function. Further, our study highlights the key role of N supply as modulator of the responses of soil variables to increased drying-rewetting cycles. While the atmospheric N deposition could minimize the impacts of the expected increase in drying-rewetting cycles with the climate change on important soil processes, this modulator effect could be stronger for the C than the N cycle. Our results emphasize that a better understanding of the interactive effect of different global change factorsis necessary in order to accurately model the effects of the global change on ecosystem processes.

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8. DISCUSIÓN GENERAL

A lo largo de esta tesis hemos analizado los efectos que los cambios en el patrón de precipitación pueden inducir a diferentes niveles de los ciclos de nutrientes, los microorganismos del suelo y la emisión de gases de efecto invernadero, desencadenando una serie de respuestas que pueden llegar a afectar en última instancia a la dinámica y el funcionamiento del ecosistema. Además, hemos determinado la gran importancia del efecto modulador tanto de la costra biológica del suelo como de la deposición atmosférica de N sobre las respuestas de las variables de estudio a los cambios en los ciclos de secado y rehumedecido del suelo. A continuación, se discutirán tanto los patrones comunes como las diferencias entre los distintos ecosistemas y tratamientos aplicados encontrados en los distintos capítulos de esta tesis.

En los capítulos 1, 2, 4 y 5 se evalúa cómo responde el pool de N y su disponibilidad a distintos cambios en el patrón de precipitación. En todos ellos encontramos una evidente respuesta de estas variables a los cambios en la humedad del suelo. Tanto en el capítulo 4 como en el 5, encontramos que estos cambios en el patrón de precipitaciones provocarían un importante efecto sobre las tasas metabólicas microbianas. Sin embargo, mientras que en el capítulo 4 se muestra una intensificación de estas tasas, en el capítulo 5 encontramos la tendencia opuesta. Probablemente estas diferentes respuestas estén debidas a las grandes diferencias que hay entre un ecosistema semiárido (zona de estudio del capítulo 4) y uno templado (zona de estudio del capítulo 5). A pesar de esta disimilitud, podemos concluir que en las tres zonas de estudio, que abarcan una amplia gama de ecosistemas, la actividad microbiana está principalmente limitada por la humedad del suelo. Esta dependencia de la actividad microbiana del contenido hídrico del suelo en diversos ecosistemas ha sido observada previamente por otros investigadores (Van Gestel et al. 1993, Jenerette & Chatterjee, 2012; Curlevski et al., 2014). Esta consistente dependencia probablemente se deba a que en todos los ecosistemas el rehumedecido del suelo supone un estrés (más o menos fuerte) para los microorganismos, obligándolos a hacer una inversión energética para sobrevivir. Estos costes energéticos pueden llegar a tener importantes consecuencias sobre el funcionamiento de los ecosistemas (Shimel et al. 2007).

Mientras que en el capítulo 1 concluíamos que un futuro con ciclos de secado y rehumedecido del suelo más intensos y frecuentes podría llegar a producir grandes

pérdidas de N inorgánico en ecosistemas mediterráneos, nuestros resultados en un ecosistema semiárido (capítulo 4) y en uno templado (capítulo 5) indican la tendencia opuesta. Debido a la similitud entre el ecosistema semiárido y los mediterráneos, esperábamos que respondieran a los cambios en el patrón de las precipitaciones de una forma parecida, y a la vez diferente al ecosistema templado. El hecho de que el capítulo 1 se haya llevado a cabo en el campo con un diseño experimental no manipulativo, y los capítulos 4 y 5 se hayan realizado en el laboratorio sometiendo a las muestras de suelo a distintos tratamientos de riego, nos lleva a pensar en la posibilidad de que estas diferencias se deban a disimilitudes en los diseños experimentales y en los tratamientos aplicados. Previas investigaciones han relacionado las diferencias en las respuestas de los procesos del suelo a los diseños experimentales manipulativos (Borken & Matzner, 2009).

