TESIS DE DOCTORADO

MICROBIAL DIVERSITY AS A BIOINDICATOR OF THE IMPACT OF FIRES

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MICROBIAL DIVERSITY AS A BIOINDICATOR OF THE IMPACT OF FIRES

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ESTA TESIS HA SIDO DESARROLLADA EN EL GRUPO DE BIOQUÍMICA DEL SUELO DEL INSITUTO DE INVESTIGACIONES AGROBIOLÓGICAS DE GALICIA (IIAG-CSIC), BAJO LA DIRECCIÓN DE MONTSERRAT DÍAZ RAVIÑA, INVESTIGADORA CIENTÍFICA DEL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

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A mi Abuela Nina,

a mis sobrinas Mencía y Nerea

y a Fiz

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RESUMEN

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1. RESUMEN

Galicia, junto con el norte de Portugal, son de las áreas del mundo más afectadas por los incendios forestales, ya que presentan el mayor número de fuegos por hectárea y habitante (Carballas *et al*., 2009). Entre los años 2006 y 2015 el 24% de los incendios forestales del país tuvieron lugar en Galicia (MAPAMA, 2015) pese a representar esta comunidad autónoma sólo el 10% de superficie forestal de todo el país. Cabe destacar el año 2006, donde la mitad de los incendios nacionales se produjeron en Galicia, con casi 100.000 ha afectadas (MAPAMA, 2015). La recurrencia constituye un gran problema en los ecosistemas forestales de Galicia, ya que en esta región los incendios se caracterizan por afectar a una misma zona año tras año. De los 100 municipios con más número de incendios entre 2001 y 2015, 72 pertenecen a Galicia, siendo A Cañiza (1.541), Viana do Bolo (1.338) y Muíños (1.226) los municipios gallegos con mayor número de incendios (MAPAMA, 2015). Además, el régimen de los incendios está cambiando como consecuencia del cambio climático que, junto con cambios en la disponibilidad del combustible, hace que nos encontremos en una quinta fase pírica, caracterizada por una nueva generación de incendios, los megaincendios (Bowman *et al*., 2011). El aumento de las temperaturas y el descenso de las precipitaciones provoca unas condiciones meteorológicas cada vez más extremas que conllevan a una alteración de los períodos de riesgo que ya no se limitan a los meses de verano, si no que se amplían de abril a octubre (WWF, 2017). Estos cambios en las condiciones meteorológicas también tienen como consecuencia que los incendios sean mayores en extensión, frecuencia y severidad. Un ejemplo que ilustra este hecho son los episodios ocurridos en Galicia en octubre de 2017, en el que se produjeron incendios muy virulentos, de alta severidad y de velocidad de propagación muy elevada, en los que en tres días se quemaron 35.500 ha (MAPAMA, 2018).
Los incendios forestales son un problema ecológico, económico y social de tal importancia que autores como Carreiras *et al.* (2014) y Pereira *et al.* (2015) han analizado las diferentes políticas de prevención y la percepción social que generan en la población. Aunque la consecuencia más aparente que producen es la destrucción de la cubierta vegetal, su efecto es mucho más amplio afectando a todos los componentes del medio ambiente (suelo, agua y atmósfera) y extendiéndose a zonas muy alejadas del lugar donde se origina el fuego (ejemplo erosión post-incendio). Los incendios forestales afectan a la fauna produciendo la muerte de aquellas especies que debido a una menor capacidad de movilidad no son capaces de escapar de las llamas, mientras que las especies que sobreviven pueden presentar una mayor sensibilidad, ya que la alteración del hábitat puede provocar falta de alimento y refugio. El fuego destruye las partes aéreas y las raíces de las plantas y los árboles. La reserva de semillas del suelo también es destruida o arrastrada por la erosión tras las primeras lluvias. El fuego altera el régimen hídrico y la calidad del agua, el clima, las propiedades del suelo y a los microorganismos que habitan en él (Neary *et al*., 2005; Carballas *et al*., 2009). El suelo es uno de los factores más importante en la biología de los ecosistemas forestales y, probablemente,

se vea más afectado en un incendio que cualquier otro componente de los ecosistemas forestales (Page-Dumroese *et al.*, 1991). El fuego modifica las propiedades físico-químicas, químicas y biológicas del suelo disminuyendo su calidad y alterando el funcionamiento (Mataix-Solera *et al*., 2002; Certini, 2005; Carballas *et al*., 2009; Díaz-Raviña *et al*., 2010; Martín *et al*., 2012; Carballas, 2014; Carballas *et al*., 2015). Los incendios producen una destrucción o reducción de la materia orgánica y la alteración de su dinámica y composición química produciendo cambios en casi todas las propiedades del suelo. La pérdida de materia orgánica provoca la disminución de la capacidad de retención de agua y la destrucción de la estructura del suelo. En general, el pH y la conductividad eléctrica aumentan debido a la producción de cenizas y a la destrucción de los ácidos orgánicos. La magnitud de los cambios va depender de la intensidad del quemado y de las características del medio como la humedad, la vegetación o el tipo de suelo. Entre los efectos indirectos más graves destacan los procesos de erosión post-incendio que se agravan a consecuencia de la desaparición de la cubierta vegetal y producen numerosos daños ecológicos, económicos y socioculturales.

Dentro del medio edáfico, uno de los componentes más afectados por los incendios forestales son los microorganismos (Acea y Carballas, 1996). La biomasa microbiana del suelo está constituida por una gran diversidad de organismos vivos, todos ellos de un tamaño inferior a 5 x 10^3 μm^3, tales como bacterias, actinobacterias, hongos, algas, protozoos y microfauna (Jenkinson y Ladd, 1981) y virus (Lynch, 1983). Estos microorganismos, que constituyen la fracción viva del suelo, no sólo utilizan el suelo como su hábitat natural, si no que intervienen en su formación y mantenimiento, tanto en ecosistemas naturales como en agrícolas, siendo los responsables de la fertilidad del suelo al llevar a cabo un gran número de transformaciones en diversas condiciones ambientales e intervenir en los ciclos del C y de los nutrientes, el ataque de las rocas y los minerales del suelo, las reacciones de complejación, etc. Su importancia se refleja en el hecho de que entre el 80 y el 90 % de los procesos que ocurren en el suelo son reacciones mediadas por los microorganismos (Coleman y Crossley 1996, Nannipieri *et al*., 2003). La microbiota edáfica, debido a ser un elemento clave en muchos de los procesos que tienen lugar en el suelo, también puede utilizarse como un bioindicador o indicador temprano de los cambios producidos en la calidad del mismo como consecuencia de procesos de degradación y/o prácticas de conservación del suelo, mucho antes de que tales cambios se detecten mediante análisis de las propiedades físicas, físico-químicas y químicas del suelo (Pankhurst *et al*., 1998). Así, cambios en número, biomasa, actividad metabólica y estructura o diversidad de la comunidad microbiana pueden ser una señal de una alteración en el ecosistema (Atlas, 1984), cambios que son fácilmente detectables con estimaciones de parámetros tales como el C biomasa, la respiración, la mineralización del N, las actividades enzimáticas del ciclo de C (β-glucosidasa), N (ureasa) y P (fosfatasa), la actividad bacteriana (Doran y Zeiss, 2000) y la estructura o composición de las comunidades microbianas (Frostegård *et al*., 2011).

Generalmente, las temperaturas que se alcanzan en la superficie del suelo durante un incendio forestal exceden a la requerida para matar a la mayoría de microorganismos (DeBano *et al*., 1998), por lo que tras un incendio la biomasa microbiana desciende notablemente llegando incluso, en los casos más extremos, a la esterilización de los primeros cm del suelo. Por otra

parte, los cambios en las propiedades del suelos producidos, de manera indirecta, por los incendios tienen también como consecuencia un efecto negativo sobre los microorganismos (Díaz-Raviña *et al*., 1992; González-Pérez *et al*., 2004), dependiendo la magnitud de ese efecto de la severidad del fuego, del ecosistema forestal (suelo y vegetación) y de las condiciones climáticas post-incendio. Los grandes cambios en las condiciones ambientales o sustratos químicos ocasionados por el fuego, pueden alterar la respiración y las actividades enzimáticas como la deshidrogenasa, ureasa, fosfatasa y la arilsulfatasa (Certini, 2005; Hamman *et al*., 2008). Sin embargo, el efecto del fuego en la microbiota no es el mismo para todos los grupos microbianos. Generalmente, se considera que la sensibilidad al calor de los hongos es mayor que la de las bacterias (Bollen, 1969; Dunn *et al*., 1985), que se ven favorecidas por el aumento de pH que sucede tras un incendio. Dentro de las bacterias, las $Gram^+$ son menos sensibles a la acción del fuego que las $Gram^-$ (Mabuhay *et al*., 2003, 2006). La diferencia de sensibilidad de los diferentes grupos de microorganismos frente a las alteraciones del medio puede producir una disminución de la diversidad microbiana que puede reducir su estabilidad (Girvan *et al*., 2005). A pesar de que los microorganimsos son unos de los agentes responsables de la funcionalidad del suelo, en los estudios sobre los incendios forestales, aspectos como su biomasa, su actividad y diversidad, particularmente esta última, no se abordan en la mayoría de investigaciones (Mataix-Solera *et al*., 2009). Por otra parte, el impacto del fuego se relaciona con la intensidad del incendio (temperatura máxima alcanzada) y no con la severidad del mismo (temperatura alcanzada y su duración).

Como indicamos anteriormente, las propiedades del suelo afectadas por el incendio van a depender de la severidad del fuego. Para el estudio de la severidad se tiene en cuenta la cantidad y la duración de la transferencia de calor (temperatura, tiempo). Dentro de estos dos factores, el componente de la severidad que resulta más destructivo para los ecosistemas edáficos es la duración del fuego (Neary, 1999). Una aproximación al estudio de la severidad es la metodología de los grados-hora, que permite la estimación la cantidad de calor aplicado al suelo considerando el efecto combinado de la temperatura alcanzada por el suelo y el tiempo de exposición del mismo a esa temperatura (Busse *et al*., 2005). En un incendio forestal en condiciones de campo, las temperaturas alcanzadas por el suelo no pueden ser medidas, además se produce un mosaico de zonas afectadas por el fuego con distintas severidades dependiendo de una multitud de factores como la intensidad, la duración, el combustible disponible, la topografía, etc. De esta manera podemos encontrar dentro de un mismo incendio áreas afectadas con distinto grado de intensidad, dificultando su estudio e imposibilitando extrapolar los resultados de un estudio a otro (Neary *et al*., 2005). Además, en condiciones de campo, controlar la severidad del incendio durante el mismo resulta imposible y determinar su magnitud después del mismo es muy difícil de evaluar (Vega *et al*., 2013). Sin embargo, los estudios de laboratorio permiten poder registrar las temperaturas máximas alcanzadas y el tiempo de duración de las mismas y, por tanto, simular diferentes severidades y tratar de aislar la influencia del fuego de la de los factores ambientales.

Las quemas prescritas son ampliamente utilizadas en el manejo de la biomasa forestal, para evitar los incendios forestales de alta severidad, o en ganadería para la generación de pastos. Aunque se conocen sus beneficios como herramientas de manejo, los estudios acerca de cómo

pueden afectar a los ecosistemas no muestran resultados concluyentes. Aunque a corto plazo se observa un aumento de los nutrientes del suelo, a largo plazo, se desconocen los efectos que estas quemas, aplicadas de manera repetitiva, tienen sobre la calidad del suelo y las comunidades microbianas. Lo mismo sucede con los tratamientos de rehabilitación post-incendio más utilizados en todo el mundo ("mulching" de paja, siembra de herbáceas); se conoce su eficacia a la hora de evitar la erosión inmediatamente o a corto plazo después del fuego, pero la información disponible sobre su impacto a corto, medio y largo sobre las propiedades del suelo, especialmente sobre la microbiota, y la vegetación (sobre todo el banco de semillas) es escasa.

Los trabajos realizados tenían como finalidad determinar la utilidad de la diversidad microbiana, taxonómica y fisiológica, para, por una parte, evaluar el impacto de diversos aspectos del fuego (severidad, recurrencia) bajo diferentes condiciones ambientales (campo, laboratorio, tipo de suelo, topografía, vegetación, época del año, tiempo transcurrido tras el incendio, etc.) y, por otra, de dos tratamientos de emergencia para controlar la erosión post-incendio ("mulching de paja" y siembra de herbáceas) sobre la calidad del suelo y la recuperación del sistema suelo-planta. Las experiencias de campo se realizaron en áreas afectadas por incendios forestales de alta o media severidad (Parque Natural das Fragas do Eume, A Coruña; Laza, Ourense; y Saviñao, Lugo) y con diferente vegetación (roble y eucalipto) También se analizó la eficacia de los dos tratamientos de rehabilitación post-incendio del mulching de paja y la siembra de herbáceas a la hora de evitar la erosión del suelo para determinar cuál de los dos es el más adecuado para aplicar en Galicia. Para determinar si procede su implementación tras el incendio se consideró no solo su eficacia sino también la existencia de efectos adversos sobre la calidad del suelo y sobre la recuperación post-incendio de la vegetación, la composición de especies, la aparición de especies no autóctonas y el efecto sobre el banco de semillas del suelo. Debido al alto coste de la implantación de estos tratamientos postincendios en condiciones de campo, se determinó la dosis (1 y 0,8 Mt/ha) y forma forma de aplicación (en franjas anchas o estrechas) más adecuadas, con el fin de optimizar su aplicación y aumentar la relación coste/beneficio. Dentro de las diferentes propiedades del suelo analizadas, se determinó la utilidad de los parámetros microbiológicos como biomasa (C biomasa y biomasa estimada mediante PLFA), actividad (respiración, actividad bacteriana y actividades enzimáticas como β-glucosidasa, ureasa y fosfatasa) y, sobre todo, la estructura de las comunidades microbianas (diversidad taxonómica y diversidad funcional) como indicadores de los efectos que tienen todos estos aspectos del fuego.

En experiencias de laboratorio, para evaluar el efecto del régimen de los incendios (severidad y recurrencia) y aislar el efecto del calentamiento de otros factores, se llevó a cabo un experimento en condiciones de laboratorio para emular la severidad del fuego. Las muestras de los suelos se sometieron a diferentes tratamientos térmicos en mufla (50, 75, 100, 125, 150, 175, 200 y 300 ºC), muestras quemadas y no quemadas de dos suelos diferentes, uno de ellos afectado por un incendio de alta intensidad (Laza, Ourense) y otro afectado por una quema prescrita de severidad baja (Estrada, Pontevedra). Para analizar el efecto de la recurrencia, se aplicaron dos tratamientos térmicos consecutivos a cada muestra tras un período de

incubación de dichas muestras reinoculadas (1% del suelo no quemado) y humetectadas a la capacidad de campo. Se midieron las temperaturas reales alcanzadas por el suelo mediante termopares (en superficie y a dos centímetros de profundidad). Con los datos obtenidos se construyeron las curvas temperatura-tiempo, se analizaron los parámetros de calentamiento obtenidos de estas curvas y se calcularon los grados-hora como una estimación aproximada de la severidad y, por último, se relacionaron los grados-hora con los cambios producidos en las propiedades del suelo. Los resultados indicaron que el fuego tiene un marcado efecto negativo sobre las propiedades del suelo, especialmente sobre las propiedades bioquímicas, como el carbono de la biomasa y las actividades enzimáticas. Esta influencia del fuego sobre la calidad del suelo, más acentuada en la superficie, puede estar enmascarada por el efecto de la vegetación. Los suelos bajo roble presentaron una mayor calidad que aquellos desarrollados bajo eucalipto (mayor contenido en materia orgánica y mayores valores de biomasa y actividad microbiana). Tal como se señaló con anterioridad, el efecto del incendio se vio atenuado por efecto de la profundidad, demostrando que el suelo no es buen conductor del calor. El fuego afectó en menor medida a las propiedades físico-químicas que a las propiedades biológicas, que mostraron una sensibilidad mucho mayor al impacto de los incendios/calentamientos. Dentro de las propiedades físico-químicas analizadas, la evaluación de la fracción lábil del carbono, a diferencia de aquellas fracciones más recalcitrantes, resultó ser muy buen indicador de los efectos del fuego y de su severidad, los cambios fueron mayores en aquellos suelos afectados por incendios de alta severidad. El carbono de la biomasa y las medidas de actividad microbiana también presentaron una gran sensibilidad al efecto del fuego. Dentro de las actividades enzimáticas, la β-glucosidasa presentó un efecto variable según el incendio forestal considerado, los valores de la actividad bacteriana medida como incorporación de leucina marcada radiactivamente aumentaban y, por último, la respiración y, especialmente, la actividad fosfatasa y la ureasa disminuyeron considerablemente en la mayoría de los casos.

