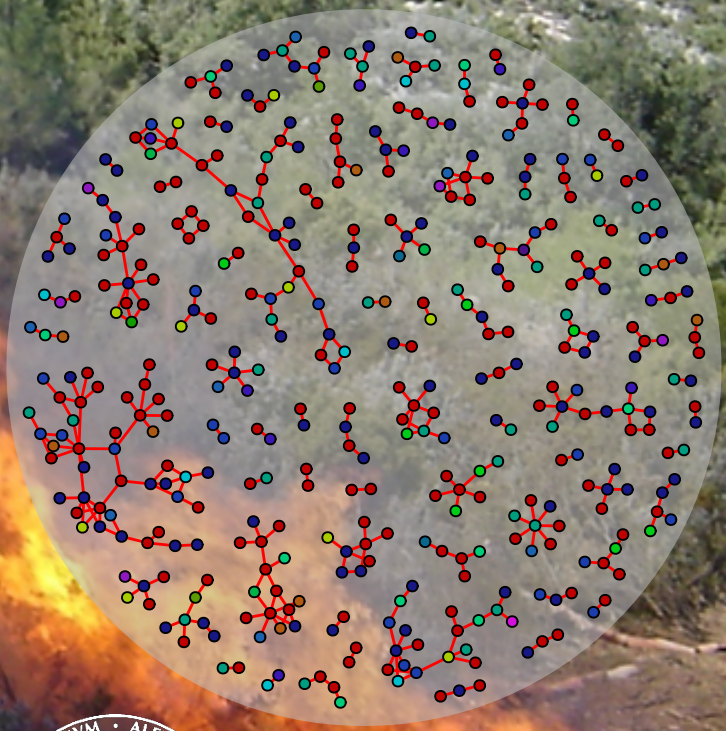
Fire and phylogenetic structure of soil microbial communities in Mediterranean ecosystems

Eduardo Pérez Valera

Tesis doctoral



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Fire and phylogenetic structure of soil microbial communities in Mediterranean ecosystems

TESIS DOCTORAL

Eduardo Pérez Valera

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Dirigida por: Dr. Miguel Verdú y Dra. Marta Goberna

Valencia, noviembre de 2017

Dr. Miguel Verdú y Dra. Marta Goberna, Investigador Científico e Investigadora Ramón y Cajal del Departamento de Ecología Vegetal del Centro de Investigaciones sobre Desertificación (CIDE)

CERTIFICAN:

Que la presente memoria titulada "Fire and phylogenetic structure of soil microbial communities in Mediterranean ecosystems", presentada por D. Eduardo Pérez Valera para optar al grado de Doctor por la Universidad de Valencia en el Programa de Doctorado en Biodiversidad y Biología Evolutiva, ha sido realizada bajo nuestra dirección en el Centro de Investigaciones sobre Desertificación –CIDE (Consejo Superior de Investigaciones Científicas, Universidad de Valencia, Generalitat Valenciana).

Considerando su nivel científico y académico, autorizamos a su presentación para optar al Grado de Doctor por la Universitad de Valencia en el Programa de Doctorado en Biodiversidad y Biología Evolutiva.

Y para que conste, firmamos el presente certificado en Moncada, a 20 de noviembre de 2017

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Index of contents

0. RESUMEN 1

1. GENERAL INTRODUCTION AND AIMS 15

1.1. INTRODUCTION 15

1.1.1. Assembly of ecological communities 15

1.1.2. Biodiversity and ecosystem functioning 18

1.1.3. Fire as an ecological disturbance 19

1.2. AIM AND OUTLINE OF THESIS 21

1.3. REFERENCES 22

2. MATERIALS AND METHODS 25

2.1. EXPERIMENTAL DESIGN AND STUDY SITES 25

2.2. ORGANISMS UNDER STUDY 27

2.3. STUDY OF SOIL MICROBIAL COMMUNITIES AND THEIR ENVIRONMENT 27

2.3.1. Physical and chemical soil analyses 28

2.3.2. Physiological and biochemical analyses 29

2.3.3. Microbial diversity 29

2.4. REFERENCES 32

CHAPTER I: Phylogenetic structure of soil bacterial communities predicts ecosystem functioning 33

3.1. INTRODUCTION 34

3.2. MATERIALS AND METHODS 36

3.2.1. Study site 36

3.2.2. Soil DNA extraction and pyrosequencing 38

3.2.3. Sequence analysis and phylogeny reconstruction 39

3.2.4. Phylogenetic community structure 40

3.2.5. Statistical analyses 41

3.3. RESULTS 42

3.4. DISCUSSION 47

3.5. ACKNOWLEDGEMENTS 49

3.6. REFERENCES 49

CHAPTER II: Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks 53

4.1. INTRODUCTION 54

4.2. RESULTS 58

4.2.1. Fire effects on the soil bacterial community 58

4.2.2. Fire effects on the soil bacterial co-occurrence networks 60

4.3. DISCUSSION 61

4.4. EXPERIMENTAL PROCEDURES 66

4.4.1. Study site and experimental fire 66

4.4.2. Soil DNA extraction and pyrosequencing 67

4.4.3. Sequence analysis and phylogeny reconstruction 68

4.4.4. Network analysis 68

4.4.5. Phylogenetic diversity 70

4.4.6. β-diversity analyses 71

4.4.7. Statistical analyses 71

4.5. ACKNOWLEDGMENTS 72

4.6. REFERENCES 73

CHAPTER III: Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities 77

5.1. INTRODUCTION 78

5.2. MATERIALS AND METHODS 80

5.2.1. Experimental design and fire effects on soil abiotic factors 80

5.2.2. DNA extraction and sequencing 81

5.2.3. Phylogeny reconstruction and phylogenetic community structure 82

5.2.4. Microbial indicators of ecosystem functioning 83

5.2.5. Statistical analyses 83

5.3. RESULTS 84

5.4. DISCUSSION 92

5.4.1. Fire and the phylogenetic structure of soil bacterial communities 92

5.4.2. Fire and microbial ecosystem functions 94

5.5. ACKNOWLEDGEMENTS 95
5.6. REFERENCES 96
CHAPTER IV: Phylogenetic diversity is resilient to fire across biological domains 101
6.1. INTRODUCTION 101
6.2. MATERIALS AND METHODS 105
6.2.1. Study area and experimental design 105
6.2.2. Plant sampling and phylogeny reconstruction 106
6.2.3. Soil sampling and sample analysis 106
6.2.4. DNA extraction and sequence processing 107
6.2.5. Sequence analysis and phylogeny reconstruction 108
6.2.6. Diversity metrics and phylogenetic composition 109
6.2.7. Statistical analysis 110
6.3. RESULTS 112
6.3.1. Fire effects on plant communities 112
6.3.2. Fire effects on soil conditions and microbial communities 113
6.3.3. Linkages between above- and belowground communities 117
6.4. DISCUSSION 118
6.5. ACKNOWLEDGMENTS 121
6.6. REFERENCES 122
7. GENERAL DISCUSSION 127
7.1. REFERENCES 130
8. CONCLUSIONS 133
APPENDICCES 135
APPENDIX A 137
APPENDIX B 153
APPENDIX C 157

List of publications derived from the thesis

- Pérez-Valera E, Goberna M, Verdú M. (2015). Phylogenetic structure of soil bacterial communities predicts ecosystem functioning. *FEMS Microbiol Ecol* **91**: fiv031.
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RESUMEN

El estudio de los procesos ecológicos implicados en el ensamblaje de las comunidades biológicas y su papel en las funciones de los ecosistemas es fundamental en la ecología de comunidades. El ensamblaje de comunidad, es decir, el conjunto de procesos mediante el cual las especies colonizan un lugar e interactúan entre sí y con su entorno para formar comunidades locales, determina su estructura y composición específica (HilleRisLambers *et al.,* 2012). Los procesos ecológicos implicados en el ensamblaje de especies (por ejemplo, filtrado de hábitat o competencia) se pueden reconocer a través de la "huella" que dejan en las comunidades (Diamond, 1975; Webb *et al.,* 2002; Mayfield y Levine, 2010). A su vez, dicha estructura de comunidad podría ser indicativa de los procesos del ecosistema que controlan los flujos de energía, nutrientes y materia orgánica (Balvanera *et al.,* 2006; Cardinale, 2013; Graham *et al.,* 2016). La comprensión de las fuerzas que controlan la diversidad biológica y, a su vez, el papel de la diversidad biológica en las funciones ecosistémicas es importante para pronosticar las consecuencias de las, cada vez más frecuentes, perturbaciones ecológicas. Esta tesis se centra en estas cuestiones fundamentales mediante el estudio de la estructura filogenética de las comunidades microbianas del suelo tras una perturbación por fuego.

Objetivos

El objetivo general de esta tesis doctoral consiste en analizar las bases ecológicas del ensamblaje de las comunidades microbianas y su relación con el funcionamiento ecosistémico, a través del análisis de la estructura filogenética de la comunidad y su resiliencia al fuego en ecosistemas mediterráneos. En concreto, los objetivos de esta tesis son los siguientes:

1. Determinar los parámetros físicos y químicos que subyacen a la estructura filogenética de las comunidades bacterianas del suelo.
2. Evaluar la estructura filogenética de las comunidades bacterianas del suelo como indicador del funcionamiento ecosistémico.
3. Investigar el fuego como fuerza ecológica que moldea el ensamblaje de las comunidades bacterianas.

4. Analizar los cambios provocados por el fuego en las funciones ecosistémicas que están mediadas por microorganismos.
5. Evaluar la resistencia o resiliencia al fuego de las comunidades ecológicas.

Materiales y Métodos

Diseño experimental y zonas de estudio

En esta tesis doctoral se analizaron un total de 27 zonas de estudio en ecosistemas de tipo mediterráneo, que se localizan en la Comunidad Valenciana (España). Los lugares de estudio incluyeron zonas de clima mediterráneo semiárido a sub-húmedo, vegetación de matorral y bosque, suelos con distintos materiales de origen como calizas, yesos y depósitos coluviales y parámetros del suelo con un amplio rango de pH, salinidad y fertilidad. El desarrollo de esta tesis doctoral requirió el uso de tres diseños diferentes.

En primer lugar, se realizó un muestreo observacional en el que se seleccionaron dos sitios con características contrastadas, tanto en las condiciones climáticas y de suelo como en la composición de plantas, con el objetivo de analizar los determinantes abióticos de la estructura filogenética de comunidades bacterianas y su relación con el funcionamiento ecosistémico (Capítulo I). En concreto, se muestrearon 10 parcelas de suelo de un matorral homogéneo (sitio 1) y 15 de parcelas de suelo situadas debajo de parches de vegetación, con sus correspondientes espacios adyacentes sin vegetación arbustiva (sitio 2). Para ello, en total se recolectaron un total de 40 muestras de suelo.

A continuación, el matorral homogéneo se sometió a un incendio experimental. Se muestrearon 10 parcelas antes y a distintos tiempos tras el fuego, incluyendo 1 día, 1 semana, 1 mes, 4.5 meses, 9 meses y 12 meses, con el objetivo de analizar las dinámicas temporales post-fuego en el ensamblaje de las comunidades microbiana (Capítulo II), y su relación con el funcionamiento ecosistémico (Capítulo III). Para ello, se obtuvieron un total de 70 muestras de suelo superficial (7 tiempos de muestreo × 10 parcelas). Las muestras recolectadas antes del incendio se usaron como control pre-fuego, con el

objetivo de reducir la heterogeneidad espacial que surge de muestrear áreas adyacentes. Además, incluimos en los modelos estadísticos la variación climática estacional (temperatura y precipitación), con el propósito de controlar dichos efectos en las dinámicas temporales de las comunidades microbianas.

Finalmente, un diseño observacional basado en cronosecuencias de fuego se utilizó con el objetivo de analizar las dinámicas post-incendio y la resiliencia al fuego de múltiples dominios biológicos (Capítulo IV). Para ello, se seleccionaron un total de 25 zonas que se vieron afectadas por un único incendio no prescrito entre los años 1994 y 2014, las cuales se agruparon en 3 cronosecuencias, de acuerdo a su localización geográfica y sus condiciones ambientales. Debido a la ausencia de muestras anteriores a los incendios, se utilizaron controles espaciales en lugar de temporales. En concreto, se utilizó un diseño pareado, en el que por cada sitio quemado se muestreó un sitio adyacente similar pero no afectado por el incendio. Se muestreó un total de 3 transectos por cada parcela quemada y control, con el objetivo de tener en cuenta la variación dentro de cada tratamiento. En total, se obtuvieron 150 muestras de suelo (25 sitios × 2 tratamientos × 3 transectos).

Organismos objeto de estudio

Las bacterias del suelo fueron el principal grupo biológico estudiado en los Capítulos I, II y III. Con el objetivo de ampliar el conocimiento sobre la resiliencia al fuego de las comunidades biológicas, se incorporaron otros organismos del suelo (hongos y arqueas) así como las comunidades de plantas en el capítulo IV.

Análisis de las comunidades microbianas y su ambiente

Las comunidades microbianas se estudiaron a distintos niveles, incluyendo su relación con el ambiente abiótico del suelo, sus efectos en las funciones ecosistémicas y el papel de las relaciones evolutivas entre linajes en múltiples dominios biológicos.

Se analizaron diversos parámetros físicos y químicos del suelo, con el objetivo de averiguar su influencia en la estructura filogenética de las

comunidades microbianas. En concreto, se analizó el pH del suelo, la humedad gravimétrica, la conductividad eléctrica y el contenido en carbono orgánico total (TOC, acrónimo en inglés de *Total Organic Carbon*), carbono oxidable por pirofosfatos, nitrógeno total, amonio (NH_4^+-N) y nitratos (NO_3^--N), siguiendo procedimientos estandarizados (ver el capítulo IV para más detalles).

Un conjunto de variables fisiológicas y bioquímicas del suelo fueron utilizadas como indicadores de funciones ecosistémicas mediadas por microorganismos. En concreto, se analizaron el carbono de la biomasa microbiana (MBC, *Microbial Biomass Carbon*), el coeficiente microbiano (la ratio MBC/TOC), la respiración basal y el cociente metabólico (qCO_2), como indicadores de actividad microbiana general. La variable MBC se utilizó como una estima de la biomasa microbiana; el coeficiente MBC/TOC como una medida de la eficiencia de conversión de carbono orgánico en carbono microbiano; la respiración basal como indicativo de la actividad de los descomponedores del suelo, que mineralizan carbono orgánico en CO_2; y el qCO_2 como respiración microbiana por unidad de biomasa (Nannipieri *et al.,* 1990). Se utilizaron tres actividades enzimáticas, es decir, β-glucosidasa (GA), fosfatasa alcalina (PA) y ureasa (UA), como indicadores de funcionalidad ecosistémica relacionados con el ciclado de C, P y N, respectivamente. En concreto, la enzima β-glucosidasa cataliza la descomposición de los compuestos de celulosa, la fosfatasa alcalina la hidrólisis de enlaces éster-fosfato liberando fósforo inorgánico y la ureasa la hidrólisis de la urea, que libera amoníaco y dióxido de carbono (Kandeler y Gerber, 1988; Tabatabai, 1994). Información adicional sobre las variables fisiológicas y bioquímicas analizadas se puede encontrar en los Capítulos I y III.

Diversidad microbiana

La diversidad microbiana se analizó mediante la secuenciación de marcadores moleculares. A continuación, se llevó a cabo el análisis a nivel de comunidad, con la ayuda de las técnicas que se detallan a continuación.

En primer lugar, se empleó metagenómica dirigida (*targeted metagenomics*) para la amplificación, por medio de PCR, de marcadores moleculares

específicos a partir de DNA extraído de muestras de suelo, con el fin de identificar y obtener las abundancias relativas de los distintos grupos microbianos del suelo. En el caso de bacterias, se utilizaron cebadores específicos que amplificaron una región del gen 16S rRNA, que codifica la subunidad pequeña del ribosoma de procariotas (Capítulos I-III). De manera similar, se utilizaron cebadores universales de procariotas para analizar de manera conjunta las comunidades de bacterias y arqueas (Capítulo IV). Finalmente, amplificamos la región del espaciador transcribible interno ribosómico nuclear (ITS) de organismos eucariotas para identificar y caracterizar las comunidades de hongos (Capítulo IV).

Las filogenias de organismos procariotas se reconstruyeron a partir de secuencias del gen 16S rRNA, que constituye un marcador filogenético ampliamente utilizado. Con el propósito de tener en cuenta la incertidumbre en la filogenia que resulta del uso de secuencias cortas, se constriñó la topología de árbol a niveles filogenéticos profundos (es decir, a nivel de filo para la mayor parte de taxones y de clase para la mayoría de Proteobacterias), de acuerdo con filogenias publicadas, construidas a partir de secuencias completas del gen 16S rRNA. Además, se utilizaron entre 3 y 5 réplicas de árboles por estudio. Los árboles filogenéticos de procariotas en los capítulos I, II y IV representan la divergencia de unidades taxonómicas operativas (OTUs, *Operational Taxonomic Units*) expresadas como sustituciones nucleotídicas. Sin embargo, hallazgos recientes en la evolución temporal de los procariotas (Marín *et al.,* 2017) nos han permitido avanzar hacia la datación de nuestras filogenias, transformando las tasas de sustitución nucleotídica en tiempo cronológico (millones de años) (Capítulo III).

Las filogenias de hongos se reconstruyeron utilizando la información publicada sobre las relaciones evolutivas a nivel de género. Sobre este árbol, injertamos los OTUs en base a la información taxonómica obtenida mediante el análisis de la región ITS. Estimamos las longitudes de las ramas de los árboles a partir de varios nodos datados de acuerdo a la literatura. Se utilizaron un total de 5 árboles filogenéticos, seleccionados al azar a partir de múltiples árboles que se construyeron con el fin de tener en cuenta la incertidumbre en la topología y la cronología.

Métricas de diversidad taxonómica y filogenética

Las comunidades microbianas se analizaron utilizando métricas taxonómicas y filogenéticas de diversidad α y β. En primer lugar, se utilizó el número OTUs por muestra como una medida de la diversidad taxonómica α (capítulos II y IV). Además, se utilizaron métricas de estructura de comunidad ponderadas por la filogenia, como el índice de parentesco neto (NRI, N*et Relatedness Index*), indicativo de la distancia filogenética media estandarizada por cada muestra. Esta métrica informa de las relaciones evolutivas de los linajes; es decir, si los linajes de una comunidad están más (agrupamiento filogenético) o menos (sobredispersión filogenética) relacionados evolutivamente de lo esperado por azar (Capítulo I). Por último, se usó -NRI como una medida de la diversidad filogenética α (capítulos II y IV).

Las métricas de divergencia taxonómica y filogenética entre comunidades (β-diversidad) se usaron adicionalmente con el objetivo de analizar el papel de los microorganismos en el funcionamiento ecosistémico (Capítulo I) y la resiliencia al fuego de las especies (Capítulos II y IV). Además, se utilizaron los componentes reemplazamiento (*turnover*) y anidamiento (*nestedness*) de la diversidad β a través del tiempo tras el fuego para evaluar las tasas de reemplazo taxonómico de especies (Capítulo II).

Las relaciones filogenéticas se incorporaron a los análisis de diversidad β, a través de i) la composición filogenética ponderada por la representatividad de los linajes (PCPS, capítulos I y III), y por medio de ii) distancias UNIFRAC, que permiten una mejor comprensión de la resiliencia ecológica ante una perturbación (Capítulo IV).

El análisis de redes de coexistencia se usó para detectar los OTUs que coexistieron más (copresencia) o menos (exclusión mutua) de lo esperado por azar (Faust y Raes, 2012). Este análisis permitió la transformación de las correlaciones en las abundancias entre pares de taxones de la comunidad en enlaces de coexistencia o exclusión mutua. La incorporación de un marco filogenético a las redes de coexistencia permitió analizar los procesos que

estructuran las comunidades microbianas antes y después de un incendio (Capítulo II).

Por último, se validó la capacidad de inferir procesos de ensamblaje a partir de patrones de coexistencia mediante simulaciones (Capítulo II, Apéndice A2). En concreto, se simularon filogenias, rasgos y abundancias de especies a partir de procesos conocidos de ensamblaje de comunidad, testando posteriormente la capacidad de la metodología para identificar correctamente el proceso de ensamblaje simulado. En el Apéndice A2 se puede encontrar una descripción detallada de los modelos de simulación (Capítulo II).

Resultados y discusión

En esta tesis doctoral se han estudiado los factores que determinan la estructura filogenética de las comunidades bacterianas del suelo y su resistencia al fuego, proporcionando información sobre los procesos implicados en el ensamblaje y su relación con el funcionamiento de los ecosistemas mediterráneos. En concreto, nuestros resultados muestran que conocer el papel que desempeñan ciertos linajes en la comunidad puede servir para comprender el ensamblaje de comunidades bacterianas y la productividad del ecosistema, lo que no resulta sencillo en los enfoques clásicos de la ecología de comunidades. Esto es posible, principalmente, gracias a la incorporación a los análisis de las relaciones evolutivas entre los linajes a nivel de comunidad.

Las propiedades abióticas del suelo, particularmente aquéllas relacionadas con la fertilidad y los niveles de carbono orgánico, determinaron la diversidad filogenética y la estructura de comunidad de las bacterias del suelo en los ecosistemas estudiados. Este resultado coincide con evidencias previas tanto observacionales como experimentales a nivel mundial, que sugieren que el carbono orgánico es uno de los principales factores que determinan la estructura de las comunidades bacterianas del suelo (Fierer, 2017). En concreto, encontramos que el aumento de los niveles de carbono orgánico, a menudo limitante en los suelos, alteró la composición de las comunidades bacterianas reduciendo su diversidad filogenética (Pérez-Valera *et al.,* 2015). Nuestros resultados son congruentes con observaciones globales que muestran que la

diversidad filogenética de las comunidades bacterianas del suelo tiende a ser menor de lo esperado por azar. Es decir, las bacterias del suelo tienden a coexistir con parientes cercanos, lo que se puede explicar por el predominio de filtros ambientales que operan a través de sus componentes bióticos y/o abióticos (Mayfield y Levine, 2010; Goberna *et al.,* 2014a). Además, los datos obtenidos sugieren que el filtro biótico prevalece como consecuencia de la sobrerrepresentación de clados extremadamente competitivos, como Proteobacterias y Actinobacterias, los cuales muestran una elevada eficacia biológica (*fitness*) en condiciones de alta disponibilidad de carbono, excluyendo completamente a otros linajes (Goldfarb *et al.,* 2011; HilleRisLambers *et al.,* 2012; Goberna *et al.,* 2014a; Pérez-Valera *et al.,* 2015). El hecho de que predominen las interacciones competitivas basadas en diferencias de *fitness* no excluye la posibilidad de que otros mecanismos actúen simultáneamente (por ejemplo, la competencia entre especies con nichos similares), incrementando la diversidad filogenética de la comunidad. Sin embargo, el efecto de las interacciones de competencia podría ser enmascarado si el filtro ambiental es el principal proceso de ensamblaje. En esta tesis, proponemos un nuevo marco para detectar los procesos de ensamblaje que operan simultáneamente mediante la combinación de herramientas de análisis filogenético y redes de coexistencia (Pérez-Valera *et al.,* 2017). En primer lugar, validamos este marco mediante simulación de comunidades y posteriormente lo aplicamos a comunidades reales, en las que observamos que tanto los taxones bacterianos que coexisten como los que se excluyen mutuamente tienden a estar filogenéticamente más emparentados de lo esperado por azar. Estos resultados coinciden con las predicciones de uno de nuestros escenarios de simulación, concretamente aquél en el que tanto el filtrado ambiental como las interacciones competitivas basadas en similitudes de nicho actúan simultáneamente para ensamblar las comunidades bacterianas del suelo (Pérez-Valera *et al.,* 2017).

El aumento detectado en la diversidad filogenética bacteriana en condiciones de baja concentración de carbono orgánico también se observó en zonas en los que el fuego disminuyó su disponibilidad (Pérez-Valera *et al.,* 2015, Capítulo IV). Los cambios en la composición bacteriana tras un incendio podrían reflejarse en las métricas de diversidad filogenética si los rasgos que

permiten la supervivencia de las especies o la superioridad competitiva están conservados filogenéticamente (Pausas y Verdú, 2010). Éste podría ser el caso de los microorganismos que poseen rasgos que les confieren tolerancia al ambiente o altas capacidades competitivas (Goberna *et al.,* 2014b; Martiny *et al.,* 2015; Goberna y Verdú, 2016). Tras los incendios estudiados, se observó un aumento generalizado en la diversidad filogenética de las comunidades de bacterias, lo que sugiere que los cambios en las comunidades bacterianas fueron filogenéticamente estructurados y, por lo tanto, reconocibles mediante el análisis de las relaciones evolutivas entre taxones que coexisten y que se excluyen mutuamente (Faust y Raes, 2012; Pérez-Valera *et al.,* 2017). De hecho, aunque el fuego impuso filtros abióticos que favorecieron los linajes microbianos con rasgos de resistencia a la temperatura, también aumentó simultáneamente las interacciones competitivas por medio de la liberación de nutrientes y/o la reducción del fuerte filtro biótico que domina en comunidades bacterianas en todo el mundo (Goberna *et al.,* 2014a; Pérez-Valera *et al.,* 2017). El análisis por medio de redes de coexistencia mostró resultados similares, lo que sugiere que los cambios en la diversidad filogenética tras el fuego es producto de alteraciones en el balance entre los procesos de filtrado ambiental y exclusión competitiva por similitud de nicho (Pérez-Valera *et al.,* 2017). Los escasos estudios que analizan el efecto del fuego sobre el ensamblaje de las comunidades microbianas revelan aumentos en la abundancia de los taxones resistentes a altas temperaturas (por ejemplo, organismos formadores de endosporas o con paredes celulares engrosadas) y de crecimiento rápido (por ejemplo, alto número de copias del operón rRNA), que se ven progresivamente desplazados competitivamente por otros organismos más eficientes en el consumo de carbono orgánico (Bárcenas-Moreno *et al.,* 2011; Jurburg *et al.,* 2017).

La incorporación de la identidad de los linajes a las métricas de estructura filogenética permitió conocer los factores abióticos que determinan la diversidad bacteriana, independientemente de la variabilidad ambiental de los ecosistemas objeto de estudio (Pérez-Valera *et al.,* 2015; Capítulo III). A diferencia de los valores promedio de distancias filogenéticas en una comunidad (es decir, NRI), que puede generar valores similares con comunidades completamente diferentes, otras métricas como el índice PCPS,

fueron útiles en todos los sitios de estudio para identificar la representatividad de los linajes (Duarte *et al.,* 2012). De hecho, nuestros resultados mostraron que el índice PCPS fue especialmente relevante para predecir las tasas de funcionalidad ecosistémica mediada por microorganismos, debido a su capacidad para capturar la "huella" que dejan los cambios ambientales en la composición de las comunidades microbianas y, a su vez, en su funcionalidad (Pérez-Valera *et al.,* 2015). De hecho, el índice PCPS detectó el papel que ciertos linajes altamente productivos (como Proteobacteria y Actinobacteria) juegan en los procesos del ecosistema (Pérez-Valera *et al.,* 2015). A pesar de que un aumento en la diversidad se relaciona con mayores tasas de productividad ecosistémica (Cardinale *et al.,* 2012), nuestros resultados mostraron una tendencia opuesta, probablemente debido al predominio de linajes altamente competitivos (que reducen la diversidad filogenética) y productivos (que aumentan las tasas de procesos del ecosistema). Este resultado subraya que conocer la identidad de los linajes es importante para comprender la relación entre funcionamiento ecosistémico y biodiversidad (Pérez-Valera *et al.,* 2015), especialmente tras una perturbación ecológica que altera las funciones principales del ecosistema.

El fuego alteró las principales funciones ecosistémicas mediadas por los microorganismos del suelo, a través de cambios en la estructura filogenética de las comunidades bacterianas. De hecho, nuestros resultados mostraron que el fuego incrementó la abundancia de ciertos linajes microbianos que respondieron al pulso de nutrientes, aumentando inmediatamente las tasas de respiración microbiana, biomasa y ciclo de nutrientes. Contrariamente a los incendios forestales, que reducen la biomasa y la actividad de las comunidades microbianas (Hernández *et al.,* 1997; Jiménez-Esquilín *et al.,* 2008), fuegos prescritos o experimentales, que son fuegos de baja intensidad, pueden provocar leves cambios o incluso aumentar la productividad microbiana y ciclado de nutrientes (p. ej. Fontúrbel *et al.,* 2012; Fultz *et al.,* 2016). Sin embargo, a diferencia de la funcionalidad ecosistémica, que recobró a medio plazo las tasas de productividad previas al incendio, la estructura filogenética de la comunidad bacteriana no se recuperó, lo que sugiere que podría existir un cierto grado de redundancia funcional (Allison y Martiny, 2008). Son necesarios más estudios para validar esta interpretación, especialmente en un contexto de

perturbación ecológica en el que la resiliencia de las especies puede ser clave para garantizar la funcionalidad del ecosistema. Explicaciones alternativas incluyen la posibilidad de que los taxones que forman parte de la comunidad tras la perturbación sean funcionalmente diferentes a los taxones en la comunidad original, pero resulten en una tasa equivalente de productividad o actividad ecosistémica a nivel de comunidad (Allison y Martiny, 2008). Estudios futuros que analicen la funcionalidad de cada taxon microbiano son necesarios para mejorar las predicciones de las tasas de los procesos ecosistémicos.

Por último, la estructura filogenética de las comunidades bacterianas en ecosistemas mediterráneos fue sensible pero resiliente al fuego en un período de dos a tres décadas. La recuperación de las comunidades bacterianas requirió el restablecimiento de las jerarquías competitivas que se establecen en las comunidades bacterianas; es decir, la recuperación del filtro biótico causado por linajes altamente competitivos (Goberna *et al.,* 2014a; Pérez-Valera *et al.,* 2017). Nuestros resultados mostraron que dicho restablecimiento fue mediado principalmente por la comunidad de plantas, mediante el aporte de carbono orgánico al suelo en forma de hojarasca y exudados. Esto sugiere que dichos aportes orgánicos, que son los responsables de la diversidad y estructura filogenética de bacterias, constituyen un factor esencial que garantiza su recuperación tras un fuego. En conjunto, nuestras observaciones confirman la capacidad que tiene la información filogenética para predecir los cambios en la composición y funcionalidad microbianas, lo que es esencial ante las crecientes tasas de cambio global.

Conclusiones

1. Las propiedades abióticas del suelo, especialmente aquéllas asociadas con la fertilidad, determinan la diversidad filogenética y la estructura de comunidad de las bacterias del suelo. Dichas métricas de diversidad, a su vez, son capaces de predecir las funciones ecosistemas mediadas por la microbiota relacionadas con las tasas de productividad, descomposición y el ciclado de nutrientes, particularmente cuando se tiene en cuenta la identidad del linaje. El signo de la relación entre la diversidad filogenética bacteriana y las funciones del ecosistema

depende de la identidad taxonómica de los principales linajes que coexisten.

2. El filtrado ambiental y la exclusión competitiva por similitud de nicho actúan simultáneamente para ensamblar las comunidades bacterianas del suelo. El fuego, a través de cambios en la riqueza y composición de especies, altera el equilibrio entre estas dos fuerzas de ensamblaje, lo que queda reflejado en los cambios en la estructura filogenética de la comunidad.

3. Las alteraciones provocadas por el fuego en las funciones del ecosistema mediadas por microorganismos son el resultado de cambios en la estructura filogenética de las comunidades bacterianas. El análisis de la contribución de los linajes microbianos a la estructura filogenética de la comunidad permite predecir cómo las funciones de los ecosistemas responden a las perturbaciones ecológicas.

4. La estructura filogenética de las comunidades biológicas de plantas y microorganismos del suelo (hongos, bacterias y arqueas) en ecosistemas mediterráneos es resistente o resiliente al fuego en un período de dos a tres décadas. La diversidad filogenética de plantas y microorganismos del suelo experimenta tendencias temporales opuestas durante el re-ensamblaje de la comunidad. La recuperación microbiana posterior al incendio, que implica el restablecimiento de grupos microbianos altamente competitivos, está mediada por las comunidades de plantas a través de cambios en las propiedades del suelo.

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1. GENERAL INTRODUCTION AND AIMS

1.1. INTRODUCTION

Which factors determine the assembly of ecological communities and how such ecological communities mediate ecosystem functions are central questions in Ecology. Community assembly, i.e. the set of processes by which species colonize a specific site and interact with each other and their environment to form local communities, determines their species composition and structure (HilleRisLambers *et al.*, 2012). The different ecological processes modulating species assembly (e.g., habitat filtering, competition) leave recognizable patterns in the structure of communities (Diamond, 1975; Webb *et al.*, 2002; Mayfield and Levine, 2010). In turn, the structure of ecological communities seems decisive in determining ecosystem processes that control the fluxes of energy, nutrients and organic matter (Balvanera *et al.*, 2006; Cardinale, 2013; Graham *et al.*, 2016). Understanding the forces that control biological diversity and ecosystem functions is, in addition, crucial to predict the consequences of ecological disturbance, which is especially relevant in view of the increasing levels of environmental change. This thesis focuses on these fundamental questions by studying soil microbial community structure after fire disturbance.

1.1.1. Assembly of ecological communities

Ecological forces determining which species, from the global species pool, eventually coexist in local communities is a classical issue that has taken renewed relevance over the last years (Adler *et al.*, 2007). A long-lasting debate exists on the relative importance of neutral vs. niche-based processes as community assembly mechanisms. Neutralists defend that stochastic processes of birth, death, colonization, and extinction (and speciation) drive the assembly of communities (Hubbell, 2001). According to Hubbell's neutral theory, species interactions do not play a major role in determining their abundance, and assumes that biological communities are random assemblages of ecologically equivalent species. On the contrary, niche-based theory proposes that differences in the species ecological niche are the primary determinants of their

coexistence and ultimately, of the community assemblage. Under these niche processes, the "competitive exclusion principle" postulates that no two species sharing the same limiting resource can stably coexist (Gause, 1934). If they did, one of them would locally conduct the other species to extinction. Before competition can take place, coexisting lineages have to overcome the filter imposed by the environmental conditions (e.g. climate, pH, light, etc.), since not all organisms can establish and persist under any environmental condition (HilleRisLambers *et al.*, 2012). Under this view, a community would be composed of species whose niches were similar enough to survive but far enough not to compete with each other.

Recent advances posed by the modern coexistence theory refine this view and establish that long-term coexistence depends on the balance between two sorts of forces: niche differences and fitness differences (Chesson, 2000). In particular, Chesson's theory states that coexistence is maximized when niche differences are high and fitness differences low (or absent). Niche differences point to differences in species traits that cause species to limit more themselves than other species (intraspecific effects) (HilleRisLambers *et al.*, 2012). Species coexistence can be explained by the absence of interspecific competition, as the ecological requirements (and hence their traits or limiting factors) among co-occurring species do not overlap. Conversely, fitness differences arise when a species is a superior competitor and limits the occurrence of other species with similar traits (HilleRisLambers *et al.*, 2012). Therefore, niche differences drive species coexistence whereas fitness differences drive competitive exclusion (Chesson, 2000).

The ecological interactions occurring between coexisting lineages are a consequence of their functional traits. How different are these functional traits is usually a product of how phylogenetically distant are the coexisting lineages (Blomberg *et al.*, 2003). This observation emanates from Darwin's ideas (1859), who first realized that taxonomically-related species tend to be more ecologically similar than non-related species. The consequence of such similitude is that closely-related species tend to compete more intensely between them than they do with distantly-related species (Violle *et al.*, 2011; Tan *et al.*, 2012). Introducing this evolutionary perspective has triggered a new

body of literature to explain how ecological communities are assembled (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009). Webb *et al.* (2002) proposed a framework in community ecology in which phylogenetic relationships are used to discern between two main assembly processes, that is, habitat filtering and competitive exclusion. They proposed several statistical metrics that "quantify the distribution of taxa in a sample relative to a pool community" from a phylogenetically-informed view, in order to test whether co-occurring lineages are more (or less) evolutionarily-related that expected by chance (Webb *et al.*, 2002). According to this view, if co-occurring species are more closely-related than expected by chance (i.e. phylogenetic clustering), habitat filtering is considered the main assembly mechanism. Close relatives survive the filter because they share traits that allow them to tolerate the abiotic conditions (Webb *et al.*, 2002; Pausas and Verdú, 2010). Conversely, if co-occurring species are less closely-related than expected by chance (i.e. phylogenetic overdispersion), competition is considered the driving mechanism as it theoretically prevents the coexistence of close relatives with similar ecological requirements (Webb *et al.*, 2002; Pausas and Verdú, 2010). It is to be noticed that these assumptions only hold when traits driving community assembly are phylogenetically conserved, i.e. when close relatives are more similar in their trait values (Webb *et al.*, 2002; Pausas and Verdú, 2010). The possibility that different assembly processes produce similar phylogenetic patterns has recently motivated alternative explanations to those suggested by Webb *et al.* (2002). Importantly, it has been suggested that competitive interactions can also cluster the phylogenetic structure of ecological communities (Mayfield and Levine, 2010; HilleRisLambers *et al.*, 2012; Goberna *et al.*, 2014; further details in Chapters I and II). This can be better understood when looked through the modern coexistence theory, as competitive exclusion might eliminate distantly related organisms when it is based on fitness (rather than niche) differences (See Figure 1 and text in Goberna *et al.* (2014) and Figure 3 in Mayfield and Levine (2010) for conceptual examples). Patterns of evolutionary relationship not only shape the assembly of ecological communities but also their functional capabilities, as closely related species tend to be more functionally similar than their distant counterparts (Cadotte *et al.*, 2008).

1.1.2. Biodiversity and ecosystem functioning

Understanding the relationship between biodiversity and ecosystem functioning is essential to predict changes in our ecosystems, particularly under the current context of diversity loss (Loreau and Hector, 2001; Cardinale *et al.*, 2012). Ecosystem functioning, understood as resource capture, biomass production, decomposition or nutrient cycling, is typically positively related to biological diversity, which is accounted for by variation in genes, species or functional traits (Cardinale *et al.*, 2012). Two mechanisms have been proposed to interpret the positive relationship between biodiversity and ecosystem functions: i) "selection effects", by which there is a higher probability of sampling productive species from a diverse pool, and ii) "complementarity effects", by which diverse assemblages of species produce more than the sum of the individual species based on a more efficient usage of the global resource (Loreau and Hector, 2001). Studies finding either negative or no effect of diversity on ecosystem functioning emphasize that processes controlling ecosystem functioning are not straightforward (Hooper *et al.*, 2005). The balance between fitness and niche differences not only influences species coexistence, as explained in the previous section, but also control the productivity and efficiency of ecological communities (Cardinale, 2013). Large niche differences allow an efficient capture of resources and biomass production, but their magnitude depends on the strength of fitness differences.