Los resultados obtenidos en el capítulo 4 sugieren que los microorganismos nitrificantes no son especialmente sensibles al estrés hídrico, sin embargo en el capítulo 5 encontramos una notable disminución en la población nitrificante en los suelos frecuentemente estresados por los ciclos de secado y rehumedecido del suelo. La bibliografía referente a este tema es controvertida. Por un lado, hay estudios que destacan el hecho de que los organismos nitrificantes son en su mayoría gram negativos (Schimel 1995b) y por lo tanto son más sensibles al estrés que otros microbios (Franzluebbers et al., 1994; Xiang et al., 2008). Otros estudios respaldan que los procesos de mineralización se ven menos afectados por el estrés hídrico que otros procesos microbianos (Reynolds et al., 1999; Smolander et al., 2005). Nuestros resultados sugieren que los microorganismos nitrificantes de ecosistemas semiáridos podrían estar más adaptados al estrés hídrico que los de ecosistemas templados, ya que están sometidos a ciclos de secado y rehumedecido del suelo más frecuentes.

Un estudio reciente desarrollado por Jenerette & Chatterjee (2012) ha sugerido la idea de que las respuestas a los ciclos de secado y rehumedecido del suelo son desencadenadas por el humedecido del suelo, pero están reguladas por la limitación de recursos. Ya que los capítulos 1, 2 y 3 de esta tesis se han llevado a cabo en dos ecosistemas mediterráneos con diferentes fertilidades, hemos querido contrastar esta hipótesis en nuestra zona de estudio. Dado que el suelo del pinar tenía una mayor disponibilidad de recursos que el del matorral, tales como C lábil y pool de nutrientes (Austin, 2011; Collins et al., 2008; Ma et al., 2012), nosotros esperábamos encontrar una mayor respuesta a los pulsos de humedad en el pinar. Sin embargo, en los capítulos 1 y

2 encontramos que la variabilidad del pool de nutrientes fue similar en los dos ecosistemas, y que por lo tanto la variabilidad de las variables de estudio no estaba asociada al tamaño del pool de C y N lábil disponible para los procesos microbianos, dando poco apoyo a esta hipótesis. Por otro lado, en los capítulos 2, 3 y 5 hemos encontrado un importante papel de la fertilidad del suelo en la respuesta de la actividad microbiana a los pulsos de humedecido. En el capítulo 2, la actividad microbiana ha resultado estar limitada por la disponibilidad de fosfato en el matorral, lo que ha minimizado el efecto desencadenador del pulso de humedad del suelo. En base al consumo de las fuentes de C en el capítulo 3, las muestras de suelo del pinar mostraron una mayor diversidad de respuestas que el matorral, probablemente debido a la menor fertilidad de los suelos del matorral, que evita que los microbios respondan de una forma más rápida y diversa a las adiciones de C. Finalmente, en el capítulo 5, encontramos una mayor diversidad funcional microbiana en los suelos tratados con N que en los suelos control, esto ha podido ayudar a los microorganismos del suelo a superar el estrés hídrico asociado a los ciclos de secado y rehumedecido facilitando la regulación de la presión osmótica. Como consecuencia, la biomasa microbiana del suelo tratado con N se ha visto menos perjudicada por la alta frecuencia de estreses hídricos que la del suelo control.

En los capítulos 1, 2 y 4 hemos utilizado resinas de intercambio iónico para estimar el efecto de los eventos de humedecido en la disponibilidad de nitrógeno del suelo. Esta técnica que estima la toma de nutrientes por parte de las plantas (Duran et al., 2013a) ha resultado ser una herramienta muy útil para determinar la mineralización de nitrógeno en el suelo. En general, tener en cuenta las tasas de difusión ha mejorado nuestro entendimiento de las respuestas del pool de N a los cambios en el patrón de precipitación. Éste método gana importancia en ecosistemas limitados por agua como es el caso de ecosistemas mediterráneos o semiáridos debido a la relevancia de la difusión del suelo para la disponibilidad de nutrientes.