El análisis de la estructura o diversidad taxonómica de la comunidad microbiana mediante el análisis de ácidos grasos de los fosfolípidos (PLFA) ha demostrado ser el método más sensible para detectar cambios producidos en la calidad del suelo inducidos por el fuego. Sin embargo, la estimación de la biomasa mediante PLFA no resultó tan útil. Dentro de las comunidades microbianas de los suelos, los diferentes grupos de microorganismos se vieron afectados por el fuego en distinto grado tal como mostraron los índices hongo/bacteria y $Gram^-/Gram^+$. Hongos y bacterias $Gram^-$ fueron los grupos más sensibles, mientras que actinobacterias, bacterias $Gram^+$ se vieron favorecidas por las condiciones post-incendio presentando concentraciones mayores tras el paso del fuego. En los diferentes experimentos analizados, en estos suelos quemados también se observó un marcado efecto estacional sobre las propiedades analizadas y, así mismo, la estructura de las comunidades microbianas varió según la época de muestreo. Los cambios producidos en la calidad del suelo se mantuvieron a medio plazo, indicando que tras un incendio los microorganismos necesitan más de 4 años para recuperarse.

Respecto a los tratamientos de rehabilitación post-incendio, el "*mulching"* resultó ser un método más efectivo que la siembra para evitar la erosiónpost-incendio, con reducciones en la

producción de sedimentos de hasta el 70-90%. En general, no se observaron cambios significativos en las propiedades del suelo analizadas por efecto del "*mulching"* de paja o de la siembra a corto plazo, aunque a medio plazo el mulching si pareció favorecer a los hongos frente a las bacterias debido al aumento de la relación C/N producido por la incorporación de la paja. El "*mulching"* favoreció la recuperación de la cobertura vegetal en la experiencia de Saviñao mientras que en Laza no se detectó dicho efecto debido, probablemente, a la diferente severidad de los dos incendios y a unas condiciones post-incendio desfavorables (1.566 m de altura, temperaturas más bajas de 0ºC no adeucadas para el desarrollo vegetal (germinación, crecimiento y regeneración). El "*mulching"* no modificó el número ni la composición de la vegetación de manera que las especies presentes en condiciones post-incendio fueron prácticamente las mismas que en las zonas adyacentes no quemadas; sin embargo, la siembra si produjo una mayor proporción de herbáceas respecto al matorral. Las especies que crecieron a partir del banco de semillas fueron las mismas entre las muestras tratadas con "*mulching*" y las no tratadas, y no aparecieron especies no autóctonas con capacidad de convertirse en invasoras en ninguno de los tratamientos aplicados.

En lo que respecta al experimento realizado en condiciones de laboratorio, los resultados demostraron que las temperaturas reales alcanzadas en el suelo fueron mucho más bajas que las indicadas en la mufla, demostrando la importancia de la medición de las mismas. La metodología de los grados-hora y los parámetros obtenidos de las curvas de temperatura-tiempo resultaron una buena herramienta como estimación de la severidad del fuego y, por tanto, para evaluar el efecto de la recurrencia en el calentamiento de las muestras. El calentamiento del suelo no ejerció un efecto significativo sobre las propiedades fisicoquímicas analizadas ni sobre el color de las muestras, independientemente de la temperatura alcanzada. Los resultados de las propiedades bioquímicas y microbiológicas analizadas nos indicó que la principal fuente de variación de las mismas era el tipo de suelo, de manera que la muestras del suelo de Laza y de la Estrada fueron muy distintas entre sí. La segunda fuente de variación de los datos era el régimen del incendio en condiciones de campo, los cambios inducidos por un incendio de alta intensidad (Laza) fueron más drásticos que los producidos por el fuego experimental de baja intensidad (Estrada). Los datos también mostraron que los cambios derivados del calentamiento del suelo y la magnitud de los mismos estaba relacionada con la severidad y recurrencia del fuego en campo y/o el calentamiento en laboratorio. La importancia de la severidad quedó reflejada de tal manera que los cambios producidos en las propiedades bioquímicas (actividades enzimáticas y actividad bacteriana) estuvieron relacionados con los grados hora aplicados a las muestras. La respuesta de estos parámetros bioquímicos frente a un nuevo calentamiento fue diferente según el historial previo de incendios, así, las muestra quemadas en campo y las calentadas previamente en el laboratorio, presentaron una sensibilidad mayor frente al estrés provocado por un nuevo tratamiento térmico. Esto demuestra el efecto negativo y acumulativo de los incendios forestales sobre las propiedades bioquímicas del suelo.

Los resultados demostraron que la diversidad taxonómica estimada mediante el análisis de los ácidos grasos de los fosfolípidos (PLFA) era un método muy efectivo para detectar cambios producidos en las comunidades microbianas del suelo como consecuencia de la severidad y de

la recurrencia del fuego/calentamiento, incluso antes que esas variaciones se vean reflejadas en otros parámetros como las propiedades fisicoquímicas del suelo. El calentamiento del suelo en durante el fuego o el tratamiento térmico el laboratorio confirmó la mayor sensibilidad de los hongos y las bacterias Gram$^-$ frente a otros grupos microbianos. La sensibilidad frente a la temperaturas de las medidas de diversidad funcional determinadas como la degradación de un total de 96 sustratos carbonados de diferente labilidad (CLPP) fue mucho menor que en el caso anterior. Por lo tanto, los cambios producidos en la diversidad taxonómica (PLFA) no se ven tan claramente reflejados en la diversidad funcional (CLPP) de los microorganismos del suelo, indicando que una parte de la comunidad microbiana del suelo puede ser funcionalmente redundantes, dado que distintos miembros de la misma pueden utilizar las mismas fuentes de carbono; si éste es el caso, la diversidad funcional puede subestimar la diversidad taxonómica. Otra posible explicación es que las dos técnicas de medida de la diversidad (taxonómica, PLFA; funcional, CLPP) proporcionan una información diferente sobre las comunidades microbianas y, por tanto, al ser complementarias, no pueden compararse.

Los resultados de todas estas experiencias, realizadas tanto en condiciones de laboratorio como de campo, muestran claramente que la determinación de la biodiversidad de las comunidades microbianas del suelo son de gran ayuda a la hora de evaluar e interpretar los resultados obtenidos sobre el impacto a corto, medio y largo plazo del fuego y de diversas prácticas de protección del suelo frente a la erosión post-incendio, sobre la calidad del medio edáfico.

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INTRODUCTION

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2. INTRODUCTION

2.1. SOIL MICROORGANISMS

Soil microbial biomass is composed of a large diversity of living organisms smaller than 5x 10^3 μm^3, such as bacteria, actinobacteria, fungi, algae, protozoa and microfauna (Jenkinson and Ladd, 1981) and virus (Lynch, 1983). The numerically dominant group are bacteria, but due to their small size, their contribution to the percentage of biomass is relatively small. The next most important group are actinobacteria, with a biomass percentage higher than or equal to bacteria due to their larger size. Alexander (1967) indicated that this group can constitute between 10 and 50% of the total biomass, reaching values of 95%. The group of fungi predominates especially in acid soils, due to less competition with other groups. Algae and protozoa are less numerous groups than the previous ones. The edaphic microfauna include nematodes, oligochaetes and arthropods, and their importance lies in their contribution to soil improvement and the control they exert over the microbial population. These microorganisms, which constitute the living fraction of the soil, not only use the soil as their natural habitat, but also participate in its formation and maintenance, both in natural, agricultural and forestry ecosystems. They are responsible for the soil fertility by conducting a large number of transformations in various environmental conditions. Its importance is reflected in the fact that between 80 and 90% of the processes that occur in the soil are mediated by microorganisms (Coleman and Crossley 1996, Nannipieri *et al*., 2003).

Regarding C cycles and nutrients, models of C, N and P transformations showed that microbial biomass is the pathway and nutrient source that controls system dynamics. In addition, once dead, they release into the environment their cellular metabolites that become available to other microorganisms and plants. The edaphic microbiota, although it only represents a small percentage of soil organic matter (between 1-3% of organic C), has a very important role as a pathway and source of mineral nutrients for plant development (Hannam *et al*., 2006). Due to their activity, they also influence certain soil properties such as structure, temperature, atmosphere composition, pH and Eh.

The edaphic microbiota is a key element in many of the processes that take place in soil. It can also be used as a bio-indicator or early indicator of changes in soil quality as a consequence of degradation processes and/or soil conservation practices, long before such changes are detected by analysis of the physical, physical-chemical and chemical properties of soil (Pankhurst *et al*., 1998). Thus, changes in number, biomass, metabolic activity and structure of the microbial community can be a sign of an alteration in the ecosystem (Atlas, 1984) and these changes are easily detectable with parameters such as C biomass, respiration, N mineralization, the enzymatic activities of the C cycle (β-glucosidase), N (urease) and P (phosphatase), bacterial activity (Doran and Zeiss, 2000) and microbial community structure (Frostegård *et al*., 2011).

Nowadays, community level physiological profiling (CLPP) and molecular biology techniques, such as the analysis of phospholipid fatty acid (PLFA), which do not involve a cultivation media, allow us to study the microorganisms at community level. Thus, by phospholipid fatty acid analysis it is possible to examine broad scale patterns in microbial composition and generally, after the application of multivariate statistical analyses, the whole community fatty acids profiles, indicate which communities are similar or different (Frostegård *et al*., 2011). Characterization of soil microbial communities by this technique gives results representing closely the *in situ* soil conditions and, hence, it is currently used for monitoring soil quality changes under wide ranges of soil types, management practices, climatic conditions and different soil perturbations (Frostegård *et al*., 1993, 1996; Zelles, 1999; Díaz-Raviña *et al*., 2006; Barreiro *et al*., 2010). The CLPP analysis is based on the premise that microorganisms vary in the pattern and the rate at which they utilize carbon sources; therefore, carbon utilization patterns can be used as measured of the microbial community structure and the functional potential (Garland and Mills, 1991; Insam, 1997).

2.2. EFFECTS OF WILDFIRES ON SOIL ECOSYSTEMS

Wildfires are the combustion of a quantity of vegetable fuel in the presence of a source of heat and oxygen. These three factors (fuel, oxygen and heat) are known as the fire triangle. Although the most evident consequence is the destruction of the vegetation cover, their effects are much more extensive involving all the environment components (soil, water and atmosphere). Wildfires affect fauna, causing the death of those more species that, due to a lower mobility, are not able to run away from flames, a decrease in sensitive species since habitat alteration can lead to a lack of food and refuge and, finally, an increase of the number of fire tolerant microorganisms. Therefore, independently of the time passed after the fire, the evaluation of microbial composition or diversity is the best indicator or fire induced-changes on soil quality. Fire destroys the roots and aerial parts of plants and trees. The soil seed bank is also destroyed or dragged away by erosion after the first rains. Fire alters the water regime and water quality, climate, soil properties and the microorganisms that inhabit it (Neary *et al*., 2005; Carballas *et al.*, 2009). Wildfires are a serious ecological, economic and social problem of such importance and, therefore, the different prevention policies and the social perception that they generate have to be analysed (Carreiras *et al*., 2014; Pereira *et al*., 2015).

Soil is one of the most important factors in the biology of forest ecosystems and probably is more affected by fire than any other component (Page-Dumroese *et al*., 1991). Fire modifies the physical-chemical, chemical and biological properties of soil, provokes soil degradation decreasing its quality and altering its functioning (Mataix-Solera *et al*., 2002; Certini, 2005; Carballas *et al*., 2009; Díaz-Raviña *et al*., 2010; Martín *et al*., 2012; Carballas, 2014; Carballas *et al*., 2015). The intensity of changes will depend on the severity of the burning and the characteristics of the environment such as moisture, vegetation or soil type. Wildfires produce a reduction or even the total destruction of the organic matter and alter its chemical composition and its dynamics. These alterations in the organic matter produce changes in almost all the soil properties. The loss of organic matter leads to a decrease in water retention capacity and the destruction of soil structure. The most labile fraction is the most sensitive

indicator of changes in organic matter, detecting these changes before than the most recalcitrant fractions or in total soil carbon (Haynes, 2000; Spielvogel *et al*., 2007). Generally, electric conductivity and pH increase due to the ash production and the destruction of organic acids. They also provoke many adverses indirect effects such as the post-fire erosion processes that are aggravated by the disappearance of the vegetation cover.

The soil components most affected by forest fires are the microorganisms (Acea and Carballas, 1996). Despite being one of the agents responsible for soil functionality, in studies about wildfires effects, aspects such as biomass, activity and diversity have not been taken into account in most investigations (Mataix-Solera *et al*., 2009). Generally, the temperatures reached on soil surface exceed those required to kill the majority of microorganisms (DeBano *et al*., 1998). After a fire, the microbial biomass decreases considerably, reaching even in the most extreme cases the sterilization of the first layers of the soil. On the other hand, changes on soil properties caused by fires indirectly have a negative effect on microorganisms (Díaz-Raviña *et al*., 1992; González-Pérez *et al*., 2004) and the magnitude of theses effects will depend on the severity of the fire and the post-fire climatic conditions. Large changes in environmental conditions or chemical substrates, such as those produced by fire, can alter respiration and enzymatic activities such as dehydrogenase, urease, phosphatase and arylsulfatase (Certini, 2005; Hamman *et al*., 2008). The response of soil ecosystems to adverses effects provoked by fire and its further recovery to pre-fire soil conditions will depend on factors such as vegetation before wildfire, depth or seasonal effects, in other words, soil quality prior to fire (Mahía *et al*., 2007; Alvárez *et al*., 2009). However, the effect of fire on the soil microorganisms is not the same for all microbial groups. Fungi are generally considered to be more sensitive to heat than bacteria (Bollen, 1969; Dunn *et al*., 1985), which are favoured by the increase in pH that occurs after a fire. Many previous studies have found that after heating, bacteria recover faster and at higher levels than fungi (Bollen 1969; Ponder *et al*., 2009) so that, decreases occur in the ratio of fungi/bacteria (Pietikainen and Fritze, 1995; Bárcenas-Moreno and Bååth, 2009; Carballas *et al*., 2009). $Gram^+$ bacteria are less sensitive to the action of fire than $Gram^-$ (Mabuhay *et al*., 2003). The difference in sensitivity of the different groups of microorganisms to environmental alterations produces a decrease in microbial diversity that can reduce the stability of the microbial community as well as its resilience and hence the soil-plant recovery (Girvan *et al*., 2005).