Phylogenetic measures, rather than taxonomic metrics such as species richness, have shown better abilities in decoding the relationship between biodiversity and ecosystem functioning (Maherali and Klironomos, 2007; Cadotte *et al.*, 2008). High ecosystem productivities can arise from elevated phylogenetic diversities, as species functional complementarity increases with the coexistence of distantly related organisms (Hooper *et al.*, 2005; Cadotte *et al.*, 2008). In contrast, it has been shown that low phylogenetic diversities can also correlate with high ecosystem productivity when competitive clades with high fitness become dominant in the community (Goberna *et al.*, 2016; Chapters I and III). This reduces the phylogenetic diversity while increasing productivity, a mechanism that seems to be particularly relevant in soil microbial communities (Chapters I and III).

Most studies tackling the biodiversity-ecosystem functioning relationship have focused on plant communities, while soil microbial communities remain rather unexplored (Van der Heijden *et al.*, 2008). However, microbial communities play an essential role as primary catalysts of nutrient cycles, transforming complex chemical forms of C, N, P or Fe into more labile compounds (Madigan *et al.*, 2015). This ability, which arises from their extraordinary metabolic diversity (Prosser *et al.*, 2007), is particularly relevant in terrestrial ecosystems. In addition, microbes intimately interact with plants, mediating their functional traits and providing them with novel nutritional and biosynthetic capabilities (Friesen *et al.*, 2011). This has also consequences in ecosystem processes, as for example microbially mediated N-fixation increases plant N content, altering the energy or nutrient fluxes and decomposition (Friesen *et al.*, 2011). In turn, during decomposition, heterotrophic microorganisms use organic compounds from plant, animal or microbes as C and energy source, incorporating some C in their biomass and releasing the rest in form of CO_2 or metabolites (Gougoulias *et al.*, 2014). Microorganisms decompose organic compounds by releasing extracellular enzymes, such as β-glucosidases that catalyze the breakdown of carbohydrates into simpler β-glucosides, phosphatases the hydrolysis of ester–phosphate bonds releasing inorganic P or ureases the conversion of urea into carbon dioxide and ammonia (Tabatabai, 1994). The rates at which microbes transform organic compounds are useful as indicators of microbially mediated ecosystem functioning (Sinsabaugh, 1994). Evidence suggests that nutrient cycles and ecosystem functioning are influenced by microbial community structure but also that, in turn, soil nutrients determine microbial structure (Graham *et al.*, 2016; Chapters I, III and IV). Understanding the mechanisms controlling the microbial diversity and its consequences in biological productivity and global cycles is key to predict how environmental changes affect ecosystems.

1.1.3. Fire as an ecological disturbance

Fire is a frequent and pervasive disturbance that affects forest ecosystems worldwide through changes in both biological communities, as well as physical, chemical and mineralogical soil properties (Certini, 2005; Mataix-Solera *et al.*, 2009; Keeley *et al.*, 2012). Fire increases the emission of greenhouse

gases and soil erosion, and modifies the abiotic and biotic components of ecosystems (Certini, 2005). Fire constitutes a main ecological force in shaping the structure of plant communities (Ojeda *et al.*, 2010). This is particularly relevant in Mediterranean ecosystems, in which plant communities have developed traits that ensure their persistence under recurrent fires (Keeley, 1986). We know that fire alters plant community assembly and decreases plant biomass (Verdú and Pausas, 2007; Keeley *et al.*, 2012), but evidence on microbial communities is scarce (Chapters II and IV).

Microbial communities submitted to an ecological disturbance like fire can experience no change (resistance), return to their pre-disturbance composition (resilience) or be sensitive and remain altered in case they are neither resistant nor resilient to disturbance (Allison and Martiny, 2008). Evidence suggests that microbial communities are sensitive to ecological disturbance, and not immediately resilient (Allison and Martiny, 2008), which seems to be the case after fire (Ferrenberg *et al.*, 2013; Xiang *et al.*, 2014). However, we have a very poor knowledge on basic questions like microbial resistance or resilience to fire and post-fire dynamics in community assembly (Chapters II and IV) and ecosystem functioning (Chapter III). The essential role of microbes in forest ecosystems emphasizes the need of studies focusing on microbial communities, which is urgent given the increasing rates of forest fires in Mediterranean ecosystems caused by temperature increase and changes in land use (Pausas, 2004; Pausas and Fernández-Muñoz, 2012).

1.2. AIM AND OUTLINE OF THESIS

The general aim of this thesis was to examine the ecological basis of soil microbial community assembly and its relationship with ecosystem functioning, by focusing on the phylogenetic structure of microbial communities and its resilience to fire in Mediterranean ecosystems. Specifically, we:

1. Investigate the physical and chemical parameters determining the phylogenetic structure of soil bacterial communities (Chapter I)
2. Evaluate the phylogenetic structure of soil bacterial communities as a predictor of ecosystem functioning (Chapter I)
3. Investigate fire as an ecological force shaping soil bacterial community assembly (Chapter II)
4. Analyse fire-induced changes in microbially mediated ecosystem functioning (Chapter III)
5. Evaluate the resistance or resilience to fire of ecological communities (Chapter IV).

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2. MATERIALS AND METHODS

2.1. EXPERIMENTAL DESIGN AND STUDY SITES

This doctoral thesis covers a total of 27 study sites in Mediterranean ecosystems in the Region of Valencia (East Spain). Climate ranges from semi-arid to sub-humid Mediterranean. Plant communities include scrublands, shrublands and woodlands. Soils have been formed on a range of parent materials including limestones, gypsum and colluvial deposits. Soil conditions are also broad in terms of pH, salinity and soil fertility. We used three different experimental designs to address the main aims of this doctoral thesis (Figure 1).

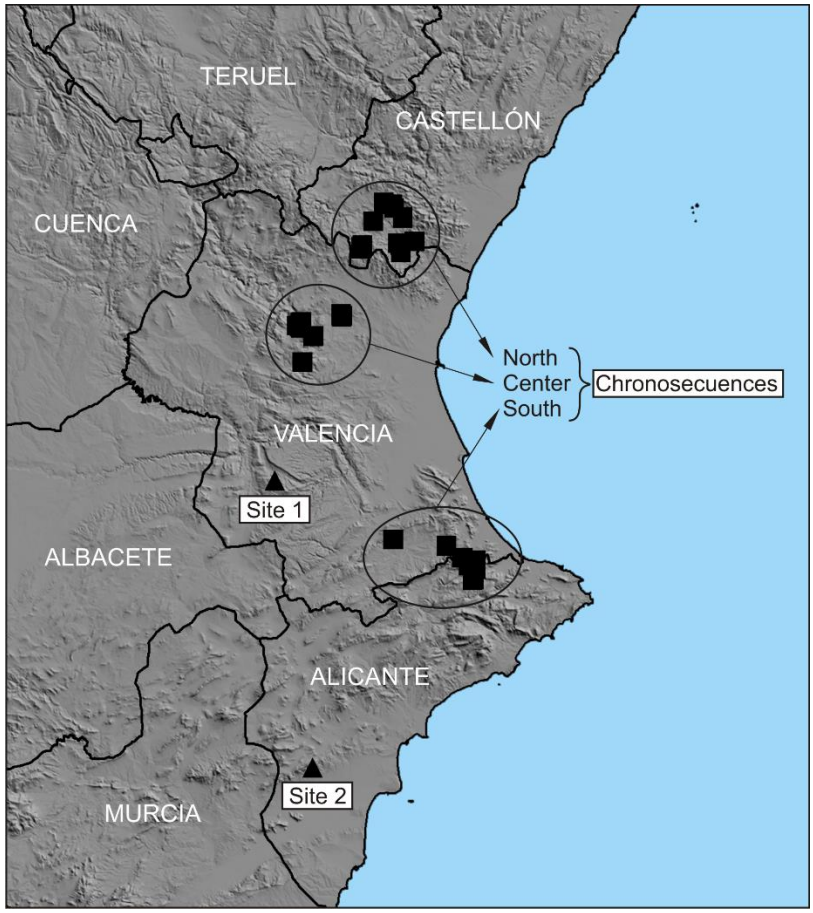

Figure 1: Geographical location of the study sites.

First, we used an observational sampling design in which we selected two contrasting sites in terms of climate, soil properties and plant composition, in order to explore the abiotic determinants of bacterial phylogenetic community structure and its relationship with the ecosystem functioning (Chapter I). Briefly, we selected a homogeneous shrubland, in which we sampled ten soil plots (Site 1), and a patchy shrub steppe, where sampling was performed underneath 15 vegetation patches and their adjacent open spaces (Site 2). Thus, we collected a total of 40 surface soil samples.

We then introduced fire disturbance by exposing a homogeneous shrubland (Site 1 in Chapter I) to an experimental burning (Figure 2). Sampling was performed in ten replicated plots before fire, and from 1 day to 1 year after fire, also including 1week, 1 month, 4.5 months and 9 months, with the aim of exploring the temporal dynamics in the post-fire microbial community assembly (Chapter II) and its relationship with ecosystem functioning (Chapter III). In this case, we collected a total of 70 surface soil samples (7 sampling times × 10 plots). Samples collected before the fire were used as the unburned control, in order to reduce the spatial heterogeneity of sampling adjacent unburned areas. To control for seasonal effects in the temporal dynamics of the microbial communities, we took into account climatic conditions (i.e., air temperature and precipitation) in our statistical models.

Finally, we explored post-fire dynamics and resilience across biological domains through replicated observational fire chronosequences (Chapter IV). We selected 25 sites, which had suffered a unique non-prescribed fire between 1994 and 2014, distributed in three chronosequences defined based on the geographic location and environmental conditions. Since pre-fire samples were not available for these wildfires, we used spatial (rather than a temporal) controls. We specifically used a paired design, by sampling adjacent sites that were similar to each burned site but that had not been exposed to fire (Figure 3). Within-treatment variability was accounted for by sampling three transects across each burned and control plot. Thus, wildfire chronosequences were characterized with a total of 150 surface soil samples (25 sites × 2 plots × 3 transects).

Figure 2: Experimental fire in the homogeneous shrubland study site.

2.2. ORGANISMS UNDER STUDY

Soil bacteria were the main biological group studied in Chapters I, II and III. To broaden our perspective on the resilience of biological communities to fire, we have incorporated other belowground (i.e. fungi and archaea) and aboveground (i.e. plants) organisms in Chapter IV.

2.3. STUDY OF SOIL MICROBIAL COMMUNITIES AND THEIR ENVIRONMENT

Microbial communities were studied at different levels, including their abiotic environment, their consequences on ecosystem functions and the role of evolutionary relationships among lineages within and across domains.

Figure 3: Site recently exposed to a wildfire and its adjacent unburned site.

2.3.1. Physical and chemical soil analyses

Several soil physical and chemical parameters were analysed in order to examine their role in the microbial phylogenetic community structure. Specifically, we quantified soil pH, gravimetric humidity (GH), total organic C (TOC), pyrophosphate oxidizable C (PPi-OC), total nitrogen (TN), electrical

conductivity (EC), NH_4^+-N and NO_3^--N following standard procedures (see Chapters I and IV for further details).

2.3.2. Physiological and biochemical analyses

We measured several soil physiological and biochemical variables as indicators of microbially-mediated ecosystem functions. Particularly, we analysed general indicators of microbial activity such as microbial biomass carbon (MBC), the microbial coefficient (MBC/TOC), basal respiration and the metabolic quotient (qCO_2). MBC was used as proxy of microbial biomass, MBC/TOC as a measure of the efficiency in converting organic C into microbial C, basal respiration as indicative of soil decomposer activity that mineralize organic C into CO_2, and qCO_2 as microbial respiration per unit of biomass indicative of microbial C that is transformed into CO_2 (Nannipieri *et al.*, 1990). Three enzymatic activities, i.e. β-glucosidase (GA), alkaline phosphatase (PA) and urease activities (UA) were used as indicators of ecosystem functions related to C, P and N cycling. In particular, β-glucosidase catalyses the breakdown of cellulose compounds, alkaline phosphatase the hydrolysis of ester–phosphate bonds and urease the hydrolysis of urea, respectively releasing simpler compounds as glucose, inorganic phosphorous or ammonia (Kandeler and Gerber, 1988; Tabatabai, 1994). Further details about these procedures can be found in Chapters I and III.

2.3.3. Microbial diversity

Microbial diversity has been analysed by sequencing molecular markers and further analyses at the community level with the help of the following techniques.

2.3.3.1. Targeted metagenomics

We used targeted metagenomics to PCR amplify specific molecular markers in soil DNA extracts, in order to identify and obtain the relative abundance of microbial groups in soil microbial communities. For bacteria, we used specific primers that amplified a region of the 16S rRNA gene, encoding the small subunit of the prokaryotic ribosome (Chapters I-III). When the whole

prokaryotic communities, i.e. bacteria and archaea, were the target we used universal primers for the 16S rRNA gene (Chapter IV). Finally, to identify and characterize fungal communities, we amplified the eukaryotic nuclear ribosomal internal transcribed spacer (ITS) region (Chapter IV).

2.3.3.2. Phylogenetic reconstruction

Prokaryotic phylogenies were reconstructed from partial 16S rRNA gene sequences, which is a widely used phylogenetic marker. To avoid the phylogenetic uncertainty that results from the usage of short sequences, we constrained the tree topology at deep levels (i.e. generally at the phylum level, and at the class level for most Proteobacteria) according to well-resolved phylogenies based on full 16S rRNA gene sequences. In addition, we worked with 3-5 replicated trees per study. Phylogenetic trees of prokaryotes in Chapters I, II and IV represent OTU divergence in terms of nucleotide substitution. However, given the recent findings in the evolutionary timescale of prokaryotes (Marin *et al.*, 2017), we have moved towards dating our phylogenies, thus transforming nucleotide substitution into chronological time (million years) (Chapter III).

Fungal phylogenies were based on published phylogenies at the genus level. We grafted each OTU into its corresponding genus according to their taxonomic information obtained from the eukaryotic ITS region (Chapter IV), which is the universal DNA barcode marker for fungi (Schoch *et al.*, 2012). Tree branch lengths were estimated from several dated nodes obtained from the literature and subsequently used to calibrate the tree. We worked with 5 phylogenetic trees, randomly selected from multiple trees that were constructed under a birth-death model in order to account for the topological and chronological uncertainty and resolve polytomies.

2.3.3.3. Taxonomic and phylogenetic diversity metrics

Microbial diversity was estimated through α and β taxonomic and phylogenetic metrics. First, we analysed the standardized number of OTUs per sample as a measure of taxonomic α diversity (Chapters II and IV). We incorporated phylogenetically-informed measures such as the Net Relatedness

Index (NRI), which corresponds to the standardized Mean Phylogenetic Distance and is indicative of coexisting lineages being more (i.e. phylogenetic clustering) or less (i.e. phylogenetic overdispersion) evolutionarily related than expected by chance (Chapter I). We used –NRI, as a measure of phylogenetic α diversity (Chapters II and IV).

Measures of taxonomic and phylogenetic divergence between communities (β-diversity) have been also used, in order to explore the particular role of microbes controlling ecosystem functioning (Chapter I), and the species resilience after fire disturbance (Chapters II and IV). In particular, we evaluated the taxonomic species replacement after fire through the nestedness and turnover components of β-diversity through time (Chapter II). Phylogenetic relationships were incorporated into β-diversity analyses through i) a fuzzy-weighted phylogenetic composition that account for the representativeness of lineages across sites (Chapters I and III), and ii) UNIFRAC distances that allow a better understanding of ecological resilience to disturbance (Chapter IV).

2.3.3.4. Microbial networks

Bacterial OTUs co-occurring more (co-presence) or less (mutual exclusion) frequently than expected by chance were detected through network analysis (Faust and Raes, 2012). By combining multiple measures of correlation and/or dissimilarity, these analyses translate taxon abundance data into links between co-occurring taxa. We phylogenetically informed our networks to detect the processes shaping microbial communities before and after the fire (Chapter II).

We validated the ability to infer assembly processes from phylogenetic co-occurrence patterns through simulations (Chapter II, Appendix A2). Briefly, we simulated phylogenies, species traits and abundances from known processes of community assembly, testing later whether our methodology had identified correctly the simulated assembly process. A thorough description of the simulation model can be found in Appendix A2 (Chapter II).

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CHAPTER I: Phylogenetic structure of soil bacterial communities predicts ecosystem functioning

ABSTRACT

Quantifying diversity with phylogeny-informed metrics helps understand the effects of diversity on ecosystem functioning (EF). The sign of these effects remains controversial because phylogenetic diversity and taxonomic identity may interactively influence EF. Positive relationships, traditionally attributed to complementarity effects, seem unimportant in natural soil bacterial communities. Negative relationships could be attributed to fitness differences leading to the overrepresentation of few productive clades, a mechanism recently invoked to assemble soil bacteria communities. We tested in two ecosystems contrasting in terms of environmental heterogeneity whether two metrics of phylogenetic community structure, a simpler measure of phylogenetic diversity (NRI) and a more complex metrics incorporating taxonomic identity (PCPS), correctly predict microbially-mediated EF. We show that the relationship between phylogenetic diversity and EF depends on the taxonomic identity of the main coexisting lineages. Phylogenetic diversity was negatively related to EF in soils where a marked fertility gradient exists and a single and productive clade (*Proteobacteria*) outcompetes other clades in the most fertile plots. However, phylogenetic diversity was unrelated to EF in soils where the fertility gradient is less marked and *Proteobacteria* coexist with other abundant lineages. Including the taxonomic identity of bacterial lineages in metrics of phylogenetic community structure allows predicting EF in both ecosystems.

3.1. INTRODUCTION

The effect of biodiversity on ecosystem functioning has been widely studied, numerous evidences indicating a positive effect but some also reporting neutral or negative relationships (Zak *et al.,* 2003; Hooper *et al.,* 2005; Balvanera *et al.,* 2006; Cardinale *et al.,* 2012). Soil bacteria are primary actors in this relationship because of their exceptional diversity and key role on ecosystem functioning, through decomposing organic matter and controlling the planetary flows of energy and nutrients (Curtis *et al.,* 2002; Wardle *et al.,* 2004; Van der Heijden *et al.,* 2008).

Species richness has been the measure of biodiversity traditionally used in studies relating biodiversity and ecosystem functioning (Cardinale *et al.,* 2012). However, this approach disregards the fact that functional similarities among species are usually determined by their common evolutionary history, and therefore phylogenetically related species tend to perform similar functions (Blomberg *et al.,* 2003; Martiny *et al.,* 2013, but see Revell *et al*., 2008 for other processes producing trait resemblance among close relatives). This is the reason why phylogenetically-informed measures of diversity tend to be more informative than traditional richness measures (Lozupone & Knight, 2007; Cadotte *et al.,* 2008). Empirical evidence on the effect of phylogenetic diversity on ecosystem functioning is widespread across the tree of life (e.g., bacteria, Gravel *et al.,* 2012; fungi, Maherali & Klironomos, 2007; plants, Cadotte *et al.,* 2008, Cadotte 2013, Navarro-Cano *et al.,* 2014). Most of these studies have found a positive relationship between phylogenetic diversity and ecosystem functioning parameters, as expected when distantly related taxa perform complementary functions. However, neutral and negative relationships have also been described, particularly in bacteria, because phylogenetic diversity and taxonomic diversity may interactively influence ecosystem functioning (Severin *et al.,* 2013; Venail & Vives, 2013).

Phylogenetic diversity of bacterial communities in soils is low compared to those in other natural environments, contrasting with their extremely high species richness and diversity (Lozupone & Knight, 2007). This paradoxical situation could be explained by adding a phylogenetic context to the modern

coexistence theory (Chesson, 2000; Mayfield & Levine, 2010; HilleRisLambers *et al.,* 2012; Godoy *et al.*, 2014). The phylogenetic structure of soil bacterial communities is primarily driven by abiotic factors, such as acidity (Jones *et al.,* 2009) and availability of organic resources (Goberna *et al.,* 2014a), that overrepresent certain clades. The composition of ecological communities is further determined by the balance between mechanisms shaping niche differences and fitness differences between lineages (Chesson, 2000). Coexistence is maximized under large niche differences (i.e. absence of niche overlap), a situation where species do not compete for resources. This increases both diversity and productivity since the functional complementarity of coexisting organisms allows a more complete usage of resources. Complementarity effects have been shown to underlie the positive relationship between bacterial diversity and productivity in simple experimental communities, but it seems to be relatively unimportant in natural communities due to the high functional redundancy of bacteria (Griffiths *et al.,* 2001; Bell *et al.,* 2005; Venail & Vives, 2013). In contrast, fitness differences between lineages tend to favor competitive exclusion because competitively superior lineages may consume too much of the resource on which other lineages depend (Chesson, 2000). Mayfield & Levine (2010) noticed that fitness differences may produce outcompetition of entire clades when competitive superiority is a phylogenetically conserved trait. The immediate consequence of competitive exclusion of entire clades is the reduction of phylogenetic diversity in ecological communities, as occurs in soil bacterial communities worldwide (Goberna *et al.,* 2014a).

Fitness differences may be produced by competitive asymmetries in which some lineages produce more per unit resource than others (Chesson, 2000). This is the case of *Proteobacteria* and *Actinobacteria*, two bacterial lineages which are extremely competitive in terms of growth response when organic carbon substrates of varying recalcitrance are supplied to the soil, which is typically carbon-limited (Goldfarb *et al.,* 2011). This competitive superiority is phylogenetically conserved and therefore competitive exclusion leads to the overrepresentation of a few, very productive, lineages resulting in phylogenetic clustering both in experimental and natural soil communities (Goldfarb *et al.,* 2011; Goberna *et al.,* 2014b). Under this scenario, highly productive

communities dominated by competitive clades would feature low phylogenetic diversity levels, leading to an inverse relationship between phylodiversity and ecosystem functioning.

Here, we selected two ecosystems contrasting in terms of environmental heterogeneity, which is a main determinant of bacterial diversity (Ramette & Tiedje, 2007). Differences between sites were particularly marked as regards the heterogeneity of resource availability, a factor that modifies the relationship between bacterial diversity and productivity (Jousset *et al.,* 2011). In both ecosystems we test whether i) soil physical and chemical parameters determine the phylogenetic structure and ii) the phylogenetic structure of bacterial communities predicts ecosystem functioning, measured through soil microbial productivity, metabolic efficiency and nutrient cycling increases, via overrepresentation of a particular productive clade.

3.2. MATERIALS AND METHODS

3.2.1. Study site

The study was carried out in two Mediterranean sites, differing in their climate, plant cover, lithology and soil type. We intentionally searched these contrasting ecosystems to test whether phylogenetic structure of soil bacterial communities predicts ecosystem function under two extremes of environmental heterogeneity. Site 1 is characterized by the presence of a dense shrubland (100% plant cover) dominated by *Rosmarinus officinalis* L. and located in Teresa de Cofrentes (Valencia, Spain). Soils are Haplic Leptosols (Calcaric, Humic) (FAO–ISRIC–IUSS, 2006) developed on limestones, mean annual rainfall is 446 mm and temperature 13.7 ºC. Topsoils (0-2 cm) were collected in ten 1 × 1 m plots located within a 150-m^2 area as described in Goberna *et al.* (2012). Site 2 is covered by a patchy shrub steppe dominated by *Ononis tridentata* L. and located in Algepsar dels Burutaus (Serra de Crevillent, Alacant, SE Spain). Soils are Leptic Regosols (Gypsiric, Calcaric) (FAO–ISRIC–IUSS, 2006) developed on gypsum, mean annual rainfall is 220 mm and temperature 20 ºC. Topsoils (0-2 cm) were collected underneath 15 vegetation patches (defined as groups of plants growing underneath the canopy of an *O. tridentata* individual) and in the adjacent open spaces, all plots being located within a 1-

ha area as described by Navarro-Cano *et al.* (2014). Sites 1 and 2, representing two extremes of environmental heterogeneity will be hereafter referred to as "non-patchy" and "patchy" ecosystems, respectively, based on the structure of their plant communities.

Plant community structure determined a low variance in the soil physical and chemical properties in the non-patchy ecosystem, which contrasted with the high variability of the same variables in the patchy ecosystem (Table 1). Further details on the soil physical and chemical environment in both sites can be found in previous studies (Goberna *et al.,* 2012; Navarro-Cano *et al.,* 2014). We characterized the soils of each plot with the scores of the first principal component (PC1-Soil) including the soil gravimetric humidity (GH), pH, electrical conductivity (EC), total organic C (TOC), pyrophosphate oxidizable C (PPi-OC), and total nitrogen (TN). PC1-Soil was then used as an abiotic predictor of phylogenetic structure of soil bacterial communities as described below. Both sites also exhibited large differences in the variability of several biochemical properties that are commonly used as proxies of ecosystem functioning, with the non-patchy ecosystem showing lower coefficients of variation compared to the patchy ecosystem (Table 1). Specifically, we used parameters that are indicators of general microbial activity and specific enzymatic activities involved in main steps of the nutrient cycles (Nannipieri *et al.,* 1990). In particular, general indicators of microbial activity included: 1) microbial biomass C (MBC) as a proxy of the microbial biomass; 2) ATP content, as an indicator of the total microbial activity; 3) basal respiration (BR), as an indicator of the activity of decomposers that mineralize organic C into CO_2; 4) microbial coefficient (MBC/TOC), which reflects the conversion efficiency of organic C into microbial C; and 5) metabolic quotient (qCO_2), which is the ratio between CO_2-C production and MBC and declines as the microbiota becomes efficient at conserving C. Specific indicators of microbial activity included 1) β-glucosidase (GA), 2) alkaline phosphatase (PA) and 3) urease activities (UA), which are hydrolytic enzymes that are respectively involved in C, P and N cycling. Further details on the soil biochemical properties in both sites can be found in previous studies (Goberna *et al.,* 2012; Navarro-Cano *et al.,* 2014).

3.2.2. Soil DNA extraction and pyrosequencing

Soil DNA from the non-patchy ecosystem was extracted within 24 h after sampling from ca. 0.25 g soil with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). Extracted DNA was checked for quality by electrophoresis in 1% agarose gels run in 0.5 × Tris–acetate–EDTA buffer. Amplifications of the 16S rRNA gene were carried out using the universal bacterial primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3'; Turner *et al.,* 1999) and 534R (5'-ATTACCGCGGCTGCTGGC-3'; Muyzer *et al.,* 1993). Each sample contained a synthetized forward primer, including a 454 sequencing adaptor (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3') and a unique 8-nucleotide barcode in its 5' end randomly selected from those published by Hamady *et al.* (2008). The reverse primer incorporated a 454 sequencing adaptor in its 5' end (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3').

Table 1: Variability among sampling plots in physical, chemical and biochemical variables in the non-patchy and patchy ecosystems (data published by Goberna *et al.,* 2012 and Navarro-Cano *et al.,* 2014).

	Non-patchy ecosystem			Patchy ecosystem		
Variable	Mean	SD	CV	Mean	SD	CV
Gravimetric humidity (%)	23.34	5.27	22.57%	2.94	1.48	50.36%
Total organic C (g kg^{-1})	43.9	5.2	11.95%	59.7	39.7	66.53%
Pyrophosphate oxidizable carbon (g kg^{-1})	15.4	6.2	40.47%	1.87	1.57	83.65%
pH	8.05	0.17	2.14%	7.18	0.16	2.26%
Electrical conductivity (µS cm^{-1})	230	35.66	15.48%	2798	334	11.95%
Total N (%)	0.39	0.13	32.32%	0.39	0.29	73.78%
Microbial biomass C (mg C kg^{-1})	469	194	41.32%	1,411	1,307	92.68%
MBC/TOC (%)	1.07	0.42	38.71%	1.89	1.11	58.95%
Basal respiration (mg C-CO_2 kg^{-1} d^{-1})	15.75	6.27	39.78%	76.26	71.88	94.25%
qCO2 (µg C-CO_2 mg $^{-1}$ MBC h^{-1})	1.91	2.19	114.57%	2.18	1.08	49.47%
ATP (ng g^{-1})	2186	493	22.53%	424	310	73.11%
β-glucosidase activity (µmol PNP g^{-1} h^{-1})	2.93	0.81	27.51%	5.88	6.07	103.28%
Phosphatase activity (µmol PNP g^{-1} h^{-1})	15.73	6.13	38.99%	16.11	14.78	91.73%
Urease activity (mg N-NH_4^+ g^{-1} h^{-1})	1.07	0.36	33.52%	2.05	1.49	72.59%

PCR reactions were performed in a Flexcycler (Analytik Jena, Jena, Germany) in 50 µl volumes. Each reaction contained a final concentration of 1 X Platinum PCR SuperMix High Fidelity (Invitrogen, Carlsbad, USA), 0.3 µM of each primer and 0.4 mg mL^{-1} bovine serum albumin. A volume of 1.5 µl DNA was directly applied to the reaction mix. Thermal cycling consisted of 5 min at 94 °C, 20 cycles including 45 s at 94 °C, 45 s at 54 °C and 90 s at 72 °C and terminated with 10 min at 72 °C. Purification of PCR products (100 µl) was carried out with the NucleoSpin Extract II Kit (Macherey-Nagel, Düren, Germany). Afterwards they were eluted in 50 µl DNAase free 1 × TE (Tris-EDTA) buffer and checked for quality and size in 2 % agarose gels run in 1 × TAE buffer (80 V, 45 min). Non-template controls followed the same procedure. Purified tagged amplicons were quantified in duplicate using the Quant-iT PicoGreen dsDNA Kit (Invitrogen, Carlsbad, USA) and pooled in equimolar amounts. Pyrosequencing was performed by GATC Biotech (Konstanz, Germany) with the Roche 454 GS-FLX system using titanium chemistry.

Similar procedures were used for DNA extraction, PCR amplification and pyrosequencing of soil samples in 30 plots from the patchy ecosystem. Details are given in Goberna *et al.* (2014b).

3.2.3. Sequence analysis and phylogeny reconstruction

For the non-patchy ecosystem, 10604 sequences were obtained. Short sequences (< 200 bp) were removed, along with those with ambiguous base calls or with homopolymers exceeding 6 bp. Primers and barcodes were trimmed. After denoising, chimeric sequences and singletons were excluded from the analysis. Operational taxonomic units (OTUs) were defined at an identity level of 97 % and taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis *et al.,* 2006). This initial sequence processing was performed by MR DNA (Shallowater, TX, USA). A final 2289 OTUs were aligned with PyNAST (Caporaso *et al.,* 2010a) by using QIIME (Caporaso *et al.,* 2010b). Then, we constructed a community matrix showing the abundance of the total 2289 OTUs in each of the 10 plots. As proposed by Kembel *et al.* (2012), the relative abundance of each OTU was corrected by the

estimated number of 16S rRNA gene copies. Bacterial phylogeny was reconstructed using RAxML 7.3.0 (Stamatakis *et al.,* 2006). We built five independent maximum-likelihood phylogenetic trees with the GTRGAMMA substitution model. Previously, hypervariable regions were removed using the Lane mask (Lane, 1991). To avoid high phylogenetic uncertainty resulting from the usage of short sequences, tree topology was constrained to match the basal relationships of the megatree built from the Silva database (Release 108, Quast *et al.,* 2013). All phylogenetic trees were selected among the best of 1000 iterations and rooted using *Archaeoglobus profundus*. Sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB6166).

In the patchy ecosystem, we worked with 24162 sequences after removal of low-quality sequences and artifacts. After excluding singletons, these were collapsed into a final 3290 OTUs. Sequence processing and phylogeny reconstruction were similar to those described above and details are given in Goberna *et al.* (2014b).

3.2.4. Phylogenetic community structure

We described the phylogenetic structure of bacterial communities by using two phylogeny-weighted metrics. First, we calculated the abundance-weighted net relatedness index (NRI), one of the most commonly used metrics in community phylogenetics, with the picante package for R (Kembel *et al.,* 2010). This computes NRI = $-(MPD_{obs} - MPD_{rand})/sd\_MPD_{rand}$, where MPD_{obs} is the average of all pairwise phylogenetic distances between the taxa in a local community, MPD_{rand} is the average of MPD calculated in *n* randomly constructed communities after shuffling all taxa in the regional pool, and sd_MPD_{rand} is the standard deviation of MPD_{rand} (Webb *et al.,* 2002). This allows examining whether co-occurring taxa are more (positive NRI) or less (negative NRI) closely related than expected by chance. Thus, positive NRI values are related to phylogenetic clustering while negative values indicate phylogenetic overdispersion.

Second, we used the phylogenetic fuzzy-weighting method proposed by Pillar & Duarte (2010). Compared to NRI, which is blind to the taxonomic identity of coexisting lineages (i.e. similar NRI´s can be obtained for communities dominated by closely-related *Actinobacteria* or for communities dominated by closely-related *Proteobacteria*), the fuzzy-weighting method identifies the representativeness of different lineages across the sites (see Duarte *et al.* (2012) for a detailed explanation). Briefly, this method calculates a matrix (matrix P), that describes the species phylogenetic composition of each plot taking into account the phylogenetic neighborhood of each OTU. To obtain matrix P, we transformed the pairwise phylogenetic distance matrix on similarities between species. Then, we used similarities to weight the species composition matrix by a fuzzy set algorithm (Pillar & Duarte, 2010). In matrix P each OTU has a value per plot that increases as the phylogenetic distance between neighboring OTUs decreases. Matrix P was calculated using the SYNCSA package implemented in R (Debastiani & Pillar, 2012). Principal components analysis with Euclidean distance was run to reduce the dimensionality of the matrix P. The loadings of each OTU indicate the relative contribution of that OTU to differentiate plots along the first principal component axis (plot scores). Consequently, each plot score captures the whole variation of species abundances weighted by phylogenetic relatedness. To identify which phyla were responsible for the phylogenetic community structure, we ran a linear model with the plot scores along the first principal component axis (PCPS1 hereafter) as the dependent variable and the relative abundance of the most abundant phyla as independent variables.

3.2.5. Statistical analyses

To check whether spatial autocorrelation in the bacterial community composition across plots should be taken into account in subsequent analyses, we correlated OTU composition and geographic distance matrices through Mantel tests in the ADE4 package for R (Mantel, 1967; Dray & Dufour, 2007). We tested whether physical and chemical soil parameters determine the phylogenetic structure of bacterial communities by performing Bayesian generalized linear models (GLMs) with NRI and PCPS1 used individually as the dependent variables and the PC1-Soil as the independent variable. The NRI

values per plot were very similar across the five phylogenetic trees in both sites ($r>0.77$; $p<0.005$ for all the correlations). Similarly, PCPS1 values per plot were very similar for all the trees ($r>0.98$; $p<0.005$ for all the correlations). Although these correlations indicate that phylogenetic uncertainty was small, we accommodated such small uncertainty by running five GLMs for each site, each one using the phylogenetic information calculated from an independent tree and integrated over the posterior samples by drawing 1000 random samples across models. The models were run with the help of MCMC techniques as implemented in the MCMCglmm package for R (Hadfield, 2010). We used the default priors and ran 13.000 MCMC iterations with a burn-in period of 3.000 iterations. Convergence of the chain was tested by means of an autocorrelation statistic. The statistical significance of the factors in the model was estimated by calculating the 95% credible interval of their posterior distribution.

Bayesian GLMs were also used to test which metrics of phylogenetic community structure predicted the ecosystem functioning more accurately. We ran five GLMs per site, using each ecosystem functioning parameter individually as the dependent variable and both NRI and PCPS1 as independent variables in the same model. The relative abundance of the most abundant clades was also used as a predictive parameter of ecosystem functioning. Clade relative abundances were estimated as the sum of the relative abundances of all OTUs that belonged to that particular clade, which were corrected based on their estimated 16S rRNA gene copy numbers (see details above). All analyses were performed using the software R 3.1.1 (R Core Team, 2014).

3.3. RESULTS

Soil bacterial communities had 602 ± 13 and 430 ± 24 OTUs per plot (mean ± SE) in the non-patchy and patchy ecosystems, respectively. *Proteobacteria* was the most dominant phylum in both ecosystems followed by *Actinobacteria* (Figure 1). There was not spatial autocorrelation across plots in the bacterial community composition (non-patchy ecosystem, $r = -0.205$, $p = 0.924$; patchy ecosystem, $r = -0.054$, $p = 0.691$; Mantel tests) nor in the phylogenetic structure measured as NRI (non-patchy ecosystem, $r = 0.04$, $p =$

0.349; patchy ecosystem, r = -0.049, p = 0.74) or PCPS1 (non-patchy ecosystem, r = 0.29, p = 0.06; patchy ecosystem, r = -0.044, p = 0.78).