En esta tesis, tanto en el capítulo 2 como en el 5, hemos evaluado la respuesta de la respiración del suelo (medida como la emisión de CO_2) a los ciclos de secado y rehumedecido. Las consecuencias de estos ciclos sobre esta variable han sido previamente estudiadas en diferentes ecosistemas (Fierer and Schimel, 2002; Mikha et al., 2005; Xiang et al., 2008). Mientras que en el capítulo 2 encontramos que durante la época lluviosa, en la que se produjeron frecuentes ciclos de secado y rehumedecido del suelo, la respiración aumentó en el pinar y disminuyó en el matorral, en el capítulo 5

encontramos la misma tendencia que en el matorral. Comparando los resultados de estos dos capítulos, podemos concluir que el efecto de los ciclos de secado y rehumedecido sobre la respiración del suelo es específica del ecosistema y que únicamente la frecuencia de los ciclos no puede explicar esta respuesta.

Dado que no todos los miembros de la comunidad microbiana del suelo son igualmente capaces de superar el estrés asociado con los eventos de rehumedecido (Lundquist et al., 1999b; McLean & Huhta, 2000), cabe esperar algún tipo de presión selectiva hacia los grupos de microorganismos que son más capaces de controlar los choques osmóticos, como por ejemplo hongos o bacterias gran positivas (Harris, 1981). Previos estudios han encontrado que ciclos de secado y rehumedecido del suelo más frecuentes con respecto al control, pueden modificar la composición de la comunidad microbiana (Chung et al. 2007; Allison & Martiny 2008; Zelikova et al. 2012). En los capítulos 3 y 5 de esta tesis, hemos estudiado la diversidad funcional microbiana mediante el índice de Shannon- Weaver. Mientras que en el capítulo 3 hemos observado como la diversidad funcional varía estacionalmente, en el capítulo 5 no hemos encontrado ninguna diferencia en esta variable en respuesta a la mayor frecuencia de ciclos de secado y rehumedecido del suelo. Dos motivos podrían explicar estos resultados. Por un lado, es posible que las comunidades microbianas no sean capaces de verse modificadas rápidamente en respuesta a un cambio brusco en el patrón de precipitaciones, si no que varíen gradualmente a lo largo de las estaciones. Por otro lado, las diferencias entre ecosistemas mediterráneos (zona de estudio del capítulo 3) y templados (zona de estudio del capítulo 5) también pueden explicar los resultados encontrados en estos capítulos.

De los dos primeros capítulos de esta tesis hemos podido deducir la gran relevancia de los ciclos de secado y rehumedecido del suelo en los ecosistemas mediterráneos, ya que tanto los nutrientes como la respiración del suelo han resultado depender en mayor medida de los eventos de secado y rehumedecido del suelo que de diferencias estacionales. Estos resultados subrayan la importancia de las consecuencias sobre los procesos del suelo que los cambios en el patrón de precipitación pueden llegar a inducir en los ecosistemas mediterráneos (Fierer & Schimel, 2002; Casals et al., 2009; Ouyang & Li, 2013)

En esta tesis hemos querido testar la hipótesis de Schwinning & Sala (2004), que sugiere que en ecosistemas semiáridos, existe una jerarquía de los eventos de los pulsos

de humedad en el suelo que se corresponde con una jerarquía de las respuestas ecológicas, de forma que pulsos cortos de humedecido desencadenan respuestas ecológicas menores y pulsos más largos desencadenan respuestas ecológicas mayores y más complejas. Para ello, en los capítulos 1, 2 y 4 hemos evaluado el efecto de la longitud de los eventos de humedecido del suelo sobre las variables de estudio. En el capítulo 1 encontramos una correlación negativa entre la longitud de los eventos de humedecido y la biomasa microbiana de suelo en el pinar, mientras que en el matorral esta correlación fue positiva para el nitrato. En ninguna de las variables estudiadas en el capítulo 2 encontramos ningún efecto de la longitud del humedecido. En el capítulo 4 observamos como los eventos de humedecido más largos produjeron un aumento del N mineral y del C orgánico en el suelo, sugiriendo un aumento de las tasas metabólicas microbianas tales como la descomposición y la mineralización. Estos distintos patrones en estos tres diferentes ecosistemas indican que los cambios en los procesos del suelo en respuesta a la longitud de los eventos de humedecido no pueden ser explicados por este único mecanismo, y que otros procesos y factores intervienen en estas respuestas.