2.3. POST-FIRE EROSION

The loss of soil and nutrients as a result of post-fire erosion is another important consequence of wildfires in Galicia. Fire produces an increase in soil erosion in this region exceeding the tolerable soil loss limits (Díaz-Fierros *et al*., 1982). The vegetation cover produced a protective effect on soil that disappeared when it is destroyed by fires. Galicia is an area of high vulnerability to post-fire erosion due to their characteristics. The geographical relief presents areas of high slope, which are frequently affected by wildfires. The climate is characterized by abundant rainfall with episodes of rain that can become very intense (Díaz-Fierros *et al*., 1987). The edaphic conditions in altered soils such as those affected by wildfire becomes soils favourables to run-off and therefore to erosion. These conditions are those

related to organic matter and texture, such as the aggregate stability and the water retention capacity that decrease after a fire due to the combustion of soil organic matter, and the increase in water repellency. These soil changes, together with slope and precipitation produce runoff and soil loss due to water erosion (Vega *et al*., 1986; Díaz-Fierros *et al*., 1987; Soto *et al*., 1997), mainly laminar (Vega *et al*., 2013). Erosive processes are considered frequent in the autumn and winter immediately after the wildfires, associated with the abundant precipitations (Díaz-Fierros *et al*., 1987; Vega *et al*., 2005). Some studies show the high values of erosion that occur in Galicia with data of up to 35 Mg/ha/year (Fernández *et al*., 2011) 20.4 Mg/ha/year (Vega *et al*., 2013) that contrast with low erosion, which practically does not exist in unburned areas (Soto *et al*., 1994, Vega *et al*., 2005) due to the protection of vegetation cover and the presence of healthly soils. These erosive processes are considered to favour the degradation of soil environment and to contribute to the loss of soil fertility (Soto *et al*., 1997; Gómez-Rey *et al*., 2013). In addition, these sediments resulting from erosion are transported by water causing serious damage to aquatic ecosystems when they are deposited in surface and subterraneas water as happened in Galicia after the wildfires of 2006 (rivers, sea).

In order to avoid soil losses on slope susceptible to erosion, emergency post-fire rehabilitation treatments of various types can be implemented. Some examples are seeding, straw mulch, different types of residues of schrub plants and trees proceding of burned proximity areas, contour trenching, log erosion barriers, use of straw or other biodegradable materials, or the application of compounds such as polyacrylamides (Robichaud *et al*., 2000). The application of these stabilization treatments should take place as soon as possible following the forest fire and just before the rain evens ocurred (Vega *et al*., 2013)

Straw mulching (which can be wheat, rye, barley, among other cereals) and seeding (mainly grass species) are considered the two most effective methods against soil erosion (Robichaud, 2009). Although, in principle, seeding has been the most commonly used method to obtain a vegetation cover in the soil, its effectiveness has not been conclusive (Robichaud *et al*., 2000). In Galicia, it was shown no effective due to steep slope (seeds can be washed away by intense rain events) and to the failure of germination and the establishment of seeded plants (unfavourable conditions such as moisture and temperature). Sediments are lost before dense vegetation cover is established (Vega *et al*., 2013). Straw mulching resulted to be the most effective post-fire treatment against erosion (Vega *et al*., 2013). Mulching consists in the application of materials such as agricultural or forest plant remains in soils recently affected by fire. The mulch application generates a protective layer on soil as occurs naturally with plant materials such as leaves and pine needles (Cerdá and Doerr, 2008). Therefore, when pine needles from pines afected by fire cover over 40% of the soil, natural mulching is produced, which makes the implementation of mulching or seeds treatments not necessary due to their high cost (Cerdá and Doerr, 2008; Prats *et al*., 2016). The efficacy of mulching is due to three processes: a) interception of the rain that does not reach the soil to the same degree as if it were naked, b) reduction of the kinetic energy of precipitation and c) limitation the surface runoff. It should be noticed that, surprising, the layer formed by straw mulching sticks, which avoid considerably its effectiveness aginast losses of soil and nutrients.

The main disadvantages of this treatment are its high cost and the possible introduction of seeds from other non authocthonous plants that may become invasive. Seeds of non-native plants can be introduced and can compete for resources with native plants and displace them. One of the greatest risks to forest ecosystems is the introduction of invasive species (Harrod *et al*., 2001) and it is known that fire facilitates their establishment (Korb *et al*., 2004; Hebel *et al*., 2009). The most effective and economical way to eliminate invasive species is prevention. Therefore, to avoid the introduction of non-native species when using any method of post-fire treatment, straw should be free of non authocthonous weeds and seeds. The application of this treatment should be performed with caution, especially in Protected Areas and Natural Parks. The use of mulching of native wheat straw can mitigate this negative effect.

In addition, information regarding the possible influence of these post-fire treatments on the ecosystem components such soil, vegetation or fauna is scarce. The information available about how mulching affects vegetation is contradictory. Authors such as Badía and Martí (2000), Peterson *et al*. (2009) and Fernández and Vega (2014) found positive effects of mulching on vegetation, while Kruse *et al.* (2004) detected a negative effect on vegetation cover recovery. Dodson and Peterson (2010) found an increase in non-native species on treated plots. Other studies showed no positive or negative effect of mulching on vegetation (Fernández *et al*., 2011; Vega *et al*., 2014). The positive effect found in some studies may be explained by the ability of the straw to retain moisture and to protect from temperature changes (Robichaud *et al*., 2000), while the negative effect may be due to the barrier effect of the straw layer against germination and seed growth. According to Dodson and Peterson (2010) these contrasting results could be attributed to environmental factors limiting plant germination and growth. On the other hand, despite the importance of the soil seed bank for vegetation recovery (Thompson *et al*., 1997) there are no studies analysing the effect that mulching produced on it. Concerning the effect of postfire rehabilitation techniques on microbial community information is scarce. Despite the important role of microbial on soil quality and the global use of mulching or seeding to avoid soil erosion, there is not information available about how this techniques influence soil microbiota in terms of biomass, activity or diversity. Stabilization treatments such as mulching have a very high cost and therefore they can not be applied in all surface burnt soil, those areas more susceptibility to suffer soil erosion close to surface or subterraneous waters should be prioritized. To reduce costs, the studies concerning the limits of surface burnt area and the minimum doses to apply without diminishing its effectiveness are of great interest.

2.4. FIRE REGIMEN: SEVERITY AND RECURRENCE

Classification of fires can be carried out according to various criteria such as the type of combustible material, the vegetal stratum affected (surface fire, crown fire or subsoil fire) and the fire spread. Generally, fires can be grouped into three types: a) uncontrolled fires, b) planned fires used for the destruction of forests for its conversion into pasture; in Galicia, fire was considered a working tool used by farmers and ranchers for agricultural purposes, and c) prescribed or controlled fires that are fires provoked for the purpose of managing forest ecosystems to control fuel and to avoid the risk of suffering high severity fires (Fernández *et*

al., 1997). Moreover, fires can be classified according their severity in high-, medium- and low- severity fires. The different severity can be estimated in field immediately after the fire directly "*in situ*" using high time consuming methods (Vega *et al.*, 2013). In low severity fires burned vegetation remains can be detected. The total destruction of vegetation cover indicates a medium-severity fire and the presence of white ashes are link to a high severity wildfire (Vega *et al*. 2013).

Intensity and severity fire terms have been used as synonyms but they reflect different concepts. Burn severity is defined as the loss of or change in ecosystem caused by fire (Keeley, 2009). It is related to fire intensity, which denotes the energy released from fire. In other words, severity is the qualitative measure that refers to the overall effect on fire on soil ecosystems (Neary *et al*., 1999). Most studies use burn severity instead of fire intensity, because it can be measured after fire (Zavala *et al*., 2014). The amount and the duration of heat transfer should be taking into account when studying the fire severity. Within these two factors (temperature and time), the component of severity that is most destructive to soil ecosystems is the duration of fire (Neary *et al*., 1999). An approximation to the study of severity is the recent degrees-hours methodology that allows the estimation of the amount of heat applied to the soil considering the combined influence of the temperature reached by soil and the time exposure to that temperature (Busse *et al*., 2005). The study of severity under field conditions results very complicated as temperatures reached by soil cannot be recorded. Furthermore, a typical fire creates a mosaic pattern leaving areas unburned and burned across a range from low to high severity depending on factors as duration, available fuel or topography (Neary *et al*., 2005). The extrapolation of the results from one field experiment to other becomes impossible. Nevertheless, studies under laboratory conditions allow to record the maximum temperature reached and the time of heating. Laboratory experiments at a range of temperatures can simulate different fire severities as well as to separate the fire effect from environmental factors.

Galicia and the North of Portugal (NW of Iberian Peninsula), is one of the area in the world most affected by forest wildfires. This region accumulates the highest number of fires per hectare and inhabitant (Carballas *et al*., 2009). During the decade 2006-2015, 24% of wildfires recorded in Spain were located in Galicia (MAPAMA, 2015), despite the fact that this area represents only the 10% of the total forest area in Spain. We could highlight the year 2006, where half of the national fires were situated in Galicia and fires burned about 4% of the total forest area in the region (MAPAMA, 2015).

Over the past several years, an alarming new kind of wildfire has emerged; the mega-fire (Williams *et al.*, 2013). Wildfires can classify by size, burnt surface area of approximatelly around 1 ha is considered aa wildfire fire while those larger than 500 ha are called large fires. The trend of large fires is shown quite stable in the last decades (San-Miguel-Ayanz and Camia, 2010). However, among these large fires, several fire episodes caused catastrophic damages and the loss of human lives (64 people killed in Portugal 2017; 102 people killed in Greece) (San-Miguel-Ayanz *et al*., 2013). The model of global pyric phases of Bowman *et al*. (2011) indicates that, nowadays, we are in the fifth pyric phase with a new generation of fires (mega fires) related with climate change and fuel modifications. The consequences of climate

change present a worse future scenario where fires present a greater virulence (WWF, 2017). The increase of temperatures and the reduction of precipitations, among other factors, provoques the extent of fire season (that is no longer limited to the summer months, it is extended from April to October) and modifies fire regimen. Furthermore, due to the extreme weather conditions, wildfire becomes virulent in extension, frecuency, severity and fire spread (Amatulli *et al*., 2013; Hinojosa *et al*., 2016), especially in areas of high net primary production, where it is expected an increase on frequency of wildfires (IPCC, 2014). Díaz-Fierros (2019) defined a megafire as a wildfire with an extension above 10.000 ha. Nevertheless, authors as Tedim *et al.* (2013) consider that megafires are not just large fires, that they are extreme events in their behaviour with difficultiess to control their impacts; thus, their size can only be considered in reference to each country. The difficulty or impossibility to control these fires explains the large extension of surface burnt areas. In Europe, megafires are not single fire events, they are the concentration in time and space of a large number of large fires.

This combination of very high amounts of fuel biomass and heat waves of long duration, accumulate drought, low humidity and high wind is the perfect scenario for extremely dangerous wildfires as happened in Portugal (2003, 2005 and 2017), Greece (2007 and 2018) and Galicia (2006 and 2017). Regarding Galicia, approximately 85% of the total burned area in the year 2006 year was due to the megafire event that lasted only 12 days with about 1.900 fires, burning approximately 78.000 ha, killing four people and causing large damages to the environment and the living organisms (European Commission, 2007). During 2017, 50.000 ha burned in 6 days and 4 people died as consequence of fire (MAPAMA, 2018). Although mega-fires usually happen in very extreme weather conditions (Williams *et al*., 2013), can appear even though the meteorological conditions are not so extreme (Tedim *et al.*, 2013). Land abandonment and the fuel accumulation are identified as factors that favour the occurrence of megafires (Moreira *et al*., 2011; Pausas and Fernández-Muñoz, 2012; Pausas and Paula, 2012). This trend has been driven by socioeconomic changes that have generated rural depopulation and changes in traditional land use (Chergui *et al*., 2017).

Galicia presents, in theory, unfavourable meteorological conditions for wildfires (temperate-humid climate) and its natural vegetation type, with oak as climax vegetation, are generally considered a low fire risk area (Díaz-Fierros *et al*., 1982). In spite of this, in the North-West of the Iberian Peninsula and the North of Portugal there are a serie of circumstances that cause that fires occurred year after year. Traditional use of fire as a tool forest management, changes in the maintenance of rural areas and incendiarism as protest have been identified as the principal causes of Galician wildfires (Fernández-Fernández, 2016). The 99% of fires are due to human factors. Within wildfires with an anthropic origin, the 80% of them are provoked by different reasons as regeneration of pastures, social conflicts, etc ... while the rest of the fires are caused either by negligence or accidents. In addition, as it was mentioned before, every few years there are extremely dry summers that are very favourable to fire spread. For all these reasons, the same areas are burned year after year, so of the 100 municipal districts with the highest number of fires between 2001 and 2015, 72 belong to Galicia, being A Cañiza (1541), Viana do Bolo (1338) and Muíños (1226) the Galician municipal district with the

highest number of fires, demonstrating the recurrence of fire in this region, as shown Figure 1 (MAPAMA, 2015).

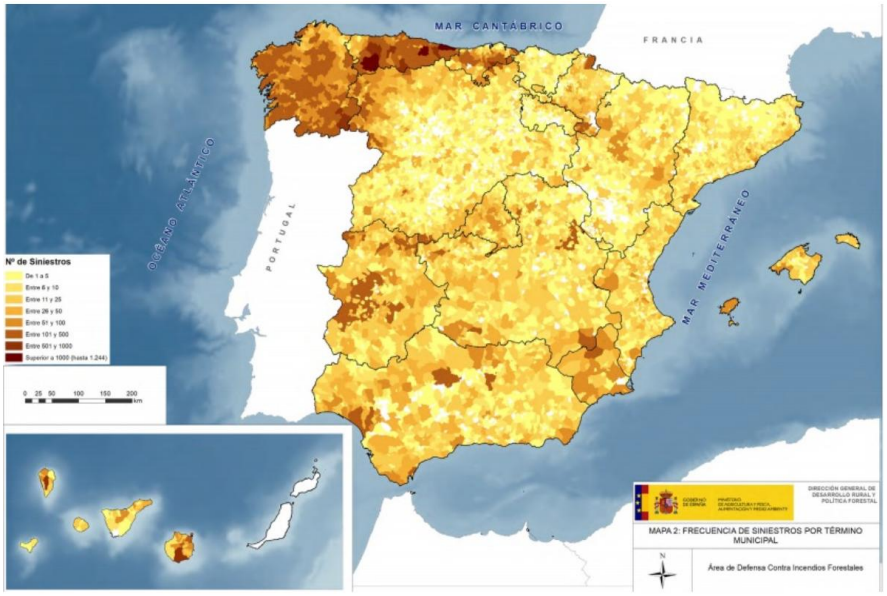

Figure 1. Fire frequency by municipalities.