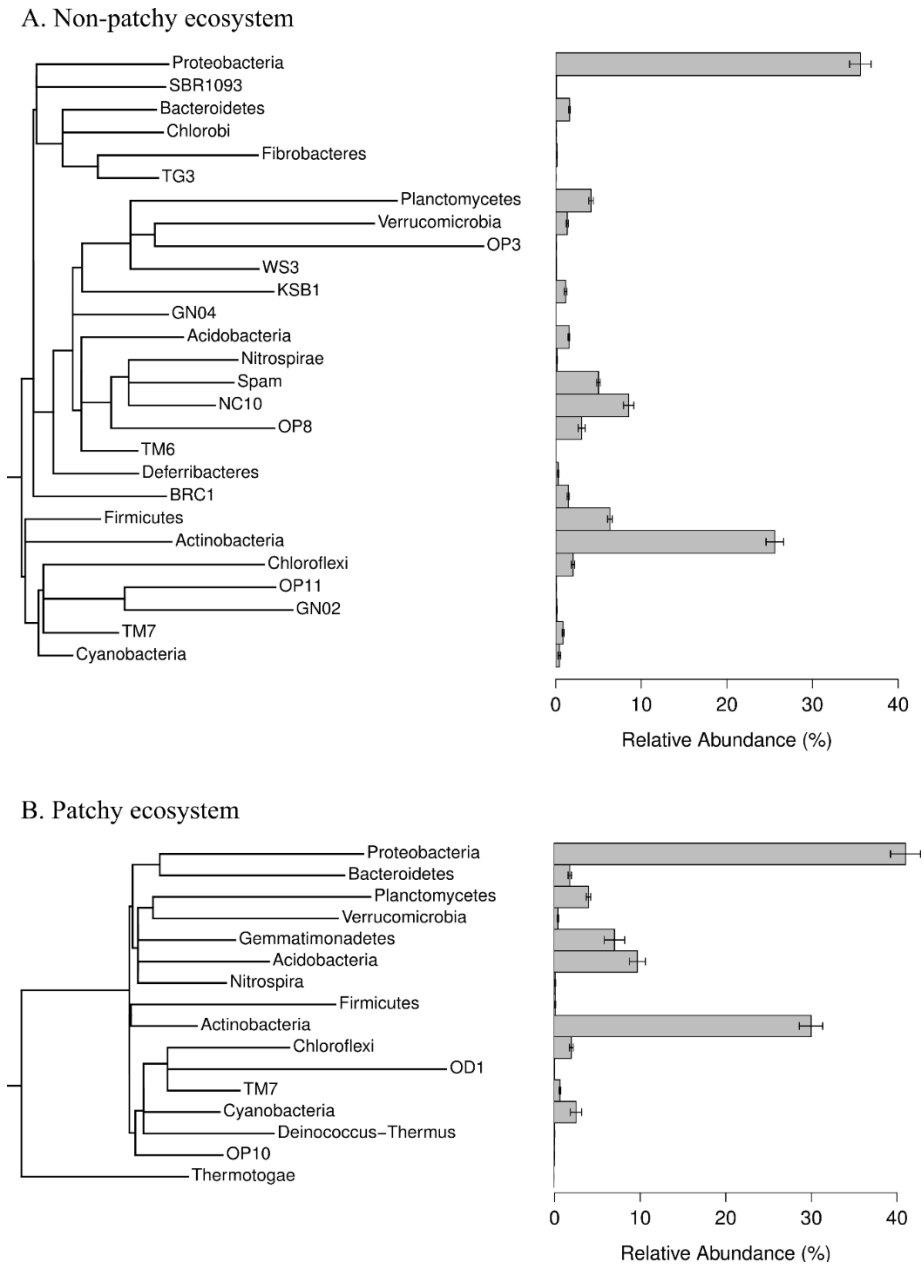

Figure 1: Phylogenetic tree of major basal groups in the non-patchy and patchy ecosystems showing the average relative abundances of each phylum across the study plots. Error bars indicate the standard error of the mean.

The phylogenetic structure of the bacterial communities in both ecosystems was clustered, as indicated by net relatedness index (NRI) significantly higher than zero (NRI post-mean estimate [95 % credible interval] = 1.70 [0.68, 2.93] for the non-patchy and NRI = 2.49 [1.37, 3.56] for the patchy ecosystems). Phylogenetically clustered plots (i.e. high NRI values) were those with higher abundances of *Proteobacteria* and/or *Actinobacteria* (see positive estimates in the NRI models in Table 2). The contribution of both phyla was significantly positive but differed in their relative importance, with *Proteobacteria* and *Actinobacteria* equally contributing in the non-patchy ecosystem but *Actinobacteria* contribution becoming non-significant in the patchy ecosystem.

Table 2: Linear model explaining the contribution (% variance) of the abundance of the dominant phyla (% of OTUs belonging to *Proteobacteria* and *Actinobacteria*) on the mean Net Relatedness Index (NRI) and on the mean plot scores along the first principal component axis of the phylogenetic community structure (PCPS1) across the five phylogenetic trees.

	NRI			PCPS		
	Estimate ± SE	t	% variance	Estimate ± SE	t	% variance
Non-patchy ecosystem						
Intercept	-11.9 ± 3.02	-3.96**		136.7 ± 36.26	3.77**	
% Proteobacteria	0.22 ± 0.05	4.49**	33.4	0.61 ± 3.64	3.64**	52.5
% Actinobacteria	0.21 ± 0.06	3.45*	41.9	-8.43 ± 0.741	-11.25***	45.2
Patchy ecosystem						
Intercept	-3.54 ± 1.35	-2.61*		-197.4 ± 7.22	-27.37***	
% Proteobacteria	0.09 ± 0.02	3.28**	35.5	5.31 ± 0.14	35.64***	97.2
% Actinobacteria	0.07 ± 0.03	2.11*	9	-0.67 ± 018	-3.56**	0.9

*p<0.05; ** p<0.01;***p<0.001

The metrics of the community structure that accounts for the variability in the taxonomic identity and the phylogenetic relatedness (PCPS1) explained 50 and 71 % of the total variance of the phylogenetic structure in the non-patchy and patchy ecosystems, respectively. The contribution of the most abundant phyla to PCPS1 differed between ecosystems, with similar contributions of *Proteobacteria* and *Actinobacteria* in the non-patchy ecosystem but with an overwhelming contribution of *Proteobacteria* in the patchy ecosystem (Table 2). Interestingly, the phylogenetic position of both phyla in distant clades

(see trees in Figure 1) was accounted for by PCPS1 and clearly segregated the plots with preponderance of *Proteobacteria* in the right extreme from those with preponderance of *Actinobacteria* in the left extreme (see positive estimates for *Proteobacteria* and negative for *Actinobacteria* in the PCPS1 linear models in Table 2).

The first axis of the PCA grouping soil physical and chemical variables (PC1-Soil) accounted for 68 and 84% of the variance in non-patchy and patchy ecosystems, respectively. The loading factors showed that PC1-Soil represented a fertility gradient of increasing oxidizable carbon and humidity contents in both ecosystems (non-patchy ecosystem: TN 0.27, EC 0.36, pH 0.40, TOC 0.43, GH 0.47, PPi-OC 0.48; patchy ecosystem: pH -0.32, EC 0.40, GH 0.40, PPi-OC 0.43, TN 0.44, TOC 0.44). While the magnitude of such a gradient was slight in the non-patchy ecosystem (e.g. TOC ranged from 3.3 to 5 % across plots), it was extremely accentuated in the patchy ecosystem (e.g. TOC ranged from 1.8 to 12.5 % across plots; Table 1). This fertility gradient could not predict the NRI in the non-patchy ecosystem where both *Actinobacteria* and *Proteobacteria* had relevant contributions (NRI *vs* PC1-Soil = 0.15 [-0.19, 0.56]). However, once the identities of both phyla and the variability in phylogenetic relatedness across plots were accounted for, the fertility gradient significantly explained the phylogenetic structure of the community (PCPS1 *vs* PC1-Soil = 11.66 [2.68, 23.19]). In the patchy ecosystem where the taxonomic relevance of a single phylum (*Proteobacteria*) was disproportionate, the fertility gradient significantly explained both NRI (NRI *vs* PC1-Soil = 0.59 [0.36, 0.84]) and PCPS1 (PCPS1 *vs* PC1-Soil = 17.61 [23.36, 11.33]).

NRI did not predict any of the ecosystem functioning variables related to soil microbial productivity, metabolic efficiency and biogeochemical cycling in the non-patchy ecosystem while PCPS1 significantly explained most of the general indicators of microbial activity. Specifically, PCPS1 was negatively associated to MBC and MBC/TOC and positively to BR and qCO_2 (Figure 2 upper panel, Model 1). Plots with high abundances of *Actinobacteria* were those with high microbial biomass (MBC) and high efficiency in converting organic C into microbial C (MBC/TOC) and conserving C (as indicated by the negative relationship with qCO_2) (Figure 2 upper panel, Model 2). Plots with abundant

Proteobacteria were those with high activity of decomposers that mineralize organic C into CO_2 (as indicated by BR) and phosphatase activity (PA) (Figure 2 upper panel Model 2). In the patchy ecosystem, most of the ecosystem functioning parameters, including indicators of both general microbial activity and specific enzymatic processes, were predicted by both NRI and PCPS1 (Figure 2 bottom panel, Model 1). All these relationships were positive and were also explained by the relative abundance of *Proteobacteria* (Figure 2 bottom panel, Model 2).

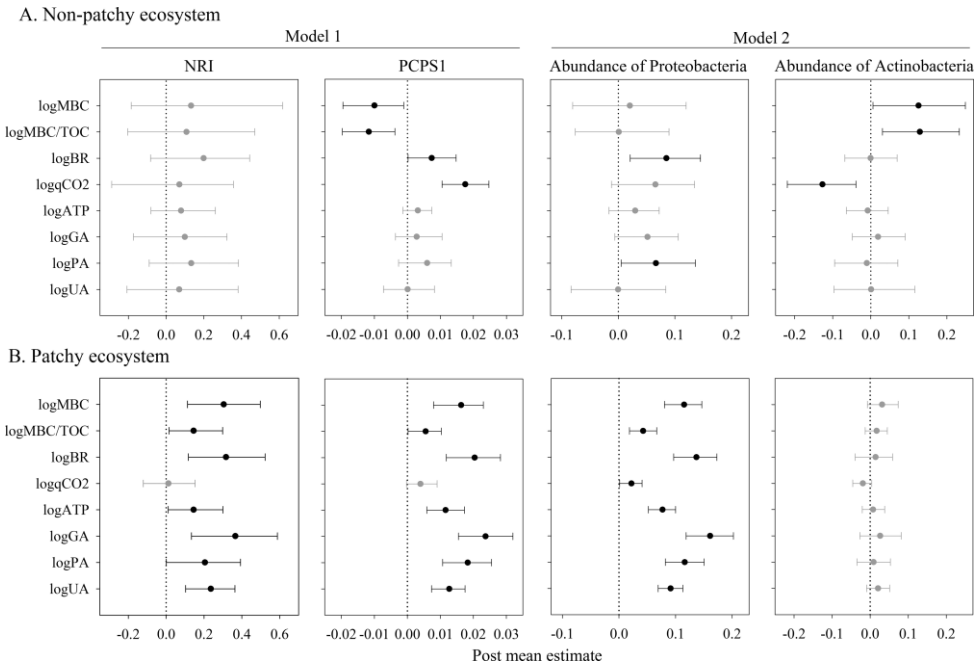

Figure 2: Bayesian post-mean estimates and their expected 95% credible intervals for the effect of NRI and PCPS1 (Model 1) and the relative abundance of *Proteobacteria* and *Actinobacteria* (Model 2) on the eight soil microbial indicators from (A) non-patchy and (B) patchy ecosystems. All variables were log-transformed to improve normality. Effects with intervals not including zero are significant (black-colored intervals), whereas those including zero are not significant (gray-colored intervals).

3.4. DISCUSSION

Our results show that the relationship between phylogenetic diversity and ecosystem functioning is dependent on the taxonomic identity of the main coexisting bacterial lineages. We show that the soil environment structures a phylogenetically clustered community and discuss the mechanisms underlying the relationship between such phylogenetic community structure and ecosystem functioning. To understand this relationship, we invoke the need to include the species identity in phylogenetic diversity metrics to account for variation in phylogenetically-weighted abundances across communities.

The soil abiotic variables determined a fertility gradient that explained the phylogenetic structure of soil bacterial communities in both ecosystems. This correlates well with previous observations showing that the amount of oxidizable substances is a good predictor of the phylogenetic community structure of soil bacteria worldwide (Goberna *et al.,* 2014a). Our ability to explain the bacterial phylogenetic community structure through abiotic factors depended on the level of environmental heterogeneity. At high environmental heterogeneity (patchy ecosystem) the abiotic environment explained the community structure regardless the inclusion (PCPS1) or not (NRI) of lineage identity, while at low environmental heterogeneity (non-patchy ecosystem) only the most complex measure of community structure including lineage identity was predicted by the abiotic environment. Future studies in other ecosystems are needed to refine the relationship between environmental heterogeneity and the power of phylogenetic metrics to detect community structure in soil bacterial communities. Another important picture emerging from the present study is that environmentally mediated changes in the composition of bacterial communities left a phylogenetic signature in the community structure with profound implications in ecosystem functions. Detecting which lineage has been overrepresented under particular environmental parameters is key to understand the meaning of the phylogenetic clustering in the communities.

Ecosystem functioning was also better predicted by the metrics accounting for the identity of the lineages. In our non-patchy ecosystem, both *Actinobacteria* and *Proteobacteria* were key components structuring productive bacterial communities (Goldfarb *et al.,* 2011). As both phyla are distantly-

related, their coexistence in more fertile plots was not translated into increased phylogenetic clustering as shown by the lack of correlation between the fertility gradient and NRI. Similarly, NRI could not predict any ecosystem function in this non-patchy ecosystem. However, the phylogenetic structure metrics accounting for the identity of both phyla predicted most of the general indicators of ecosystem functioning. On the other side, in the patchy ecosystem we found that communities phylogenetically clustered because of the overrepresentation of a particular clade (*Proteobacteria*) were the most productive. In this case, the coexistence of closely-related *Proteobacteria* in fertile plots was translated into increased phylogenetic clustering, and therefore NRI could also predict high ecosystem functioning at low phylogenetic diversities.

Our results contrast with the common findings in 'macro'organisms that indicate that phylogenetic diversity is positively related to ecosystem functioning (Cadotte *et al.,* 2008; Flynn *et al.,* 2011; Cadotte, 2013). They agree, however, with other lines of evidence showing variable responses of ecosystem functioning parameters to bacterial phylogenetic diversity. In simple experimental communities, positive and neutral responses of community productivity to increasing levels of phylogenetic diversity have been described (Gravel *et al.,* 2012; Venail & Vives, 2013). In some instances, positive responses could be experimentally attributed to complementarity effects based on the overyielding of the mixtures compared to their constituent species (Venail & Vives, 2013), but this pattern is not consistent in the literature (Gravel *et al.,* 2012). In more complex microcosms, bacterial productivity showed mostly negative, but also neutral and positive responses, to phylogenetic diversity (Severin *et al.,* 2013). These authors suggest that negative responses are mediated by the overrepresentation of productive *Betaproteobacteria* with the ability to consume an aromatic carbon compound. Similarly, our results in natural soil communities indicate that fast growing, competitively superior clades in the presence of soil organic carbon outcompete other clades, thus reducing phylogenetic diversity but rising indicators of ecosystem functioning. These results are consistent with fitness differences as the predominant mechanism causing high productivity at low phylodiversity through competitive exclusion (Mayfield & Levine, 2010; Carroll *et al.,* 2011; HilleRisLambers *et al.,* 2012).

In short, microbially mediated ecosystem functions can be predicted by the phylogenetic structure of soil bacterial communities because this metrics contains information on both the outcome of the ecological processes determining species coexistence and the functionality of these coexisting lineages. We suggest that outcompetition of big clades by very competitive and productive lineages explains both the phylogenetic diversity patterns of bacterial communities and the relationship between diversity and ecosystem functioning. Capturing the ecological and evolutionary idiosyncrasies of the soil bacterial communities is crucial to understand the relationship between diversity and ecosystem functioning. The improvement in our prediction ability of the ecosystem functions performed by soil bacteria is of paramount importance given the relevance of these processes (i.e. biogeochemical cycling of nutrients, decomposition of organic matter, etc.) at the planetary level.

3.5. ACKNOWLEDGEMENTS

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CHAPTER II: Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks

SUMMARY

Fire alters ecosystems by changing the composition and community structure of soil microbes. The phylogenetic structure of a community provides clues about its main assembling mechanisms. While environmental filtering tends to reduce the community phylogenetic diversity by selecting for functionally (and hence phylogenetically) similar species, processes like competitive exclusion by limiting similarity tend to increase it by preventing the coexistence of functionally (and phylogenetically) similar species. We used co-occurrence networks to detect co-presence (bacteria that co-occur) or exclusion (bacteria that do not co-occur) links indicative of the ecological interactions structuring the community. We propose that inspecting the phylogenetic structure of co-presence or exclusion links allows to detect the main processes simultaneously assembling the community. We monitored a soil bacterial community after an experimental fire and found that fire altered its composition, richness and phylogenetic diversity. Both co-presence and exclusion links were more phylogenetically related than expected by chance. We interpret such a phylogenetic clustering in co-presence links as a result of environmental filtering, while that in exclusion links reflects competitive exclusion by limiting similarity. This suggests that environmental filtering and limiting similarity operate simultaneously to assemble soil bacterial communities, widening the traditional view that only environmental filtering structures bacterial communities.

4.1. INTRODUCTION

Fires are important disturbances that affect forest ecosystems through the combination of effects that are initially triggered by heat (Certini 2005; Bárcenas-Moreno and Bååth 2009). The consequences of fire on the soil environment are complex, including the removal of plant cover and changes in physical and chemical parameters (Certini 2005; Smith *et al.* 2008; Goberna *et al.* 2012; Xiang *et al.* 2014). Fire affects soil microbial communities both directly by high temperatures inducing mortality or cell damage (Daniel and Cowan 2000) and indirectly through the combustion of organic matter, increase in available nutrients, destruction of the soil physical structure and shifts in soil pH, humidity, or electrical conductivity, among others (Certini 2005), although the magnitude of these effects depends on fire intensity (Bárcenas-Moreno and Bååth 2009). In turn, the composition and community structure of soil microbial communities is highly dependent on the environmental parameters that are altered by fire (Fierer and Jackson 2006; Smith *et al.* 2008; Goberna *et al.* 2012; Xiang *et al.* 2014). Some microbial groups can benefit from fire-altered conditions, while others are harmed. For example, fire increases the abundance of both endospore-forming *Firmicutes* in low to moderate fires following the peak temperature that triggers germination (Smith *et al.* 2008; Ferrenberg *et al.* 2013) and clades like *Betaproteobacteria* in response to changed environmental conditions (Ferrenberg *et al.* 2013; Xiang *et al.* 2014). Conversely, other taxa such as *Nitrobacter* seem to be more heat-sensitive and thus less abundant after a fire (Janzen and Tobin-Janzen 2008). Fluctuations in community composition induced by fire concomitantly change the phylogenetic structure of the community (e.g., Xiang *et al.* 2014). This observation agrees with empirical and conceptual models of temporal changes in microbial community structure, which postulate that niche-based assembling processes like environmental filtering and competition increase its relative importance after a perturbation (Ferrenberg *et al.* 2013, Dini-Andreote *et al.* 2015).

The way a community is phylogenetically structured provides clues about its main assembling mechanisms (Webb *et al.* 2002; HilleRisLambers *et al.* 2012). Environmental filtering decreases functional and phylogenetic diversity, both through the existence of: i) abiotic filters, which can be only

surpassed by species sharing certain traits (Webb *et al.* 2002), and ii) biotic filters, by which one (or a few) clade of strong competitors outcompete distantly-related lineages (Mayfield and Levine, 2010). In contrast, processes like competitive exclusion by limiting similarity increase the phylogenetic diversity of the communities by preventing the coexistence of species that are too functionally (and phylogenetically) similar (Pausas and Verdú 2010; Mayfield and Levine 2010). This community phylogenetics framework relies on two assumptions. First, traits are phylogenetically conserved, i.e. evolutionarily related species tend to be functionally similar, which has been recently demonstrated for microbes (Martiny *et al.* 2013, 2015; Goberna and Verdú 2016). Second, community patterns unequivocally reflect ecological processes, which is not straightforward in the traditional framework (Mayfield and Levine 2010; Narwani *et al.* 2015). Here, we try to overcome this limitation by i) incorporating to the traditional framework the ideas by Mayfield and Levine (2010), i.e. expanding the concept of environmental filtering to include biotic filters, and ii) suggesting a new approach that incorporates network analysis to detect the contribution of assembly processes operating simultaneously. Specifically, we propose to evaluate the phylogenetic community structure in co-occurrence microbial networks, which allow separately investigating the patterns of co-presence (microbes that co-occur) and exclusion (microbes that do not co-occur).

The study of communities from a network-based approach has been dealt with for a long time, comprising numerous studies in food-webs, plant-animal interactions or host-parasite systems (e.g. Solé and Montoya, 2001; Bascompte *et al.* 2003; Gómez *et al.* 2013). Ecological networks show complex relationships between nodes (species) connected by links (interactions), which inform about the composition and ecological interactions taking place in biological communities. Improvements of sequencing techniques in environmental samples have made also possible the inference of microbial co-occurrence networks from sequence data (Faust and Raes 2012). Co-occurrence networks may detect pairs of microbes that co-occur more (co-presence links) or less often (exclusion links) than expected by chance. Co-presence links may be reflecting shared niches while exclusion links suggest niche segregation (Barberán *et al.* 2012; Faust and Raes 2012). Applying the

community phylogenetics framework described above to co-presence and exclusion links, we can test whether environmental filtering alone (scenario A in Figure 1), competitive exclusion by limiting similarity alone (scenario B in Figure 1) or both mechanisms simultaneously (scenario C in Figure 1) are assembling the soil bacterial communities. Environmental filtering, by favouring the coexistence of functional (and phylogenetically) similar species, will reduce the phylogenetic diversity of co-presence links (dark grey boxes in scenarios A and C, Figures 1 and Appendix A2). Following the same rationale, environmental filtering, by excluding distantly related species, will increase phylogenetic diversity of exclusion links (light grey box in scenario A, Figures 1 and Appendix A2). The other main assembling mechanism -competition by limiting similarity- will prevent the coexistence of closely related species, resulting thus in high phylogenetic diversity of co-presence links (the dark grey box in scenario B, Figures 1 and Appendix A2). For the same reason, non-coexisting species under limiting similarity will be those that are functional (and phylogenetically) similar and therefore, exclusion links will have low phylogenetic diversity (light grey boxes in scenarios B and C, Figures 1 and Appendix A2). Simulations to validate this theoretical framework are provided in Appendix A2 (Figures A2.1 and A2.2).

Here, we analyse the temporal changes of soil bacterial communities before and after (from one day to one year) an experimental fire by focusing on the phylogenetic structure of co-presence and exclusion links. Because fire may impose filters to some microbial lineages unable to survive high temperatures and, at the same time, favour other lineages that are able to take advantage of nutrient release, we hypothesise that both environmental filtering and competitive exclusion by limiting similarity are simultaneously assembling post-fire soil bacterial communities.

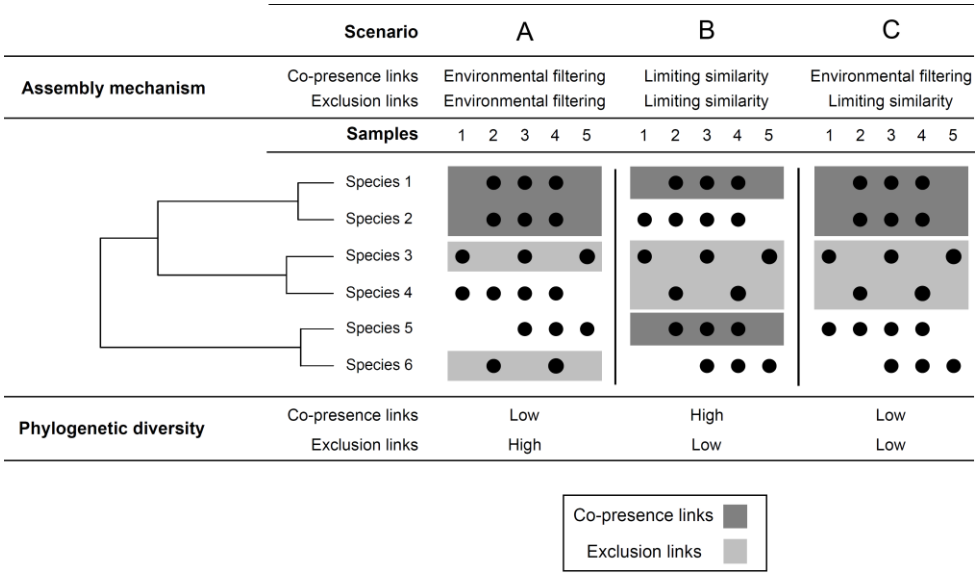

Figure 1: Schematic representation of the phylogenetic structure of co-occurring species as a result of two assembly mechanisms operating simultaneously in the community. Species co-occurrence is represented as an incidence matrix (i.e. presence-absence) of six species in five plots, where ● is drawn when a species is present in a sample. The species whose abundance patterns are positively-correlated (e.g. species 1 and 2 in scenario A) form co-presence links (shaded by a dark grey background) whereas those species whose abundance patterns are negatively-correlated (e.g. species 3 and 6 in scenario A) form exclusion links (shaded by a light grey background). Species with uncorrelated-abundance patterns are not shaded. Assuming trait conservatism (Goberna and Verdú, 2016), three different scenarios are possible (A-C), depending on how members of the co-presence and exclusion links are phylogenetically related: A and C correspond to scenarios in which two phylogenetically close species (species 1 and 2) in a co-presence link co-occur as the result of an environmental filter, while B corresponds to a scenario in which competitive exclusion by limiting similarity causes the coexistence of phylogenetically distant species (species 1 and 5). Simultaneously, not co-occurring species in exclusion links would be phylogenetically related (species 3 and 4, scenarios B and C) as the result of competitive exclusion by limiting similarity whereas they would be distantly related (species 3 and 6, scenario A) as a consequence of environmental filtering.

4.2. RESULTS

4.2.1. Fire effects on the soil bacterial community

Fire altered most soil physical and chemical properties (Figure A1.1). Some variables showed a significant increase as soon as 1 day after fire, e.g. the inorganic forms of nitrogen (NO_3^--N and NH_4^+-N) and electrical conductivity (EC). Others exhibited a delayed response to fire, such as soil humidity, which started decreasing after 1 week. Total organic carbon (TOC) doubled its levels after 1 month with the associated decrease in pH and increase in the C:N ratio. Total nitrogen (TN) tended to increase in response to fire, but differences were not significant due to a high inter-plot variation (Figure A1.1). Generally, soil parameters differed the most from the pre-fire levels after 1 and 4.5 months (Figure A1.1). Pre-fire soil properties were recovered after 12 months except for soil humidity, TOC and the C:N ratio (Figure A1.1). PCoA showed that bacterial community structure differed the most from pre-fire conditions after 1 and 4.5 months based on the separation of these plots along axis 1 (Figure A1.2A). TOC, NH_4^+-N and EC were positively correlated with axis 1, while soil humidity and pH had a negative correlation with the same axis (Figure A1.2A, Table A1.1). A similar temporal trajectory in the community composition space was observed across plots (Figure A1.2B).

Bacterial richness before fire was 602 ± 13 OTUs (mean ± SE) and significantly decreased 1 month after fire but recovered 1 year later (Figure 2). Fire reduction of bacterial richness was significant even when seasonal climatic variation was taken into account (Table 1). Fire also produced a high turnover of species (Table 2). Indeed, a substantial proportion of species at different time points after fire had not been present at the previous time point (Table 2). Fire also shifted the relative abundance of relevant taxonomic groups (Figure A1.3). Specifically, fire immediately (1 day after burning) increased the relative abundances of candidate division KSB1 and *Bacilli* while decreasing those of *Alphaproteobacteria* and candidate division NC10 (Figure A1.3). The relative abundance of *Bacilli*, whose initial increase was mainly due to that of the genus *Bacillus*, decreased along the year, while *Alphaproteobacteria* recovered its pre-fire levels after 9 months. Interestingly, *Betaproteobacteria* almost tripled its pre-fire values between 1 and 4.5 months since fire due to the increased abundance of

the genus *Massilia* (Figure A1.3). The analysis of bacterial community composition through OTU-based distance metrics revealed that soils harboured significantly different bacterial communities immediately after the fire (PERMANOVA: $F_{6,61} = 2.1$, $P < 0.001$, $R^2=0.17$). Furthermore, pairwise comparisons showed that pre-fire composition had not been recovered at any time point after the fire (all $P < 0.01$, data not shown).

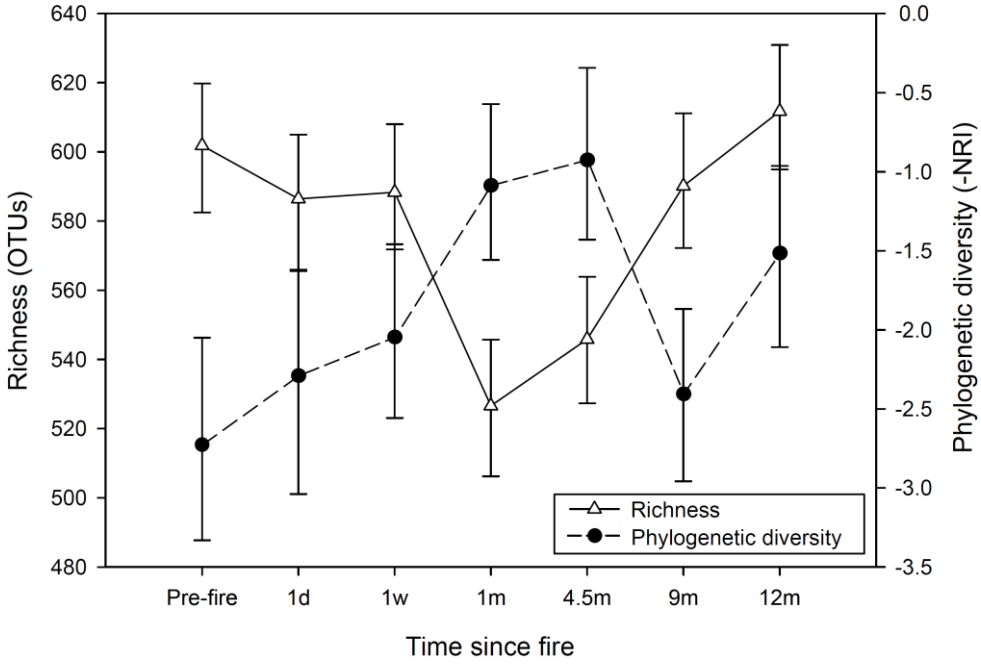

Figure 2: Post-mean estimates and credible intervals (95%) of the OTU richness and phylogenetic diversity of the soil bacterial community regarding time since fire. Negative values of phylogenetic diversity indicate phylogenetic clustering.

Changes in the composition of the bacterial community were translated into changes in the phylogenetic diversity of the bacterial community (Figure 2). The high phylogenetic clustering showed by the pre-fire bacterial community was relaxed with time after fire, reaching values close to randomness at 1 and 4.5 months and fluctuating later (Figure 2). Fire effects on phylogenetic diversity were significant after controlling for climatic conditions (Table 1).

Table 1: Post-mean estimates and their expected credible intervals (95%) for the fire-driven effect on the part of richness and phylogenetic structure (residues) that were not explained when climatic variables (temperature) were taken into account. Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are in bold.

	Richness residuals	Phylogenetic diversity (-NRI) residuals
Pre-fire (Intercept)	14.95 [-3.46, 34.61]	-0.68 [-1.19, -0.14]
1d	-16.18 [-43.73, 9.90]	0.43 [-0.30, 1.17]
1w	-13.83 [-39.76, 14.21]	0.67 [-0.05, 1.40]
1m	**-44.57 [-75.02, -20.93]**	**0.78 [0.02, 1.48]**
4.5m	-10.08 [-34.23, 22.08]	**0.77 [1×10^{-3}, 1.50]**
9m	**-37.91 [-63.37, -6.91]**	**1.05 [0.02, 1.82]**
12m	13.60 [-12.60, 40.57]	**1.05 [0.28, 1.89]**

Table 2: β-diversity analysis and number of shared and not shared (lost and new) species between pairs of samples at different time points. Lost (new) species are those present (absent) in the first time point and absent (present) in the second time point. Average values (± SD) of 10 plots are provided.

Time points		**β-diversity**			**Species**		
Initial	**Final**	**Turnover**	**Nestedness**	**Total**	**Shared**	**Not shared (Lost)**	**Not shared (New)**
Pre-fire	1d	0.62±0.03	0.02±0.02	0.64±0.02	213±32	396±28	349±39
1d	1w	0.60±0.03	0.02±0.01	0.62±0.02	221±30	341±39	383±29
1w	1m	0.62±0.03	0.02±0.01	0.64±0.02	211±19	397±39	344±38
1m	4.5m	0.60±0.02	0.03±0.02	0.62±0.03	217±27	338±35	376±61
4.5m	9m	0.60±0.02	0.04±0.02	0.64±0.03	192±25	400±73	296±35
9m	12m	0.60±0.05	0.06±0.03	0.66±0.03	182±26	306±52	403±82

4.2.2. Fire effects on the soil bacterial co-occurrence networks

The main topological parameters describing our study networks, including the number of nodes and the number and ratio of co-presence and exclusion links, were similar before and after the fire (Table 3). Networks were dominated by co-presence links, which accounted for approximately 60% of the links (Tables 3 and A1.2, Figure A1.4).

OTUs belonging to the same link, either co-presence or exclusion, tended to be more evolutionarily related than expected by chance, as indicated by a phylogenetic diversity significantly lower than zero (Figure 3). A significant interaction between time since fire and interaction type occurred ($F_{6,7636} = 2.5$, $P = 0.021$, Figure 3) because the phylogenetic diversity of co-presence links was initially higher than that of exclusion links but the opposite trend occurred 1 month later, and both link types had similar values after 4.5 months.

Table 3: Overall characteristics of the microbial networks regarding the fire event.

	Co-presence nodes	Exclusion nodes	Co-presence links	Exclusion links	Co-presence links / total links
Pre-fire	566	474	606	456	0.57
1d	543	439	727	499	0.59
1w	584	450	630	438	0.59
1m	545	426	617	402	0.61
4.5m	592	423	637	431	0.60
9m	479	385	677	436	0.61
12m	563	427	656	438	0.60

4.3. DISCUSSION

Our results show that fire did not alter general network parameters describing the soil bacterial co-occurrence patterns but changed the richness, composition and consequently the phylogenetic diversity of the community. Delving into the phylogenetic signature left in the network by species that co-occur and by those that do not co-occur helps us to discern the mechanisms assembling soil bacterial communities after fire.

Fire changed the soil abiotic environment as has been previously described (Certini 2005). The combustion of organic matter provoked an immediate increase in the inorganic compounds of nitrogen and electrical conductivity whereas the complete depletion of the plant cover reduced the soil humidity. The massive input of burned debris into the soil, which doubled the TOC contents, cannot be attributed to plant recovery that was very slight one month after fire. Seasonality might have also altered the levels of several

parameters, such as TOC, humidity or pH, but the magnitude of seasonal effects is lower than that detected here as we previously described in nearby Mediterranean ecosystems (Goberna *et al.* 2007). Even if the use of an unburned control in an adjacent area could have helped us to partly account for the influence of seasonal effects during this study, it would have not been without the presence of other confounding factors such as the environmental heterogeneity (e.g. presence of a natural plant cover, differences in soil properties) or the spatial distance which are a remarkable source of variation in microbes (Ramette and Tiedje 2007). Instead, we have directly controlled for seasonal climatic variation in our statistical models to test fire effects on microbial community parameters.

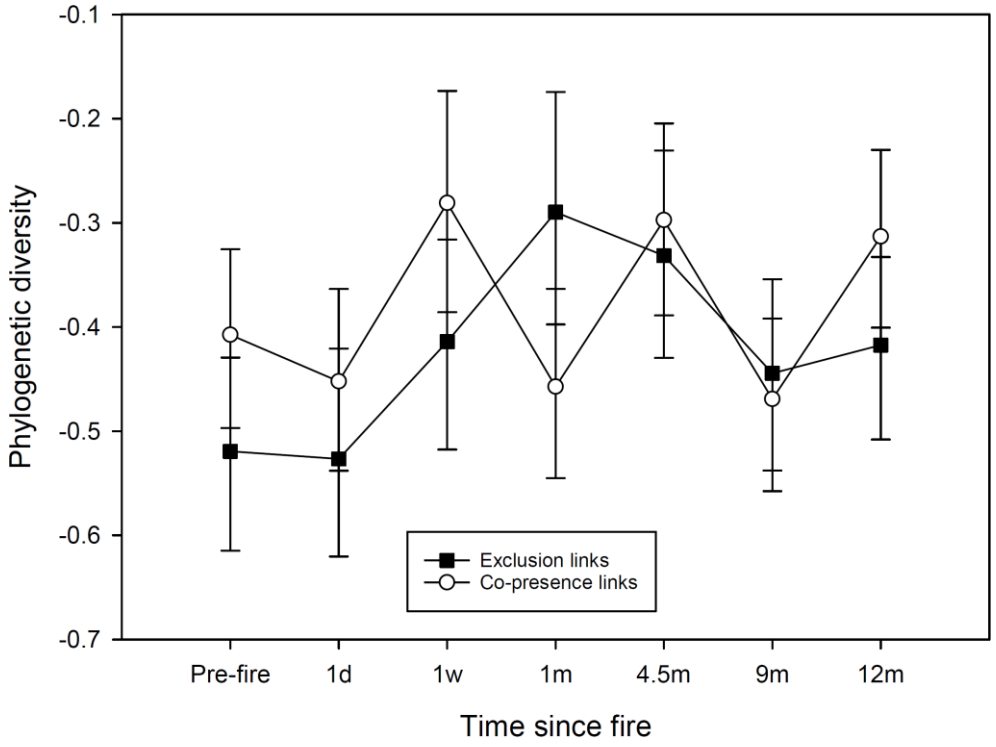

Figure 3: Post-mean estimates and credible intervals (95%) of the average phylogenetic diversity of co-presence and exclusion links regarding time since fire. Negative values indicate phylogenetic clustering of links.

Fire dramatically altered the specific composition of the soil bacterial community, showing particular shifts in some groups with a range of potential strategies that respond differentially to fire. In particular, it has been found that *Firmicutes*, which contains species able to form spores whose germination is triggered by high temperatures (Dworkin 2006), benefit from post-fire soil conditions in different environments (Yeager *et al.* 2005; Smith *et al.* 2008). In contrast, other groups (e.g. *Alphaproteobacteria*) decrease after fire (e.g. Smith *et al.* 2008; Xiang *et al.* 2014), suggesting that they could either be more sensitive to heating or harmed by the post-fire conditions. Temporal fluctuations in the community composition were not restricted to the immediate days following fire but continued to occur several weeks later. Notably, *Betaproteobacteria* experienced an important increase mainly caused by the rise of *Massilia*, a root-colonizing copiotrophic genus which is related to both early stages of microbial succession and plant development (Ofek *et al.* 2012).

Changes in the bacterial composition should be reflected in changes in the phylogenetic structure of the community if the traits allowing survival or competitive superiority are phylogenetically conserved (Pausas and Verdú 2010). This seems to be the case of traits conferring either environmental tolerance or competitive abilities in soil bacterial communities (Goberna *et al.* 2014a). Our results show that the community phylogenetic structure was always clustered, which could indicate the prevalence of environmental filtering in the community assembly (Webb *et al.* 2002; Mayfield and Levine 2010). However, fire reduced the richness while increasing the phylogenetic diversity at the community level as soon as 1 month after fire. These concomitant changes in richness and phylogenetic diversity could indicate that missing species after fire were phylogenetically related as a consequence of other mechanisms like competition by limiting similarity. Alternatively, it could also indicate that the communities are being stochastically re-assembled through other mechanism like dispersal. This could be the case if 1) the contribution of turnover with respect to nestedness were high and 2) the phylogenetic patterns in the community structure were erased, as our simulations confirm (Appendix A2, Figure A2.3). While we found a strong role of the species turnover after fire, this process did not erase the phylogenetically clustered pattern across communities, suggesting thus that dispersal was phylogenetically structured.