En los capítulos 4 y 5 de esta tesis se estudia el papel modulador de la costra biológica del suelo y de la adición de nitrógeno respectivamente. Mientras que en el capítulo 4 hemos observado cómo la costra modula las tasas metabólicas microbiana del suelo, en el capítulo 5 hemos encontrado un efecto tampón de la adición de N al aumento en la frecuencia de los ciclos de secado y rehumedecido del suelo, así como un incremento de la diversidad funcional microbiana. Nuestros resultados indican que los dos factores evaluados cambiaron las respuestas de los procesos del suelo estudiados a los cambios en el patrón de precipitación, evidenciando de esta forma su relevancia como moduladores de las respuestas del suelo. Por lo tanto, la prevista disminución en la cobertura de la costra biológica del suelo (Maestre et al. 2013) y el aumento en la deposición atmosférica de nitrógeno (Schlesinger 2013) pueden llegar a tener consecuencias sobre los balances de los ciclos de C y N en el suelo.

En general, todos los capítulos de esta tesis coinciden en que dado que el contenido hídrico determina la actividad microbiana del suelo (Austin et al, 2004; Morillas et al. 2013), un cambio en el patrón de precipitaciones como el que los modelos del cambio climático predicen, puede llegar a modificar la estructura y la función de la comunidad microbiana del suelo, así como el balance de C y N y la emisión de gases de efecto invernadero no sólo en ecosistemas limitados por la

disponibilidad de agua (Rustad et al. 2000; Easterling et al. 2000), sino también en otro tipo de ecosistemas como los templados.

9. CONCLUSIONES GENERALES

1. En los cuatro ecosistemas que se han estudiado en esta tesis, la actividad microbiana ha demostrado estar principalmente limitada por la humedad del suelo. Dado que las comunidades microbianas, en última instancia, son las que determinan los ciclos biogeoquímicos, los cambios en el patrón de precipitaciones podrían llegar a afectar al balance de los ciclos del N y del C.

2. Los diseños experimentales manipulativos comparados con los no manipulativos pueden producir resultados inconsistentes e incluso contradictorios, probablemente debido a variaciones en el diseño experimental, temperaturas y humedades de incubación, propiedades del suelo y tratamientos.

3. Los microorganismos nitrificantes de ecosistemas semiáridos podrían estar más adaptados al estrés hídrico que los de ecosistemas templados, ya que están sometidos a ciclos de secado y rehumedecido del suelo más frecuentes.

4. Tomar en consideración las tasas de difusión mediante el uso de resinas de intercambio iónico ha mejorado nuestro entendimiento de las respuestas del pool de N a los cambios en el patrón de precipitación, especialmente en ecosistemas limitados por agua debido a la relevancia de la difusión del suelo para la disponibilidad de nutrientes.

5. El efecto de los ciclos de secado y rehumedecido sobre la respiración del suelo es específica del ecosistema, y únicamente la frecuencia de los ciclos no puede explicar esta respuesta.

6. Es posible que las comunidades microbianas no sean capaces de verse modificadas rápidamente en respuesta a un cambio brusco en el patrón de precipitaciones, si no que varíen gradualmente a lo largo de las estaciones.

7. En los ecosistemas mediterráneos, los procesos del suelo parecen depender en mayor medida de los eventos de secado y rehumedecido del suelo que de diferencias estacionales, subrayando la importancia que los cambios en el patrón de precipitaciones pueden llegar a tener en estos ecosistemas.

8. La costra biológica del suelo modifica la respuesta del pool de N a los eventos de humedecido, produciendo mayores o más rápidas respuestas microbianas en comparación con el suelo desnudo. Estos resultados evidencian su efecto modulador y subrayan la relevancia de la costra como un factor clave en los ciclos del C y N en ecosistemas semiáridos.

9. Mientras que la deposición atmosférica de N puede minimizar el impacto sobre los procesos del suelo del aumento previsto de los ciclos de secado y rehumedecido con el cambio climático, este efecto modulador puede ser mayor para en ciclo del C que para el del N.

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