Although information concerning the impact of both wildfires and prescribed fires on various components of forest ecosystems is available, it is not being fully utilized in planning prescribed burning for management purposes (Tiedemann *et al*., 2000). The role of prescribed fire on reducing the effect of high severity wildfire is wellknown, reduction of the amount of fuel available on forest and controlling the tolerance of forest to fire (Fernández *et al*., 1997; Choromanska and DeLuca, 2001; Alcañiz *et al*., 2018). Nevertheless, despite the importance of forest productivity, climate regulation, C sequestration, wildlife, biodiversity, and other resources and values on the forest health equation, the long term effect of fire on those parameters is unknown. Prescribed burning has the potential to affect soil microorganisms because of the direct effect of heat or indirect effect due to changes in chemical and physical properties (González-Pérez *et al*., 2004), so prescribed burning may be a source of damage (Fernandes *et al*., 2013), particularly when applied repeatedly in the same area. Information about how fire recurrence can affect ecosystems at long-term are very scarce but it is known that the effects of a single fire on soil ecosystems differ from those of repeated fires (Vance and Henderson, 1984). Studies concerning the effect of repeated prescribed fires as management tool on soil are non conclusives. Although prescribed fires produce, at short-term, an increased in nutrient availability (De Marco *et al*., 2005; Úbeda *et al*., 2005), other studies at medium and long term showed a negative effect on physicochemical properties as N, S, C and P dynamics and nutrient availability, microbiological properties and even on soil erosion (Binkley *et al*., 1992; Ojima *et al*., 1994; Monleon *et al*., 1997; Wright and Hart, 1997; DeLuca and Sala, 2006; Eugenio *et al*., 2006). In addition, not only recurrence but frequency of burning had a negative impact on soil quality such as reductions in forest nutrients (Guinto *et al*., 2001; Muqaddas *et al*., 2016). Fire recurrence have also a negative effect on soil microorganisms (Banning and Murphy, 2008; Guénon *et al*., 2011; Fultz *et al*.,

2016; Rodríguez *et al*., 2017), on vertebrates (Darraqc *et al*., 2016), vegetal production (Ferrán *et al*., 2006) or the initial pine seedling establishment (Taboada *et al*., 2017). These negative effects may require more than a decade after the fire to disappear (Fritze *et al*., 1993; Mack *et al*., 2008; Goulden *et al*, 2011; Oliver *et al*., 2015). Variations on the effect of fire in soils suggest that fire response may be site specific (Mikita-Barbato *et al*., 2015), I mean, the impact of prescribed fires differs greatly depending on the initial soil characteristic and vegetation type (Alcañiz *et al*., 2018).

Tiedemann *et al.* (2000) in a review about how prescribed fires and their frequency can negatively affect the productivity of forest ecosystems, concluded that they should be used with caution and that a deeper understanding of their effects on ecosystems is required before they can be implemented globally. Regarding the soil microorganisms, evaluating the long-term effect of recurring prescribed fires on soils microbial communities is important to properly manage forest to avoid compromising their composition and function as its important role on plant productivity (Wardle *et al*., 2004). The use of prescribed fires to control biomass, together with the characteristics of fires in Galicia that cause them to occur repeatedly in the same areas, and the future scenario with wildfire increasingly severity, makes it vitally important to study how fire recurrence and severity can affect forest ecosystems.

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OBJECTIVES

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3. OBJECTIVES

Wildfires are a major problem in Galician forest ecosystems, where fires are recurrent, affecting the same area year after year. Their severity is increasing because of climate change and hence, every now and then, virulent fires of high severity and rapid fire spread ravage this region. Prescribed burning is widely used as a management tool to prevent high-severity fires or to generate grassland for cattle farming. However, information on the effect this repetitive and periodic burning has on microbial communities and, hence, on soil quality is scarce. The effectiveness of post-fire rehabilitation techniques for reducing the soil erosion is evident, but their effects on soil properties and vegetation, particularly on the soil seed bank, are unknown.

Several fields and laboratory experiments were performed to fulfill the main objectives of the present work, which are the following:

-To evaluate the direct and indirect effects of medium and high severity forest fires on the soil quality (physical, chemical, biochemical and microbiological properties) as well as to determine the relative importance of some factors involved in this process (soil type, vegetation prior to the fire, depth, post-fire climatic conditions, etc.) as well as in the recovery of the soil-plant system.

-To evaluate the effectiveness of two emergence post-fire rehabilitation treatments (seeding and straw mulching) as well as their effects on both, soil quality recovery (soil properties, including several aspects of microorganisms) and the regeneration of the vegetation.

-To analyse the usefulness of the degrees-hour methodology and the temperature-time curves as estimation of the severity of fire (temperature reached and its duration) as compared with the fire intensity (Tmax reached) to evaluate the fire impacts on soil environment.

-To assess the influence of severity and recurrence on physical-chemical, biochemical and microbiological properties, especially on the aspect of the biodiversity (taxonomic and functional).

-To determine the usefulness of various microbial parameters such as biomass, activity and, especially the taxonomic (PLFA) or physiological (CLPP) diversity of soil microbial communities as bioindicators of soil quality as a consequence of fire regimen (severity, occurrence) and the soil protection practices against the post-erosion fire.

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MATERIAL AND METHODS

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4. MATERIAL AND METHODS

4.1. DESCRIPTION OF FIELD EXPERIMENTS

Soil samples were collected in different areas of Galicia affected by medium and high severity wildfires. We selected forest ecosystems formed by acid soils, rich in organic matter, developed on acid rocks and under native shrub and tree vegetation (*Quercus robur*) and non-native tree (*Eucalyptus*) with different types of understorey. These types of soils were selected as they occupy a large amount of the surface of the territory and are strongly affected by forest fires, especially shrub and *Eucalyptus* ecosystems. In Fragas do Eume Natural Park (A Coruña), the effect of a wildfire in a soil with different type of vegetation on soil quality was studied. In the Laza (Ourense) and Saviñao (Lugo) field experiments, we analysed the effects of wildfires and post-fire stabilization techniques on the soil quality and the vegetation as well as the effectiveness of these postfire treatments.

4.1.1. Fragas do Eume

The study was performed in the Fragas do Eume Natural Park (A Coruña) declared Natural Park in 1997, with 9.000 ha. The Natural Park includes the municipalities of Pontedeume, Cabanas, A Capela, Monfero and As Pontes (Figure 2).

Figure 2 View of the area affected by a wildfire in Fragas do Eume Natural Park (Author: Serafín Prieto González)

The Natural Park is situated along the valley of the river Eume and is considered as one of the best preserved riverside forests in Europe and therefore was declared as Site of Community Importance (SCI) according to the Habitat Directive of the European Commission (92/43/ECC). The climax vegetation is a mix of species such as *Quercus robur, Corylus avellana, Castanea sativa, Betula alba, Laurus nobilis, Ulmus glabra, Salix atrocinerea, Fraxinus excelsior, Fraxinus angustifolia* and *Alnus glutinosa*. The protected area also included vegetation dominated by non-autochthonous species, such as *Eucalyptus* and to a lesser extent *Pinus radiata*. On March 2012 a wildfire affected 1.100 ha of the Natural Park, 750 ha dominated by *Eucalyptus* and 350 ha by autochthonous climax vegetation (*Quercus*). The severity wildfire was estimated as medium to high severity due to the combustion of the organic layer and the ash deposition (Vega *et al*., 2013). In order to evaluate the effect of the fire, 16 plots of 1.000 m^2 each were established in an area with slopes of between 30 and 70%. Of these 16 plots, 8 correspond to soil dominated by autochthonous vegetation and the other 8 to non-autochthonous vegetation. For each type of vegetation, 4 plots were established in unburned zone and 4 in burned zone. The samples were collected three months after the fire in horizon A, at two different depths, from 0 to 2.5 cm and from 2.5 to 5 cm.

4.1.2. Laza

The study was in an area of special interest near the Serra do Invernadeiro Natural Park in Laza (Ourense).

Figure 3: View of the area affected by a high severity wildfire in Laza (Ourense). (Author: Serafín Prieto González)

This area was affected by a wildfire in September 2010. Approximately 1.700 ha were burned in an area with high susceptibility to soil erosion (Figure 3). The dominant vegetation in the area is scrub with species such as *Erica* spp., *Vaccinium myrtillus, Pterospartum tridentatum* and *Cistus* spp.reforested with *Pinus sylvestris*. An area of 0.5 ha with a 30% slope was selected to establish plots for the study of fire effect and post-fire treatments on soil properties and post-fire erosion. The prevalence of black and white ashes and the total consumption of the ground plant communities (vegetation and litter layers) suggested that fire severity had been moderate to high in the study area (Chandler *et al*., 1983). Twelve experimental plots of 80 m^2 (4 x 20 m) each was established: 3 unburned control plots, 3 plots of burned soil to determine the effect of fire on soil properties. To evaluate the effectiveness of post-fire rehabilitation techniques as mulching and seeding and its effect on soil quality, 3 plots of burned soil with rye seeding (0.1 Mg ha^{-1}) and 3 plots of burned soil with straw mulching (2.5 Mg ha^{-1}) were established (Figure 4). Samples were collected from the top layer (0-2 cm) 1 and 16 weeks after the wildfire and 8, 12 and 48 months after the wildfire of the first 2 centimeters of soil. Additionally, samples were collected from 2 to 5 cm at 48 months in the same surface than those collected at 0-2 cm.

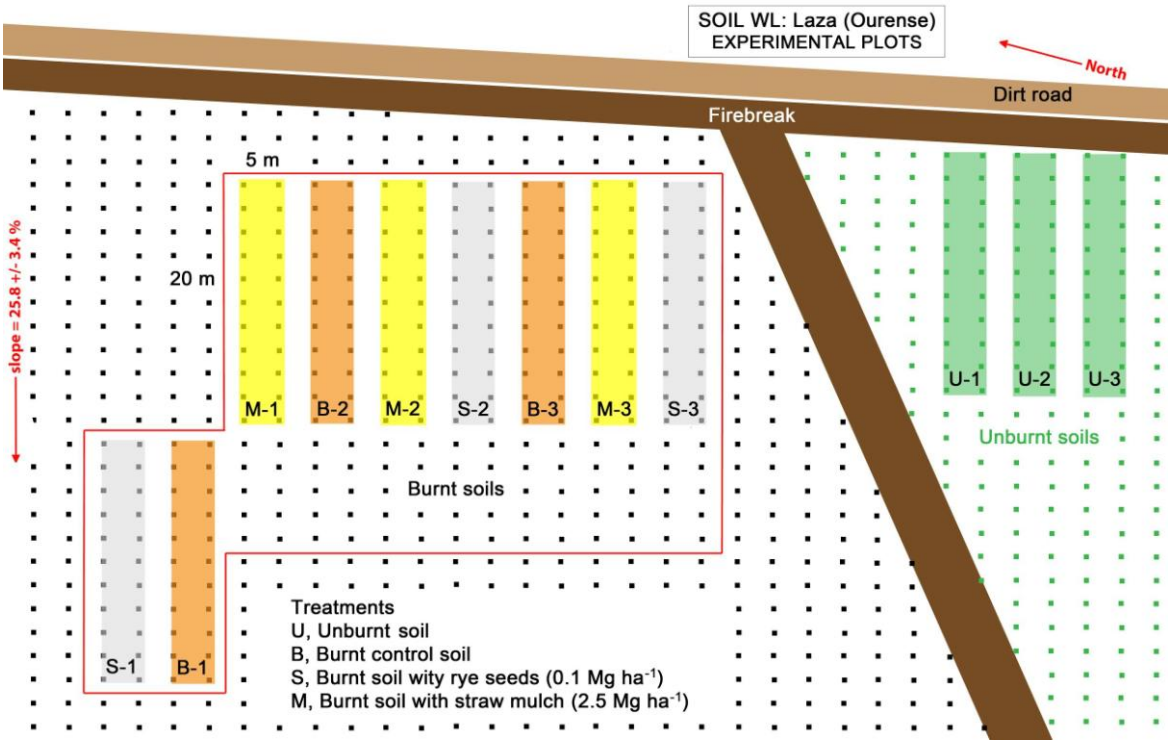

Figure 4: Scheme of the macro-plots established in experimental area in Laza (Ourense). (Author: Serafín Prieto González).

4.1.3. Saviñao

The following experience took place in a shrubland ecosystem with species such as *Erica arborea, Ulex europaeus, Pterospartum tridentatum* with some dispersed individual of *Quercus Pyrenaica* located in O Saviñao (Lugo).

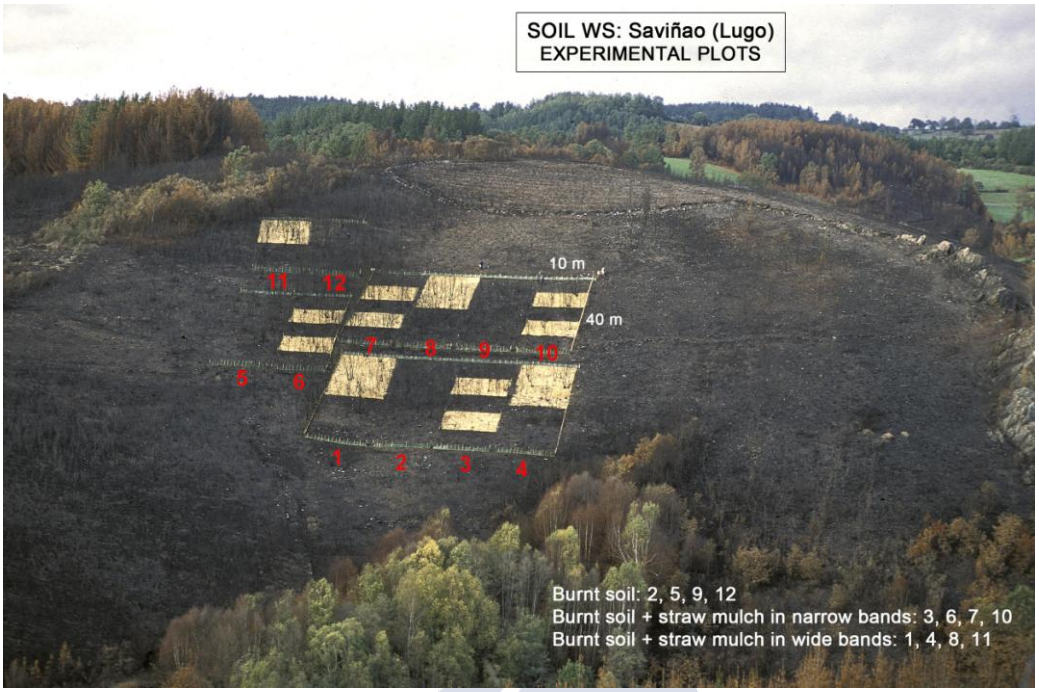

Figure 5 and 6: View of the macro-plots in the area affected by a wildfire. (12 experimental plots, 10 × 40 m each), and detail of straw mulch application. Treatments: B, burned soil (2, 5, 9, 12); B1S+M, treatment with 1 straw strip per plot, soil with straw mulching; B1S, treatment with 1 straw strip per plot, bare soil (1, 4, 8, 11); B2S+M, treatment with 2 straw strips per plot, soil with straw mulching; B2S, treatment with 2 straw strips per plot, bare soil (3, 6, 7, 10). (Author: Serafín Prieto González).

This area was affected by a forest fire in September 2012, in which 85 ha were burned. This is an area with high slope and therefore high risk of post-fire erosion (Figure 5). Visually, the affected area was classified as of medium-high severity due to the presence of white ash (Vega *et al*., 2013). A sampling area was delimited in 12 plots of 10 m x 40 m (40

m^2) each: 4 plots of unburned control soil, 4 plots of burned soil with straw mulching in a wide band of 20 meters (dose of straw: 1 Mg ha^{-1}) and 4 plots of burned soil with straw mulching in two narrow bands of 8 meters (dose of straw: 0.8 Mg ha^{-1}) (Figure 6). A total of 5 different samples were collected, control not burned, burned with wide mulching in the straw area, burned with wide mulching in the open area, burned narrow mulching in the straw area and burned narrow mulching in the open area. For the study of fire and straw mulching effects on the properties of the soil in the short and medium term, as well as to determine the dose and the most suitable way of application of straw mulching, samples of the first 2.5 centimetres of the surface layer of the soil were taken the first week after the fire and at 3, 6, 9 and 12 months.

4.2. DESCRIPTION OF THE LABORATORY EXPERIMENTS

Two Galician soils were used, one affected by a medium-high severity wildfire (Laza forest fire described above) and a low-intensity prescribed fire carried out in Estrada (Pontevedra).