This raises the possibility that other mechanisms like competition by limiting similarity are also acting.

Co-occurrence networks allow a deeper analysis of the ecological processes structuring microbial communities, identifying patterns that could be indicative of environmental filtering (e.g. Levy and Borenstein, 2013; Pascual-García *et al.* 2014) but also other processes (e.g. competitive exclusion by limiting similarity) that would be indistinguishable at the community level if environmental filtering dominates (e.g. Horner-Devine and Silver, 2007; Steele *et al.* 2011; Faust and Raes, 2012). Positively and negatively correlated co-occurrence patterns indicated by co-presence and exclusion links respectively could be interpreted in terms of either niche preferences or ecological interactions (Faust and Raes, 2012; Barberán *et al.* 2012; Pascual-García *et al.* 2014). For instance, co-presence links could be the result of species sharing niche (i.e. species that exhibit abiotic or biotic abilities allowing its growth in similar environments) and/or interacting through cross-feeding, co-aggregation or co-colonization whereas exclusion links could arise because species have different niche and/or are involved in interactions like amensalism, competition or predation (Faust and Raes 2012). By phylogenetically informing the co-presence and exclusion links we have tried to shed light on the relative contribution of two types of processes (niche preference vs. competitive ecological interactions) after fire. The phylogenetic analysis of our network links supports the hypothesis that both processes are acting because co-presence and exclusion links were phylogenetically clustered, which agrees with environmental filtering determining co-presence and competition by limiting similarity favouring exclusion (see Figure 1, scenario C).

Closely-related species co-occurring more often than expected by chance is a common result that has been mainly attributed to environmental filtering in bacterial communities across a wide range of environments (Chaffron *et al.* 2010; Faust *et al.* 2012; Stegen *et al.* 2012; Levy and Borenstein 2013; Pascual-García *et al.* 2014). Levy and Borenstein (2013) found that metabolic competition was positively correlated to microbial co-presence in the human microbiome, suggesting that despite closely-related species being more

likely to share nutritional profiles and therefore to compete more, they tended to co-occur frequently probably because they also share other traits allowing them to survive a strong environmental filter. In agreement with the predominant evidence of environmental filtering determining bacterial co-occurrence, our co-presence links were populated with closely related species suggesting environmental filtering once more. This is not to say that ecological interactions like competition are not operating in bacterial communities (Levy and Borenstein 2013). In fact, our exclusion links also showed a phylogenetically clustered structure. We interpret this as the result of competitive exclusion by limiting similarity, where non-coexisting species belonging to an exclusion link were closely related species competing and excluding each other. In brief, both assembly processes occur at the same time and do not necessarily involve the same bacterial taxa. For example, immediately after fire, the co-presence links involving the most closely related taxa occurred between *Bacilli* species, suggesting that fire filtered the sporulation character. However, the exclusion links involving closely related taxa occurred between *Alphaproteobacteria*, indicating their role in competitive interactions (Goberna *et al.* 2014b). Other assembly processes (e.g. priority effects) could be relevant to the community after a disturbance (Nemergut *et al.* 2013). However, the fact that temporal trajectories in community composition after fire were similar across plots in addition to the phylogenetic patterns not being erased after the fire suggests that initial taxonomic composition, and therefore priority effects, were not determinant.

Fire changed the relative importance of niche-based assembling mechanisms over time, as postulated by empirical and conceptual models of microbial community succession (Ferrenberg *et al.* 2013; Dini-Andreote *et al.* 2015). This was suggested by the temporal variation in the phylogenetic diversity of both co-presence and exclusion links after fire indicating that this perturbation alters the contribution of environmental filtering and competition by limiting similarity. Ferrenberg *et al.* (2013) showed that soil bacterial community assembly in burned sites one month after fire was significantly more stochastic compared to the control, the reverse trend appearing several weeks later. We detected a very similar trend in our community, with phylogenetic diversity values approaching randomness one month after fire and

the low phylodiversity values indicative of environmental filtering (*sensu* Mayfield and Levine 2010) recovering later. By carefully inspecting the phylogenetic diversity of co-presence and exclusion links, we interpret this temporal fluctuation at the community level as the result of the balance between environmental filtering and competition by limiting similarity pushing towards low or high phylogenetic diversities. Species sharing a link might represent common life strategies to cope with the environmental conditions imposed by the great diversity of microhabitats contained in the soil (Raynaud and Nunan 2014; Koeppel and Wu 2014; Pascual-García *et al.* 2014). Examples of these strategies could include the ability to sporulate, the early colonization of the environment (e.g. by fast-growing copiotrophic organisms), or the use of the newly available forms of mineral nitrogen by denitrifiers, able to thrive in low-oxygen microniches that can be found in any aerobic soil. Those strategies, which involve traits related to either environmental tolerance (e.g. endospore formation) or competitive abilities (e.g. denitrification), are phylogenetically conserved with a varying strength (Goberna *et al.* 2014a). Ultimately, the phylogenetic signatures at the community level will be the result of both the evolutionary conservatism and the importance of these traits to survive post-fire conditions. Thus, combining phylogenetic and functional analyses will provide a better understanding of the post-fire community assembly mechanisms.

In conclusion, we suggest that despite the weak changes showed in the general parameters of the co-occurrence networks, fire altered community assembly mechanisms by changing species richness and composition. By phylogenetically informing co-presence and exclusion links of co-occurrence networks, we detected that fire altered the relative importance of environmental filtering and competitive exclusion by limiting similarity.

4.4. EXPERIMENTAL PROCEDURES

4.4.1. Study site and experimental fire

An experimental fire was ignited on 22 April 2009 in a 500 m^2 area of a dense shrubland dominated by *Rosmarinus officinalis* L. in eastern Spain (Teresa de Cofrentes, Valencia). Fire completely burned the plant cover that started

slightly recovering 4 months later (Figure A1.5). Soils are Haplic Leptosols (Calcaric, Humic) (FAO–ISRIC–IUSS, 2006) developed on limestones. The mean annual rainfall in the study site is 446 mm and mean annual temperature 13.7 ºC (Figure A1.5). Surface soil samples (0-2 cm) were taken from ca. 1×1 m georeferenced plots (n=10), which were randomly located at 1 to 3 m apart from each other within a 150 m^2 area. A total of 70 topsoil samples (i.e. 10 plots x 7 time points) were collected immediately before fire, and 1 day, 1 week, 1 month, 4.5 months, 9 and 12 months after the fire. To reduce the spatial heterogeneity that results from sampling an adjacent unburned area, the pre-fire samples were considered as the unburned control. Soils were transported to the laboratory on ice, immediately sieved (<2 mm) and stored at 4 ºC. Soil samples (approximately 300g) were analysed for their physical and chemical properties, including pH, gravimetric humidity, total organic carbon (TOC), electrical conductivity (EC), total nitrogen (TN), nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) using standard procedures as described by Goberna *et al.* (2012).

4.4.2. Soil DNA extraction and pyrosequencing

Soil DNA was extracted within 24 h after sampling from ca. 0.25 g soil with the PowerSoil® DNA isolation kit (MO BIO Laboratories, Carlsbad, California), which directly extracts the DNA after the physical and chemical lysis of cells. After a quality check of DNA extracts, the bacterial 16S rRNA gene was amplified using primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3'; Turner *et al.* 1999) and 534R (5'-ATTACCGCGGCTGCTGGC-3'; Muyzer *et al.* 1993), including each sample a 454 sequencing adaptor (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3') and a barcode in its 5'-end randomly selected from those published by Hamady *et al.* (2008). Pyrosequencing was performed by GATC Biotech (Konstanz, Germany) with the 454 GS-FLX platform (Roche). Further details of PCR conditions and purification can be found in Pérez-Valera *et al.* (2015).

4.4.3. Sequence analysis and phylogeny reconstruction

The initial sequence processing was performed by MR DNA (Shallowater, TX, USA) where short sequences (< 200 bp) were removed, primers and barcodes trimmed, and chimeric sequences excluded. After the initial processing, a total of 69143 sequences were obtained, with 1016.81 ± 198 (Mean ± SD) sequences per sample (Table A1.3). Two samples (belonging to 1d and 9m time points) were discarded because they failed to amplify. Operational taxonomic units (OTUs) were defined at an identity level of 97% and, after removing singletons, 3464 OTUs were aligned with PyNAST 1.2.2 in QIIME 1.8.0 (Caporaso *et al.* 2010a; Caporaso *et al.* 2010b). After manually checking the alignments and removing the hypervariable regions in QIIME, maximum likelihood phylogenetic trees were built with the GTRGAMMA substitution model using RAxML 7.3.0 (Stamatakis 2006). We constructed three independent trees to account for the uncertainty of the phylogenetic reconstruction. The topology of the basal relationships in the trees was constrained to match that of the megatree built from the Silva database (Release 108, Quast *et al.* 2013). Then, we constructed an OTU x plot abundance matrix showing the abundance of the total 3464 OTUs in each of the 68 samples. In order to reduce the potential bias caused by the differential sequencing depth between samples, rarefied richness was calculated (at 1023 sequences per sample) through an individual-based multinomial model which uses ten randomized samplings without replacement to estimate richness as in Colwell *et al.* (2012). The relative abundance of each OTU was corrected by the estimated number of 16S rRNA gene copies (Kembel *et al.* 2012). Further details about the sequence analysis along with sequences from the pre-fire conditions are available in Pérez-Valera *et al.* (2015). Post-fire sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB9090).

4.4.4. Network analysis

OTUs co-occurring more (co-presence) or less (exclusion) often than expected by chance were detected through co-occurrence network analysis. Co-presence and exclusion interactions were identified using an ensemble-based

network approach, which captures links from two measures of correlation (Pearson and Spearman) and dissimilarity (Bray Curtis and Kullback-Leibler) to cover a wide range of relationships (e.g. linear or non-linear), to deal with noise and outliers and thus, to reduce the impact of choosing a single measure (Faust and Raes 2012). Links detected by several correlation/dissimilarity measures in the same pair of OTUs were considered as a single link. The interaction sign was used to distinguish between co-presence and exclusion links. The analyses were run with the help of CoNet 1.0b6 (Faust *et al.* 2012; Faust and Raes 2012) and the script available at http://psbweb05.psb.ugent.be/conet/cmdline.php. Seven networks, one per time point, were constructed from the OTU x plot relative abundance matrix. Before network construction, samples were filtered such that OTUs present in less than 1/3 of the samples, i.e. low-abundant OTUs which could cause artefactual associations (Faust and Raes, 2012), were removed. The sum of the filtered OTUs was kept to preserve taxon proportions. Next, samples were normalized by calculating the relative abundance of each OTU. Then, networks were computed with the 1000 initial top- and bottom-scoring links for each measure. Statistical significance was tested by obtaining the link- and measure-specific p-value as the mean of the permutation distribution under the bootstrap distribution, using 1000 iterations for each distribution. In order to deal with the compositionality bias caused by the data normalization, that is, an increase in the absolute abundance of an organism implies a decrease in the relative abundance of all other, we re-normalized the data in each permutation (Faust *et al.* 2012). Thus, the null model captures the effect of data normalization (Faust *et al.* 2012). Dissimilarity measures (i.e. Bray Curtis and KullBack-Leibler) were not re-normalized because they are not affected by this bias (Lovell *et al.* 2010; Faust *et al.* 2012; Weiss *et al.* 2016,). Prior to computation, each row was divided by its sum for Bray Curtis calculations. Unstable links with scores not within the 95% confidence interval of the bootstrap distribution (e.g. outliers) or those with an opposite interaction sign were removed. P-values of different correlation/dissimilarity measures supporting the same link were merged using Brown's method and corrected for multiple testing using Benjamini-Hochberg's procedure (Brown 1975; Benjamini and Hochberg 1995). Finally, networks were filtered to keep only links with an adjusted merged p-value below 0.05. In order to reduce the

number of spurious and artefactual relationships, only those links supported by at least two correlation and/or dissimilarity measures were kept. We run sensitivity analyses to different parameters involved in network construction. Specifically, we modified data normalization (yes/no), number of correlation/dissimilarity measures (1/2), initial top- and bottom-scoring links (1000/2000) and minimal species occurrence (2/6) and results were not altered (data not shown).

4.4.5. Phylogenetic diversity

Phylogenetic diversity (PD) of the whole bacterial community was calculated as the mean pairwise distances between OTUs standardized by the expectation of a null model. This is equivalent to -1 times the abundance-weighted Net Relatedness Index (NRI):

$$PD = -NRI = (MPD_{obs} - MPD_{rand})/sd\_MPD_{rand}$$

where MPD_{obs} is the mean pairwise phylogenetic distances between the OTUs coexisting in a sampled plot, MPD_{rand} is the average of MPD calculated in *n* randomly constructed communities after shuffling the distance matrix labels of all the OTUs in the community, and sd_MPD_{rand} is the standard deviation of MPD_{rand} (Webb *et al.* 2002). Phylogenetic diversity of the links was calculated as the phylogenetic distance of each species pair against the phylogenetic distance of two randomly selected species (999 iterations). This procedure allows examining whether OTUs belonging to co-presence or exclusion links are more (negative values) or less (positive values) closely related than expected by chance. Thus, negative values of phylogenetic diversity indicate phylogenetic clustering while positive values indicate phylogenetic overdispersion. Calculations were run with the picante package for R (Kembel *et al.* 2010). Significance was tested by an across-sample (link) analysis (Hardy 2008). That is, we tested if the sets of communities (links) within a time point (link type) were significantly different from zero by calculating a Bayesian mean over sites with the help of the MCMMglmm package for R (Hadfield 2010). Phylogenetic (i.e. patristic) distances were computed using the *cophenetic* function for R.

4.4.6. β-diversity analyses

Nestedness and turnover components of temporal β-diversity (i.e. through time) were computed in order to test whether species after fire were a subset of the previously present species or, conversely, the loss and gain of species were more relevant after fire. The β-diversity analysis was performed between pairs of samples of adjacent time points using incidence matrices and the *beta.temp* function (with the Sorensen dissimilarity index) of the betapart package for R (Baselga *et al.* 2013). We also calculated the number of shared and not shared (lost and new) species between such samples using the *betapart.core* function of betapart.

4.4.7. Statistical analyses

Changes in the OTU composition of the bacterial communities after the fire were tested by permutational multivariate analysis of variance (PERMANOVA) using Bray Curtis dissimilarity matrices. This analysis was carried out with the *adonis* function using pairwise orthogonal contrasts comparing the pre-fire OTU x plot abundance matrix with all the post-fire matrices in the vegan package for R (Oksanen *et al.* 2015). Principal coordinates analysis (PCoA) of the Bray Curtis dissimilarity matrix was used to analyse and visualize the spatial differences in the community structure among plots over time in R. Physical and chemical parameters were fitted onto the ordination with the *envfit* function in the vegan package for R, showing only the variables that were significantly ($P < 0.05$) correlated to either axis.

Post-fire changes in OTU richness and phylogenetic diversity were calculated through a Bayesian generalized linear model using time since fire as a categorical independent factor. To account for temporal variation in diversity parameters due to seasonal climatic conditions (i.e. air temperature and precipitation, Figure A1.5), we used as the dependent variable of the model the residuals of a previous model including climatic conditions as independent factors. Both OTU richness and phylogenetic diversity were significantly correlated with air temperature (Richness post-mean estimate [95% credible interval]: -5.34 [-8.14, -2.89]; PD: 0.12 [0.06, 0.19]) but not with precipitation

(Richness post-mean estimate: -0.08 [-0.33, 0.19]; PD: 3×10^{-3} [-3×10^{-3}, 1×10^{-2}]). Thus, temperature was the only climatic variable taken into account to obtain the statistical residuals. To accommodate the topological and chronological uncertainty of the trees in the phylogenetic diversity model, we ran three models with three independent trees and integrated over the posterior samples by drawing 1000 random samples across models.

Post-fire changes in the phylogenetic diversity of co-presence and exclusion links were analysed following the same steps described above. In this case, the GLM had phylogenetic diversity as dependent variable and time since fire and link type (i.e. co-presence vs. exclusion links) as crossed independent factors. Neither temperature nor precipitation explained the phylogenetic diversity of co-presence links (temperature post-mean estimate [95% credible interval]: 5×10^{-3} [-5×10^{-3}, 0.01]; precipitation: 5×10^{-4} [-6×10^{-4}, 2×10^{-3}]). The phylogenetic diversity of exclusion links was correlated with air temperature (post-mean estimate: 0.01 [1×10^{-3}, 0.02]) but not with precipitation (post-mean estimate: 4×10^{-4} [-8×10^{-4}, 0.01]). Therefore, in this case we used the residuals from the climatic model.

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CHAPTER III: Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities

ABSTRACT

1. The ecosystem functions performed by soil microbial communities can be altered by ecological disturbances that deeply modify abiotic factors. Fire, a widespread disturbance in nature, is well known to alter soil abiotic properties but we still ignore how these shifts are translated into changes in the structure of soil microbial communities and their ecosystem functions. The phylogenetic structure of soil bacterial communities has been shown to be a good predictor of ecosystem functions, and therefore we used it as a measure linking the temporal variation of soil abiotic properties and ecosystem functions caused by an experimental fire in a Mediterranean shrubland.
2. Fire immediately favoured one phylogenetic clade containing lineages that are able to thrive with high temperatures and to take advantage of the post-fire nutrient release. Later changes in the phylogenetic structure of the community were dominated by phyla from another basal clade that show competitive superiority coinciding with high levels of oxidizable carbon in soil. The phylogenetic structure of the bacterial community significantly explained not only microbial biomass, respiration and specific enzymatic activities related to C, N and P cycles but also the mean number of rRNA copies in the community, an integrative proxy of different ecosystem functions.
3. While most of the ecosystem functions recovered one year after the fire, this was not the case for the structure of bacterial community, suggesting that functionally equivalent communities might be recovering the pre-disturbance levels of ecosystem functions.

5.1. INTRODUCTION

Microbial communities are an essential component of ecosystems, involved in many processes that shape the energy and nutrient flows and determine their productivity (Van der Heijden *et al.* 2008; Bardgett & van der Putten 2014). Soil bacteria are an extraordinarily diverse group of organisms with enormous functional capabilities that are fundamental for ecosystem performance, including mineral weathering, primary production and organic matter decomposition (Van der Heijden *et al.* 2008; Schimel & Schaeffer 2012; Bardgett & van der Putten 2014). Soil abiotic factors are main drivers of microbially-mediated ecosystem processes (Graham *et al.* 2014; López-Poma & Bautista 2014; Graham *et al.* 2016), and these processes can be better predicted by incorporating measures of microbial community structure (Graham *et al.* 2016). An increasing body of evidence suggests that including phylogenetic information to the measures of community structure improves the prediction of ecosystem functions (EF), since common evolutionary history defines shared functional abilities (Maherali & Klironomos 2007; Cadotte *et al.* 2008; Srivastava *et al.* 2012). This statement holds true for soil bacterial communities (Gravel *et al.* 2012; Venail and Vives 2013; Pérez-Valera *et al.* 2015), as prokaryotic traits that are relevant both to community assembly and EF are phylogenetically conserved (Martiny *et al.* 2015; Goberna & Verdú 2016; Morrissey *et al.* 2016).

Wildfires alter the functioning of forest ecosystems through changes in their biotic and abiotic components (Certini, 2005; Hart *et al.* 2005; Mataix-Solera *et al.* 2009; Keeley *et al.* 2012). Fire exposes soil microbial communities to extremely high temperatures and shifts their abiotic environment, thus altering their taxonomic and phylogenetic composition (Chapter IV). Fire tends to favour those lineages with heat-resistance capacities (e.g. spore-formers) and/or fast-growth strategies (Smith *et al.* 2008; Bárcenas-Moreno *et al.* 2011; Ferrenberg *et al.* 2013). Since microbial traits conferring capabilities to cope with fire exhibit a phylogenetic signal, i.e. closely related taxa tend to be more similar in their trait values (Goberna & Verdú 2016), changes in the community are phylogenetically structured (Pérez-Valera *et al.* 2017). That is to say, the probability of taxa to survive and thrive after fire are determined by their

evolutionary history. From an ecological perspective, fire also alters the competitive relationships among bacterial community members by shifting the availability of soil resources, mainly organic matter, nutrients and water (Pérez-Valera *et al.* 2017). Such variations in the competitive interactions can shift the dominance of main lineages, ultimately conditioning the overall microbial productivity (Knelman & Nemergut 2014; Pérez-Valera *et al.* 2015). Indeed, fire-induced shifts in the communities of soil microbes change microbial biomass, total activity and the rates at which organic compounds are decomposed and hydrolysed (Hernández *et al.* 1997; Choromanska & DeLuca 2002; Fontúrbel *et al.* 2012; Goberna *et al.* 2012).

The phylogenetic composition of soil bacterial communities in Mediterranean ecosystems is resilient to fire (Chapter IV), but the effects of post-fire community assembly on ecosystem performance have not been explored. By assembling bacterial communities through immigration experiments, Tan *et al.* (2012) showed that the initial phylogenetic relatedness among lineages determines the final composition of assembled communities. In these experimental communities, phylogenetic diversity was systematically related to ecosystem functioning, but the assembly history determined EF depending on the identity of community members (Tan *et al.* 2012). For instance, the assembly history of *Staphilococcus* communities determined both bacterial productivity and decomposition, while that of *Bacillus* communities influenced only productivity. These enticing experiments suggest that surveying the relationship between microbial diversity and EF needs of phylogenetically-informed metrics that take taxon identity into account. This is the case of the measures of phylogenetic community structure, such as that proposed by Pillar and Duarte (2010), which is able to identify the lineages linked to shifts in the phylogenetic structure of communities across environmental gradients (Duarte *et al.* 2016) and predict microbially-driven EF (Pérez-Valera *et al.* 2015). In addition, by showing the differential response of bacterial productivity and decomposition to community composition, the experiments by Tan *et al.* (2012) encourage using a battery of microbial indicators of ecosystem functioning. Community-level EF indicators that have been traditionally used include microbial biomass, activity, carbon use efficiency (i.e. organic carbon transformed into microbial biomass), as well as the rates of organic matter

decomposition and enzymatic hydrolysis of carbon, phosphorous and nitrogen organic compounds (Zak *et al.* 2003; Maestre *et al.* 2012; Goberna *et al.* 2012; Navarro-Cano *et al.* 2014). More recently, the rRNA operon copy number has been shown to bear several attributes that turn it into a good proxy of EF, since it correlates to microbial growth and sporulation efficiency and is negatively related to carbon use efficiency and protein yield (Lauro *et al.* 2009; Yano *et al.* 2013; Nemergut *et al.* 2016; Roller *et al.* 2016). Therefore, it could be expected that the immediate burst of nutrients caused by fire (Certini 2005) leads to the dominance of bacteria adapted to high resource availability showing high rRNA operon copy numbers, but low carbon use efficiency and reduced rates of enzymatic activity.

We speculated that the post-fire evolution of the phylogenetic composition of soil bacterial communities would drive the ecosystem functions related to microbial productivity, decomposition and nutrient cycling. To test this hypothesis, we analysed the phylogenetic structure of soil bacterial communities and measured microbial indicators of ecosystem functioning immediately before and during one year after an experimental fire in a Mediterranean ecosystem. We also tested i) which soil abiotic properties that are altered by fire drive the recovery of the phylogenetic structure of soil bacterial communities, and ii) whether the phylogenetically structured shifts in the soil bacterial communities determine the post-fire recovery of indicators of microbial biomass, growth rate, carbon use efficiency, organic matter decomposition, as well as carbon, phosphorous and nitrogen cycling.

5.2. MATERIALS AND METHODS

5.2.1. Experimental design and fire effects on soil abiotic factors

This study was carried out in a Mediterranean ecosystem that was exposed to an experimental fire in April 2009. The vegetation, a dense shrubland dominated by *Rosmarinus officinalis* L., was completely burned out. Soils were Haplic Leptosols (FAO–ISRIC–IUSS 2006), mean annual rainfall 446 mm and temperature 13.7ºC. Further details about the site, experimental fire and sampling can be found in Goberna *et al.* (2012). Briefly, surface soil samples (0-2 cm) from about 1 × 1 m were randomly selected (n=10), 1-3 m

apart from each other within a 150 m^2 area. Seventy samples (10 plots x 7 time points) were collected (~300g) prior to fire and 1 day, 1 week, 1 month, 4.5 months, 9 and 12 months after the fire. Pre-fire samples were considered as the unburned control to minimize the environmental and spatial heterogeneity that results from sampling an adjacent unburned area. The effect of the seasonal variation in the climatic conditions throughout the experiment were accounted for in the statistical analyses (see below). Samples were transported to the laboratory on ice, sieved (2 mm) and stored at 4ºC. Several physical and chemical variables were analysed using standard procedures (see Goberna *et al.* (2012) and Pérez-Valera *et al.* (2017) for further details). Briefly, fire triggered an immediate (1 day) pulse in inorganic forms of N (i.e. NO_3^--N and NH_4^+-N) and electrical conductivity (EC) (Figure B1). In addition, fire significantly increased total organic C (TOC) and decreased humidity and pH after 1 week to 1 month (Figure B1). Despite NO_3^--N or NH_4^+-N returned to pre-fire levels after several months, TOC, humidity, pH or EC did not recover during the study period (Figure B1).

5.2.2. DNA extraction and sequencing

A thorough description of DNA extraction, purification and pyrosequencing procedures is given in Pérez-Valera *et al.* (2017). Briefly, DNA from soil samples was extracted within the first 24 h after sampling from ca. 0.25g of soil with the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, California). After quality check of DNA fragments, 16S rRNA genes were PCR amplified using universal bacterial primers, and amplicons purified and sequenced using the Roche 454 GS-FLX platform. Raw DNA sequences were processed in order to remove low-quality, chimera and singleton sequences. A total of 3,474 operational taxonomic units (OTUs) were obtained after grouping sequences at the 97% sequence similarity level. We then calculated the relative abundance of OTUs as the ratio between absolute reads per OTU and the total number of sequences per sample. We estimated the number of 16S rRNA gene copies for each OTU using ancestral state reconstruction methods following Kembel *et al.* (2012), and used it to correct the relative abundance of each OTU. This correction was obviously not used to calculate community-weighted means of rRNA operon copy numbers (see

details below). We have previously described how fire altered the relative abundance of main phyla (Pérez-Valera *et al.* 2017). Briefly, *Firmicutes* increased immediately after fire, and recovered their pre-fire levels after 4.5 months (Table B1). On the contrary, *Proteobacteria* was initially reduced, due to the decline in *Alphaproteobacteria*, but surpassed its pre-fire values after 1 month caused by a peak of root-colonizing *Betaproteobacteria* (Table B1). A delayed response was detected in the relative abundance of *Bacteroidetes* and *Actinobacteria*, which respectively showed increased and reduced levels one month and one year after fire compared with pre-disturbance levels (Table B1).

5.2.3. Phylogeny reconstruction and phylogenetic community structure

Sequences representative of each OTU were PyNAST-aligned, manually checked, and the hypervariable regions removed (Pérez-Valera *et al.* 2017). To avoid the uncertainty produced by reconstructing phylogenies from short DNA sequences, we i) constrained the topology of the basal nodes accordingly to the OTU taxonomy and the SILVA database (Release 108, (Quast *et al.* 2013)) and ii) constructed three phylogenetic trees using the maximum likelihood algorithm in RAxML 7.3 (Stamatakis 2006). All trees were calibrated so as branch lengths represent chronological time (in million years) by using the function *chronos* in APE 4.0 (Paradis *et al.* 2004) for R (R Core Team 2017). Such a function uses a penalized likelihood approach to estimate the divergence times through a "correlated" model, which allocates similar diversification rates to closely-related tips. Phylogenetic trees were calibrated by using six dated nodes at the phylum-level (Table B2) according to Marin *et al.* (2017).

The phylogenetic structure of soil bacterial communities was estimated through the phylogenetic fuzzy-weighted method originally described by Pillar and Duarte (2010). This procedure calculates an OTU x plot matrix (matrix P) that describes the phylogenetic composition of the community by taking into account the abundance and the pairwise phylogenetic relatedness of each OTU with every other OTU in the community. The more diverse the phylogenetic neighbourhood of an OTU in a sample, the lower its value in matrix P. Second, the method reduces the dimensionality of matrix P through principal

coordinate analysis (PCoA) and extracts the loadings of each taxon to the principal coordinates of phylogenetic structure (PCPS). We calculated the contribution of each lineage to the first (PCPS1) and second (PCPS2) axes of the PCoA by averaging the OTU loadings *per* phylum. While PCPS1 accounts for differences at the basal nodes of the phylogeny, PCPS2 and all subsequent axes tend to catch shallower phylogenetic levels in the tree (Duarte *et al.* 2012, 2016). Both matrix P and PCoA calculations were run with the PCPS package for R (Debastiani & Duarte 2014).

5.2.4. Microbial indicators of ecosystem functioning

We measured five soil biochemical or physiological variables as in Goberna *et al.* (2012). Microbial biomass C (MBC) was quantified by the fumigation-extraction procedure as a proxy of microbial biomass. Basal respiration (BR) was measured during a 28d aerobic incubation experiment as an indicator of the activity of decomposers in mineralizing organic C into CO_2. Enzymatic assays (β-glucosidase, alkaline phosphatase and urease activities) were performed to respectively estimate the rates of C, P and N cycling. Two indices, the microbial carbon use efficiency (MBC/TOC ratio, microbial biomass C per unit organic C) and the metabolic quotient (qCO_2, C respired per unit microbial biomass) were respectively calculated as indicators of C use efficiency and C conservation efficiency (Anderson & Domsch 1990; Wardle & Ghani 1995). Finally, we calculated a community-weighted mean of the rRNA operon copy numbers by taking the product of the estimated 16S rRNA gene copies per OTU and its relative abundance (see details above), and summing the values across all OTUs in a plot.

5.2.5. Statistical analyses

We evaluated the post-fire evolution of the soil bacterial phylogenetic community structure, by testing the effect of time since fire on the two principal coordinates of phylogenetic structure (PCPS) using Bayesian generalized linear models (GLMs). Since the sampling covered time points varying in the climatic conditions, we initially tested the effect of the air temperature and precipitation on PCPS1 and PCPS2 in two separate Bayesian GLMs (data on the climatic conditions are given in Pérez-Valera *et al.* (2017)). We then used the residuals

of the 'climatic' model as the dependent variable in a second Bayesian GLM in which time since fire was used as a continuous independent variable. In this second model, we tested independently the effect of time since fire (as a continuous variable) on the residuals of PCPS1 and PCPS2. We also used the square of time since fire in the model to test for quadratic relationships. In all Bayesian GLMs, we accounted for the uncertainty of phylogenetic reconstructions by running three GLMs, each one using a PCPS calculated from an independent tree, and integrated over the posterior samples by drawing 1.000 random samples across models in the MCMCglmm package in R (Hadfield 2010). We used default priors, with 130.000 MCMC iterations, a burnin period of 30.000 iterations and a thinning of 100.

We tested whether taxa, PCPS and EF microbial indicators in post-fire communities differed significantly from pre-fire values by fitting GLMs with taxa abundances, PCPS or EF indicators as dependent variables and time since fire as a categorical independent factor. In this case, we also took into account the seasonal variations in the climatic conditions as above.

We then tested whether changes in soil abiotic properties determined the phylogenetic community structure of bacterial communities using PCPS1 or PCPS2 as the dependent variable and the soil abiotic factors as independent variables. Finally, we evaluated the effect of the phylogenetic community structure (PCPS1 and PCPS2) on each EF microbial indicator. Time since fire was included as a random factor in all models and seasonal variation in climatic variables was accounted for as above.

5.3. RESULTS

We described the phylogenetic composition of the bacterial communities through matrix P, in which each OTU has a value per sample that describes its phylogenetic neighbourhood. We show average matrix P values for all phyla detected before and after an experimental fire together with the phylogenetic relationships among phyla (Figures 1A and 1B). Under pre-fire conditions, OTUs belonging to *Actinobacteria*, *Proteobacteria* and the phylogenetic clade containing *Nitrospirae* and *Acidobacteria* showed high matrix P values

indicating that they tend to coexist with abundant close relatives (Figure 1B). Conversely, *Thermi* and *Cyanobacteria* exhibited low matrix P values, suggesting that OTUs within these lineages share their neighbourhood with more distantly related bacteria. Fire altered matrix P values distinctly depending on the lineage (Figure 1B). The clade including *Proteobacteria* and *Bacteroidetes* had lower matrix P values 1 day after fire and progressively higher values towards the end of the study period, whereas the opposite tendency was detected for the clade containing *Actinobacteria*, *Firmicutes*, *Thermi* and *Cyanobacteria*.

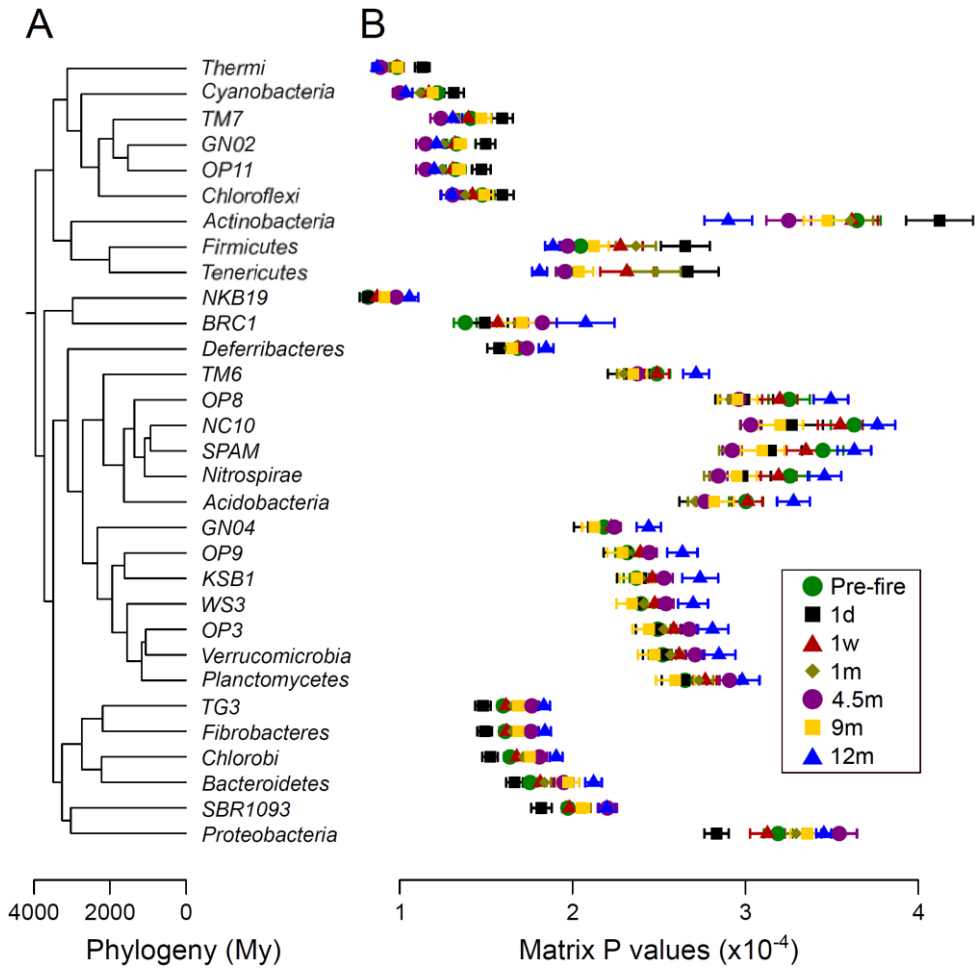

Figure 1: A) Phylogenetic relationships of main bacterial phyla, B) Matrix P values per phylum and sampling time. Bars indicate SE.

Variations in matrix P values with fire were translated into shifts in the two principal coordinates of phylogenetic structure (PCPS). According to the taxon loadings on PCPS1, this axis segregated two clades at the deepest phylogenetic level (Figure 2). One of these basal clades, including *Actinobacteria*, *Firmicutes*, *Thermi* and *Cyanobacteria* (Figure 1A), contributed to the negative pole of PCPS1 (Figure 2). The second clade, including *Proteobacteria*, *Bacteroidetes*, *Planctomycetes* and *Deferribacteres* (Figure 1A), had positive loadings on PCPS1 (Figure 2). PCPS1, which explained 40% of the total variance, was linearly correlated with time since fire (post-mean estimate [95% credible interval] = 4×10^{-4} [2×10^{-4}, 6×10^{-4}]) after accounting for climatic oscillations (Figure 3A). PCPS1 scores 1 day after fire were significantly lower than pre-fire scores, and reached significantly higher values 1 year later. PCPS2 (11% of total variance) was also significantly correlated with time since fire, once the climatic variations were considered, following a quadratic model (post-mean estimate of time = 4×10^{-4} [-2×10^{-5}, 9×10^{-4}]; time^2= -1×10^{-6} [-2×10^{-6}, -4×10^{-8}]) (Figure 3B). PCPS2 scores were significantly higher than pre-fire scores 1 month after fire, and then recovered pre-fire levels (Figure 3B). *Proteobacteria* had the highest loadings on PCPS2 (Figure 2).

Fire-induced shifts in the phylogenetic structure of soil bacterial communities were determined by changes in main soil abiotic properties (Figure 4). Specifically, the levels of NH_4^+-N and pyrophosphate extractable C (i.e. a measure of the total amount of oxidizable C) were the main predictors of PCPS1, whereas EC significantly explained PCPS2 (Figure 4, Table 1). In turn, the phylogenetic community structure of soil bacteria determined microbial EF indicators. PCPS1 correlated negatively with the 16S rRNA copy number and positively with respiration and qCO_2. PCPS2 significantly explained MBC, MBC/TOC ratio and enzymatic activities related to C, P and N cycling (Figure 4, Table 2).