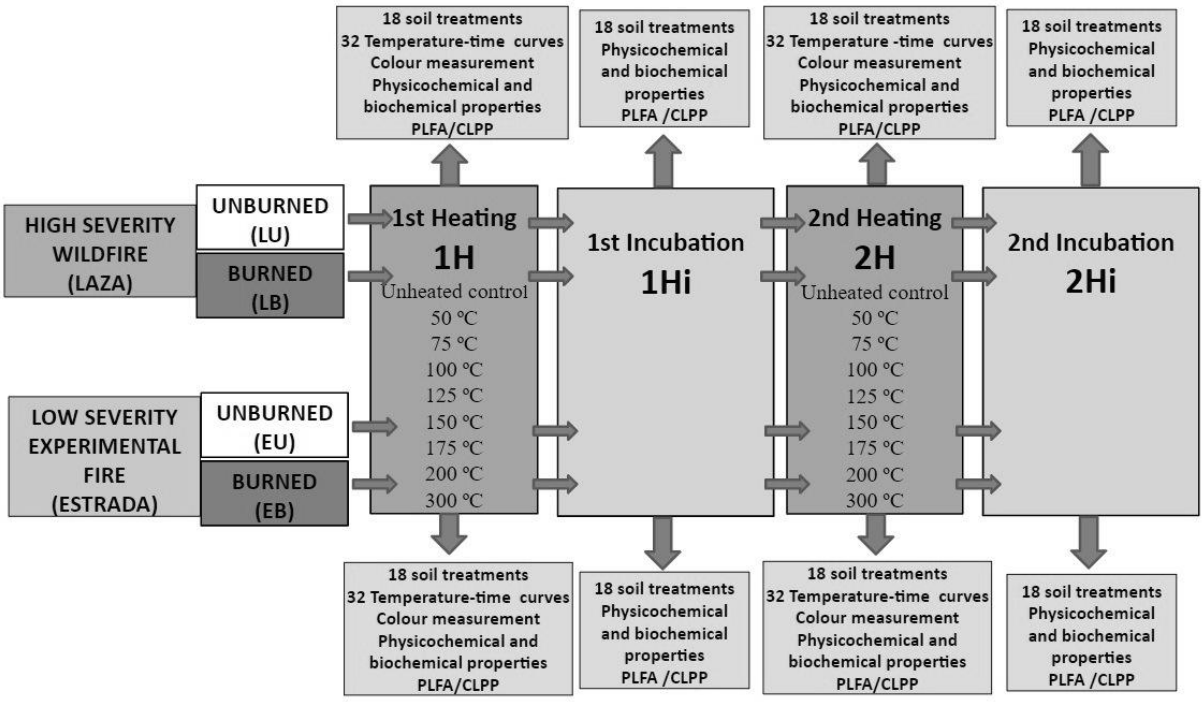

Figure 7. Scheme of the experimental design for laboratory heating. LU, unburned Laza; LB, burned Laza; EU, unburned Estrada; EB, burned Estrada.

The study area is a granite soil with scrub vegetation with species such as *Ulex* sp., *Pteridium aquilinum*, *Daboecia cantabrica*. The shrub was cut and put on the surface to favour its combustion and facilitate heat transmission. The propagation speed was low (0.3- 0.33 m/min) and the temperatures reached, which were measured with thermocouples, were moderate on the surface (mean 153ºC, range 48ºC-420ºC) and low at two centimetres depth (mean 34ºC, range 22ºC-43ºC). Under laboratory conditions, unburned and burned samples from both soils were subjected to two consecutive heat treatments in the muffle, with an

intermediate incubation period of one month (Figure 7). Eight different heat treatments were applied to simulate different burn intensities. (50ºC, 75ºC, 100ºC, 125ºC, 150ºC, 175ºC, 200ºC and 300ºC). A subsample of each soil (Laza unburned and burned, Estrada unburned and burned) was maintained at room temperature (21ºC) during the period of the experiment to use them as control. Samples from each soil (Laza and Estrada) burned and not burned (8 subsamples) of 200 g of fresh soil were separated, which were arranged in a layer of 2 cm in a metallic tray to apply the heat treatment and another subsample to maintain as control soil. (8 subsamples x 8 thermal treatments, 64 samples in total). After reaching the selected temperature they were kept at that temperature for a period of 15 minutes.

The actual temperature reached by the soil was measured every minute with two thermocouples located 1 cm deep and on the surface. These subsamples were divided into two parts, one part was used to analyse soil properties and the other was rewetted to 75-80% of field capacity, re-inoculated with the same 1% fresh soil and incubated under laboratory conditions (21ºC and darkness). One month later the subsample was again divided into two parts, one to analyse the effect of incubation on soil properties and the other was submitted to a new heat treatment at the same temperature as the previous treatment. After the second heating treatment samples were incubated again one month. A total of 144 different samples were finally obtained (4 soils x 9 temperatures x 2 heating cycles x 2 incubation cycles).

4.3. METHODS USED FOR SOIL CHARACTERISATION

Soil samples were sieved (< 2 mm), homogenized and stored at 4ºC no more than one month before the analysis of physicochemical and chemical properties.

4.3.1. Time-temperature curves and calculation of degrees-hour (fire severity)

With the data obtained from the two thermocouples located in the samples, the temperature-time curves were constructed. The exponential equation $y = ae^{bx}$ was used to model the temperature-time curves, where "a" is the ascending slope, "b" is the maximum temperature (Tmax) peak reached by the soil. These two parameters were used to define the heating curve and analyse the susceptibility of soil to heating. The model was adjusted using Origin Pro 8 software. The temperature recorded during the heating treatments was used to calculate the degrees-hour (DH) reached by soil as an estimate of the amount of heat applied to each soil or fire severity. The DH were calculated using the formula modified by Cancelo-González *et al*. (2012), $DH = \Sigma (Tx-Ti)/60$, where Tmax is the temperature in ºC measured every minute and Ti is the initial temperature (room temperature).

4.3.2. Physico-chemical and chemical properties

The granulometric composition, texture, pH in water and KCl, electrical conductivity, and water retention capacity were characterized according to the classical methods described in Guitián-Ojea and Carballas (1976). Soil water repellency was determined using the ethanol molarity test (MED) (Roy and McGill, 2002). The procedure described by Kemper and Rosenau (1986) was used to study the stability of the aggregates. Total C, total N, C^{13} and N^{15} were measured with an elementary analyser (Carbo Erba CNS 1508) coupled in line with a

mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). The labile fractions of organic matter, soluble carbon and carbohydrates were analysed using a modification of the method described by Ghani *et al*. (2003). The carbon and hydrosoluble carbohydrates were extracted with distilled water at 22 ºC (2 hours) and 80 ºC (16 hours) successively, with soil:water ratio of 1:5 (Haynes, 2000); the amount of carbon and carbohydrates extracted was colorimetrically estimated following the methods of Sims and Haby (1971) and Doutre *et al*. (1978), respectively.

Soil colour was measured with a portable spectrophotometer (Konica Minolta CM-700d) equipped with the software CM-S100w (Spectramagic TM NX) described in Cancelo-González *et al*. 2014 considering the CIELAB colour system. The mean values of Cartesian (L*a*b*) and cylindrical (L*C*ab h_{ab}) coordinates are calculated. The parameters L and h_{ab} are considered as they show significant changes after thermal stress.

4.3.3. Biochemical and microbiological properties

Soil respiration was determined by incubation of fresh soil at 75% field capacity for 10 days at 22 ºC, measuring CO_2 trapped in a NaOH solution titrated with HCl (Díaz Raviña *et al*., 1993). Microbial carbon was estimated by the fumigation-extraction method (Díaz-Raviña *et al*., 1992). After the soil fumigation with $CHCl_3$, during 24 hours, the carbon was extracted from the samples fumigated and not fumigated with K_2SO_4 0.05 M and digested with H_2SO_4, for its later colorimetric determination. The microbial biomass values were calculated applying the formula: C biomass = 2.64 x C extractable, with extractable carbon being the difference between the carbon of fumigated and non-fumigated samples. The specific respiration rate or metabolic quotient (qCO_2) was calculated from the respiration rate and the microbial C (μg CO_2 mg^{-1} Cmic h^{-1}) (Anderson and Domsch, 1990).

The measurement of enzymatic activities characteristic of the cycles of C (β-glucosidase), N (urease) and P (phosphatase) were used as indicators of soil microbial activity. β-glucosidase was estimated according to the method of Eivazi and Tabatabai (1988), which determines the remaining *p*-nitrophenol after an incubation of 2 hours at 37ºC with a solution of *p*-nitrophenyl glucosidase. Urease activity was estimated by incubating the samples with a solution of urea for 2 hours at 37°C; the released NH_4^+ was measured by a colorimetric reaction with indophenol (Kandeler and Gerber, 1988). The phosphatase activity was estimated following the method described by Trasar-Cepeda *et al*. (1985), which determines the remaining p-nitrophenol after 30-minutes incubation at 37°C with *p*-nitrophenyl phosphate. The bacterial activity was estimated using the method of incorporation of leucine radioactively marked with tritium in a bacterial extract obtained from fresh soil after its homogenization-centrifugation (Bååth *et al*., 2001).

The fatty acid profile of phospholipids (PLFA) was performed following the method described by Frostegård *et al*. (1993). In synthesis, the PLFAs were extracted from the soil with a mixture of chloroform, methanol and citrate buffer, separated in the organic phase (chloroform) and fractionated in salicylic acid columns to separate the phospholipids (polar lipids) from the remaining lipids (neutral lipids and glycolipids). Finally, these phospholipids were subjected to methanolysis to obtain methyl esters of the acids, which were quantified by

Gas Chromatography based on their retention times relative to those of internal standards (e.g., 19:0 and 13:0). Total microbial biomass is determined as the sum of all PLFAs. The sum of PLFAs considered to be of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:1ω8, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) is used as the bacterial biomass index and the amount of fatty acids 18:2ω6, 18:1ω9 and 16:1ω5 as the fungal biomass index. PLFAs i14:0, a15:0, i16:0 and 10Me18:0 are found mainly in gram-positive bacteria (G^+) and PLFAs cy17:0, cy19:0, 16:ω7c are characteristic of gram-negative bacteria (G^-). The amount of fatty acids 10Me18:0, 10Me17:0 and 10Me16:0 is used as an indicator of actinobacteria biomass (Díaz-Raviña *et al*., 2006).

The physiological profile of the soil microbial community (CLPP), using different carbon substrates, was carried out to determine the functional diversity of the soil microbial community, using Biolog® plates. A 1:10 mixture of soil and water was agitated in a Waring mixer; 25 ml of the suspension was then taken and centrifuged at 10.000 g. 10 ml of the supernatant was transferred into 250 ml bottles containing 90 ml of milli-Q water. The plates were inoculated with 130 µl of the suspension and incubated at 28°C in darkness. Optical density was measured (at 590 nm) every 24 hours for one week on an automatic plate reader and plate readings were recorded after 72 hours of incubation. The normalized optical density data, corrected with the developed mean colour value (AWCD), were used as the activity index (Garland and Mills, 1991). Microbial richness was expressed as the number of oxidized carbon substrates of the 31 different present in the microplates (Zak *et al*., 1994). The available substrates are: CA, 7 carboxylic acids; AMN, 2 amines/amides; AAC, 6 amino acids; CH, 10 carbohydrates; PHE, 2 phenolic compounds and POL, 4 polymers. The Shannon-Webber diversity index was calculated as $H' = - \Sigma pi \, (\ln pi)$, where pi is the ratio of the activity in each substrate to the sum of the activity in all substrates.

4.3.4. Erosion measurements

Soil erosion was measured using metallic sediment collectors (Soto and Díaz-Fierros, 1998). The sediments were taken to the laboratory and the amount retained in each collector was calculated. In addition, the physicochemical properties of the sediments were measured using the same procedures.

4.3.5. Vegetation monitoring

The percentage of soil covered by vegetation in the experimental area of Laza was estimated at 10 sub-plots of 1 m^2 evenly distributed. Photographs were taken immediately, and 4, 8 and 12 months after the fire. The images were analysed in the laboratory using Photoshop 5.0. In each image was established a rack with 10 x 14 squares of which were recorded those completely covered by mulching of straw and plants. From each plot the average percentage of vegetation cover of the dominant herbaceous and shrub species was calculated.

4.3.6. Study of the seed bank

The composition of the seed bank in Saviñao was estimated using the emergency seed technique (Brown, 1992). Soil samples (0.2 m x 0.25 m wide and 5 cm deep) were collected immediately after mulching so that the soil structure remained intact. Bulbs and roots were

discarded and kept in greenhouses with natural photoperiod and samples moistened every two days. During the study period the relative humidity varied between 60-80% and the temperature between 12ºC and 21ºC. Greenhouse conditions were used to favour seed germination and avoid the effect of environmental variability and animals on seed germination to compare with field data. Vegetation was measured in two 1 m x 1 m sub-plots one year after the fire. All plant species were identified and the number of seedlings of each species was counted. The nativity of the species was defined according to Sanz *et al*. (2004). The similarity of plant composition between greenhouse and field samples was determined by the Sorensen similarity index (Sorensen, 1948).

4.3.7. Statistical analysis

The data were analysed by ANOVA variance analysis to determine the effect of fire, depth and vegetation. In addition, a major component analysis was performed using SPSS 15.0 software with data obtained from PLFA analysis, physical-chemical and biological properties analysed, and substrate utilization data obtained from microplates analysis.

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RESULTS AND DISCUSSION

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5. RESULTS AND DISCUSSION

5.1. FIELD EXPERIMENT

5.1.1. Wildfire effect on the quality of one soil with different vegetation. Laza experiment.

The physicochemical properties analysed are shown in Table 1. Fragas do Eume soil had an acid pH. Values were 3.53-4.09 under *Eucalyptus* vegetation and 3.37-3.91 under *Quercus* vegetation. The moisture (M) and the water retention at field capacity (WR) were elevated in both soils, but significant higher in *Quercus* soil samples (M: 38-58; WR: 765-1635 g water kg^{-1}) than in *Eucalyptus* soil samples (M: 33-45; WR: 674-1026 g water kg^{-1}). Soils under both vegetation types showed an elevated content in organic matter, (Total C and extractable C). Soil under *Eucalyptus* showed total C values about 71-240 g kg^{-1} and soil under *Quercus* about 111-400 g kg^{-1}. Although the total C content was higher in *Quercus* soil samples, the percentage of extractable C was significant higher in *Eucalyptus* soil samples (19%) than *Quercus* soil samples (17%).

Table 1. Minimum and maximum values of physicochemical properties analysed in soil samples from Fragas do Eume Natural Park (n=16, 8 unburned and 8 burned samples). For each parameter, ANOVA3 (V, vegetation; B, burning; D, depth) was performed, but only the proportions of a variance explained by the significant factors (p< 0.05 level) are indicated.