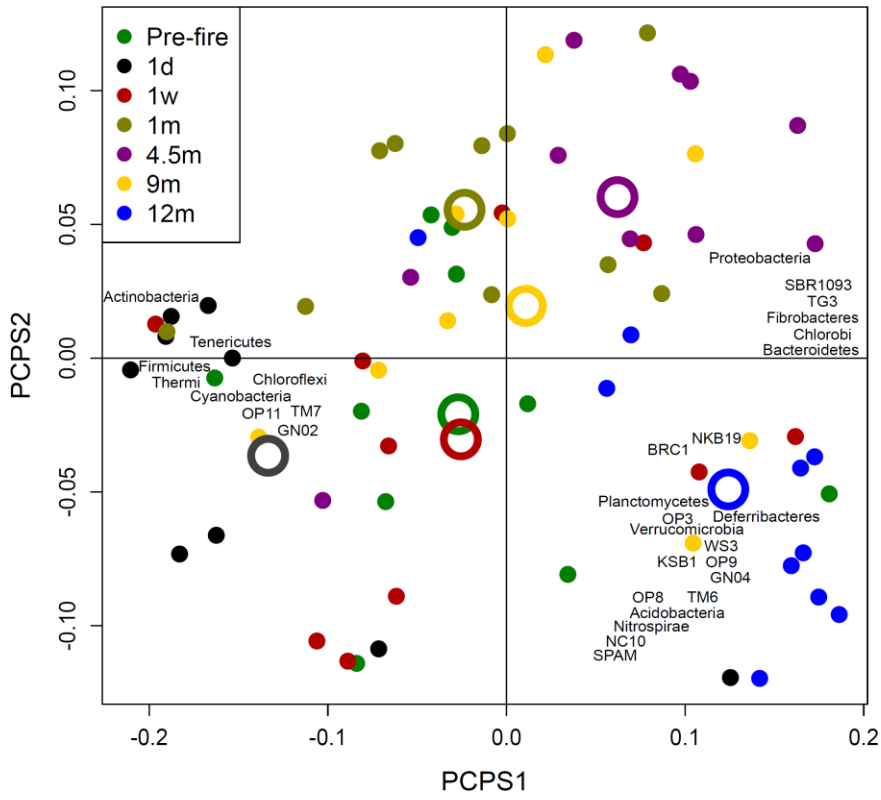

Figure 2: Ordination biplot of the two first principal coordinates of phylogenetic structure (PCPS) of bacterial communities before and after an experimental fire. Taxon names indicate loading factors of bacterial phyla on PCPSs. Open circles represent average PCPS scores per time point.

Table 1: Bayesian post-mean estimates and their expected 95% credible intervals for the effect of soil abiotic properties on the phylogenetic structure of bacterial communities. Significant values are shown in bold type.

	PCPS1	PCPS2
Total organic C	5.3×10^{-3} [-6.7×10^{-3}, 1.7×10^{-2}]	2.6×10^{-3} [-3.3×10^{-3}, 9.2×10^{-3}]
Total N	-1.3×10^{-2} [-3.1×10^{-1}, 2.7×10^{-1}]	1.2×10^{-1} [-4.8×10^{-2}, 2.7×10^{-1}]
pH	4.8×10^{-2} [-1.5×10^{-1}, 2.4×10^{-1}]	4.6×10^{-2} [-7.3×10^{-2}, 1.5×10^{-1}]
Gravimetric humidity	-5.8×10^{-3} [-1.3×10^{-2}, 2.0×10^{-3}]	2.3×10^{-3} [-1.6×10^{-3}, 6.2×10^{-3}]
NO_3^--N	-8.2×10^{-4} [-1.6×10^{-3}, 1.2×10^{-5}]	-2.7×10^{-4} [-7.3×10^{-4}, 2.0×10^{-4}]
NH_4^+-N	**-9.0×10^{-3} [-1.6×10^{-2}, -3.1×10^{-3}]**	-1.7×10^{-3} [-5.0×10^{-3}, 2.2×10^{-3}]
Pyrophosphate oxidizable C	**1.1×10^{-5} [2.8×10^{-6}, 2.1×10^{-5}]**	-4.1×10^{-6} [-9.4×10^{-6}, 2.7×10^{-7}]
Electrical conductivity	-3.6×10^{-4} [-7.9×10^{-4}, 1.3×10^{-4}]	**5.8×10^{-4} [2.9×10^{-4}, 8.5×10^{-4}]**

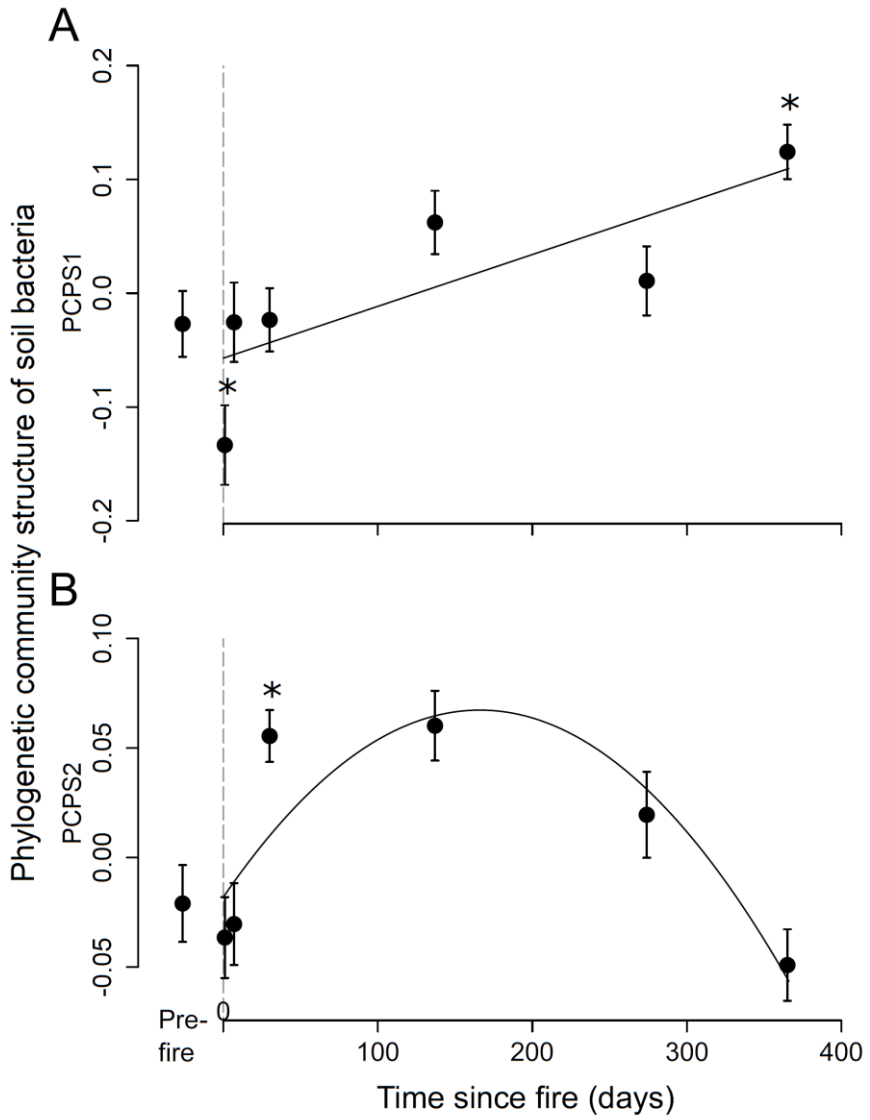

Figure 3: Evolution of the phylogenetic structure of soil bacterial communities before and after an experimental fire considering A) PCPS1 and B) PCPS2. Experimental fire was performed at Time 0. Solid lines indicate linear (PCPS1) and quadratic (PCPS2) regressions as a function of time since fire. Bars indicate SE for n=10. Asterisks indicate significant differences between each time point and the pre-fire level after accounting for the seasonal variation of climatic conditions.

Fire produced both immediate and mid-term effects on microbial EF indicators once changes explained by climatic variations were accounted for (Figure 5). Fire initially (1 day to 1 week) increased the levels of microbial biomass C (MBC), MBC/TOC ratio, 16S rRNA copy numbers, basal respiration (BR), β-glucosidase and phosphatase activities, whereas it did not alter the metabolic quotient (qCO_2) and decreased urease activity. Most of the initial peaks were reverted 1 month after fire, some variables such as MBC/TOC and phosphatase activity significantly decreasing even below pre-fire levels. While fire-driven changes in MBC, 16S rRNA copy number and enzymatic activities recovered pre-fire values within the first year, the shifts in BR and MBC/TOC were long-lasting (Figure 5).

Table 2: Bayesian post-mean estimates and their expected 95% credible intervals for the effect of bacterial phylogenetic structure (PCPS1 and PCPS2) on ecosystem function indicators. Significant values are given in bold type.

	PCPS1	PCPS2
Microbial biomass C	90 [-218, 433]	**1039 [334, 1672]**
MBC/TOC ratio	-0.1 [-0.8, 0.5]	**1.3 [0.3, 2.4]**
16S rRNA copy number	**-1.0 [-1.8, -0.5]**	1.2 [-0.1, 2.3]
qCO_2	**3.0 [0.9, 5.4]**	-1.0 [-5.8, 3.2]
Basal respiration	**22.6 [4.0, 40.8]**	36.1 [-0.4, 70.8]
β-Glucosidase activity	1.1 [-0.9, 3.3]	**8.2 [4.6, 12.1]**
Phosphatase activity	7.0 [-5.9, 19.9]	**39.9 [16.8, 65.6]**
Urease activity	0.1 [-0.3, 0.7]	**-1.1 [-2.1, -0.1]**

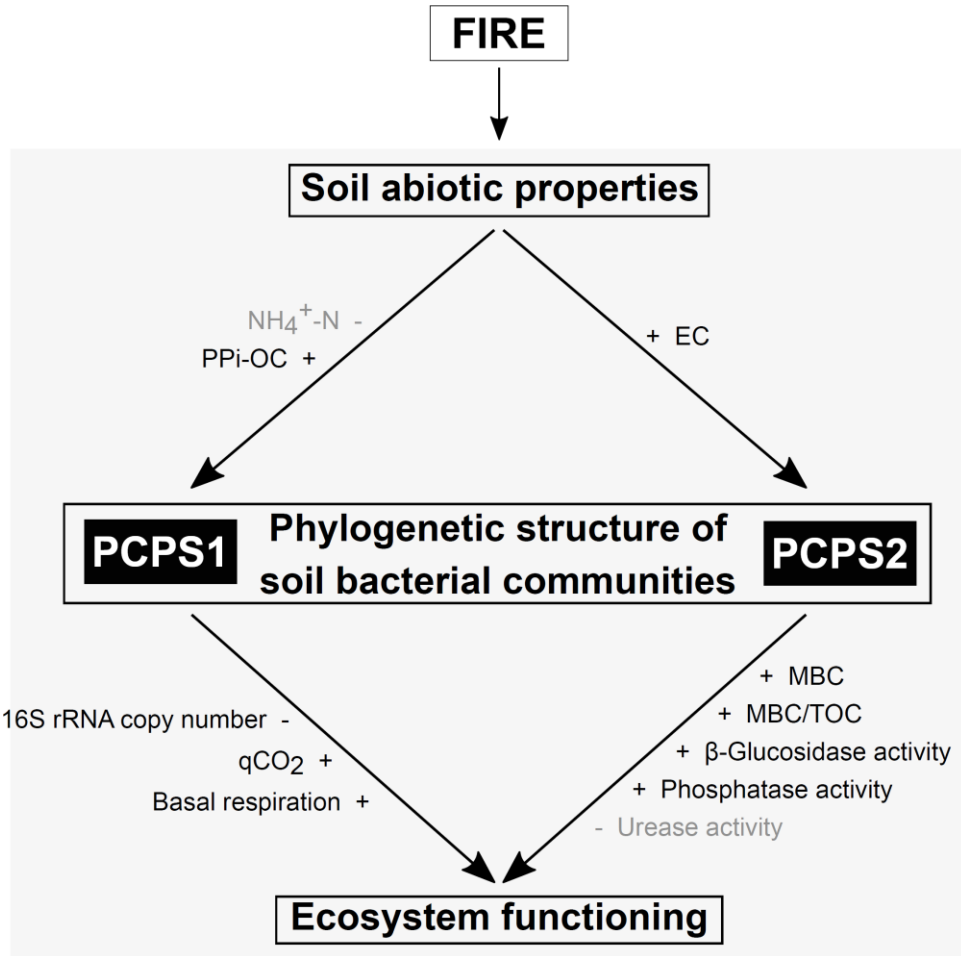

Figure 4: Fire-induced shifts on ecosystem functions are driven by changes in the soil abiotic environment that ultimately modifies the phylogenetic structure of soil bacterial communities. Positive and negative significant relationships are respectively shown in black and grey. Post-mean estimates and credible intervals (95%) are given in Tables 1 and 2.

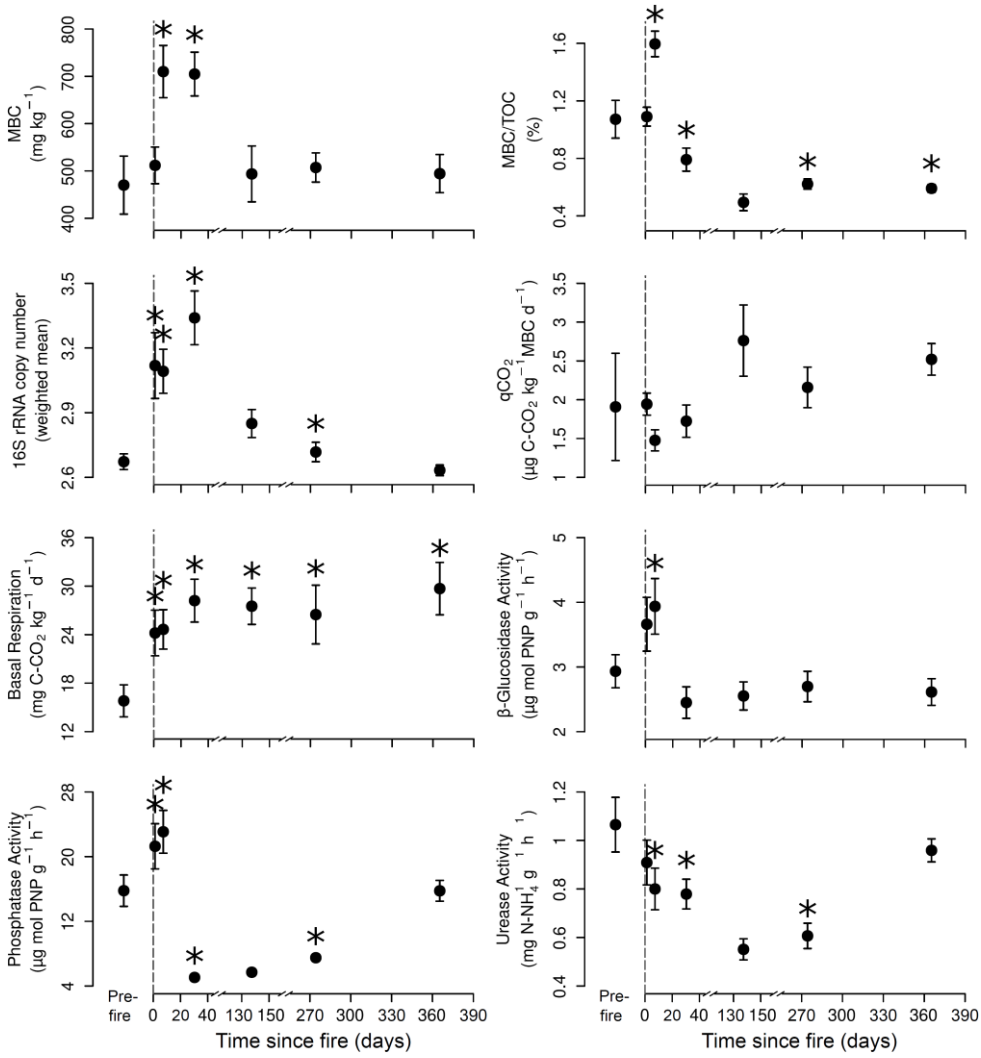

Figure 5: Post-fire evolution of microbial parameters indicative of biomass, growth, organic matter decomposition, carbon use efficiency, and C, N and P cycling. Asterisks indicate significant differences between each time point and the pre-fire level after accounting for the seasonal variation of climatic conditions.

5.4. DISCUSSION

Our results show that fire, by modifying soil abiotic properties, shifted the phylogenetic structure of bacterial communities and modified ecosystem functions related to microbial productivity, decomposition and nutrient cycling. Fire distinctly affected the two principal components (PCPS) that accounted for half of the variation in the phylogeny-weighted bacterial OTU composition. While PCPS1 scores increased in a linear fashion during post-fire evolution, those of PCPS2 followed a hump-shaped curve and recovered pre-fire levels. Scores of either PCPS responded to different soil abiotic parameters and eventually determined specific ecosystem functions. Although the bacterial phylogenetic community structure did not completely recover within the first year, most ecosystem functions returned to pre-disturbance levels.

5.4.1. Fire and the phylogenetic structure of soil bacterial communities

Fire instantly altered the phylogenetic structure of soil bacterial communities. As soon as one day after fire we detected significantly lower PCPS1 scores, a pattern that was driven by the response of organisms within the same basal clade in the bacterial phylogenetic tree. Many bacteria in these lineages are able to cope with high temperatures, either by producing resistance structures such as endospores (*Firmicutes*), spores (*Actinobacteria*) and akinetes (*Cyanobacteria*) or because of their thick cell walls (*Thermi*) (Dworkin 2006). The immediate response to fire of organisms belonging to this basal clade was most likely promoted by high temperatures, which stimulate spore germination (Dworkin 2006) and the ephemeral pulse in ammonium nitrogen, a direct product of combustion (Certini 2005). Indeed, we found that ammonium nitrogen correlated with PCPS1, suggesting that heat-resistant microbes thriving immediately after fire might have taken advantage of the burst in mineral nitrogen (Smith *et al.* 2008; Bárcenas-Moreno *et al.* 2011). Despite heat-resistant bacterial lineages had different dominance in the community (ranging from <1% to 25% of the total community for *Thermi* and *Actinobacteria*, respectively) and their relative abundances shifted distinctly after fire (Pérez-Valera *et al.* 2017), the response of their phylogenetic neighbourhood to fire was similar. That is, they tended to coexist with closer relatives immediately

after fire. This observation suggests that fire acts as an environmental filter that promotes the heat-resistance traits shared by these evolutionarily related organisms. The fact that such syndromes were captured by PCPS1, a metrics that accounts for differences at the most basal phylogenetic nodes, is consistent with those traits being deeply conserved in the phylogeny (Goberna & Verdú 2016).

The phylogenetic structure of the soil bacterial communities changed permanently during the study period. Our results suggest that such a shift was driven by organisms that belong to the second basal clade in the bacterial phylogeny, such as *Proteobacteria* and *Bacteroidetes*. These lineages include organisms that respond to the availability of organic carbon in soils (Fierer *et al.* 2007). In addition, *Proteobacteria* have been shown to exhibit a delayed response to abrupt environmental changes and competitively displace rapid responding (stress-tolerant) bacteria in laboratory experiments (Placella *et al.* 2012; Jurburg *et al.* 2017). The dominance and shifts in relative abundance of *Proteobacteria* and *Bacteroidetes* in response to fire were not alike. However, their neighbourhood shifted similarly during post-fire recovery, as they all bore higher phylogenetic resemblance to neighbouring OTUs towards the end of the study period. This pattern underlay the significant increase in PCPS1 scores one year after fire and is therefore responsible for the fact that PCPS1 did not recover pre-fire levels. This trend was linked to the total levels of oxidizable carbon in soil, which we found to be positively correlated with PCPS1. This observation is in agreement with the fact that *Proteobacteria* respond to organic carbon producing changes in the community that are phylogenetically structured (Goldfarb *et al.* 2011; Goberna *et al.* 2014; Morrissey *et al.* 2016). *Proteobacteria* were also key determinants of the second component of phylogenetic structure (PCPS2), to which this taxon contributed with the highest loadings. The post-fire evolution of PCPS2 scores, peaking from 1 to 4.5 months after fire and then returning to pre-disturbance values specifically resembles that of root-colonizing *Betaproteobacteria* (Pérez-Valera *et al.* 2017). The promotion of these organisms was likely supported by the temporary increase in the availability of inorganic ions in the soil solution, which is common after fire (Certini 2005), as PCPS2 scores were significantly explained

by the electrical conductivity. Shifts in the phylogenetic structure of soil bacterial communities were reflected in microbial EF indicators.

5.4.2. Fire and microbial ecosystem functions

Fire initially increased soil microbial biomass, C use efficiency and mineralization rates, as well as the cycling of organic C and P compounds. However, in the short term fire hampered the hydrolysis of organic N compounds, most likely due to product (ammonium N) inhibition of urease activity (Hoare & Laidler 1950). Contrarily to wildfires that significantly reduce microbial biomass and activity (Hernández *et al.* 1997; Jiménez-Esquilín *et al.* 2008), prescribed or experimental fires, with their lower intensity, have been shown to induce light shifts (even increases) in microbial productivity and nutrient cycling activities (González-Pérez *et al.* 2004; Fontúrbel *et al.* 2012; Fultz *et al.* 2016; Muñoz-Rojas *et al.* 2016).

We also detected an immediate increase in the community weighted mean rRNA copy numbers, indicating that fire favoured microbial lineages with an elevated number of copies of the 16S rRNA gene. Our results therefore support the observation that bacterial communities during the first stages of succession feature high rRNA operon copy numbers, as has been previously detected both in experimental and natural communities (Shrestha *et al.* 2007; Nemergut *et al.* 2016). Multiple rRNA operons have been suggested to be a discriminative genomic feature of the copiotrophic strategy (Lauro *et al.* 2009) and have been shown to determine cell growth and sporulation efficiency (Yano *et al.* 2013). Thus, in the first stages of succession, bearing an elevated 16S rRNA copy number is thought to provide a selective advantage by increasing the ability to rapidly respond to nutrient inputs and/or to form spores (Nemergut *et al.* 2016). We could specifically attribute the increase in the rRNA to the initial rise of *Firmicutes* (Figure B2), basically within the class *Bacilli* (Pérez-Valera *et al.* 2017). This peak lasted for the first month after fire, when the community weighted mean rRNA copy number was still abnormally high, but C use efficiency, and the rates of C, P and N cycling had significantly dropped to (or below) pre-disturbance levels. These patterns fit well with the idea that organisms with high numbers of the rRNA operon can exhibit high

reproductive rates but low levels of C use efficiency and protein yield (Roller *et al.* 2016).

Most microbial EF indicators returned to pre-fire levels during the study period, specifically those related to microbial biomass, rRNA operon copy number, and the rates of C, N and P cycling. Therefore, the recovery of most microbially-driven ecosystem functions was faster than that of the phylogenetic community structure. This opens the possibility that bacterial communities were not fully recovered, but replaced to a certain extent by another functionally equivalent community. Although functional redundancy has been suggested to operate in experimental bacterial communities (Bell *et al.* 2005), this is currently difficult to test in natural communities based on our still low knowledge on the contribution of specific microbial groups to ecosystem processes (Allison & Martiny 2008). Alternatively, taxa in the post-fire scenario could be taxonomically and functionally different to those prior to disturbance, but result in the same process rates measured at the community level (Allison & Martiny 2008). In addition, we detected a certain degree of functional dissimilarity between pre- and post-fire communities, as not all microbial EF indicators recovered original levels throughout the study period. Microbial respiration and carbon use efficiency pointed to faster rates of organic carbon mineralization into carbon dioxide and a reduced conversion into microbial biomass one year after fire. Higher respiration rates correlate well with the delayed promotion of *Betaproteobacteria* and *Bacteroidetes*, whose relative abundance significantly explains C mineralization rates in soils (Fierer *et al.* 2007). The observation that EF indicators had dissimilar post-fire trajectories depending on the relative abundance of particular phylogenetic lineages emphasize the importance of incorporating evolutionary information to understand how ecological disturbances may alter the relationship between biodiversity and ecosystem functioning.

5.5. ACKNOWLEDGEMENTS

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CHAPTER IV: Phylogenetic diversity is resilient to fire across biological domains

ABSTRACT

Fire alters the structure and composition of above- and belowground communities with concurrent shifts in phylogenetic diversity. It is unknown whether phylogenetic diversity across biological domains is resilient to fire, what would preserve the evolutionary history represented in our ecosystems. While fire reduces plant phylogenetic diversity based on the recruitment of evolutionarily-related species with post-fire seed persistence, it increases that of soil microbes by limiting soil resources and changing the dominance of competing microbes. During community reassembly plant and soil microbes might experience opposing temporal trends in their phylogenetic diversity that are linked through changes in soil conditions. We tested this hypothesis by investigating post-fire evolution of plant and soil microbial (fungi, bacteria and archaea) communities across three 20-year chronosequences. Plant phylogenetic diversity increased with time since fire as pioneer seeders facilitate the establishment of distantly-related late-successional shrubs. The post-fire increase in plant phylogenetic diversity fostered plant productivity, eventually ameliorating soil organic C. These shifts in the soil conditions explained the post-fire reduction of fungal and bacterial phylogenetic diversity, suggesting that evolutionarily-related taxa with high relative fitness recover their competitive superiority during community reassembly. Our results suggest that phylogenetic diversity across biological domains is resilient to fire.

6.1. INTRODUCTION

Ecological disturbance can disassemble biological communities by changing their structure and composition, a topic of prime relevance in the face of the current unprecedented rates of environmental change (Keeley, 1986; Cairney and Bastias, 2007; Mikita-Barbato *et al.*, 2015). Ecological communities can, however, experience no significant changes due to disturbance (resistance) or be capable of returning to their pre-disturbance structure and composition (resilience). The processes of community reassembly in resilient communities

can be better studied by using phylogenetic metrics of diversity, which inform on the evolutionary relationships among community members (Webb *et al.*, 2002). This is due to the fact that phylogenetically related organisms tend to respond similarly to disturbance (Verdú and Pausas, 2007; Amend *et al.*, 2016), since functional resemblances among species can be predicted using their common ancestry (Blomberg *et al.*, 2003; Goberna and Verdú, 2016). Furthermore, community resilience depends on the set of initial conditions, including the phylogenetic diversity of the species pool from which the community is reassembled (Tan *et al.*, 2012).

The phylogenetic diversity of plants and soil microbes is governed by sequentially operating assembly rules (Keddy, 1992; Goberna *et al.*, 2014a). Abiotic filtering is a pervasive community structuring force across biological groups, and biological interactions further fine-tune the community structure (HilleRisLambers *et al.*, 2012; Goberna *et al.*, 2014b). Both assembly mechanisms determine the phylogenetic structure of plant and soil microbes, which in turn show intricate linkages. Plant phylogenetic diversity, which increases biomass production through species complementarity (Cadotte *et al.*, 2008; Cadotte, 2013), has been observed to either have a positive or a negative reflection on soil microbial phylogenetic diversity (Barberán *et al.*, 2015; Goberna *et al.*, 2016). These divergent patterns can be theoretically explained by two alternative mechanisms of community assembly (HilleRisLambers *et al.*, 2012; Goberna *et al.*, 2016). First, diverse plant assemblages may supply a higher diversity of organic substances to the soil (Steinauer *et al.*, 2016) leading to higher microbial phylogenetic diversity through niche differences. Second, diverse plant assemblages can supply more organic substances to the soil (Lange *et al.*, 2015), thus increasing the competitive dominance of a few clades with high relative fitness that exclude entire lineages and lower microbial phylogenetic diversity. In addition to these top-down effects, evidence suggests that belowground diversity may affect plant diversity by changing herbivory, pathogenesis or soil nutrient availability, among others (Bardgett and van der Putten, 2014). The study of the phylogenetic diversity of biological communities has detected abiotic filters and biotic interactions also operate simultaneously to reassemble communities after an ecological disturbance (Verdú and Pausas, 2007; Pérez-Valera *et al.,* 2017).

Fires are widespread disturbances worldwide that disrupt the composition and phylogenetic structure of biological communities (Verdú and Pausas, 2007; Xiang *et al.,* 2014; Pérez-Valera *et al.*, 2017). Different lineages have evolved a wealth of ecological strategies to cope with heat-induced mortality or cell damage resulting in contrasting disassembly processes. Plant species may persist in a population after fire by recruiting from a fire-resistant seed bank (i.e. seeders) or by the vegetative regrowth of adults (i.e. resprouters) (Keeley, 1986). High fire intensity, especially in arid ecosystems, acts as an abiotic filter favouring the seeder over the resprouter strategy (Pausas and Keeley, 2014). Because seeding is a phylogenetically conserved trait, the high abundance of seeders after fire often results in the overrepresentation of closely related species (Verdú and Pausas, 2007). Thus, the phylogenetic fingerprint of plant community disassembly produced by fire, although it depends on the pre-fire proportion of seeders and resprouters, is generally the loss of phylogenetic diversity (Verdú *et al.*, 2009).

Soil microbes also have functional traits related to heat resistance. Archaea are the most tolerant to high temperatures given their characteristic cell wall and membrane lipid structure, based on ether bonds instead of the ester linkages found in most bacteria and eukaryotes (Stetter, 1999; Rothschild and Mancinelli, 2001). Bacterial living cells are generally not as heat-resistant, except for some groups of thermophiles, but many bacteria are able to produce resistant structures (e.g. spores, cysts, akinetes) that can withstand high temperatures, desiccation, radiation and other extreme abiotic conditions (Dworkin, 2006). Fungal cells are sensitive to heating (Rothschild and Mancinelli, 2001). However, fungi may produce thick-walled spores in hypogeous fruiting bodies or highly-compacted mycelia that provide them with fire resistance (Horton *et al.*, 1998; Tedersoo *et al.*, 2006). Since microbial functional traits are phylogenetically conserved (Martiny *et al.*, 2013; Goberna and Verdú, 2016; Kia *et al.*, 2017), it could be expected that, similar to plants, the overrepresentation of heat-resistant microbes would reduce soil microbial phylogenetic diversity after fire. However, existing evidence for bacteria and fungi points the opposite way, as fire increases the phylogenetic diversity of soil microbial communities (Rincón *et al.,* 2014; Pérez-Valera *et al.,* 2017). This increase in phylogenetic diversity could be attributed to a stronger competitive

exclusion between closely related species with similar niches and/or a reduced competitiveness of dominant taxa from entire clades with high relative fitness (Mayfield and Levine 2010; Goberna *et al.,* 2014b; Rincón *et al.,* 2014; Pérez-Valera *et al.,* 2017). Specifically, fire can increase competition by limiting the availability of soil moisture and organic substances (Neary *et al.*, 1999; Certini, 2005; Hart *et al.*, 2005; Mataix-Solera *et al.*, 2009).

The high resilience of Mediterranean plant communities to fire has been attributed to the fact that fire alters species abundance rather than composition and therefore recovery only involves the return to pre-fire abundances (Lavorel, 1999). Post-fire recovery of soil bacterial and fungal communities, which are fire-sensitive and predominantly heterotrophic microbes, require the amelioration of soil conditions (Treseder *et al.*, 2004; Cairney and Bastias, 2007; Xiang *et al.*, 2014). Soil archaea, generally heat-tolerant and including many chemolithotrophic organisms, seem to be more resilient to fire although scarce and contrasting results have been described (Goberna *et al.*, 2012; Mikita-Barbato *et al.*, 2015). Incorporating phylogenetic information to post-fire diversity trends would allow a better understanding of the assembly mechanisms driving the resilience of ecological communities across biological groups. We hypothesise that the phylogenetic diversity of plant communities in Mediterranean ecosystems will be resilient to fire, consequently triggering the reassembly of soil microbial communities through changes in soil conditions. Specifically, we test whether plant community reassembly enhances plant phylogenetic diversity up to pre-fire levels, fostering plant biomass (Cadotte *et al.*, 2008; Cadotte, 2013) and in turn soil fertility (Goberna *et al.*, 2016), thus ultimately decreasing the phylogenetic diversity of soil microbes (Goberna *et al.*, 2014a). These opposing phylogenetic temporal trends during the post-fire recovery of plants and soil microbes would be coherent with the recovery of competitive microbial clades with high relative fitness. To test these hypotheses, we analysed the post-fire evolution of the phylogenetic diversity of plant, and soil fungal, bacterial and archaeal communities across three 20-year fire chronosequences.

6.2. MATERIALS AND METHODS

6.2.1. Study area and experimental design

This study was carried out in three fire chronosequences that were located in the North, Centre and South of Valencia (E Spain; Figure C1). Each chronosequence included 8 to 9 sites, making a total of 25 sites that had experienced a single wildfire event during the last 20 years (between 1994 and 2014). In the study area, the fire regime has changed in the last 130 years, fire recurrence dropping from 397 to 49 years around the early 1970s (Pausas and Fernández-Muñoz, 2012). For the selection of the sites, we identified numerous burned areas using a database provided by the Valencian Government that included the dates and burned perimeter of all fires. According to this database only 1.7% of the total surface burned between 1994 and 2014 in the Valencian Community (ca. 5,300 out of 320,000 hectares) has experienced two or more fires. In order to reduce the environmental heterogeneity across sampling sites, we restricted the potential sites based on their similarities in lithology, slope orientation, and plant cover with the help of lithological maps (Gabaldón, 1994), topographical maps (CNIG, 2014a) and orthophotographs (CNIG, 2014b) using QuantumGIS 2.2 (QGIS Development Team, 2016). Sampling sites were finally established after an extensive field inspection. All sites had calcareous lithologies and we selected S-SW oriented slopes with <36° where typical Mediterranean scrublands develop (see details below). The main features of all sites are given in Table C1.

To further control for the environmental variability, we established a paired experimental design, each site having a burned and an unburned plot. We considered as long unburned plots (hereafter referred to as "unburned plots") those that had similar environmental conditions and land-use history than its paired burned plot, but had no historical fire register. Burned and unburned plots were located on average at (mean ± SE) 435 ± 49 meters away, ensuring the avoidance of fire edge effects. Plant and soil sampling was carried out in spring 2014 along three linear 25 m transects *per* plot, thus making a total of 150 transects (i.e., 25 sites × 2 plots × 3 transects). Transects were drawn in the direction of the slope and located ca. 10 meters apart.

6.2.2. Plant sampling and phylogeny reconstruction

Plant cover of each species was estimated through the line-intercept sampling method in the three 25 m transects in each plot (Canfield 1941; Butler and McDonald 1983). In each transect, we measured the horizontal distance of the interception of each plant individual. Plant cover was estimated by adding the intercept distances per species and expressing it over the total transect distance (25m). Plant height was measured for each individual intercepting each transect, and its biomass estimated as plant height × horizontal interception. This quantifies the plant area that intercepts the transect, which we used as an estimate of the aboveground biomass supplying organic inputs to the soils that were sampled along the transect (details below).

To reconstruct plant phylogeny, we grafted our study species in the family-level angiosperm tree derived from the Angiosperm Phylogeny Group III (https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20120829.new) by using the phylomatic package in Phylocom 4.2 (Webb *et al.*, 2008) (Figure C2). The ages of 29 nodes in our tree were obtained from literature (Table C2) and subsequently used to calibrate the tree under a birth-death model with the BEAST 1.5.4 (Drummond and Rambaut, 2007) and the PolytomyResolver script (Kuhn *et al.*, 2011). We also used this procedure to simultaneously resolve polytomies and generate many independent trees in such a way that topological and chronological uncertainty could be included in subsequent analyses. We generated 11,112 phylogenetic trees, discarded the first 25%, and randomly selected five phylogenetic trees. Further details about this procedure can be found in Verdú and Pausas (2013).

6.2.3. Soil sampling and sample analysis

Surface soil samples (0-5 cm) from the 150 transects were collected with a hand shovel after removing the surface layer that included ashes (for burned plots), litter, mosses and stones. Along each transect, one composite sample was taken that consisted of 10 regularly distributed subsamples of ca. 100 g. Samples were transported into an isothermal icebox to the laboratory, sieved (<2mm) and kept at 5ºC. Soil moisture content (gravimetric humidity), pH and

electrical conductivity (EC) were analysed with standard procedures as in Goberna *et al.* (2012). Total C (TC) and N (TN) were determined by dry combustion at 500ºC using a TruSpec C/N analyzer (Leco Corp., MI, USA). Total organic C (TOC) was also quantified after a 55 °C acidic (HCl) treatment of the samples, and total inorganic C estimated as the difference between TC and TOC. Ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) were quantified spectrophotometrically using the Nessler's reagent (0.09 M solution of K_2HgI_4 in 2.5 M KOH) and after reducing it to NO_2^--N.

To assess the adequacy of our paired samples, we checked that the contents of total inorganic C did not significantly differ between pairs of burned and unburned transects ($t_{71} = 0.77$, $P = 0.44$). Total inorganic C, mainly corresponding to carbonates in our study soils, is not expected to be affected by fire unless temperature exceeds 1000ºC (Certini 2005).

6.2.4. DNA extraction and sequence processing

Soil DNA was extracted from ca. 0.25 g soil with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). DNA quality was checked by electrophoresis in 1% agarose gels run in 0.5 × Tris–acetate–EDTA buffer. Amplifications of fungal ITS region were performed using the ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') primers (Gardes and Bruns, 1993; White *et al.*, 1990). Amplifications of the bacterial and archaeal 16S rRNA gene were performed using the universal prokaryotic primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3') (Caporaso *et al.*, 2012). Each sample contained a unique 8-nucleotide barcode in its 5' end. A single-step 30 cycle PCR was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: denaturation at 94ºC for 3 min, followed by 28 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC for 40 s, and elongation at 72ºC for 1 min, and a final elongation step at 72ºC for 5 min. PCR products from all samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Pyrosequencing was performed by MR DNA (Shallowater, TX,

USA) using Roche 454 FLX titanium instruments and reagents, and following manufacturer's instructions.

6.2.5. Sequence analysis and phylogeny reconstruction

Fungal ITS amplifications produced 1,649,877 DNA sequences. Primers and barcodes were trimmed and short sequences (<150 bp) removed. Sequences with homopolymers exceeding 6 bp and those with ambiguous base calls were removed. The sequence processing workflow included denoising, chimera and singleton removal. Operational taxonomic units (OTUs), defined at an identity level of 97%, were taxonomically classified using BLAST and the UNITE database v.7 (Kõljalg *et al.*, 2013). After this initial processing, 1,080,311 sequences were grouped into 6,620 OTUs. The OTU × transect community matrix, initially constructed from absolute read counts, was standardized by dividing the abundance of each OTU between the total number of reads *per* transect. In order to reconstruct the fungal phylogeny, we first constructed a genus-level tree from the literature that included all possible fungal genera, families, orders, classes or phyla found in our study (Figure C3). Then, OTUs were grafted into this tree according to their taxonomic information. Tree branch lengths were estimated from 42 dated nodes obtained from the literature, as for plants (Table C3). In order to resolve the polytomies, 580 trees were generated with BEAST after running the PolytomyResolver script. Five phylogenetic trees were randomly selected after removing the first 143 trees (25% of burnin), which were used for subsequent analyses. Since fungal ITS region is highly variable within and between species (Nilsson *et al.,* 2008), we tested the robustness of our results after delimiting fungal OTUs at a cut-off of 99% sequence similarity. Post-fire recovery in richness, phylogenetic α and β diversity, as well as relative abundance of main phyla remained the same (data not shown).

The 16S rRNA amplifications produced 2,547,644 sequences, which were processed as the ITS DNA sequences. After initial processing, 1,280,728 sequences were grouped into 7,003 bacterial OTUs and 38,503 sequences into 26 archaeal OTUs. OTUs were taxonomically classified using BLASTn and a curated database based on GreenGenes, RDPII and NCBI (DeSantis *et al.*,

2006) and aligned with PyNAST (Caporaso *et al.*, 2010a) in QIIME 1.9.1 (Caporaso *et al.*, 2010b). We constructed a separated OTU × transect abundance matrix for bacteria and archaea, and calculated relative abundance as above. We corrected the relative abundances based on the estimated number of 16S rRNA gene copies (Kembel *et al.*, 2012). Bacterial and archaeal phylogenies were separately reconstructed using RAxML 8.2.4 (Stamatakis, 2014) on the Cipres Portal (http://www.phylo.org), using the Maximum-Likelihood algorithm with 1,000 bootstraps. A constrained topology at the phylum level, and at the class level for Proteobacteria, was used for all monophyletic clades in accordance with the SILVA database (Release 123, Quast *et al.*, 2013). To account for the uncertainty of the phylogenetic reconstruction from short DNA sequences, five independent phylogenetic trees were constructed for bacteria and archaea. DNA sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB13469).

6.2.6. Diversity metrics and phylogenetic composition

Plant richness was calculated as the sum of species *per* transect. Fungal, bacterial and archaeal richness was estimated by an individual-based multinomial model using QIIME, in order to reduce the bias due to the differential sequencing depth across samples. This model samples without replacement at a given sampling depth as in Colwell *et al.* (2012).

Phylogenetic α diversity of plants, fungi, bacteria and archaea was calculated as the abundance-weighted standardized mean phylogenetic distance ($_{std}$MPD) with the picante package for R (Kembel *et al.*, 2010):

$$\text{Phylogenetic } \alpha \text{ Diversity (P}\alpha\text{D)} = {}_{std}\text{MPD} = (\text{MPD}_{obs} - \text{MPD}_{rand})/\text{sd\_MPD}_{rand}$$

where MPD_{obs} is the mean pairwise phylogenetic distance of species or OTUs *per* transect, MPD_{rand} is the mean (n=999) of the community phylogenetic distance after randomly shuffling the distance matrix labels of all the species or OTUs, and sd_MPD_{rand} is the standard deviation of MPD_{rand} (Webb *et al.*, 2002). Positive values of $_{std}$MPD indicate that the community is composed by

organisms more distantly related than expected by chance, whereas negative values indicate the opposite situation. Plant cover and OTU relative abundance matrices were used to weight the $_{std}$MPD of above- and belowground communities, respectively.

Fire-driven changes in the phylogenetic composition between each pair of burned and unburned transects (i.e., phylogenetic β diversity) were evaluated by using UniFrac distances (Lozupone and Knight, 2005). In order to have an estimate of the natural compositional similarity that occurs under unburned conditions, we also calculated phylogenetic β diversities for pairs of unburned transects within each site. Weighted UniFrac distances were calculated by using the GUniFrac package in R (Chen, 2012).

6.2.7. Statistical analysis

In order to test the effects of geographic distance on both soil abiotic properties and community composition, Mantel tests were run by using the ade4 package for R (Dray and Dufour, 2007). Correlations were only calculated between unburned transects to avoid the potential confounding effect in the burned plots caused by the time elapsed since fire at short sampling distances. We performed Mantel tests between soil or composition dissimilarity (Bray Curtis) matrices and geographic distance (Euclidean) matrices using 1,000 randomizations. To correct for multiple testing, significance of Mantel tests was assessed using the Benjamini-Hochberg procedure implemented in the p.adjust function in R. Neither soil abiotic parameters nor plant, fungal, bacterial or archaeal community composition showed spatial autocorrelation (Table C4).

We tested the existence of short-term fire effects by comparing soil properties, species richness and phylogenetic α and β diversities between plots that had burned 0-3 years ago and their unburned transects through paired t-tests in R. To estimate the post-fire recovery of all variables, we used the difference (Δ) between the value of each variable in paired burned and unburned transects, or directly the value from phylogenetic β diversity, as the dependent variable and time since fire as the independent variable in a Bayesian

generalized linear model (GLM). The models were run with the help of the MCMCglmm package for R (Hadfield, 2010). We calculated the recovery times (average and 95% credible intervals) for all variables by interpolation or extrapolation through the equation of the fitted model.

We explored the relationships between the post-fire recovery in plant phylogenetic diversity, plant biomass, the soil conditions and the soil microbial phylogenetic diversity. To do this, we performed a series of Bayesian GLMs, whose directionality was posed based on a priori knowledge as follows. First, to test whether the recovery of plant phylogenetic α diversity fosters plant biomass (Cadotte, 2013), we performed a GLM with ΔPlant PαD as independent and ΔPlant biomass as dependent variables including time since fire as a random factor in the model. Second, we analysed the effects of plant biomass on soil conditions and vice-versa, after reducing the variability of soil parameters through a principal component analysis (PCA) that included the differentials (Δ) of soil pH, TOC, TN, moisture, NO_3^--N, NH_4^+-N and EC between pairs of burned and unburned transects (Figure C4). We interpreted PC1 and PC2 respectively (52% and 21% of total variance) as gradients of recovery of soil organic matter and mineral N (Figure C4). We analysed the effects of plant biomass on the post-fire recovery of soil organic matter (PC1) since soil organic matter essentially comes from plant inputs (i.e. litter and exudates). In contrast, we analysed the effects of soil mineral N (PC2) on the post-fire recovery of plant biomass, since the forms of mineral N available to plants are generated either by the combustion or by the microbial mineralization of organic N. Finally, we analysed the effects of soil conditions on microbial phylogenetic α diversity and vice-versa. Since the vast majority of soil microbes are heterotrophic, and their contribution to the total pool of soil organic carbon is generally very low (<3% in nearby Mediterranean ecosystems, Goberna *et al.*, 2012; Navarro-Cano *et al.*, 2014), we evaluated whether the post-fire recovery in soil organic matter (PC1) determines ΔFungal PαD, ΔBacterial PαD and ΔArchaeal PαD in three separate models. In contrast, since soil microbes are both consumers (e.g. heterotrophs) and producers of mineral N (e.g. nitrifying microbes), we tested the bidirectional relationship between the post-fire recovery of soil mineral N (PC2) and microbial phylogenetic diversity.

6.3. RESULTS

6.3.1. Fire effects on plant communities

Fire significantly decreased aboveground plant cover and biomass (Figure 1, Table 1). In particular, fire decreased the plant cover of main families under unburned conditions, which were dominated by *Fagaceae* (plant cover % ± SE, 29 ± 3%), *Poaceae* (28 ± 3%) and *Lamiaceae* (18 ± 1%) (Figure C5). While the reductions in plant cover were large for *Fagaceae* (-21 ± 6%), *Poaceae* (-18 ± 5%) and *Pinaceae* (-17 ± 3%), those for *Lamiaceae* (-10 ± 6%), *Fabaceae* (+1 ± 3%) and *Cistaceae* (+2 ± 2%) were less pronounced or even increased after fire (Figure C5). Both plant cover and biomass significantly tended to recover with time after fire (plant Δcover post-mean estimate [95% credible interval] = 4×10^{-3} [3×10^{-3}, 6×10^{-3}]; Δbiomass = 0.14 [0.06, 0.23]). Plant cover recovered after 17 [11, 27] years while the recovery of the plant biomass was not reached during the time spanned in the study, but extrapolated to 22 [10, 69] years.

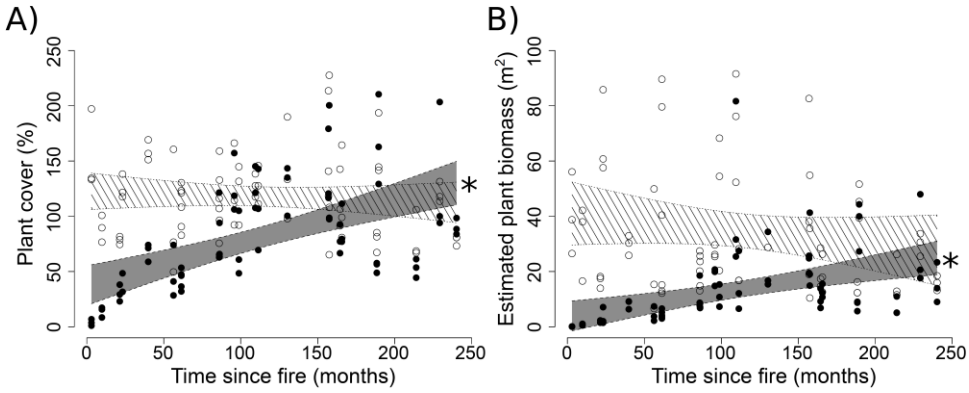


Figure 1: Post-fire trends of A) plant cover and B) biomass after fire. Filled circles indicate burned transects and unfilled circles unburned transects. Shaded and hatched areas show the confidence intervals of linear regressions among burned and unburned plots, respectively. Asterisks indicate the existence of a significant post-fire temporal trend of the studied parameter measured as the paired difference (Δ) between burned and unburned transects ($P < 0.05$). See the Results section for statistical details.

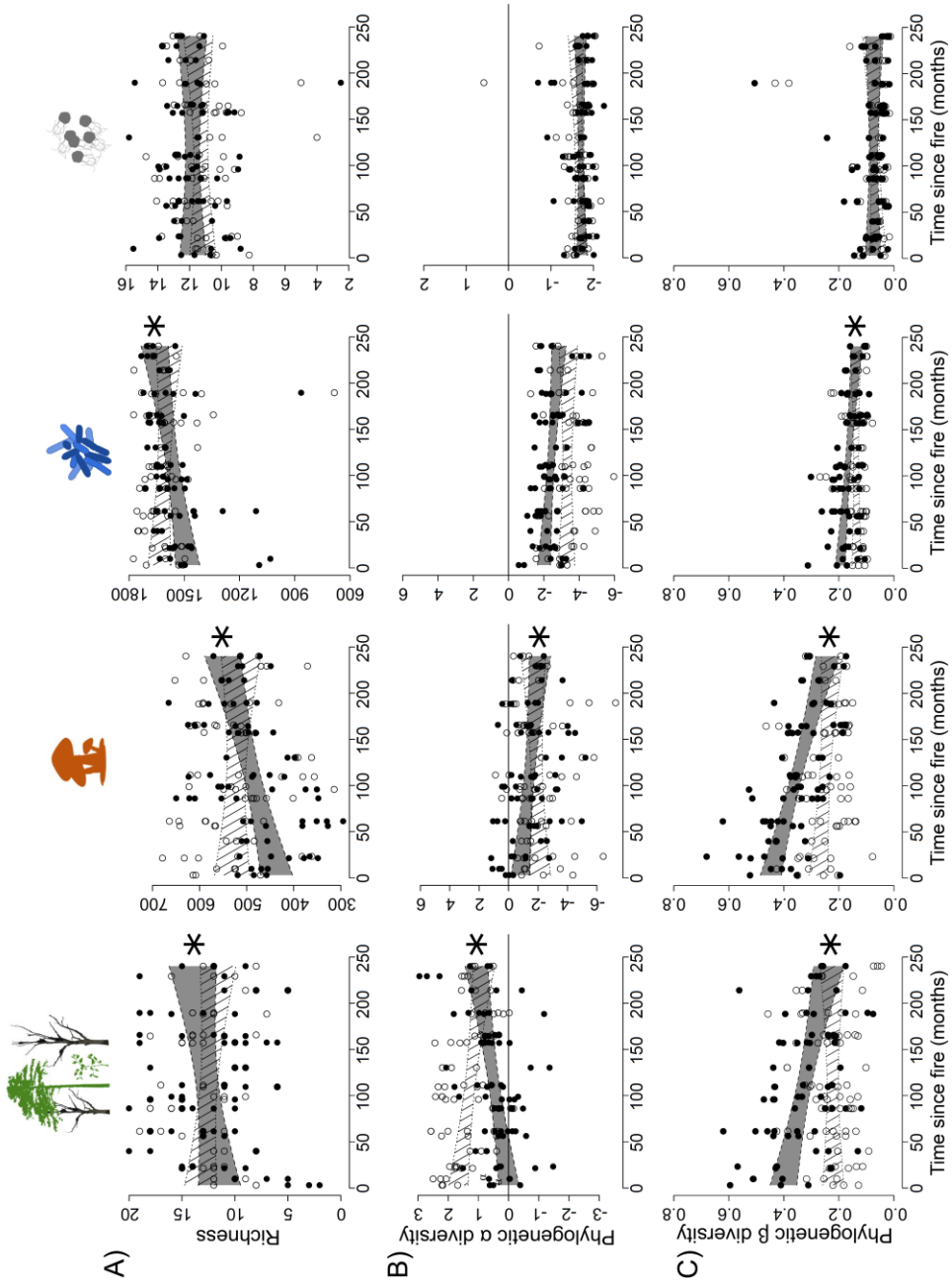
Fire did not significantly alter richness, but decreased the phylogenetic α diversity of plant communities (Figures 2A and 2B, Table 1). In addition, the plant phylogenetic β diversity between pairs of burned and unburned transects (shaded area, Figure 2C) was higher than that between unburned transects

(hatched area, Figure 2C, Table 1). That is, plant communities exposed to fire were more phylogenetically dissimilar to unburned plots than expected based on non-disturbed communities. Differences in plant richness, phylogenetic α and β diversities between burned and unburned plots were significantly reduced with time since fire, as significant temporal trends indicate (Δrichness = 0.02 [2.8×10^{-3}, 0.04]; ΔPαD = 8.2×10^{-3} [2.8×10^{-3}, 1.4×10^{-2}]; PβD = -6.9×10^{-4} [-1.0×10^{-3},-3.0×10^{-4}]; asterisks in Figure 2). Plant phylogenetic α and β diversities recovered to unburned levels after ca. 17 years (ΔPαD post-mean [95% credible interval] = 17 [5, 85]; PβD = 22 [11, 65]).

6.3.2. Fire effects on soil conditions and microbial communities

Fire decreased soil TOC, TN, moisture and the C/N ratio, while it increased pH and NO_3^--N in the short term (Figure C6, Table 1). In the 20 years spanning our study, these initial changes only reverted for NO_3^--N and pH, whose unburned values recovered after approximately 12 [3, 53] and 18 [6, 74] years, respectively. Fire-driven changes in soil TOC, TN and moisture decreased with time since fire but according to the extrapolations they would require ca. 26 years (TOC = 28 [9, 168]; TN = 24 [9, 105]; moisture = 24 [9, 88]) to reach the unburned level. It should be noticed that some of the estimates of the maximum recovery times might be biologically unrealistic since the limits of the credible intervals expanded very rapidly after the time period for which we have observations.

The effects of fire on the belowground communities were group-dependent. Archaea, which belonged mainly to *Crenarchaeota* (99 ± 0.3%) under unburned conditions, were resistant to fire as shown by the similar values in burned and unburned plots for all diversity metrics during the whole study period (Figures 2 and C5; Table 1). However, fire initially decreased both fungal and bacterial richness (Figure 2A, Table 1). Richness recovered with time since fire (fungi = 0.56 [0.20, 0.93]; bacteria = 0.98 [0.50, 1.43]), and differences


A)
B)
C)
Richness
Phylogenetic α diversity
Phylogenetic β diversity
Time since fire (months)
0 50 100 150 200 250
0 5 10 15 20
-3 -2 -1 0 1 2 3
0.0 0.2 0.4 0.6 0.8
300 400 500 600 700
-6 -4 -2 0 2 4 6
600 900 1200 1500 1800
2 4 6 8 10 12 14 16
-2 -1 0 1 2

between burned and unburned plots diminished after 14 [4, 60] and 13 [6, 36] years (Figure 2A). Fire provoked an initial increase in the phylogenetic α diversity of both fungi and bacteria, contrarily to plants (Figure 2B). Fungal phylogenetic α diversity significantly decreased with time since fire (-7.5×10^{-3} [-1.5×10^{-2}, -1.1×10^{-3}]), reaching unburned levels after 15 [3, 174] years. Bacterial phylogenetic α diversity also tended to decrease but changes were not significant during the study period. Both fungal and bacterial communities exposed to recent fires showed higher phylogenetic β diversity than expected based on the variability of non-disturbed communities (Figure 2C, Table 1). The phylogenetic β diversity between burned and unburned plots decreased significantly with time since fire (fungi = -8.7×10^{-4} [-1.2×10^{-3}, -6.0×10^{-4}]; bacteria = -2.5×10^{-4} [-3.9×10^{-4}, -1.3×10^{-4}]). Both fungal and bacterial communities recovered their pre-fire phylogenetic β diversity composition after 19 years (fungi = 19 [12, 34]; bacteria = 19 [9, 51]). Compositional changes within fungi resulted from increases in *Ascomycota*, which altered the balance between *Ascomycota* (% DNA sequences ± SE, 52 ± 2%) and *Basidiomycota* (47 ± 2%) after recent fires (Figure C5). Fire did not alter in the short-term the relative abundance of main bacterial phyla, i.e. *Actinobacteria* (27 ± 0.9%) followed by *Proteobacteria* (22 ± 0.4%), *Planctomycetes* (21 ± 0.5%) and *Acidobacteria* (9 ± 0.5%), although significant decreases in *Actinobacteria* and *Proteobacteria,* and increases in *Acidobacteria* were found after 5 years (Figure C5).

Figure 2: Post-fire trajectories of A) richness, B) phylogenetic α diversity and C) phylogenetic β diversity of plants, soil fungi, bacteria and archaea (from left to right). Filled circles indicate burned transects and unfilled circles unburned transects. In A) and B), shaded and hatched areas show the confidence intervals of linear regressions among burned and unburned plots, respectively. In C), shaded areas indicate confidence intervals of the phylogenetic β diversity between each burned and unburned plot, and hatched areas between pairs of unburned transects. Asterisks indicate significant post-fire temporal trends of the paired difference (Δ) between burned and unburned transects ($P < 0.05$). See the Results section for statistical details. Silhouettes represent plant and soil fungal, bacterial and archaeal communities. Original images (from http://www.phylopic.org and http://www.silhouettevectorstock.com) have been slightly modified and are licenced for use either under the Public Domain Mark 1.0 (fungi) or under a Creative Commons Attirbution-ShareAlike 3.0 Unported license (pine, MM Tobias; bacteria and archaea , M Crook).

Table 1: Short-term (0-3 years) fire effects on plant and soil microbial communities and soil parameters. t-tests (df=14) comparing burned and unburned plots are shown. Significant differences (P<0.05) between burned and unburned plots are indicated in bold.

	Variable	Burned plots	Unburned plots	t_{14}	*P*
PLANTS	**Cover**	**0.31±0.06**	**1.21±0.10**	**-9.048**	**<0.001**
	Biomass	**2.87±0.85**	**37.4±5.23**	**-6.656**	**<0.001**
	Species richness	10.4±1.40	11.1±0.60	-0.577	0.573
	Phylogenetic α diversity	**0.30±0.20**	**1.70±0.10**	**-5.576**	**<0.001**
	Phylogenetic β diversity	**0.38±0.04**	**0.19±0.02**	**5.004**	**<0.001**
FUNGI	**OTU richness**	**454±14**	**536±23**	**-2.844**	**0.013**
	Phylogenetic α diversity	**-0.50±0.32**	**-2.29±0.44**	**3.136**	**0.007**
	Phylogenetic β diversity	**0.43±0.03**	**0.29±0.02**	**3.532**	**0.003**
BACTERIA	**OTU richness**	**1492±48**	**1621±26**	**-3.442**	**0.004**
	Phylogenetic α diversity	**-2.14±0.21**	**-3.06±0.25**	**2.389**	**0.032**
	Phylogenetic β diversity	**0.18±0.01**	**0.12±0.01**	**4.394**	**<0.001**
ARCHAEA	OTU richness	11.8±0.5	10.9±0.4	1.370	0.192
	Phylogenetic α diversity	-1.76±0.05	-1.68±0.05	-1.555	0.142
	Phylogenetic β diversity	0.08±0.01	0.07±0.01	1.542	0.145
SOIL PARAMETERS	**TOC (g/100g)**	**7.1±0.4**	**13.8±1.8**	**-3.808**	**0.002**
	TN (g/100g)	**0.5±0.04**	**0.8±0.09**	**-3.928**	**0.002**
	pH	**8.0±0.02**	**7.6±0.08**	**4.731**	**<0.001**
	Moisture (%)	**5.5±0.5**	**10.2±1.0**	**-5.652**	**<0.001**
	NO_3^--N (mg/kg)	**94±18**	**41±13**	**3.309**	**0.005**
	NH_4^+-N **(mg/kg)**	2.5±1.1	2.8±0.9	-0.132	0.897
	C/N ratio	**13.7±0.5**	**16.6±0.6**	**-3.470**	**0.004**
	EC (μS/cm)	247±18	235±20	0.673	0.512

Plant cover is expressed as the fraction of the plot that is covered by one or more plant species. Note that the plant cover fraction can be greater than 1 if there are overlapping canopies. Biomass is expressed as the total sum of the biomass per plant species.

6.3.3. Linkages between above- and belowground communities

To test our hypothesis we performed sequential Bayesian GLMs, whose main results are schematized in Figure 3. First, we confirmed that the post-fire increase of plant phylogenetic α diversity leads to the recovery of plant biomass (post-mean=9.2 [2.6, 14.8]). Further, the recovered plant biomass partly accounted for the amelioration of soil organic matter as reflected by its significant positive effect on the first axis (PC1) of a PCA performed on soil abiotic parameters (0.02 [0.007, 0.04]). In turn, changes in soil PC2, which we

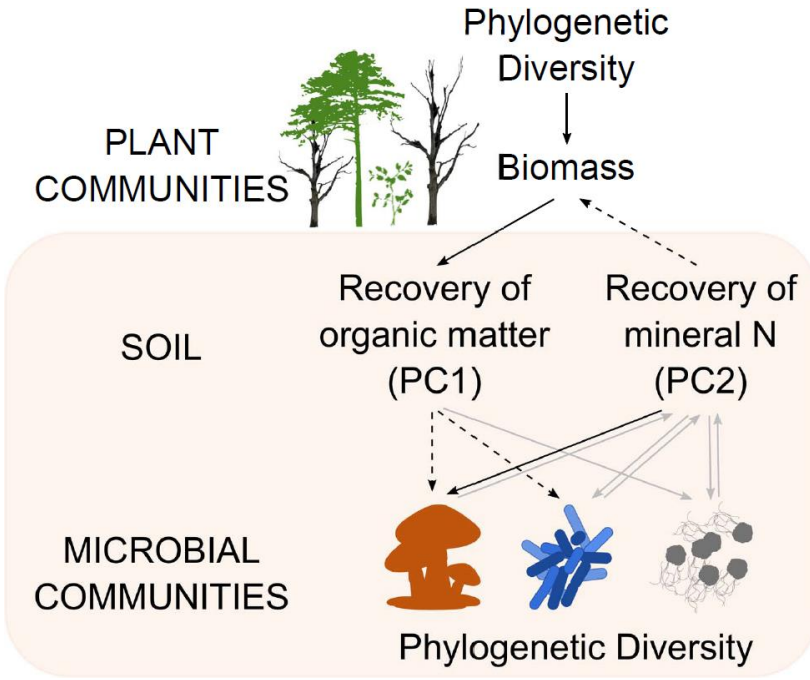


Figure 3: The recovery of plant phylogenetic diversity after fire significantly fosters plant biomass, which in turn ameliorates soil organic matter (PC1). The recovery of soil organic matter leads to a reduction of the fungal and bacterial phylogenetic α diversity. Plant biomass and fungal phylogenetic α diversity shift significantly associated with the recovery in soil mineral nitrogen. Black arrows indicate significant effects of Bayesian GLMs, with either positive (solid lines) or negative effects (dotted lines). Grey arrows indicate non-significant effects. Silhouettes represent plant and soil fungal, bacterial and archaeal communities as in Figure 2.

interpreted as a gradient of recovery of mineral N, significantly impacted plant biomass (-5.3 [-10.6, -0.3]) (Figures 3 and C4). Finally, we detected that post-fire changes in the soil organic matter drove the recovery of soil fungal and bacterial phylogenetic α diversity, while that of archaeal phylogenetic α diversity was unrelated to the shifts in soil organic C or mineral N (PC1 = -0.06 [-0.03, 0.06]; PC2 = 0.02 [-0.05, 0.08]). In particular, both fungal and bacterial phylogenetic α diversity significantly decreased to pre-fire levels with soil organic C (fungi=-0.3 [-0.6, -0.03]; bacteria=-0.4 [-0.5, -0.18]), while PC2 only contributed to explain the shifts in fungal PαD (fungi = 0.48 [0.02, 0.93]); bacteria = -0.01 [-0.33, 0.32]). Changes in microbial PαD did not significantly explain PC2 (fungi=0.19 [-0.02, 0.18]; bacteria=-0.08 [-0.23, 0.10]; archaea=0.08 [-0.34, 0.69]) (Figure 3).

6.4. DISCUSSION

Our results showed that phylogenetic diversity is either resistant or resilient to fire across biological domains. Fire had opposing effects on plant and soil microbial (fungal and bacterial) phylogenetic α diversity, while it did not alter archaeal diversity. By favouring evolutionarily related fire-prone species, fire reduced plant phylogenetic α diversity which was restored after two decades. These shifts triggered the recovery of soil conditions that, in turn, drove the community reassembly of soil microbes. Fungal and bacterial phylogenetic α diversity, which increases after fire as a result of an altered competitive hierarchy, returned to pre-disturbance conditions after two to three decades.

Fire did not change the richness but altered the composition of plant communities, lowering their phylogenetic α diversity by favouring closely related plants. The resprouting of adult plants and the rapid emergence of seedlings from the seed bank after fire can explain the unaltered levels of plant richness, which can even increase due to the colonization by new species (Keeley *et al.*, 2012). Similar species richness after fire can be obtained with different species composition and consequently, with different phylogenetic diversity, which tend to decrease in plant communities since fire favours the evolutionarily-conserved seeder phenotype that is present in a few families

(Verdú and Pausas, 2007). Here, we found a reduction in phylogenetic diversity to levels indistinguishable from the random expectation. In Mediterranean ecosystems, this pattern has been attributed to the combination of two counteracting strategies, i.e. those of seeders belonging to a few families and resprouters spread across several families, that respectively push towards low and high phylogenetic diversities finally generating a random phylogenetic pattern (Verdú *et al.*, 2009). Our results are in line with this explanation as seeders were slightly affected (*Lamiaceae*) or even favoured (*Cistaceae* and *Fabaceae*) by fire, while resprouters (*Fagaceae* and *Poaceae*) were harmed but not excluded from the community. Plant phylogenetic diversity significantly increased with time after fire, reaching the unburned level after ca. 17 years. Such an increment has been attributed to the nurse effect of pioneer seeders that facilitate the recruitment of late-successional evolutionarily-distant species (Verdú *et al.*, 2009). At later stages, facilitation can turn into competition, further increasing the phylogenetic diversity of plant communities (Castillo *et al.*, 2010). In the long term, however, the prolonged absence of disturbance could favour the dominance of few highly competitive species declining the phylogenetic diversity of plant communities (Verdú *et al.*, 2009). Experimental evidence shows that phylogenetically diverse plant assemblages produce higher biomass through species complementarity (Cadotte, 2013). Similarly, we found that the post-fire increase in plant phylogenetic α diversity significantly drives plant productivity in terms of biomass, and this has further reflection on soil processes.

Main fire-induced changes in soil abiotic parameters included the reduction in organic substances and moisture, and a pulse in mineral nitrogen as has been widely reported in the literature (Certini, 2005). These parameters are major determinants of soil microbial composition and diversity in post-fire scenarios (Hart *et al.,* 2005; Goberna *et al.,* 2012; Liu *et al.*, 2015; Mikita-Barbato *et al.,* 2015; Pérez-Valera *et al.,* 2017). Belowground microbial communities exposed to fire showed specific responses that were group-dependent. Soil archaea were non-responsive to fire in terms of richness, composition or phylogenetic diversity, suggesting that they are highly resistant both to heating and to the concomitant changes in soil parameters. In a previous study searching for immediate fire effects on soil microbiota in Mediterranean

ecosystems, we detected shifts in archaeal diversity one day after fire that recovered as soon as one week later (Goberna *et al.*, 2012). However, studies in other ecosystems point to shifts in archaeal communities that persist for at least two years (Mikita-Barbato *et al.*, 2015). Fungal and bacterial communities showed parallel responses to fire, in spite of their enormous physiological and ecological differences such as heat tolerance or response to changes in organic compounds that suggest that fungi could be more sensitive to fire than bacteria (Hart *et al.*, 2005; Cairney and Bastias, 2007; Mataix-Solera *et al.*, 2009). Specifically, fire decreased richness, altered the community composition, and increased the phylogenetic α diversity of both fungal and bacterial communities. This is consistent with multiple studies that report fire-driven reductions in soil microbial richness (Visser 1995; Smith *et al.,* 2008; Kipfer *et al.,* 2010; Ferrenberg *et al.,* 2013; Rincón *et al.,* 2014; Xiang *et al.,* 2014, 2015; Pérez-Valera *et al.,* 2017), although contrasting patterns have been also reported (Hamman *et al.*, 2007; Rincón and Pueyo, 2010; Holden *et al.*, 2013; Buscardo *et al.*, 2014; Sun *et al.*, 2015; Shen *et al.,* 2016). In Mediterranean ecosystems, the increment in phylogenetic α diversity has also been observed both for fungal and bacterial communities. Rincón *et al.,* (2014) detected this trend in soil ectomycorrhizal fungi in response to the overrepresentation of *Ascomycetes* after fire, which we also detected. This phylum contains species able to produce fruiting flushes, resistant spores and hydrolytic and phenol oxidizing enzymes that could help them utilize the newly released nutrients and colonize post-fire emerged plants (Cairney and Bastias, 2007; Rincón *et al.*, 2014). In bacteria, we attributed the increase in phylogenetic α diversity after an experimental fire to a shift in the competitive hierarchies (Pérez-Valera *et al.,* 2017). Similarly, the fire-induced reduction in organic resources here detected can diminish the competitiveness of dominant taxa from entire clades that have high relative fitness under carbon-enriched conditions (Goldfarb *et al.*, 2011), leading to an increased phylogenetic α diversity. This is supported by the fire-induced reduction in the relative abundance of both *Proteobacteria* and *Actinobacteria*, lineages that possess high competitive abilities for organic carbon substrates (Goldfarb *et al.,* 2011). In addition, the alleviation of such a biotic filter sets the conditions for a stronger competition between closely related taxa with similar niches, e.g. fast-growing bacteria that benefit from the pulse in mineral N,

which would further increase the phylogenetic diversity of the bacterial community (Mayfield and Levine, 2010).

Fungal and bacterial phylogenetic diversities were resilient to fire, recovering after ca. two decades. The decreasing phylogenetic trends in α diversity of soil microbes contrast with the increasing phylogenetic α diversity trend of plant communities after disturbance. The main driver of the recovery of microbial phylogenetic α diversity was the restoration of soil organic matter supplied by an increasingly productive and phylogenetically diverse plant community. These results suggest that the evolutionarily-related microbial taxa that dominate under high soil fertility recover their competitive strength as communities reassemble. Therefore, we can speculate that, even under the current accelerated fire regimes (Pausas and Fernández-Muñoz, 2012), our studied communities had enough time to recover. Further studies are needed to corroborate these results in other ecosystems, since ours are restricted to Mediterranean ecosystems and specific environmental conditions. The resilience of plant and soil microbes to current fire regimes guarantees the conservation of the old evolutionary legacy represented by all the biological domains in the tree of life. However, the increasing rates of disturbance the Earth is now facing could dramatically reduce the resilience of these biological lineages by eroding the phylogenetic diversity from which the communities are reassembled (Tan *et al.*, 2012).

6.5. ACKNOWLEDGMENTS

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7. GENERAL DISCUSSION

The global aim of this thesis is to delve into the factors that determine the phylogenetic structure of soil bacterial communities and its resilience to fire, eventually providing insights into the processes that drive bacterial community assembly and its relationship with the functioning of Mediterranean ecosystems. Essentially, by incorporating the evolutionary relationships among bacteria our findings provide support to the role that particular lineages have on the bacterial community assembly and ecosystem productivity, which is not straightforward under the classical approaches of community ecology.

Soil abiotic properties, especially those related to fertility such as the levels of organic carbon, determined the phylogenetic diversity and community structure of soil bacteria in the ecosystems under study. This agrees with both observational and experimental evidence suggesting organic C as one of the main drivers structuring bacterial communities worldwide (Fierer, 2017). In particular, we found that increasing levels of organic C, which is typically limiting in soils, altered the bacterial community composition and reduced its phylogenetic diversity (Pérez-Valera *et al.*, 2015). This agrees with the widespread observation that the phylogenetic diversity of soil bacterial tends to be lower than expected by chance, i.e. soil bacteria tend to coexist with close relatives, a pattern that has been explained by the dominance of environmental filters operating via biotic and/or abiotic components (Mayfield and Levine, 2010; Goberna *et al.*, 2014a). Our data point to a dominant biotic filter that is due to the overrepresentation of extremely competitive clades, mainly *Proteobacteria* and *Actinobacteria*, which show high fitness under carbon enriched conditions and exclude entire lineages (Goldfarb *et al.*, 2011; HilleRisLambers *et al.*, 2012; Goberna *et al.*, 2014a; Pérez-Valera *et al.*, 2015). The dominance of competitive interactions based on fitness differences does not exclude the possibility that other mechanisms that would increase the phylogenetic diversity of the community (e.g. limiting similarity via competition between species with similar niches) could be operating simultaneously. However, the effect of such processes would be indistinguishable when analysed at the community level if environmental filtering prevails. We propose in this thesis a

new approach that combines phylogenetic tools with co-occurrence network analysis to detect the contribution of assembly processes that operate simultaneously (Pérez-Valera *et al.*, 2017). After validating this framework with simulated communities, we applied it to real communities and found that both co-existing and mutually excluding bacterial taxa tend to be phylogenetically more closely related than expected by chance. This conforms with the simulated scenario in which environmental filtering and competitive interactions based on niche similarities concur to assemble soil bacterial communities (Pérez-Valera *et al.*, 2017).

Our results showing that bacterial phylogenetic diversity increases at low levels of organic resources also applied to burned sites in which the availability of organic C decreased after fire (Pérez-Valera *et al.*, 2015; Chapter IV). Changes in the post-fire bacterial composition may be reflected in phylogenetic community measures if traits that allow species survival or competitive superiority are phylogenetically conserved (Pausas and Verdú, 2010). This seems to be the case of microbial organisms bearing traits that confer environmental tolerance or competitive abilities (Goberna *et al.*, 2014b; Martiny *et al.*, 2015; Goberna and Verdú, 2016). We found that the post-fire increases in bacterial phylogenetic diversity were consistent across studied fires, suggesting that changes in bacterial communities were phylogenetically structured and hence, recognizable by exploring the evolutionary relationships between coexisting and non-coexisting taxa (Faust and Raes, 2012; Pérez-Valera *et al.*, 2017). Indeed, while fire imposed abiotic filters that favored microbial lineages bearing heat-resistance traits, it simultaneously increased competitive interactions via releasing a burst of nutrients and/or alleviated the strong biotic filter that operates in bacterial communities worldwide (Goberna *et al.*, 2014a; Pérez-Valera *et al.*, 2017). Interestingly, our network analyses pointed in the same direction, suggesting that fire increased the phylogenetic diversity as result of the altered balance between environmental filtering and competitive exclusion based on niche similarities (Pérez-Valera *et al.*, 2017). Evidence on altered microbial assembly after fire is rather scarce, but suggests increases in taxa showing heat-resistance (e.g. endospores, thickened cell walls) and fast-growth strategies (e.g. high rRNA operon copy numbers) that are

progressively outcompeted by strong competitors for organic carbon (Bárcenas-Moreno *et al.*, 2011; Jurburg *et al.*, 2017).

Metrics of phylogenetic structure that account for lineage identity allowed examining the abiotic drivers of bacterial diversity in ecosystems regardless of their environmental variability (Pérez-Valera *et al.*, 2015; Chapter III). Compared to the average phylogenetic distances (i.e. NRI), which are blind to the taxonomic identity of coexisting lineages, fuzzy-weighting community metrics such as PCPS identify the representativeness of different lineages across sites (Duarte *et al.*, 2012). We found that this is particularly relevant to predict microbially mediated ecosystem functions, as PCPS captures the signature that environmental changes leave in the composition of bacterial communities and consequently in their functionality (Pérez-Valera *et al.*, 2015). Indeed, PCPS is able to capture the weight that highly productive lineages such as *Proteobacteria* and *Actinobacteria* have in the ecosystem processes (Pérez-Valera *et al.*, 2015). Despite numerous pieces of evidence indicating positive effects of diversity on ecosystem functioning (Cardinale *et al.*, 2012), we found the opposite trend, resulting from the dominance of lineages that are both competitive (and thus reduce phylogenetic diversity) and productive (and thus increase the rates of ecosystem processes). This emphasizes that focusing on lineage identity is necessary to understand the biodiversity-ecosystem functioning relationship (Pérez-Valera *et al.*, 2015), especially after ecological disturbance that disrupts main ecosystem functions.

Fire-induced alterations in ecosystem functions related to microbial metabolism were the result of shifts in the bacterial phylogenetic community structure. Indeed, fire favored the abundance of microbial lineages that responded to the nutrient pulse, immediately increasing the rates of microbial respiration, biomass and nutrient cycling. Contrarily to wildfires that reduce the biomass and activity of microbial communities (Hernández *et al.*, 1997; Jiménez-Esquilín *et al.*, 2008), low intensity fires, such as those that are experimental or prescribed, might lead to slightly shifts or increases in microbial productivity and nutrient cycling activities (e.g. Fontúrbel *et al.*, 2012; Fultz *et al.*, 2016). However, while those trends were mostly recovered at the mid-term, that was not the case for the bacterial phylogenetic community structure,

suggesting that it could be some degree of functional redundancy (Allison and Martiny, 2008). Further research is needed to validate this interpretation, especially in a context of ecological disturbance in which species resilience could be key to guarantee the ecosystem functionality and nutrient cycling. This even includes, but does not restrict to, the possibility that taxa in the altered community are also functionally different to those prior to disturbance, but process rates are the same at the community level (Allison and Martiny, 2008). Future studies that deepen in the traits that define the functionality of particular microbial taxa are necessary to improve predictions of ecosystem process rates.