	ANOVA3			*Quercus*		*Eucalyptus*	
	V	B	D	Unburned	Burned	Unburned	Burned
pH_{water}	10 %	49 %		3.37-3.91	3.71-4.50	3.53-4.09	3.88-4.81
pH_{KCl}		38 %	10 %	2.55-3.27	2.93-3.59	2.56-3.35	2.84-3.56
Moisture (%)	31 %	22 %	9 %	38-58	31-49	33-45	24-35
Water retention at field capacity (g water kg^{-1})	31 %	15 %	10 %	765-1635	646-1175	674-1026	461-751
Total C (g kg^{-1})	11 %	29 %		111-400	111-262	71-240	82-255
Electric conductivity (µS cm^{-1})	14 %	17 %		110-180	74-148	70-164	68-130
Al_2O_3 (g kg^{-1})			20 %	5.7-14.5	8.7-15.9	8.7-14.3	7.5-18.2
Fe_2O_3 (g kg^{-1})				21.4-56.6	22.0-59.6	34.1-71.1	19.4-58.8
Extractable C (mg kg^{-1})		68 %		23.0-53.4	12.3-23.1	25.0-42.6	9.0-22.9

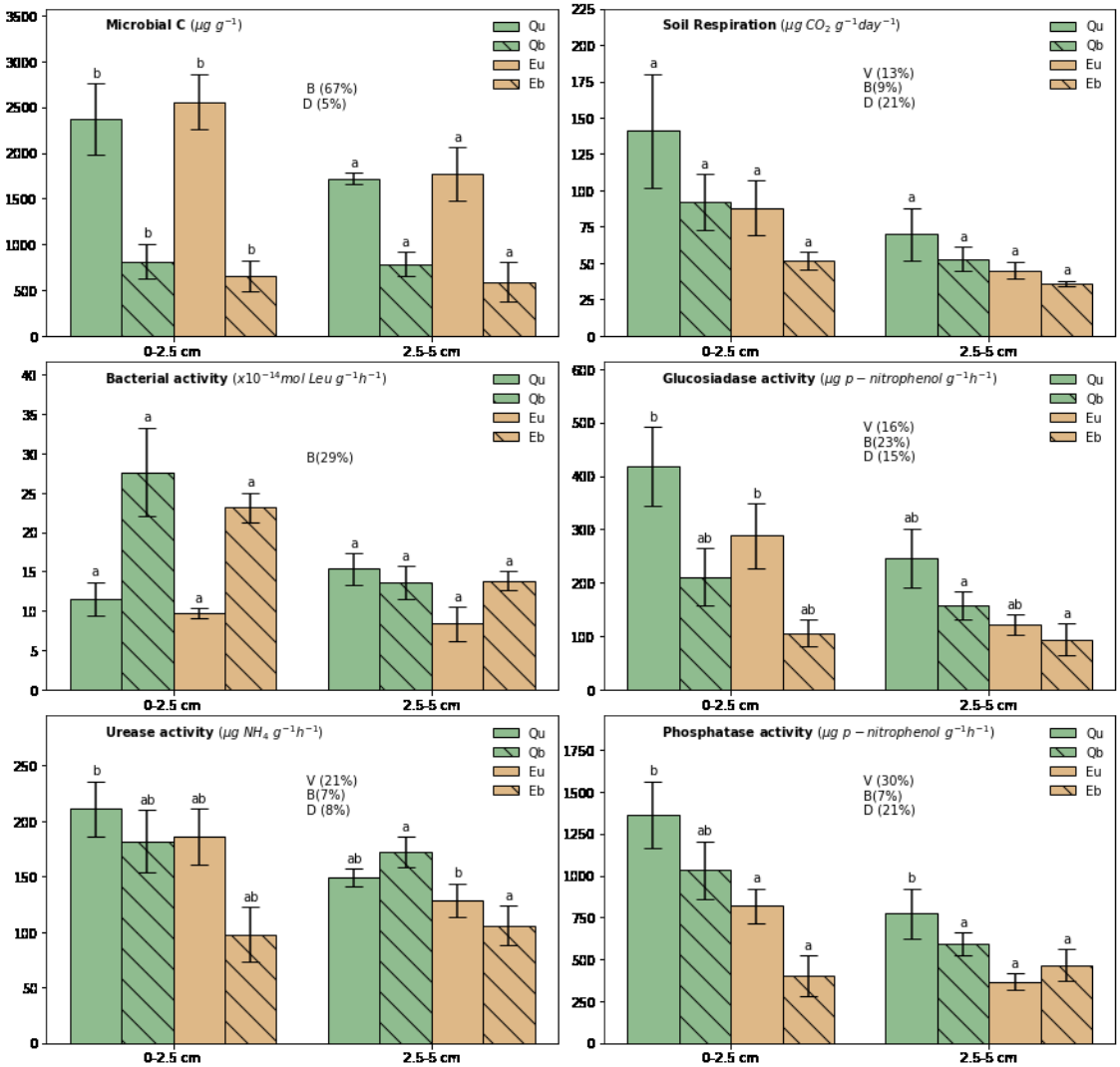

Figure 8: Biochemical properties of the studied soils in the unburned and burned soil samples studied under different vegetation properties at two depths (1, 0-2.5 cm; 2, 2.5-5 cm). (Mean value ±SE; n=4).For each depth, different letters show significant differences (ANOVA1, p<0.05 level). For each parameter ANOVA3 was performed, but only the proportions of a variance explained by the significant factors (p<0.05 level) are indicated.

The ANOVA3 (V, vegetation; B, burning; D, depth) analysis established that pH_{KCl}, moisture, aluminium oxides and water retention at field capacity were significant influenced by depth (10%, 9%, 20% y 9% respectively), Values were higher at 2.5 to 5 cm depth, except the moisture and water retention, that were lower at greater depth. Although pH raised as consequence of fire, the rest of physicochemical properties (total C, electric conductivity, water retention, extractable C) showed lower values than unburned soil samples, and these differences were significant. These results proved the negative effect of wildfires on soil quality. The percentages of explained variance due to fire were 49% for pH, 38% for pH_{KCl}, 22% for moisture, 15% water retention, 17% electric conductivity and 29% for Total C. Wildfire did not affect the iron and aluminium oxides. The vegetation before the fire had a significant influence in moisture and water retention (31% of variance explained by vegetation in both soil properties). However, the rest of physicochemical properties were more influenced by fire than by the previous vegetation of soil samples. The variance

explained by the previous vegetation in Total C, pHw and electric conductivity were 10-14% since extractable carbon and aluminium and iron oxides didn't show differences due to this factor. The values of these soil properties obtained were coincident with those observed in acid soils with high content on organic matter representative of forest soils developed on acid rocks in the Atlantic humid temperate zone of the NW of Spain. These characteristics become these soils in very productive and support the establishment of goods forests (González-Prieto *et al*., 1996; Carballas *et al*., 2009; Martín *et al*., 2012).

Values of biomass and microbial activity were expressed by gram of organic matter (Data not shown). As happened with extractable C, the percentage of biomass C from total C was higher in *Eucalyptus* soil samples (1.39%) than *Quercus* soil samples (0.91%). Soil respiration in soil samples under *Quercus* vegetation was 292±35 gCO_2 g^{-1} OM day^{-1}, while respiration values in *Eucalyptus* samples was lower (268±23 gCO_2 g^{-1} OM day^{-1}). Urease activity in soil under *Quercus* was 494±80 µg NH_4^+ g^{-1} OM h^{-1} and in the soil under *Eucalyptus* was 623±146 µg NH_4^+ g^{-1}OM h^{-1}. The β-glucosidase and phosphatase activity in *Quercus* samples were 940 µg *p*-nitrophenol g^{-1} OM h^{-1} and 3081 µg p-nitrophenol g^{-1} OM h^{-1}, respectively. These values were significantly higher than values obtained in *Eucalyptus* soil samples, especially for phosphatase activity (β-glucosidase: 875 µg *p*-nitrophenol g^{-1} OM h^{-1}; phosphatase: 2.588 µg *p*-nitrophenol g^{-1} OM h^{-1}).

Biomass and activity values were elevated, higher than those reported for arid and semiarid zones (Hernández *et al*., 1997; Bárcenas-Moreno *et al*., 2011). These results were due to the high organic matter content of Galician forest soils (Trasar-Cepeda *et al*., 2000; Basanta *et al*., 2002; Mahía *et al*., 2006; Álvarez *et al*., 2009; Barreiro *et al*., 2015), as those biological parameters are mainly determined by both quantity and quality of the organic matter. This idea is supported by the positive correlation found between organic matter content of soil samples and biochemical properties analysed (Table 2).

Table 2. Correlation between organic matter content of soil samples and biochemical properties analysed. (p<0.001, n=32)

Microbial C	Respiration	Glucosidase	Urease	Phosphatase
0.57	0.91	0.84	0.49	0.89

In order to calculate the ANOVA3 (V, vegetation; B, burning; D, depth) absolute values from biomass and activity were used (Figure 8). Although biomass, respiration and enzymatic activities values were higher in *Quercus* samples than *Eucalyptus* ones, differences due to vegetation only were significant for respiration (13% variance explained) and the enzymatic activities (β-glucosidase, 16% variance explained). The parameters most affected by vegetation were urease and phosphatase activities (21% y 30% of variance explained) which showed more differences due to vegetation than the effect of fire. Regarding depth, except for bacterial activity, biomass values and enzymatic activity values were lower at greater depth. The percentages of explained variance by depth were 5% for biomass C, 21% for respiration, and for enzymatic activities 15%, 8% y 21% respectively. Differences between *Quercus* samples and *Eucalyptus* samples on these biochemical properties could be caused by the higher content on organic matter and moisture in *Quercus* samples or due to the toxic effect of

some chemical components of *Eucalyptus* leaves, which provoke a direct and negative effect on soil microorganisms (Dellacassa *et al*., 1989; Animon *et al*., 1999), or an indirect effect on vegetation (Behera and Sahani, 2003; Alvárez *et al*., 2009).

Biomass C was strongly affected by the wildfire in both types of vegetation. Biomass values in *Quercus* samples were reduced by 45%, while in *Eucalyptus* samples biomass values were reduced by 30%, demonstrating that the biomass C analysis is a good index to detect the fire effect on soil quality (Prieto-Fernández *et al*., 1998; Basanta *et al*., 2004; Villar *et al*., 2004). The decline in biomass values was due to the direct mortality of microorganisms by fire and the indirect effects derived of changes in physicochemical properties, as modifications in the organic matter. The biochemical properties exhibit lower values in the unburnt soil samples than in the corresponding unburnt ones, indicating that fire had a negative effect on the soil quality (Figure 8). The ANOVA3 (V, vegetation; B, burning; D, depth) analysis shown that all the biochemical properties were affected by fire. Microbial biomass was the parameter most influenced by wildfire (67% of explained variance). Microbial activity shown different sensibility to fire; urease, phosphatase and microbial respiration showed lower values of variance explained by fire (7%,7% and 9% respectively), while bacterial activity and β-glucosidase were more affected by fire (29% and 23% variance explained).

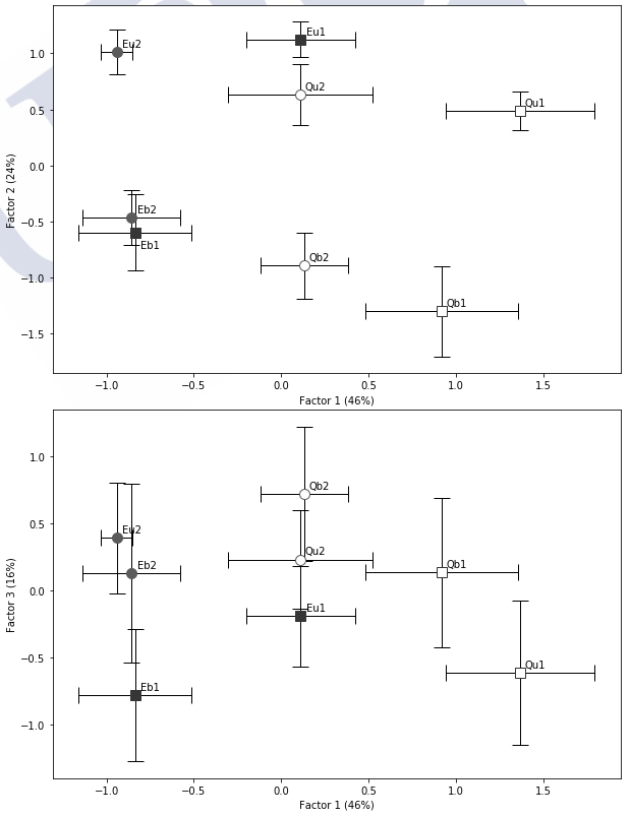

Figure 9. Distribution of the soil samples (mean ± SE; n = 4 field plots) in the plane defined by Factors 1 and 2 (A) and 1 and 3 (B) from principal component analysis performed on the physicochemical, chemical and biochemical properties from the unburned (U) and burned (B) soil samples studied under *Quercus* (Q) and *Eucalyptus* (E) at two depths (1, 0-2.5 cm; 2, 2.5-5 cm).

In order to compare the soil quality in the samples collected in the Fragas do Eume, all the physicochemical and biochemical properties should be used together. Consequently, the principal component analysis was used to analyse the 15 soil variables of the whole data set of the samples analysed (n = 32 samples) (Figure 9). The main three factors identified accounted for 86% of the variance altogether, and allowed to separate samples according the vegetation (Factor 1: 46% of explained variance) and between unburned and burned samples (Factor 2: 24% variance explained). The Factor 3 (16% of variance explained) allowed separating samples according soil depth. The data confirmed that the major factor influencing the soil quality was the type of vegetation, which can be mainly attributed to the influence of tree specie on the C dynamics in the forest ecosystems (tree biomass, forest floor and mineral soil) (Alvárez *et al*., 2009; Martín *et al*., 2011; Pérez-Cruzado *et al*., 2012 Yarwood *et al*., 2015; Bárcenas-Moreno *et al*., 2016).

No significant differences were detected on biomass values estimated by means of phospholipid acid due to the dominant vegetation before the fire or burned, but total biomass and biomass of specific groups showed a slight decrease as consequence of fire (Table 3). Therefore, biomass estimations by means of phospholipids fatty acids are not adequate to determine the short-term fire impact on these forest ecosystems. Bárcenas-Moreno and Bååth (2009) and Bárcenas-Moreno *et al*. (2011) have also detected problems in using biomass estimates by means of phospholipid fatty acid analysis to evaluate the effects of wildfire. This is due to their lower sensitivity to temperature comparing with other microbial indices (heating only destroys the PLFAs at very high temperatures), as well as the presence of confounding factors (a decrease in PLFAs due to the death and degradation of organisms and an increase in PLFAs resulting from the emergence of a new community growing under post-fire conditions). Nevertheless, biomass was affected by depth, showing samples collected at greater depth biomass values higher that superficial soil samples. Except for fungi, which were not affected by depth, the variance explained was from 13% to 19% in the different groups of microorganisms (Table 3).

Table 3. Minimum and maximum values of total biomass and biomass of specific groups (nmol g^{-1}) estimated by means of phospholipid fatty acid analysis in soil samples from Fragas do Eume Natural Park (n=16, 8 unburned and 8 burned samples). For each parameter (mean value) ANOVA3 (V, vegetation; B, burning; D, depth) was performed, but only the proportions of a variance explained by the significant factors (p< 0.05 level) are indicated.

	ANOVA3	*Quercus*		*Eucalyptus*	
		Unburned	**Burned**	**Unburned**	**Burned**
Total PLFA	D (15%)	285-832	306-688	405-673	319-777
Fungal PLFA		50-122	42-108	48-97	39-98
Bacterial PLFA	D (17%)	115-402	148-298	175-287	133-390
Actinomycetes PLFA	D (19%)	39-76	31-67	41-84	37-78
Gram-negative PLFA	D (13%)	60-258	95-200	102-165	78-265
Gram-positive PLFA	D (19%)	39-76	31-67	36-65	30-69

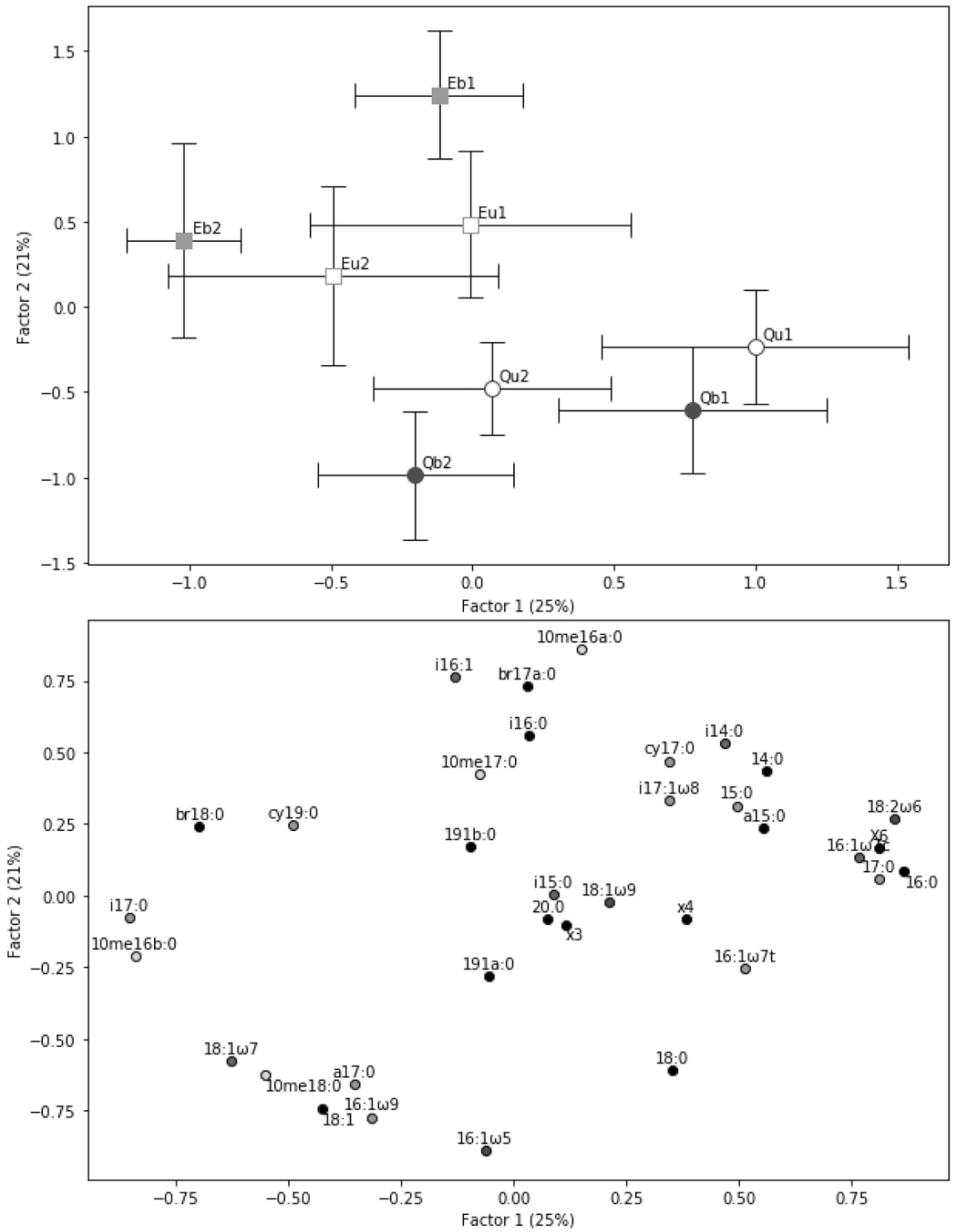

Figure 10. Score (mean ± SE; n = 4 field plots) and loading plots from principal components analysis performed on the PLFAs of the unburned (u) and burned (b) soil samples studied under Quercus (Q) and Eucalyptus (E) at two depths (1, 0-2.5 cm; 2, 2.5-5 cm). (mean ± SE; n = 4 field plots).

The principal component analysis performed with the data from phospholipid fatty acid analysis (Figure 10) showed that Factor 1 and Factor 2 accounted the 46 % of the variance and allowed us to differentiate among microbial communities according to tree species, soil depth and burning. Factor 1, explaining 25% of the variance, was defined on its positive part by the PLFA 18:2ω6, 16:1ω7c, 16:1ω7t, 16:0, 17:0, a15:0 and x6, and on its negative part 10Me16b:0, i17:0, br18:0, cy19:0, 18:ω7 and 10Me 18:0. F2, explaining 21% of the variance,

was defined by the PLFAs 10Me16a:0, i16:1, br17:0 and i16:0 at its positive part and by 16:1ω5, 16:1ω9, 18:1, 18:0, a17:0, 18:1ω7 and 10Me18:0 at its negative part.

Soil samples under the same vegetation are grouped together and separated according to the soil depth. The *Quercus* soil samples (having positive values along Factor 1 and negative values in Factor 2) were mainly characterized by high concentration of PLFA indicative of fungi (18:1ω6 and 16:1ω5) and Gram- bacteria (16:1ω7, 16:1ω9 and 18:1ω7). The *Eucalyptus* soil samples (having negative values in Factor 1 and positive values en Factor 2) had relatively high concentrations of PLFA characteristic of actinobacteria (10Me16:0, 10Me17:0 and 10Me18:0) and PLFAs indicative of Gram positive bacteria (br17:0, i16:1, i16:0 and i17:0). For the same vegetation, higher values along Factor 1 were exhibited by the 0-2.5 cm layer samples with respect to the 2.5-5 cm layer; and by the unburned soil samples compared with the burned samples.

The results showed that the relative importance of the three factors considered on the PLFA pattern was as follows: soil vegetation < soil depth = soil burning. The results of the biochemical properties indicating that the type of vegetation before fire is one of the most important factors for determining variance of the parameters related with the microbial activity (13–30% of the variance explained) seem to support these data (Figure 10). This is also consistent with earlier investigations showing that the microbial component can vary greatly depending on the type of vegetation (Grayston *et al*., 1998; Priha *et al*., 2001; Mahía *et al*., 2006; Alvárez *et al*., 2009) and soil depth (Fritze *et al*., 2000; Fierer *et al*., 2003; Mahía *et al*., 2007; Matinizadeh *et al*., 2008; Yang *et al*., 2010; González-Prieto *et al*., 2013). Likewise, other studies also showed immediate or short-term fire induced changes in microbial community structure were attributed mainly to variations in the soil environment, particularly soil pH and C and nutrient availability, following soil heating, prescribed fires or wildfires (Bååth *et al*., 1995; Barreiro *et al*., 2010; Díaz-Raviña *et al*., 2006; Bárcenas-Moreno e*t al*., 2011).

The data of PCA along with values of soil properties analysed indicated that soil properties mainly depend on the type of vegetation and its effect on soil quality is so important that persist after an impact as a wildfire, masking the consequences of burned on soil properties. Soil developed under *Quercus* vegetation showed a higher quality than those developed under *Eucalyptus*, and the microbial community was different between them. This is coincident with results obtained in previous studies where a same soil developed under *Quercus* showed higher values of biomass and enzymatic activities that the soil developed under *Eucalyptus* or grassland (Lombao, 2011).

The dominant vegetation is the key for the development of soils, and the different plant residues (Hart *et al*., 2005) and root exudates (Grayston *et al*., 1998) influence properties as C, P, N and pH. The role of vegetation and the leaf litter as source of C in soils determine the quantity and quality of the soil organic matter and determine the biomass, activity and structure of soil microbial communities (Wardle, 1992; Grayston *et al*., 1998; Garbeva *et al*., 2004).

5.1.2. Effects o ffire and post-fire treatmets (mulching and seeding) on soil quality

Values obtained in Laza soil for the physico-chemical properties analysed before the wildfire are showed in Table 4.

Table 4. Mean values ±SE of selected physicochemical properties of the studied soil at different sample times (1 week and 4,8, 12 and 48 month after the fire). Treatments: U, unburned soil; B, burned soil; B+S, burned soil with seeding treatment; B+M, burned soil with mulching treatment. For each variable and sampling time different letters denotes significant differences (p<0.005) among treatments (ANOVA1)

	Time	U	B	B+S	B+M
	1w	18±1 a	28±1 b	28±2 b	28±0 b
	8m	25±3 a	30±2 a	29±5 a	32±1 a
Sand (%)	12m	24±5 a	30±2 a	31±3 a	31±6 a
	1w	61±1 b	55±1 a	55±1 a	55±0 a
	8m	56±3 a	53±3 a	54±5 a	51±3 a
Silt (%)	12m	57±1 a	54±1 a	53±3 a	52±5 a
	1w	21±2 b	17±1 a	17±2 a	17±1 a
	8m	20±0 a	17±1 a	17±1 a	17±2 a
Clay (%)	12m	20±2 b	17±1 a	16±1 a	17±1 a
	1w	23±1 b	9±2 a	8±2 a	9±1 a
	4m	45±1 b	32±1 a	34±4 a	32±2 a
	8m	35±2 a	29±2 a	31±6 a	31±1 a
	12m	26±2 b	13±3 a	14±4 a	19±3 ab
	48m	40±1 b	15±1 a	19±4 a	17±3 a
Moisture (%)	48m (2-5 cm)	36±2 b	19±1 a	22±3 a	20±2 a
	1w	94±2 a	95±2 a	96±1 a	91±1 a
	4m	93±2 a	91±3 a	94±1 a	91±2 a
	8m	96±1 b	84±8 a	87±2 ab	92±1 ab
Aggregate stability (%)	12m	96±2 a	90±4 a	89±3 a	90±7 a
	1w	Very severe	Very severe	Very severe	Very severe
	4m	Very severe	Severe	Severe	Severe
	8m	Very severe	Severe	Severe	Severe
Water repellence	12m	Very severe	Severe	Severe	Very severe
	1w	899±53 b	603±15 a	577±73 a	590±44 a
	4m	924±32 b	612±9 a	679±81 a	623±43 a
	8m	872±29 b	537±28 a	565±49 a	579±65 a
	12m	770±74 b	501±33 a	460±83 a	488±45 a
	48m	1035±13 c	530±26 a	622±65 ab	687±60 c
Water Retention (g kg^{-1})	48m (2-5 cm)	958±39 c	636±19 a	698±34 ab	699±64 c
	1w	3.7±0.1 a	4.2±0.0 b	4.1±0.0 b	4.1±0.0 b
	4m	3.9±0.0 a	4.5±0.0 b	4.5±0.0 b	4.6±0.0 b
	8m	3.4±0.1 a	4.2±0.2 b	4.2±0.1 b	4.0±0.1 b
	12m	3.7±0.0 a	4.1±0.1 b	4.0±0.1 b	4.4±0.0 b
	48m	3.6±0.0 a	4.3±0.0 bc	4.1±0.0 b	4.0±0.0 c
pH_{water}	48m (2-5 cm)	3.5±0.0 a	4.0±0.0 b	3.9±0.0 b	4.0±0.0 b
	1w	2.8±0.0 a	3.2±0.0 b	3.2±0.0 b	3.2±0.0 b
	4m	2.8±0.0 a	3.1±0.0 b	3.1±0.0 b	3.1±0.0 b
	8m	2.5±0.0 a	2.9±0.1 b	2.9±0.1 b	2.9±0.1 b
	12m	2.6±0.0 a	2.9±0.0 b	2.9±0.0 b	2.9±0.0 b
	48m	2.3±0.0 a	2.8±0.0 b	2.8±0.0 b	2.6±0.0 b
pH_{KCl}	48m (2-5 cm)	2.4±0.0 a	2.6±0.0 a	2.5±0.0 a	2.6±0.0 a
	1w	16±1 a	102±10 b	102±12 b	102±9 b
	4m	30±5 a	28±3 a	29±0 a	38±7 a
	8m	51±5 b	27±1 a	25±4 a	26±2 a
	12m	23±2 a	25±3 a	30±1 a	30±5 a
	48m	74±2 b	22±2 a	31±8 a	26±2 a
Electric conductivity (µS cm^{-1})	48m (2-5 cm)	82±4 b	29±2 a	35±1 a	27±5 a
	1w	11.6±1.8 b	8.3±0.9 a	8.8±0.9 a	8.9±0.4 a
	4m	11.7±1.5 b	8.3±0.5 a	8.9±0.9 a	9.8±1.9 a
	8m	12±1 b	8±1 a	9±1 a	9±1 a
	12m	10±1 b	8±1 a	7±1 a	8±1 a
	48m	11±0 b	6±0 a	7±1 a	8±0 a
Total N (g kg^{-1})	48m (2-5 cm)	8±0 b	5±0 a	7±0 ab	6±0 a
	1w	218±8 b	154±7 a	155±12 a	155±21 a
	4m	233±15 b	147±4 a	158±15 a	162±24 a
	8m	239±11 b	143±12 a	154±13 a	157±20 a
	12m	216±24 b	140±12 a	132±26 a	139±20 a
	48m	266±4 b	121±13 a	154±2 a	171±1 a
Total C (g kg^{-1})	48m (2-5 cm)	195±17 b	116±9 a	129±2 a	136±2 a

Values were characteristic of shrubland Galician soil developed over acid and metamorphic rocks (Leirós *et al*., 2000; Martín *et al*., 2011; Varela *et al*., 2010). Laza soil was acid (3.80), with elevated content of organic matter (218±11 g C kg^{-1} and 11.6±1.8 g N kg^{-1}) and low electrical conductivity (23±10 µScm^{-1}). Soil properties related with the organic matter as water retention at field capacity (911±18 g water kg^{-1} soil), aggregate stability (93±1%) and water repellency (very severe) were high (Table 4).

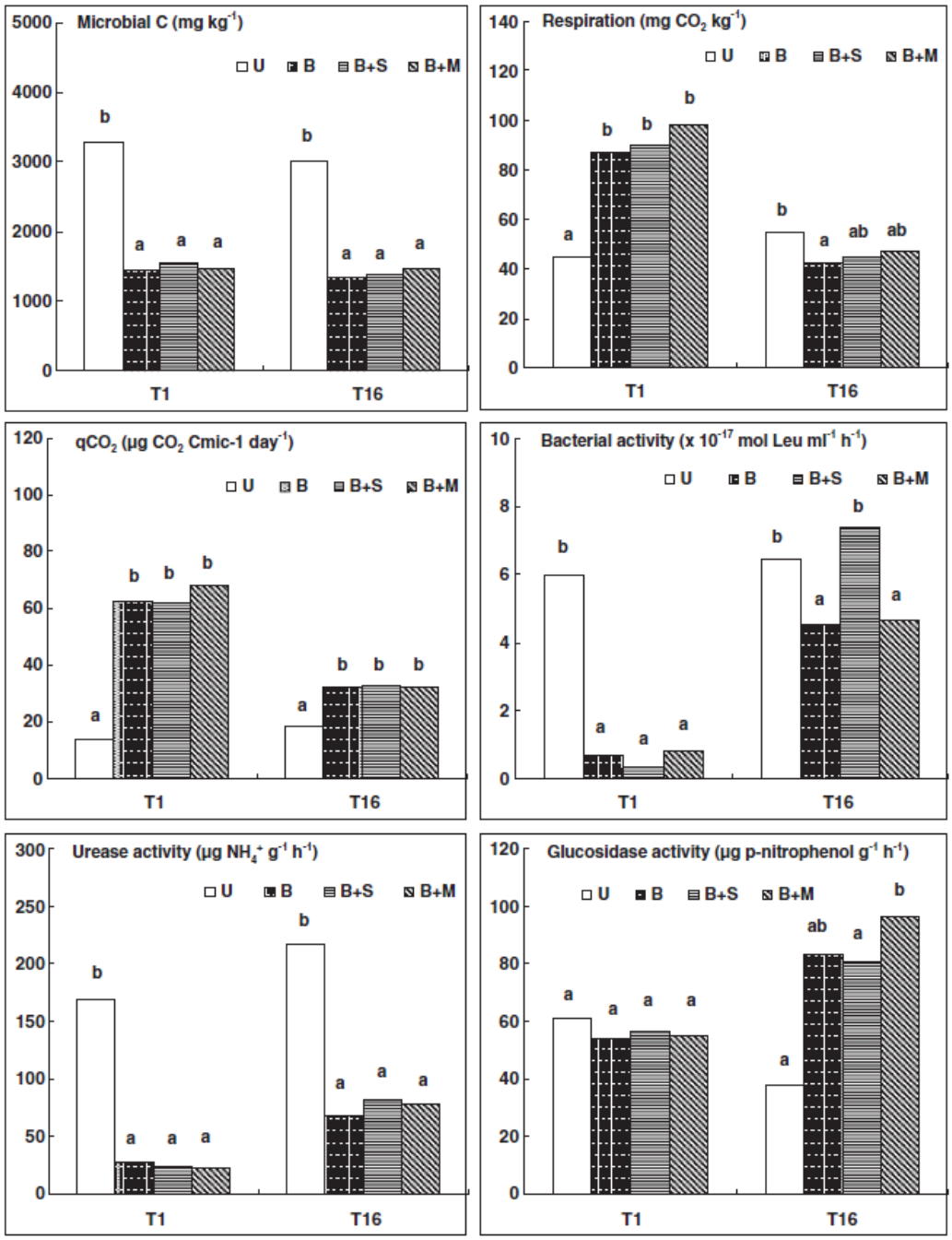

Figure 11. Soil biochemical properties in the different soil treatments 1 and 16 weeks after the wildfire (mean values of three field replicates). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw addition. For the same sampling time different letters denote significant differences (P<0.05) among treatments (ANOVA1).