Finally, the phylogenetic structure of bacterial communities was sensitive but resilient to fire in a period of two to three decades in our Mediterranean ecosystems. In turn, the recovery of bacteria after fire involved the reestablishment of the competitive hierarchies that operate in bacterial communities, that is, the biotic filter caused by competitive lineages (Goberna *et al.*, 2014a; Pérez-Valera *et al.*, 2017). This process was mainly mediated by the plant communities through organic C inputs, likely in the form of litter and exudates. Therefore, those organic inputs constitute an essential factor that guarantees microbial resilience to fire, ultimately determining the bacterial phylogenetic structure and diversity. This is particularly important given the increasing rates of environmental change to which our ecosystems are exposed. Altogether, our findings highlight the capacity that the phylogenetic information has for predicting shifts in microbial composition and functioning, which is essential in the face of global change.

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8. CONCLUSIONS

1. Soil abiotic properties, most notably those associated with fertility, determine the phylogenetic diversity and community structure of soil bacteria. These diversity metrics, in turn, predict microbially-mediated ecosystem functions related to microbial productivity, decomposition and nutrient cycling, particularly when lineage identity is taken into account. The sign of the relationship between bacterial phylogenetic diversity and ecosystem functions depends on the taxonomic identity of the main coexisting lineages.

2. Soil bacterial communities are simultaneously assembled through environmental filtering and competitive exclusion by limiting similarity. Fire alters the balance between these assembly forces through changes in species richness and composition, which are ultimately reflected in the phylogenetic structure of the community.

3. Fire-induced alterations in microbially-mediated ecosystem functions are the result of shifts in the bacterial phylogenetic community structure. Exploring the contribution of microbial lineages to the phylogenetic structure allows predicting how ecosystem functions respond to ecological disturbance.

4. The phylogenetic structure of both above (plants) and belowground (fungi, bacteria, archaea) biological communities is either resistant or resilient to fire in a period of two to three decades in Mediterranean ecosystems. Plants and soil microbes experience opposing temporal trends in phylogenetic diversity during community reassembly. The post-fire microbial recovery involves the reestablishment of highly competitive clades, a process mediated by plant communities through changes in the soil environment.

APPENDICES

APPENDIX A

CHAPTER II: Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities

Figure A1.1: Post-mean estimates and credible intervals (95%) of several soil physical and chemical properties regarding time since fire. Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are indicated with *.

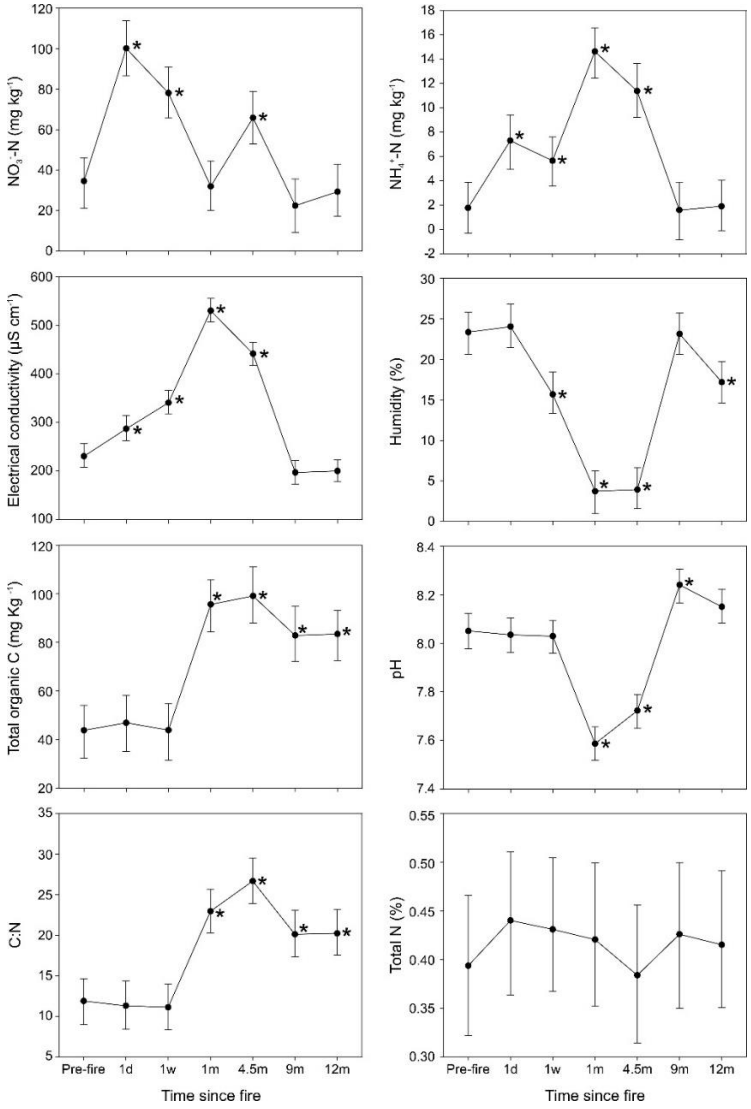

Figure A1.2: PCoA based on Bray Curtis distances of the bacterial community showing differences in the OTU composition among samples and time since fire. Soil environmental parameters that were significantly correlated with changes in the community composition (Axis 1 and/or Axis 2) are shown in A). Individual trajectories of each plot over time after fire are linked by solid lines in B), where arrows indicate the final time point. Dashed lines indicate an indirect trajectory due to a missing intermediate sampling point. Abbreviations: *TOC* total organic C, *EC* electrical conductivity, *NH_4^+-N* ammonium-N.

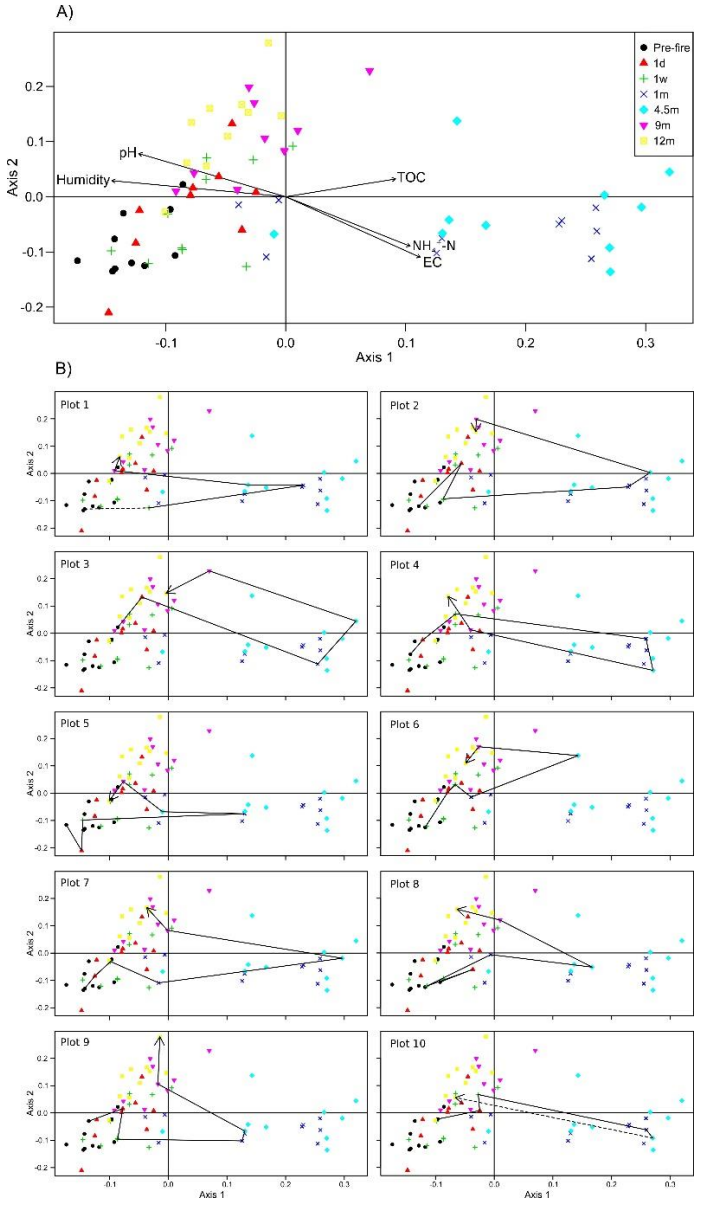

Figure A1.3: Relative abundance (post-mean and credible intervals [95%]) of the ten most abundant classes before and after the experimental fire. Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are indicated with *.

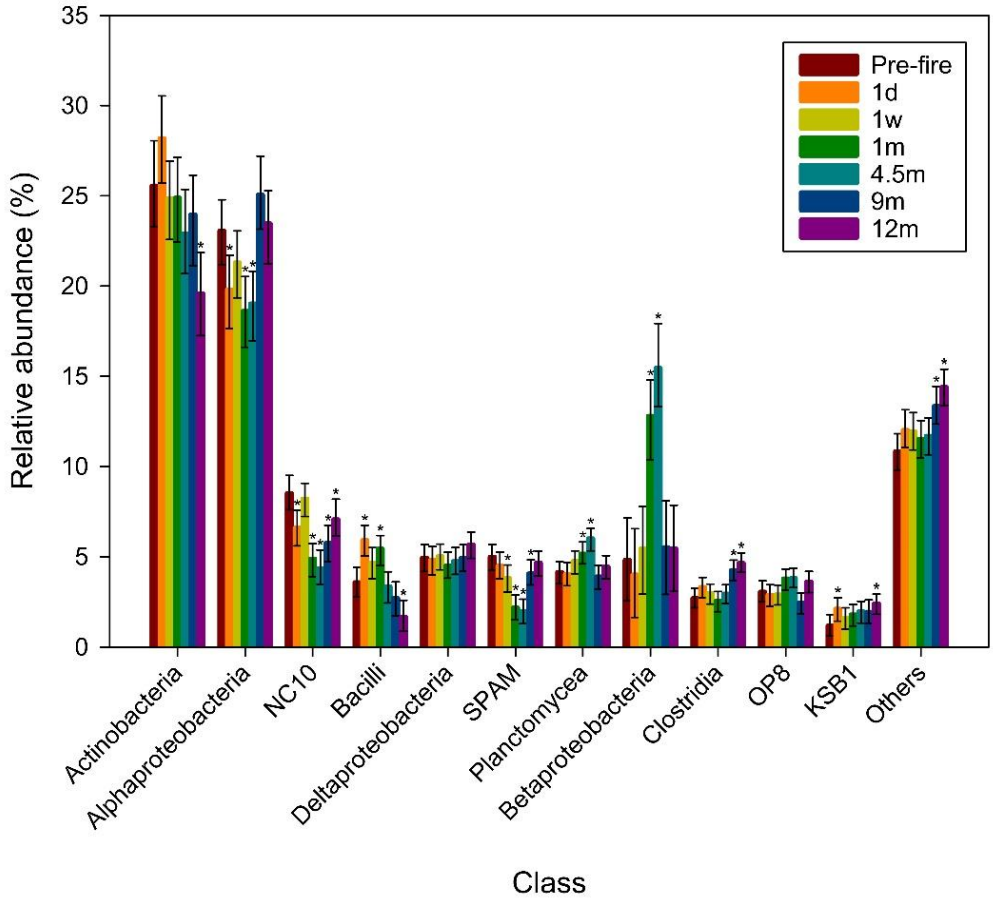

Figure A1.4: Co-occurrence networks supported by positively (A) and negatively (B) correlated abundance patterns at the OTU level for the pre-fire time point. Each node belongs to a phylum following the colour code shown in the phylogenetic tree in such a way that phylogenetically related OTUs share similar colours.

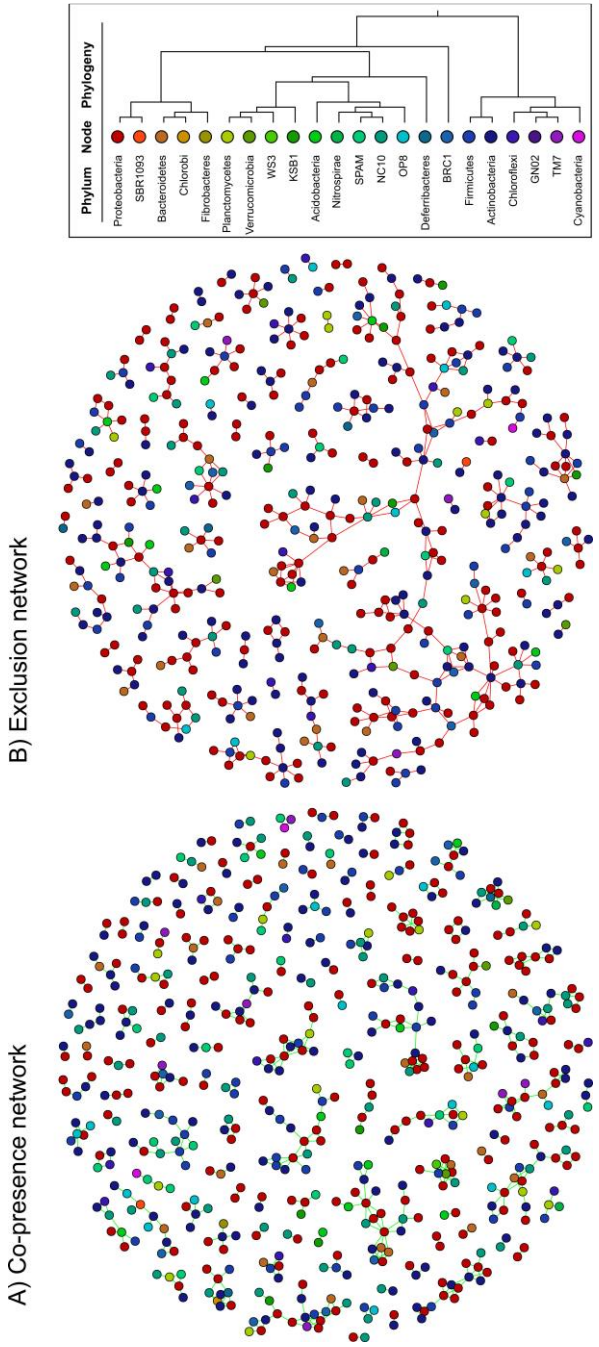

Figure A1.5: Monthly accumulated precipitation (expressed in mm), mean monthly temperature (in ºC) and plant cover (in %) over the study period (CEAM-UMH, 2009, 2010). The arrows indicate the experimental fire and time since fire.

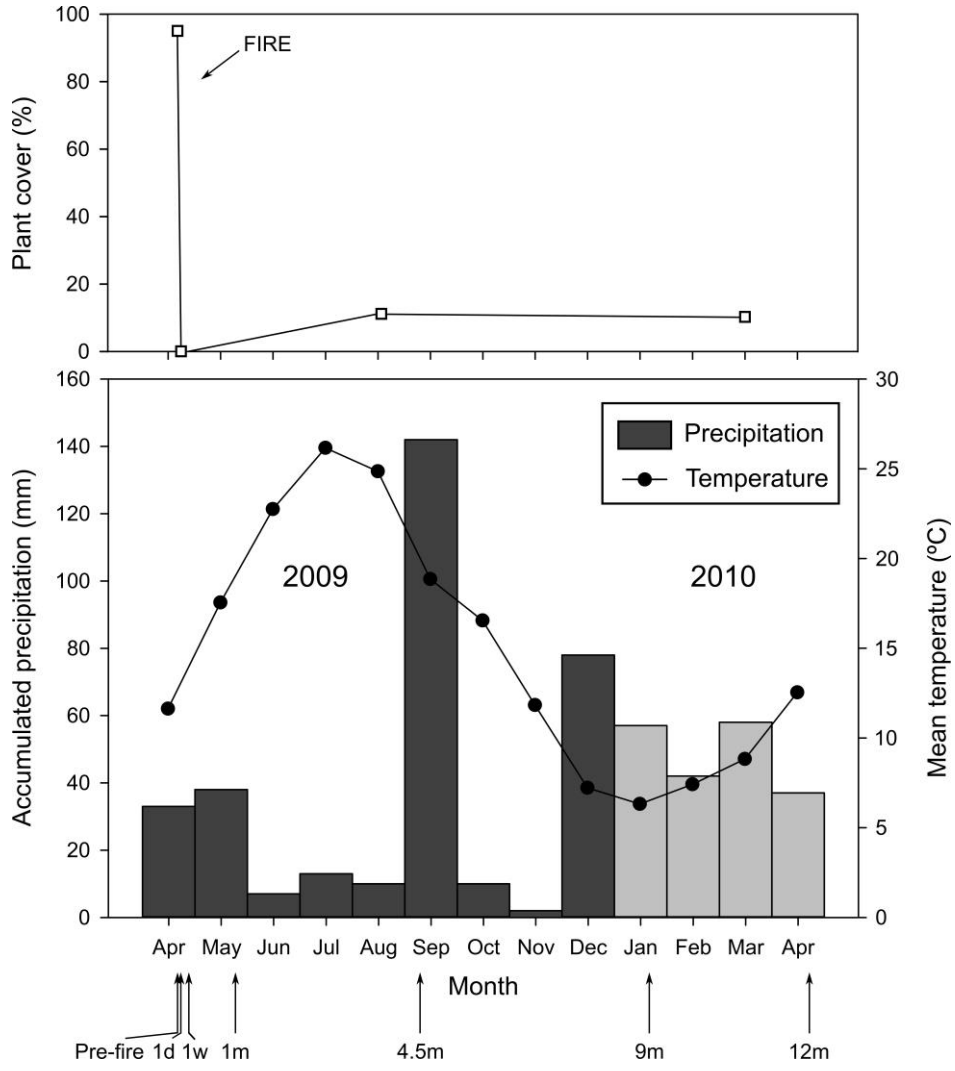

Table A1.1: Soil variables and their correlations with the axis 1 of the PCoA.

Variables	t	r	*P*
pH	**-7.639**	**-0.685**	**<0.001**
EC	**7.073**	**0.656**	**<0.001**
TOC	**4.607**	**0.493**	**<0.001**
TN	-1.240	-0.151	0.220
NH_4^+-N	**6.061**	**0.598**	**<0.001**
NO_3^--N	0.235	0.029	0.815
Humidity	**-9.790**	**-0.769**	**<0.001**

Table A1.2: Edge lists of the pre- and post-fire networks.

This table can be found (as Table S2) at
http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13609/full

Table A1.3: Number of sequences and OTUs *per* sample and time since fire.

	Plot	Nº Sequences	Nº OTUs
Pre-fire	**F1**	865	533
	F2	1086	595
	F3	900	582
	F4	978	616
	F5	1139	619
	F6	1081	604
	F7	966	541
	F8	1203	660
	F9	1088	654
	F10	973	613
1d	**F12**	988	590
	F13	700	448
	F14	861	542
	F15	1122	640
	F16	948	571
	F17	862	543
	F18	1067	587
	F19	1186	635
	F20	946	506
1w	**F21**	1256	646
	F22	1128	668
	F23	922	532
	F24	931	546
	F25	1108	623
	F26	900	551
	F27	1125	615
	F28	1163	634
	F29	1170	667
	F30	1073	602
1m	**F31**	1321	621
	F32	993	491
	F33	1391	554
	F34	1211	569

	F35	1294	613
	F36	837	512
	F37	1044	569
	F38	938	539
	F39	938	519
	F40	1236	562
4.5m	**F41**	948	525
	F42	1144	575
	F43	1214	616
	F44	1461	609
	F45	1234	674
	F46	674	413
	F47	1200	580
	F48	1286	648
	F49	1277	687
	F50	1273	603
9m	**F51**	761	467
	F52	747	506
	F53	697	436
	F54	874	528
	F55	712	497
	F56	741	462
	F57	995	596
	F58	736	444
	F59	722	453
12m	**F61**	1037	623
	F62	1118	674
	F63	1066	646
	F64	966	624
	F65	1343	668
	F66	941	601
	F67	537	374
	F68	815	529
	F69	885	529
	F70	801	514

APPENDIX A2: Simulations to validate the conceptual scenarios of community assembly (Chapter II, Figure 1).

The ability to infer assembly processes from phylogenetic co-occurrence patterns was validated by simulations. Specifically, we used a simulation model that structures communities from an assembly process (e.g. environmental filtering, limiting similarity or both), testing later if co-occurring and not co-occurring species showed the phylogenetic patterns expected in Figure 1. That is, if co-presence and exclusion links showed either clustered (i.e. they were more phylogenetically related than expected by chance) or overdispersed phylogenetic patterns (i.e. they were less phylogenetically related than expected by chance).

The simulation protocol involves four main steps, which are explained below. A graphic example is provided in Figure A2.1.

1. Creating the species pool

We first simulated i) a pool of 300 species, ii) five traits, and iii) a phylogenetic tree in such a way that the phylogenetic signal of the traits ranged from 5 to 6 (Blomberg's K). A pool of 300 species was selected so as to produce enough edges in the downstream network analysis while keeping our ability to detect phylogenetic patterns at the community level (Kraft *et al.* 2007). We also worked with five highly conserved traits because such conditions produce strong phylogenetic patterns as suggested by Kraft *et al.* (2007). Random pure-birth phylogenetic trees were created with the *pbtree* function in the picante package for R (Kembel *et al.* 2010). Species traits were then assigned with the *rTraitCont* function by using the "OU" evolution model in phytools for R (Revell 2012). Phylogenetic signals were tested with the function *phylosignal* of picante. Those traits whose phylogenetic signal was not high enough were recalculated until values between 5 and 6 were obtained.

2. Generating the ecological communities

Second, we generated individual communities by removing species from the initial pool until the final community size (100 species) was reached, by using the "assembly_community.R" script provided by Kraft *et al.* (2010). This script removes species on the basis of the species trait value and the chosen assembly process. For example, if environmental filtering is selected, those species whose trait value is distant from the niche optimum (i.e. a point in trait space which refers to the more appropriate trait value in the community) will be progressively (i.e. one by one) removed. On the contrary, if competition is chosen, those species pairs whose trait value is closer to each other will be progressively selected, and one of them randomly removed. If the

community is the result of both processes acting simultaneously, some species will be first removed by environmental filtering, and the remaining species then subjected to competition until the final richness is reached. We set the final richness to 100 species in such a way that the community size (the initial pool / final richness ratio) was set to 30%. This increases the probability of detecting phylogenetic patterns (Kraft *et al.* 2007). The order in which assembly processes were applied was chosen to mimic natural communities, which are sequentially submitted to an environmental filter and then to the filter of biotic interactions (Keddy 1992).

In order to adapt this simulation model to our conceptual framework, we complemented the script by Kraft *et al.* (2010) with further information in order to 1) generate multiple communities, and to 2) assign species abundances. First, the modification to generate multiple communities consisted on running the assembly script on the same species pool as many times as communities are required (in our case, 200 communities). When environmental filtering was selected, the niche optimum for each community was randomly selected from a normal distribution (mean = trait median, SD = 1/20 * trait range). Second, in order to generate species abundance data, we extracted from a log-normal distribution (mean=3, SD=1) as many random values as species were present in the final community. Then, these numbers were arranged by descending order for a posterior rank-based assignation. For example, for communities entirely assembled by environmental filtering, those surviving species whose trait was more similar to the niche optimum got a better position and thus the highest abundances. Conversely, those surviving species whose trait was less similar to the niche optimum got worse positions and thus the lowest abundances. We calculated a species deviation index as the sum of the differences of each species trait with the niche optimum. The lower the species deviation index, the higher the species abundance. For limiting similarity, the species ranking was established as follows. We first obtained a distance matrix of all possible species from their trait (or traits) values. Then, we calculated a species similarity index as the average value of the paired distances between each species and the rest. Because species competitive abilities depend on the degree of niche overlap, species abundance data were assigned based on the species similarity index. The lower the species similarity index, the higher the species abundance. For communities assembled by both environmental filtering and limiting similarity, we assigned the species abundance data on the basis of limiting similarity, which is the process that assembles the community once it has been subjected to an environmental filter.

Finally, in order to increase the sensitivity of the networks, we constructed a total of 200 communities, filtering out those 190 communities whose similarity in terms of shared species (i.e. Jaccard index) with the first community was low (*sensu* Berry and Widder 2014).

3. Co-occurrence network construction

Co-occurrence networks were constructed using the same parameters as for the construction of the pre- and post-fire networks (see description in the Methods section of the main text) with two changes: 1) we considered links when they were supported by at least one correlation/dissimilarity measure and 2) the initial top- and bottom-scoring links that was set to 100, in order to achieve an enough number of links and maintain high levels of correlation/dissimilarity between species, respectively.

4. Phylogenetic analysis

Co-presence and exclusion links were analyzed following the same protocol we used for pre- and post-fire bacterial communities (see description in the manuscript). The values provided in Figure A2.2 are Bayesian means of 10 independent runs for each assembly process.

Simulation results

We found that co-occurrence links showed the phylogenetic patterns expected in Figure 1, depending on the assembly process (Figure A2.2). Specifically, we found that simulation-based co-presence and exclusion links under environmental filtering (scenario A, Figure 1) showed an opposite phylogenetic pattern, such that co-occurring species were phylogenetically close whereas not co-occurring species were phylogenetically distant, as expected by our predictions (Figures 1 and A2.2). At the community level, we found a phylogenetically-clustered pattern (NRI post-mean estimate [95% credible interval] = -23.4 [-24.2, -22.7]). Our scenario B (Figure 1), which was entirely assembled by competitive exclusion by limiting similarity, partially agreed with our predictions (Figures 1 and A2.2). In particular, exclusion links were phylogenetically close, as theoretically expected (Figure 1), whereas co-presence links did not show any phylogenetic pattern (Figure A2.2). Two non-mutually exclusive explanations could underlie the lack of detection of a phylogenetic pattern in the co-presence links. First, under this theoretical scenario in which competition is acting alone and species occurrence is determined by their degree of niche overlap, the number of indirect links (e.g., by higher-order correlations) increases. That is, two species could indirectly co-occur as a consequence of true interactions (i.e. exclusions) with a third species (Faust and Raes 2012, Berry and Widder 2014), and this process would erase any phylogenetic pattern in the co-presence links. Second, the power to detect limiting similarity by this standardized phylogenetic distance is low (Kraft *et al.* 2007). At the community level, in scenario B we detected a phylogenetically-

overdispersed pattern (NRI post-mean estimate [95% credible interval] = 0.85, [0.95, 0.75]). Finally, we found that co-presence and exclusion links under both (at 50% each) environmental filtering and limiting similarity (scenario C, Figure 1) showed lower phylogenetic diversity than expected by chance, which is consistent with our pre- and post-fire results (Figures 1 and A2.2). Notably, we detected a phylogenetically-clustered pattern at the community level (NRI post-mean estimate [95% credible interval] = -2.4 [-2.9, -2.1]), which would be interpreted as environmental filtering acting alone in the community under the traditional phylogenetic framework (Webb *et al.* 2002).

Our simulation-based results, thus, suggest that the phylogenetic clustering found in both pre- and post-fire co-presence and exclusion links unequivocally reflects the action of both environmental filtering and competition acting simultaneously. We emphasize that the detected phylogenetic pattern at the community level does not necessarily reflect the processes that could actually be occurring. This requires a deeper phylogenetic co-occurrence analysis.

Simulations to test the effect of a random re-assembly on phylogenetic structure of communities and networks

We also used simulations to test the effect of a random re-assembly (i.e. through dispersal) on the phylogenetic structure of both communities and co-occurrence links. To do that, we first assembled communities in which the 36% of the species composition was determined by an environmental filtering, being the rest 64% then randomly chosen from the original pool (accordingly to the species turnover occurring in our real communities). We maintained the previously used species pool and community sizes. We found that random re-assembly through dispersal erased any phylogenetic pattern at the community (NRI post-mean estimate = -1.02 [-2.45, 0.34]) and co-occurrence level (Figure A2.3).

References

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Figure A2.1: Simulation example of microbial communities depending on the assembly process.

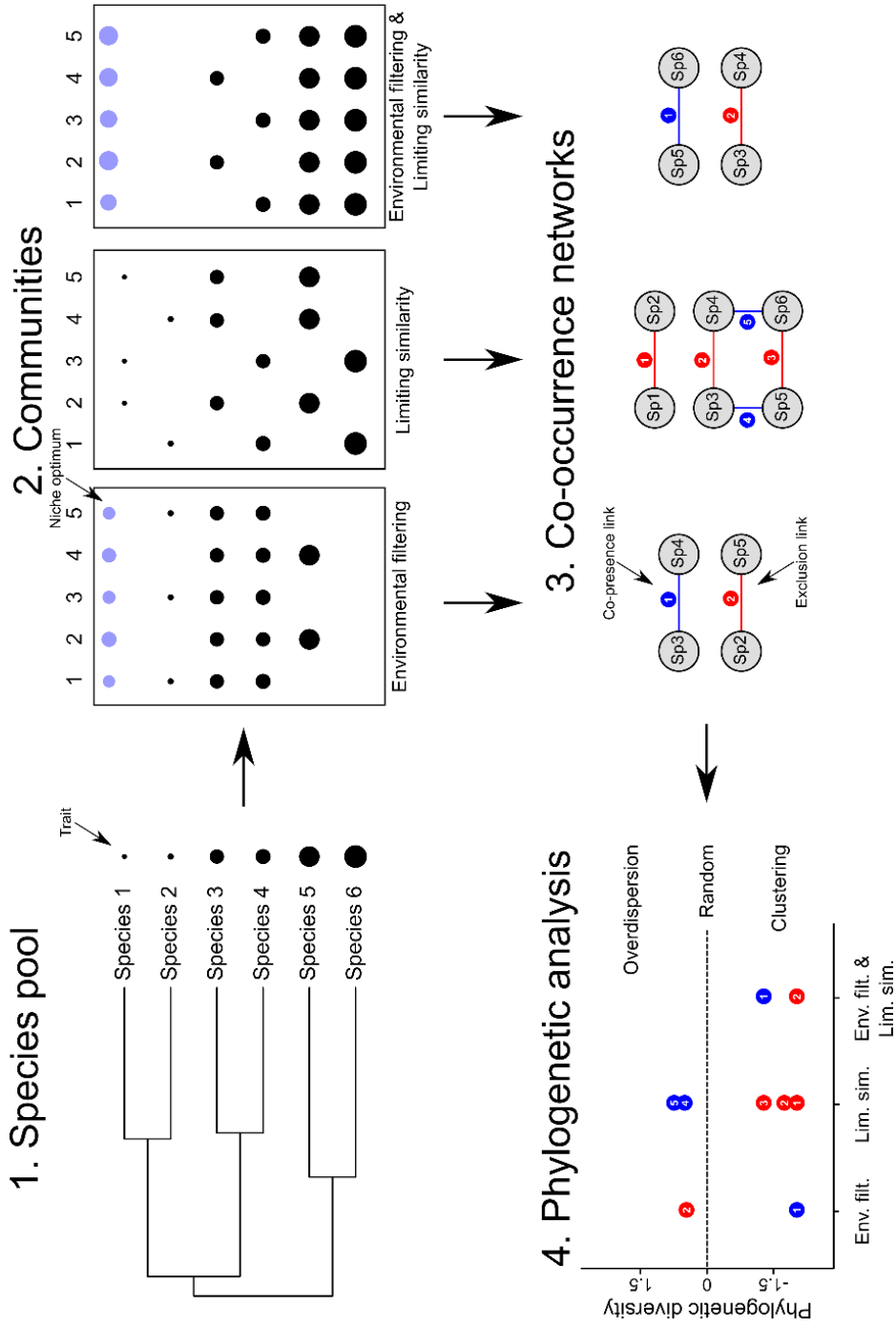


Figure A2.2: Phylogenetic diversity (mean and the 95% credible intervals) of co-presence and exclusion links from simulated data in accordance with the conceptual framework proposed in Figure 1. Negative values indicate overdispersion whereas positives values indicate phylogenetic clustering. Represented values are a Bayesian mean of 10 independent runs.

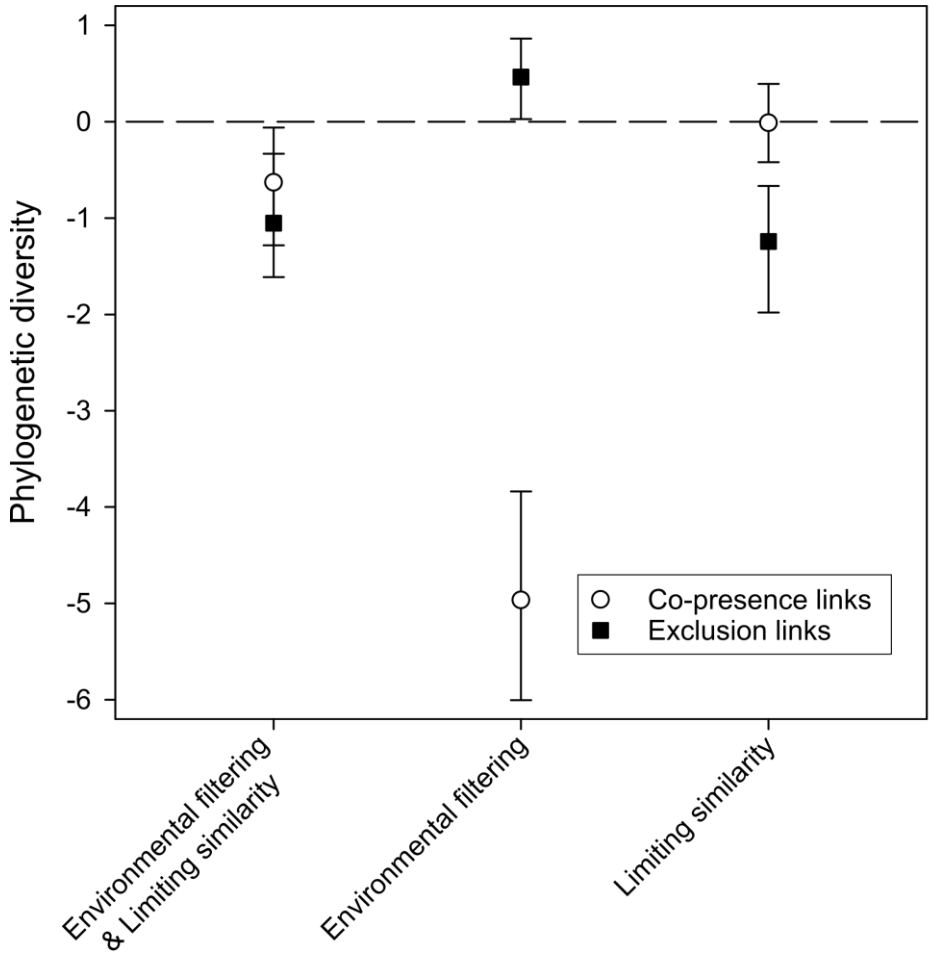
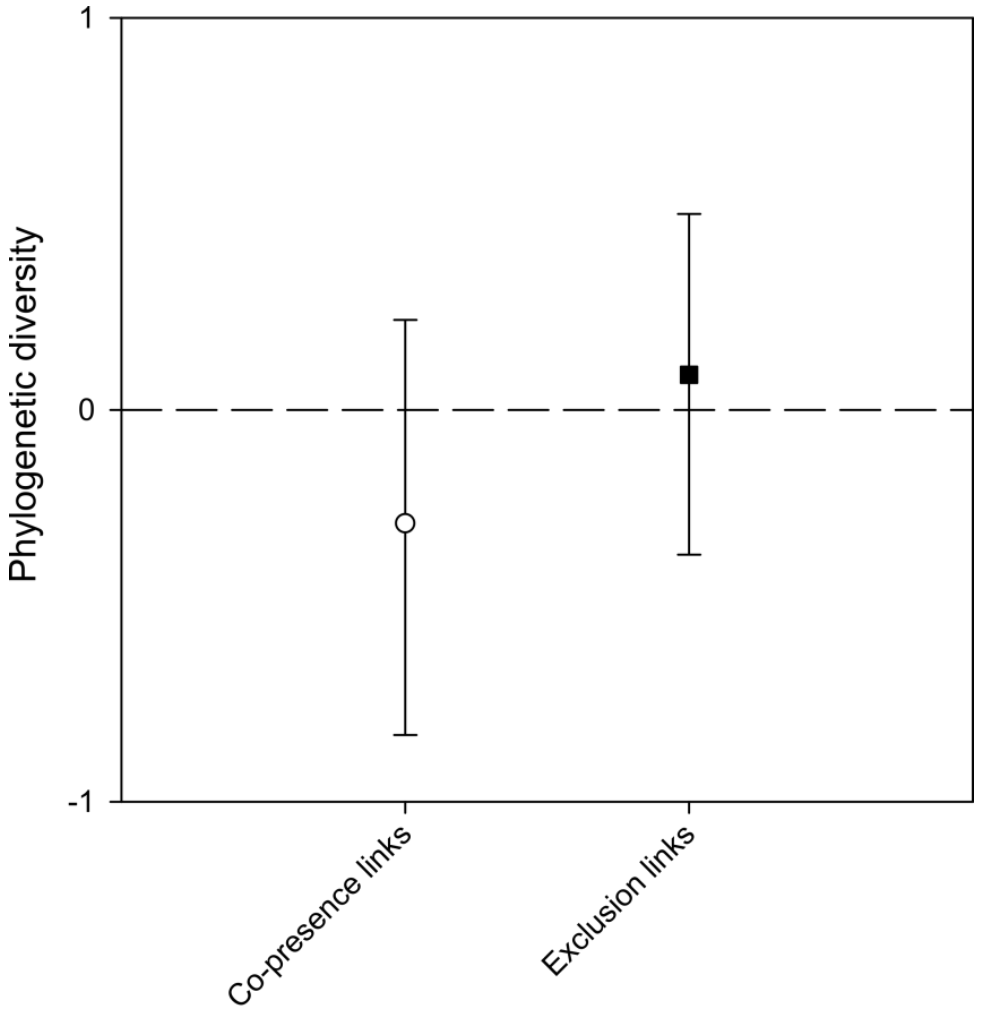


Figure A2.3: Post-mean estimates and credible intervals (95%) of the average phylogenetic diversity of co-presence and exclusion links after simulating several steps (64%) of stochastic reassembly processes (i.e. dispersal). Represented values are a Bayesian mean of 10 independent runs.



APPENDIX B

CHAPTER III: Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities

Figure B1: Post-fire evolution of soil abiotic properties. Bars indicate SE for n=10. Asterisks indicate significant differences between each time point and the pre-fire level after accounting for the seasonal variation of climatic conditions. Grey horizontal lines indicate the pre-fire level.

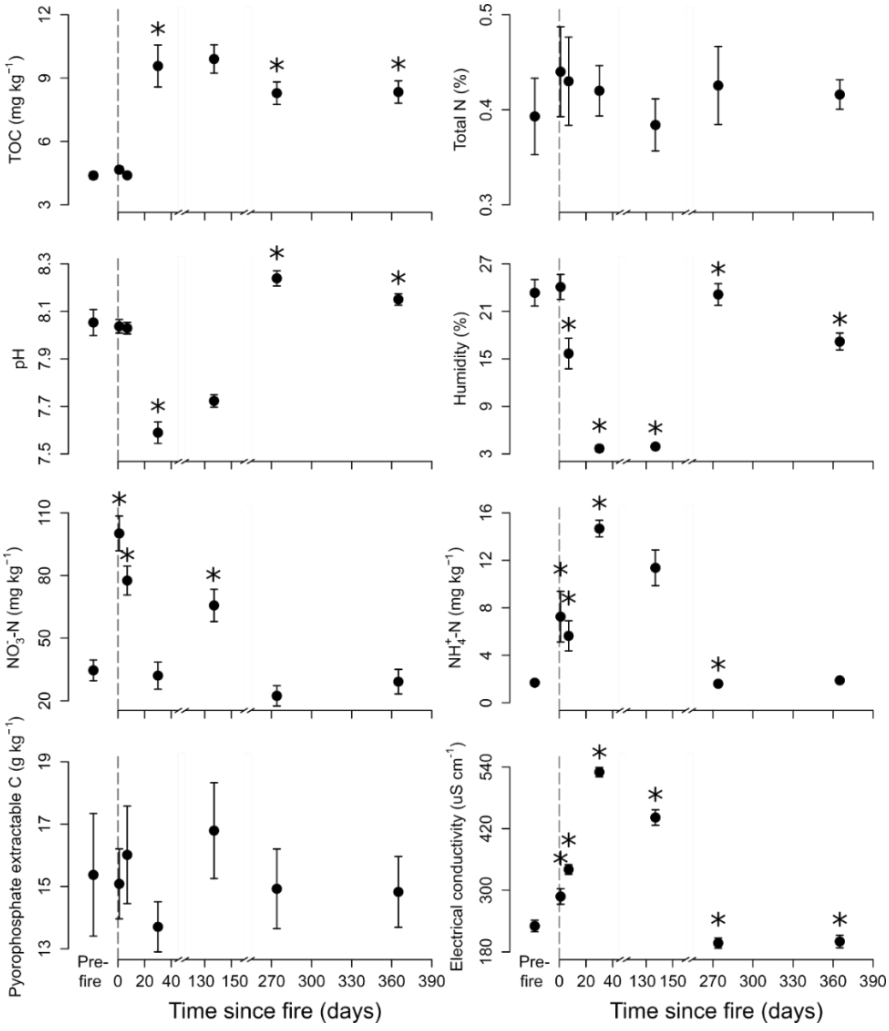


Figure B2: Relative contribution per phyla to the 16S rRNA copy number before and after the experimental fire.

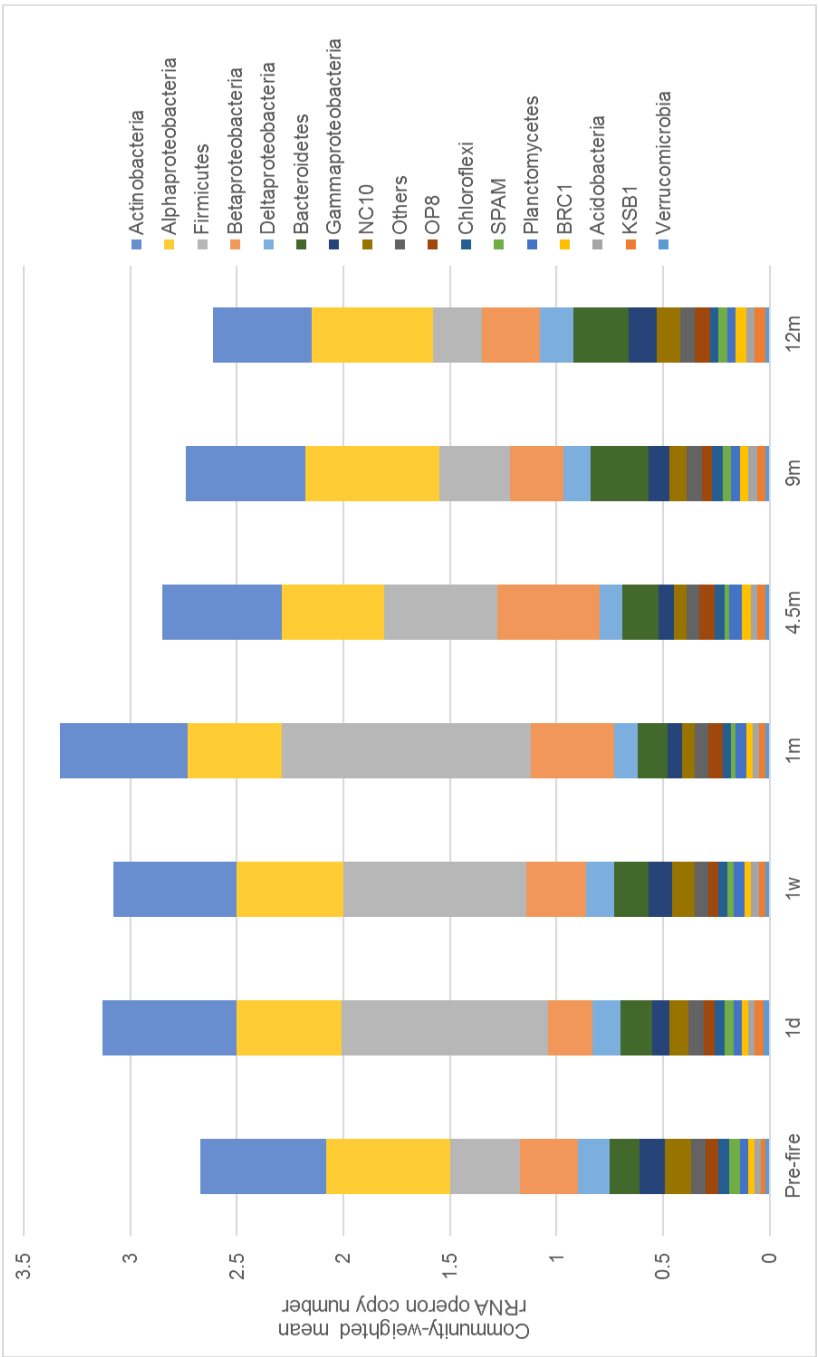


Table B1. Relative abundance (mean ± SE) of the most abundant phyla (classes for *Proteobacteria*) before and after the experimental fire. Significant differences between each time point and the pre-fire level after accounting for seasonal variation are indicated in bold.

Taxon	Pre-fire	1d	1w	1m
Alphaproteobacteria	23.0 (1.1)	**19.8 (0.9)**	21.3 (1.3)	18.7 (1.0)
Betaproteobacteria	4.8 (0.3)	4.0 (0.3)	5.4 (0.3)	**12.8 (2.1)**
Gammaproteobacteria	2.7 (0.2)	**2.0 (0.2)**	2.5 (0.2)	**1.8 (0.1)**
Deltaproteobacteria	4.9 (0.4)	4.8 (0.3)	5.0 ±0.3)	4.5 (0.3)
Actinobacteria	25.6 (1.0)	28.1 (1.5)	24.9 (1.1)	24.9 (1.1)
NC10	8.5 (0.6)	**6.6 (0.4)**	8.2 (0.6)	**4.9 (0.2)**
Firmicutes	6.3 (0.3)	**9.3 (0.6)**	**7.7 (0.6)**	**8.1 (0.7)**
SPAM	5.0 (0.2)	4.5 (0.6)	**3.8 (0.4)**	**2.2 (0.1)**
Planctomycetes	4.1 (0.3)	4.0 (0.3)	4.8 (0.3)	5.2 (0.3)
OP8	3.0 (0.4)	2.9 (0.3)	2.9 (0.2)	3.8 (0.3)
Chloroflexi	2.0 (0.2)	1.8 (0.2)	1.7 (0.2)	1.8 (0.2)
Bacteroidetes	1.6 (0.1)	1.9 (0.2)	2.1 (0.3)	**1.9 (0.1)**
Acidobacteria	1.6 (0.1)	1.4 (0.2)	**2.1 (0.1)**	**1.9 (0.1)**
BRC1	1.5 (0.1)	1.8 (0.2)	1.8 (0.2)	2 (0.2)
Verrucomicrobia	1.4 (0.1)	**2.3 (0.4)**	1.5 (0.1)	1.7 (0.3)
KSB1	1.2 (0.2)	**2.1 (0.4)**	1.6 (0.2)	1.8 (0.2)
Others	2.6 (0.7)	2.6 (0.7)	2.4 (0.5)	2.2 (0.6)

Taxon	4.5m	9m	12m
Alphaproteobacteria	19.1 (0.7)	25.0 (1.0)	23.5 (0.7)
Betaproteobacteria	15.5 (2.1)	5.5 (0.8)	5.4 (0.5)
Gammaproteobacteria	1.8 (0.2)	**2.2 (0.2)**	2.9 (0.3)
Deltaproteobacteria	4.7 (0.4)	4.9 (0.4)	5.7 (0.5)
Actinobacteria	22.8 (1.1)	23.9 (1.2)	**19.6 (1.2)**
NC10	4.3 (0.2)	**5.7 (0.6)**	**7.1 (0.5)**
Firmicutes	6.3 (0.2)	6.9 (0.5)	6.4 (0.3)
SPAM	2.0 (0.2)	**4.1 (0.5)**	4.7 (0.3)
Planctomycetes	6.0 (0.3)	3.9 (0.5)	4.4 (0.3)
OP8	3.8 (0.3)	2.5 (0.3)	3.6 (0.2)
Chloroflexi	1.9 (0.2)	2.1 (0.2)	1.7 (0.2)
Bacteroidetes	**2.0 (0.2)**	**2.9 (0.3)**	**3.0 (0.2)**
Acidobacteria	1.9 (0.1)	**2.2 (0.3)**	**2.4 (0.2)**
BRC1	2.2 (0.2)	2.0 (0.2)	**2.6 (0.3)**
Verrucomicrobia	1.6 (0.2)	1.5 (0.1)	1.8 (0.2)
KSB1	2.0 (0.3)	1.9 (0.3)	**2.4 (0.5)**
Others	2.0 (0.6)	2.5 (0.7)	2.6 (0.6)

Table B2: Divergence times (million years) and confidence intervals used for calibrating the bacterial phylogenies.

Group1	Group2	Estimated	Confidence interval
Archaea	Bacteria	4187	4199-4163
Firmicutes	Actinobacteria	2908	3041-2755
Cyanobacteria	Chloroflexi	2761	2920-2592
Betaproteobacteria	Alphaproteobacteria	2504	2630-2371
Bacteroidetes	Chlorobi	2099	2261-1932
Gammaproteobacteria	Betaproteobacteria	1993	2099-1894

APPENDIX C

CHAPTER IV: Phylogenetic diversity is resilient to fire across biological domains

Figure C1: Location of the 25 sites in the study area.

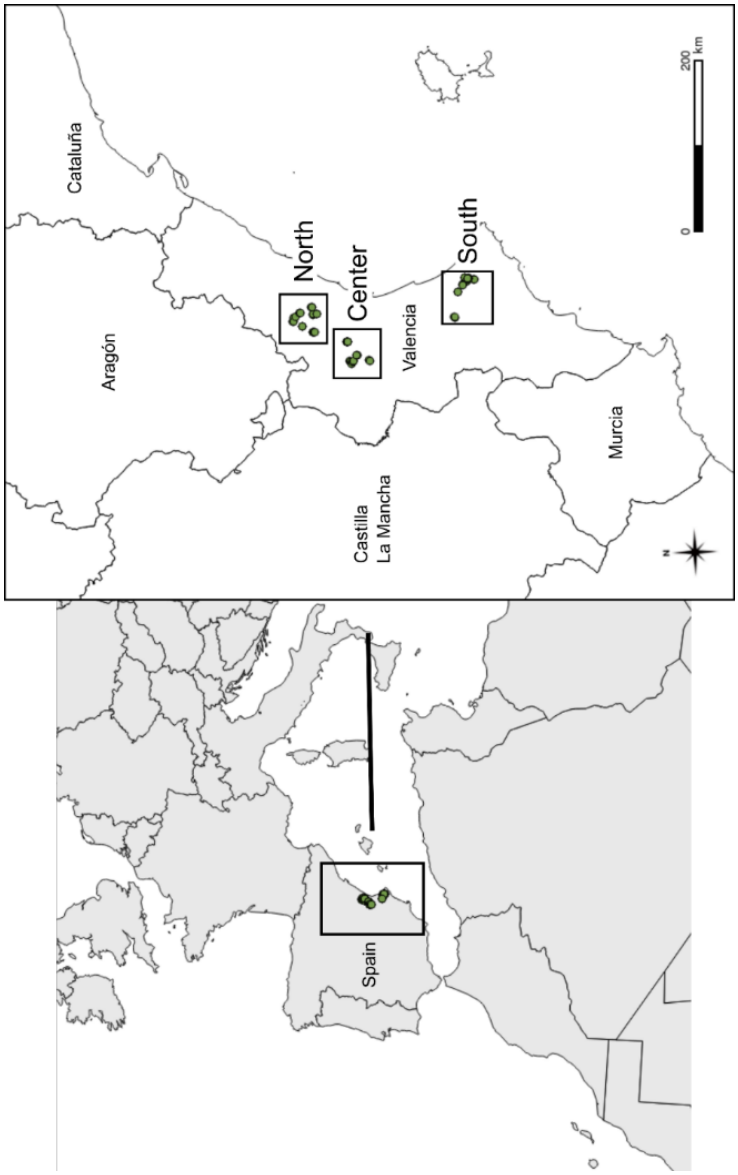


Figure C2: Topology of the plant phylogenetic tree. Node numbers indicate dated nodes according to Table C2.

This figure can be found at:
https://nuvol.uv.es/owncloud/index.php/s/HfsnoCTXQNaneQ5

Figure C3: Topology of the fungal phylogenetic tree. Node numbers indicate dated nodes according to Table C3.

This figure can be found at:
https://nuvol.uv.es/owncloud/index.php/s/QhncQBYS0QIVghJ

Figure C4: PCA biplot of the difference in soil parameters between burned and unburned plots. Arrows indicate the factor loadings on each axis. We interpret that soil conditions recover after fire with increasing PC1 and decreasing PC2 values as follows.

PC1 interpretation: TOC and TN decrease after fire and significantly increase with time since fire, while pH shows the opposite pattern (Figure C6). High values in ΔTOC and ΔTN, and low values in ΔpH associated with the positive pole of PC1 indicate similar levels in burned and unburned plots. We interpret this axis as the recovery of soil organic matter.

PC2 interpretation: NO_3^--N increases after fire and significantly decreases with time since fire, while moisture shows the opposite pattern (Figure C6). Low values in ΔNO_3^--N, and high values in Δmoisture associated with the negative pole of PC2 indicate similar levels in burned and unburned plots. We interpret this axis as the recovery of the levels of soil mineral nitrogen.

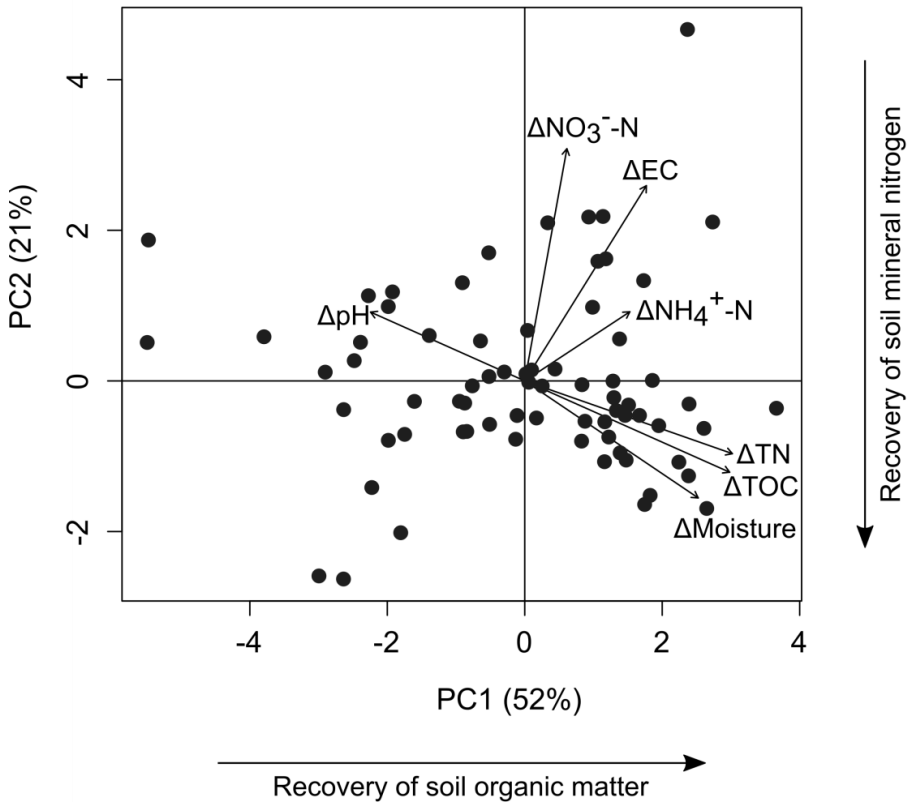


Figure C5: Fire effects on the relative abundance of the most abundant families (plants) or phyla (soil fungi, bacteria and archaea). Asterisks indicate significant differences between the abundances in burned and unburned plots ($P < 0.05$). Error bars indicate standard errors.

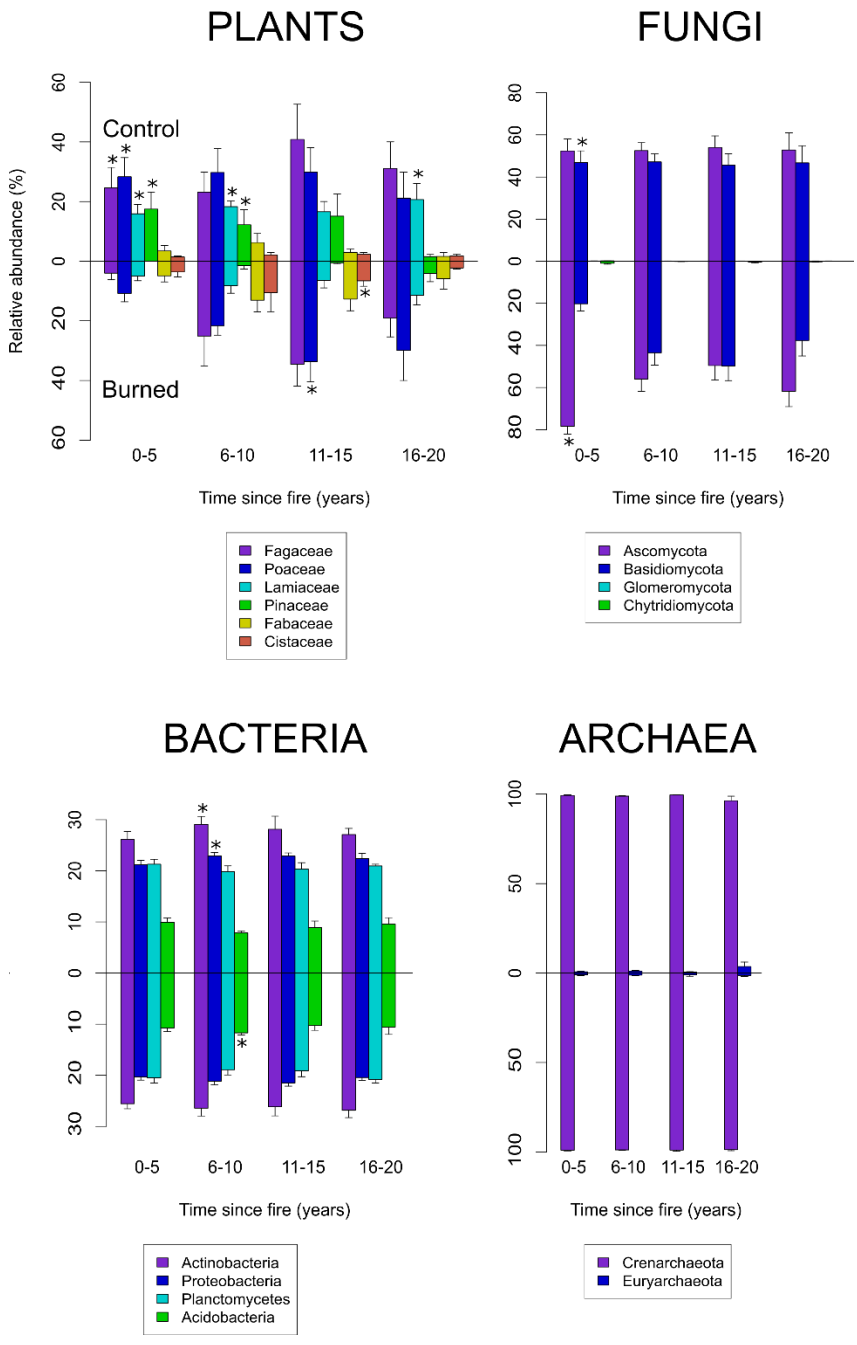


Figure C6: Temporal trends of soil chemical variables after fire. Filled circles indicate burned transects and unfilled circles unburned transects. Shaded and hatched areas show the confidence intervals of linear regressions among burned and unburned plots, respectively. Asterisks indicate significant post-fire temporal trends of the paired difference (Δ) between burned and unburned transects ($P < 0.05$). See the Results section for statistical details.

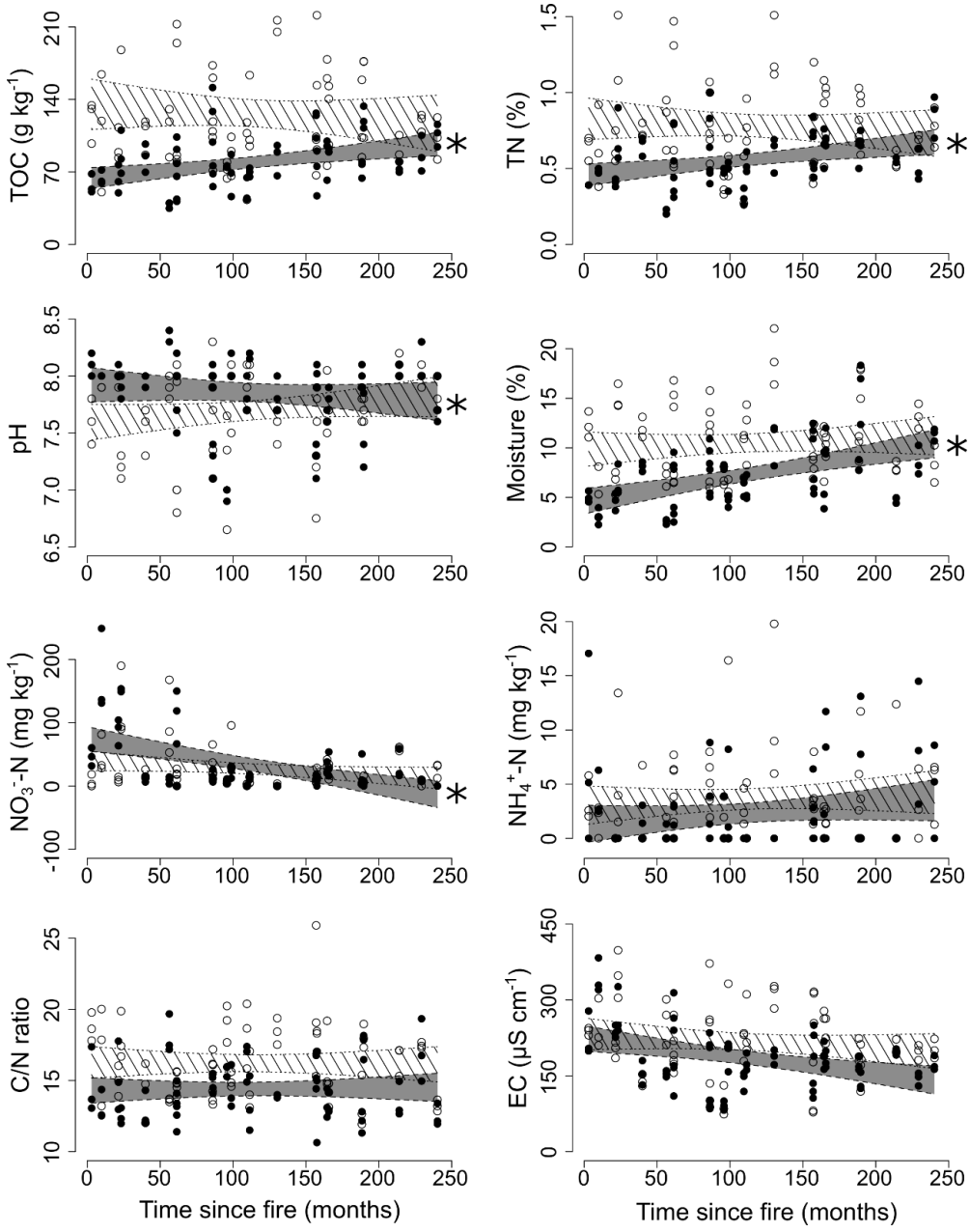


Table C1: Main features of burned and control sites.

Transect	Chronosequence	Slope °	Treatment	Fire date	Altitude	UTM 30-N (X)	UTM 30-N (Y)
A94C1	North	10	Unburned		701	705066,16	4409062,40
A94C2	North	14	Unburned		701	705075,50	4409071,56
A94C3	North	20	Unburned		701	705085,21	4409081,30
A94P1	North	26	Burned	7/4/1994	794	704975,49	4408308,66
A94P2	North	20	Burned	7/4/1994	794	704980,37	4408315,65
A94P3	North	24	Burned	7/4/1994	794	704985,16	4408322,62
A96C1	North	<5	Unburned		294	722508,33	4410756,59
A96C2	North	<5	Unburned		294	722497,55	4410754,17
A96C3	North	<5	Unburned		294	722487,97	4410753,98
A96P1	North	<5	Burned	07/07/1996	296	722593,84	4410895,91
A96P2	North	<5	Burned	07/07/1996	296	722596,39	4410885,85
A96P3	North	<5	Burned	07/07/1996	296	722602,27	4410875,84
A00C1	North	<5	Unburned		585	712474,39	4423491,29
A00C2	North	<5	Unburned		585	712475,96	4423500,95
A00C3	North	<5	Unburned		585	712477,88	4423510,19
A00P1	North	10	Burned	21/08/2000	603	712344,38	4423021,86
A00P2	North	10	Burned	21/08/2000	603	712351,24	4423026,98
A00P3	North	<5	Burned	21/08/2000	603	712357,90	4423031,46
A01C1	North	32	Unburned		571	708902,03	4417484,09
A01C2	North	26	Unburned		571	708904,01	4417475,41
A01C3	North	29	Unburned		571	708906,10	4417465,76
A01P1	North	21	Burned	23/04/2001	584	708947,11	4417410,21
A01P2	North	22	Burned	23/04/2001	584	708947,93	4417398,07
A01P3	North	19	Burned	23/04/2001	584	708947,69	4417386,94
A05C1	North	22	Unburned		648	718507,48	4418836,25
A05C2	North	18	Unburned		648	718503,37	4418845,76
A05C3	North	19	Unburned		648	718499,33	4418854,86
A05P1	North	10	Burned	24/01/2005	650	718698,71	4418386,45
A05P2	North	13	Burned	24/01/2005	650	718698,63	4418375,29
A05P3	North	14	Burned	24/01/2005	650	718699,15	4418363,76
A07C1	North	27	Unburned		593	715273,81	4422963,83
A07C2	North	17	Unburned		593	715288,00	4422963,15
A07C3	North	27	Unburned		593	715299,55	4422962,67
A07P1	North	27	Burned	07/03/2007	593	715892,45	4421806,40
A07P2	North	23	Burned	07/03/2007	593	715897,59	4421797,05

A07P3	North	24	Burned	07/03/2007	593	715910,77	4421787,49
A09C1	North	17	Unburned		394	716961,68	4410193,51
A09C2	North	18	Unburned		394	716972,73	4410188,66
A09C3	North	24	Unburned		394	716982,48	4410183,98
A09P1	North	20	Burned	26/08/2009	338	717797,15	4409670,27
A09P2	North	21	Burned	26/08/2009	338	717806,55	4409669,99
A09P3	North	27	Burned	26/08/2009	338	717815,76	4409671,73
A12C1	North	21	Unburned		837	705306,84	4409561,49
A12C2	North	21	Unburned		837	705312,70	4409567,90
A12C3	North	21	Unburned		837	705320,22	4409573,85
A12P1	North	17	Burned	29/06/2012	842	705443,98	4409654,27
A12P2	North	19	Burned	29/06/2012	842	705451,85	4409660,51
A12P3	North	21	Burned	29/06/2012	842	705460,99	4409665,76
A14C1	North	12	Unburned		455	717934,72	4407446,15
A14C2	North	9	Unburned		455	717926,41	4407451,36
A14C3	North	10	Unburned		455	717917,56	4407456,76
A14P1	North	14	Burned	04/02/2014	474	718066,62	4407214,19
A14P2	North	15	Burned	04/02/2014	474	718074,66	4407219,72
A14P3	North	17	Burned	04/02/2014	474	718082,91	4407224,96
B94C1	Center	12	Unburned		597	685212,01	4382407,61
B94C2	Center	15	Unburned		597	685218,54	4382416,93
B94C3	Center	36	Unburned		597	685224,37	4382424,92
B94P1	Center	17	Burned	04/07/1994	514	683698,50	4383232,46
B94P2	Center	17	Burned	04/07/1994	514	683704,68	4383241,32
B94P3	Center	15	Burned	04/07/1994	514	683708,97	4383253,40
B98C1	Center	7	Unburned		527	685180,16	4382365,22
B98C2	Center	15	Unburned		527	685184,80	4382374,02
B98C3	Center	8	Unburned		527	685192,16	4382382,53
B98P1	Center	16	Burned	25/10/1998	524	684723,16	4382819,38
B98P2	Center	17	Burned	25/10/1998	524	684721,81	4382828,70
B98P3	Center	13	Burned	25/10/1998	524	684720,55	4382837,34
B00C1	Center	23	Unburned		490	689432,45	4379789,97
B00C2	Center	18	Unburned		490	689433,00	4379800,49
B00C3	Center	22	Unburned		490	689433,76	4379810,04
B00P1	Center	20	Burned	16/09/2000	491	689122,62	4379686,35
B00P2	Center	15	Burned	16/09/2000	491	689113,73	4379683,96
B00P3	Center	20	Burned	16/09/2000	491	689104,74	4379678,94

B03C1	Center	19	Unburned		773	685633,16	4371157,85
B03C2	Center	10	Unburned		773	685645,99	4371146,48
B03C3	Center	9	Unburned		773	685653,95	4371138,65
B03P1	Center	<5	Burned	28/08/2003	773	685756,33	4371211,95
B03P2	Center	<5	Burned	28/08/2003	773	685743,37	4371223,17
B03P3	Center	<5	Burned	28/08/2003	773	685732,34	4371233,47
B06C1	Center	20	Unburned		210	698715,79	4386371,33
B06C2	Center	18	Unburned		210	698706,82	4386370,62
B06C3	Center	20	Unburned		210	698696,34	4386371,51
B06P1	Center	24	Burned	12/03/2006	208	698641,52	4385931,49
B06P2	Center	20	Burned	12/03/2006	208	698631,97	4385921,99
B06P3	Center	20	Burned	12/03/2006	208	698622,90	4385913,60
B09C1	Center	21	Unburned		182	698431,56	4386915,92
B09C2	Center	17	Unburned		182	698421,45	4386919,55
B09C3	Center	18	Unburned		182	698412,55	4386923,34
B09P1	Center	21	Burned	25/04/2009	177	698428,94	4386840,61
B09P2	Center	17	Burned	25/04/2009	177	698436,38	4386848,35
B09P3	Center	17	Burned	25/04/2009	177	698442,10	4386855,37
B12C1	Center	18	Unburned		404	684955,70	4384223,68
B12C2	Center	18	Unburned		404	684962,43	4384231,45
B12C3	Center	15	Unburned		404	684969,22	4384239,67
B12P1	Center	7	Burned	22/09/2012	408	685320,25	4384485,20
B12P2	Center	5	Burned	22/09/2012	408	685318,24	4384475,57
B12P3	Center	10	Burned	22/09/2012	408	685311,67	4384465,57
B13C1	Center	28	Unburned		200	698728,63	4386357,00
B13C2	Center	31	Unburned		200	698730,71	4386366,34
B13C3	Center	21	Unburned		200	698725,21	4386371,54
B13P1	Center	25	Burned	12/08/2013	206	698504,93	4386423,32
B13P2	Center	12	Burned	12/08/2013	206	698497,30	4386431,65
B13P3	Center	15	Burned	12/08/2013	206	698491,47	4386439,97
C95C1	South	25	Unburned		366	742619,75	4306203,17
C95C2	South	24	Unburned		366	742611,47	4306199,78
C95C3	South	23	Unburned		366	742597,98	4306198,96
C95P1	South	30	Burned	01/07/1995	374	742635,81	4306251,64
C95P2	South	27	Burned	01/07/1995	374	742630,91	4306255,94
C95P3	South	25	Burned	01/07/1995	374	742623,04	4306259,18
C98C1	South	17	Unburned		164	737936,89	4307071,14

C98C2	South	20	Unburned		164	737932,24	4307065,82
C98C3	South	22	Unburned		164	737926,57	4307058,26
C98P1	South	27	Burned	19/10/1998	148	738311,66	4307083,77
C98P2	South	29	Burned	19/10/1998	148	738308,19	4307084,25
C98P3	South	22	Burned	19/10/1998	148	738304,22	4307084,93
C01C1	South	<5	Unburned		555	741985,47	4303755,65
C01C2	South	<5	Unburned		555	741991,46	4303773,45
C01C3	South	<5	Unburned		555	742000,35	4303781,68
C01P1	South	9	Burned	09/08/2001	594	742594,01	4303180,14
C01P2	South	11	Burned	09/08/2001	594	742604,62	4303192,31
C01P3	South	10	Burned	09/08/2001	594	742576,91	4303173,91
C05C1	South	2	Unburned		189	715506,16	4312988,99
C05C2	South	3	Unburned		189	715518,11	4312984,18
C05C3	South	6	Unburned		189	715529,13	4312976,04
C05P1	South	<5	Burned	22/06/2005	189	715420,72	4312973,18
C05P2	South	<5	Burned	22/06/2005	189	715432,31	4312955,36
C05P3	South	<5	Burned	22/06/2005	189	715440,40	4312947,17
C06C1	South	<5	Unburned		577	741939,61	4303760,92
C06C2	South	<5	Unburned		577	741952,79	4303756,73
C06C3	South	7	Unburned		577	741965,71	4303761,82
C06P1	South	6	Burned	14/09/2006	598	742385,79	4303484,39
C06P2	South	6	Burned	14/09/2006	598	742397,71	4303488,61
C06P3	South	13	Burned	14/09/2006	598	742412,50	4303491,48
C07C1	South	<5	Unburned		567	740223,16	4304473,81
C07C2	South	<5	Unburned		567	740231,20	4304483,67
C07C3	South	<5	Unburned		567	740245,13	4304505,10
C07P1	South	6	Burned	31/05/2007	571	740308,90	4304519,47
C07P2	South	7	Burned	31/05/2007	571	740326,41	4304519,28
C07P3	South	8	Burned	31/05/2007	571	740344,49	4304528,56
C09C1	South	9	Unburned		624	741909,65	4299884,14
C09C2	South	6	Unburned		624	741916,51	4299882,12
C09C3	South	6	Unburned		624	741930,43	4299882,27
C09P1	South	<5	Burned	25/07/2009	648	741588,30	4299695,05
C09P2	South	<5	Burned	25/07/2009	648	741589,99	4299693,70
C09P3	South	<5	Burned	25/07/2009	648	741602,61	4299688,85
C11C1	South	9	Unburned		230	732962,70	4310867,13
C11C2	South	9	Unburned		230	732968,48	4310861,44

C11C3	South	8	Unburned		230	732981,27	4310866,96
C11P1	South	7	Burned	10/04/2011	222	732990,83	4310905,60
C11P2	South	11	Burned	10/04/2011	222	733001,75	4310904,85
C11P3	South	6	Burned	10/04/2011	222	733011,15	4310904,22

Table C2: Nodes ages used for plant phylogenetic calibration.

Node Number	Age (Million years)
1	400
2	161
3	48
4	12
5	56
6	76
7	81
8	24
9	14.8
10	4.3
11	2.3
12	1.5
13	6.2
14	2
15	26
16	23
17	8.6
18	8.1
19	2.19
20	18.8
21	6.4
22	9.1
23	47
24	71
25	56
26	44
27	33
28	37
29	43.66

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Table C3: Node ages used for fungal phylogenetic calibration.

Node Number	Age (Million years)
1	1300
2	812
3	648
4	500
5	425
6	153
7	347
8	294
9	203
10	181
11	149
12	108
13	121
14	84
15	115
16	213
17	273
18	382
19	542
20	527
21	458
22	309
23	260
24	246
25	244
26	169
27	184
28	353
29	315
30	239
31	143
32	138
33	244
34	336
35	189

36	362
37	350
38	230
39	430
40	413
41	373
42	770

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Table C4: Results of the spatial autocorrelation tests (Mantel correlations) for both biotic (similarity in the specific composition) and abiotic properties. Adjusted P-values after correction for multiple testing following the Benjamini-Hochberg procedure are shown.

Variables	r	*P*	*P-adjusted*
Plants	0.024	0.369	0.492
Fungi	0.173	0.014	0.168
Bacteria	0.016	0.336	0.492
Archaea	0.007	0.359	0.492
pH	0.210	0.036	0.216
TOC	0.019	0.294	0.492
TN	0.104	0.141	0.441
Moisture	0.079	0.147	0.441
NO_3^--N	-0.033	0.365	0.492
NH_4^+-N	-0.038	0.533	0.533
C/N ratio	-0.016	0.514	0.533
EC	-0.024	0.532	0.533