Biomass C values in the unburned samples were 3146±213 mg kg^{-1} and microbial activity values were 50.2±6.92 mg kg^{-1} for respiration, 6.2±0.34x10^{-17} mol Leu h^{-1} for bacterial activity, 49.45±16.6 µg *p*-nitrophenol $g^{-1}h^{-1}$ for β-glucosidase and 193.25±34 µg NH_4^+ g^{-1} h^{-1} for urease activity (data not showed). The total biomass estimated by PLFAs (Table 5) was 358 nmol kg^{-1} soil and the biomass of specific groups were 84, 127, 71 y 33 nmol g^{-1} soil for fungi, bacteria and G^- and G^+ bacteria respectively. These values are coincident with ranges obtained by other authors in the same region (Díaz-Raviña *et al*., 1996; 2006; Basanta *et al*., 2002; Mahía *et al*., 2006; Barreiro *et al*., 2010; 2015).

After a wildfire, most properties suffered a significant shift. Regarding psychochemical properties (Table 4) fire caused important changes in the size particle distribution. The sand content increased and the lime and clay content decreased, indicating that fire was of high severity and temperatures reached were elevated, so changes in granulometry are produced above 400ºC (Giovannini *et al*., 1990; Ulery and Graham, 1993). Fire produced a decrease of soil organic matter (organic C and N). Nevertheless, no significant changes were produced in aggregate stability or water repellency. These results can be explained by the magnitude order of the values obtained for the unburned soils (aggregate stability 90%, very severe water repellency). Earlier studies performed with soils from the same region showed that fire induces repellency in soils that are wettable, increases repellency in soils that are slightly to moderately repellent and leaves it basically unaltered in soils that are strongly or very strongly repellent (Varela *et al*., 2005; 2010), as happened in Laza soil. Wildfire also produced a rise in pH water and pH KCl values and in the electric conductivity.

The biochemical properties were also affected by wildfire (Figure 11). Wildfire produced a positive effect on microbial respiration which raised by 50% after the fire (Figure 11). Likewise, in the burned samples the metabolic quotient (qCO_2) was around 4 times higher than that in the reference site; this increase in qCO_2 can be considered as indicative of a detrimental effect of the fire since the microbes are force to utilize a large part of their energy budget for their maintenance to support the stress provoked (Anderson and Domsch, 1990). Biomass, bacterial activity and urease activity suffered a marked decrease, while no changes were detected on β-glucosidase activity due to fire.

Table 5. Total biomass and biomass of specific groups (nmol g^{-1}) estimated by means of phospholipid fatty acid after the wildfire (mean values±SD). Treatments: U, unburned soil; B, burned soil; B+S, Burned soil with seeding; B+M, Burned soil with straw. For the same variable, different letters denote significant differences (p<0.05) among treatments (ANOVA1).

	U	B	B+S	B+M
Total PLFA	358±26 b	245±14 a	256±46 a	223±27 a
Fungal PLFA	84.2±7 b	49.7±3 a	50.7±11 a	44.7±5 a
Bacterial PLFA	123±9 b	87.1±5 a	87.6±16 a	76±9 a
Gram- PLFA	70.6±5 b	44.9±3 a	45.3±8 a	40.5±4 a
Gram+ PLFA	33.4±2 b	25.2±2 a	26±5 a	22.4±3 a
FungPLFA/ BactPLFA	0.68±0.01 b	0.57±0.01 a	0.57±0.02 a	0.59±0.01 a
Gram-/Gram+	2.11±0.05 b	1.8±0.01 a	1.74±0.09 a	1.83±0.08 a

The total biomass and the biomass of specific groups estimated by PLFA data decreased after a fire. Fungi/bacteria and G^{-}/G^{+} ratio were 0.68 and 2.11 in unburned samples and 0.57 and 1.8 in burned samples, proving that fungi and G^{-} bacteria showed a high sensibility at fire or they cannot proliferate in post-fire conditions (Carballas *et al*., 2009; Bárcenas-Moreno *et al*., 2011; Holden *et al*., 2013; Barreiro *et al*., 2015). These results agree with studies performed in the same area about the effect of medium and high severity fire (Villar *et al*., 2004; Carballas *et al*., 2009; Martín *et al*., 2009; Vega *et al*., 2013; Barreiro *et al*., 2015). Four months after the wildfire, values obtained from burned samples were different than values from burned ones, demonstrating that the negative effect remained at least at short term. The negative effect on biomass, urease and bacterial activity remained but was attenuated with time. Nevertheless, the positive effect detected immediately after the fire on soil respiration disappeared and a positive effect appeared on β-glucosidase activity. The results of the experiment indicated that the different soil properties analysed showed a different sensibility to fire. Some of them were not affected while others were drastically modified as a consequence of fire.

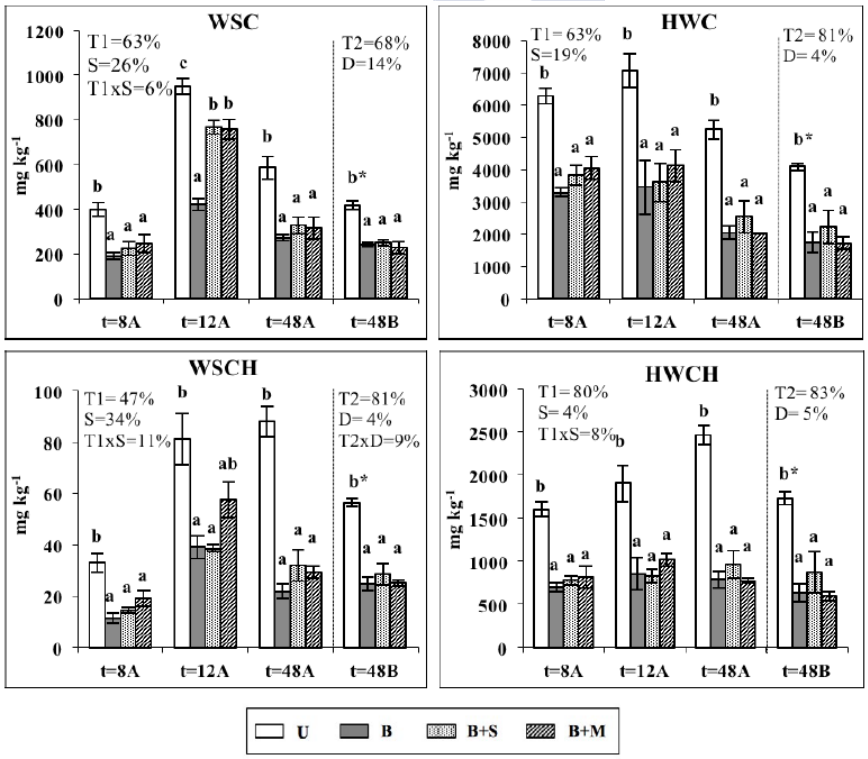

Figure 12. Organic matter labile fractions [(water soluble and extractable carbon (WSC and HWC) and carbohydrates (WSCH and HWCH)] in the different soil treatments 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values ± SE). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA 2 (T2, treatment; D, depth; T2xD, interaction treatment x depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p < 0.05$) between different depths.

In order to analyse the effect of fire and sampling time on soil quality at medium term, samples were collected in Laza 8, 12, and 48 months after the wildfire.

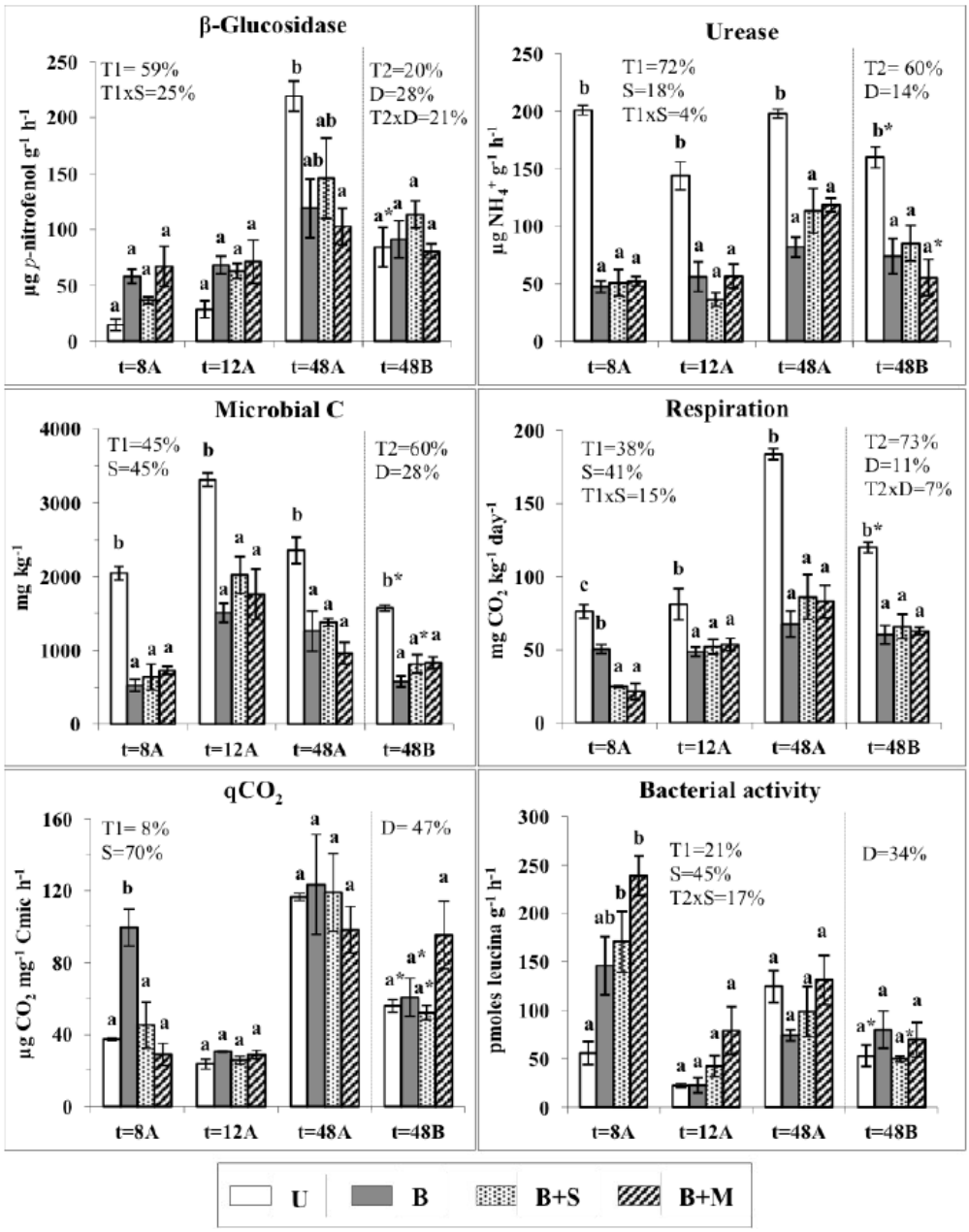

Figure 13. Soil biochemical properties in the different soil treatments 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Percentages of variance explained by significant factors (p < 0.05 level) for samples collected at 0-2 cm depth, according to an ANOVA2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors (p < 0.05 level) for samples collected 48 months after the fire, at two depths, 0-2 cm (A) and 2-5 cm (B) according to an ANOVA2 (T2, treatment; D, depth; T2xD, interaction treatment x depth). For the same sampling time different lowercase letters denote significant differences (p < 0.05) among treatments. For samples collected 48 months after the fire, * denotes significant differences (p < 0.05) between different depths.

The results obtained showing that changes induced by fire persist 4 years after, except for

texture, which tended to recuperate to the unburned values. The fire decreased moisture, water retention, electrical conductivity while C and N content was not affected. The rise of pH values in 0.5 units lasted (Table 4). The labile fraction of the carbon pool were reduced drastically (50%) in burned soils (Figure 12), due to changes in vegetation and microbial activity (Nannipieri *et al*., 2003; Certini *et al*., 2005) and erosive process (Gómez-Rey *et al*., 2013). The different soil treatments (unburned, burned, burned soil plus mulching and burned soil plus seeding) explained between 47-80% of variance and sample time between 4-34%. The values obtained before the wildfire did not recover after 4 years, as happened on previous studies in forest soils affected by fire of different severity with cellulosic and non-cellulosic carbohydrates (Martín *et al*., 2009) and microbial C and extractable C and N (Prieto-Fernández *et al*., 1998).

Biochemical properties remained affected by fire after four years (Figure 13). As happened at short term (4 months after fire) each soil property showed a different behavior. Biomass, urease activity and respiration did not recover, while bacterial activity and qCO_2 did it. As it was said before, this behavior could be due to the different information obtained from each soil parameter and their different sensibility to fire impact (Certini, 2005; Mataix-Solera *et al*., 2009; Díaz-Raviña *et al.*, 2010). Soil treatments (unburned, burned, burned soil plus mulching and burned soil plus seeding) explained between 8-72% of variance while sampling time explained between 18-70% of variance. Properties most affected by fire according the ANOVA2 were urease (72%), β-glucosidase (59%), and microbial C (45%), whereas qCO_2 (70%), microbial C (45%) and respiration (41%) were influence by sampling time.

Regarding the total biomass and biomass of specifics groups estimated by means of PLFA, the reduction produced by fire remained (Figure 14), while the negative effect on fungal/bacterial and G^{-}/G^{+} index disappeared (Figure 15). Burned soil samples collected 48 months after a fire showed high values of fungal biomass, indicating that those microorganisms play an important role in soil recovery after a wildfire. The importance of fungi can be explained because they contribute more than bacteria to the microbial biomass, increase C sequestration (Six *et al*., 2006) and soil aggregation (Helfricht *et al*., 2015), which will be specially important after a high severity wildfire when a reduction of C and a loss of structure occurs. In general, a significant effect of the sampling time was also observed, associated to vegetation and climatic conditions (Díaz-Raviña *et al*., 1993; 1995). These changes were of higher magnitude than changes due to fire, as indicate by the percentages of variance explain by ANOVA2 (T1, treatments; D, depth) (sampling time: 9-94% of variance explained, soil treatments: 3-69% of variance explained).

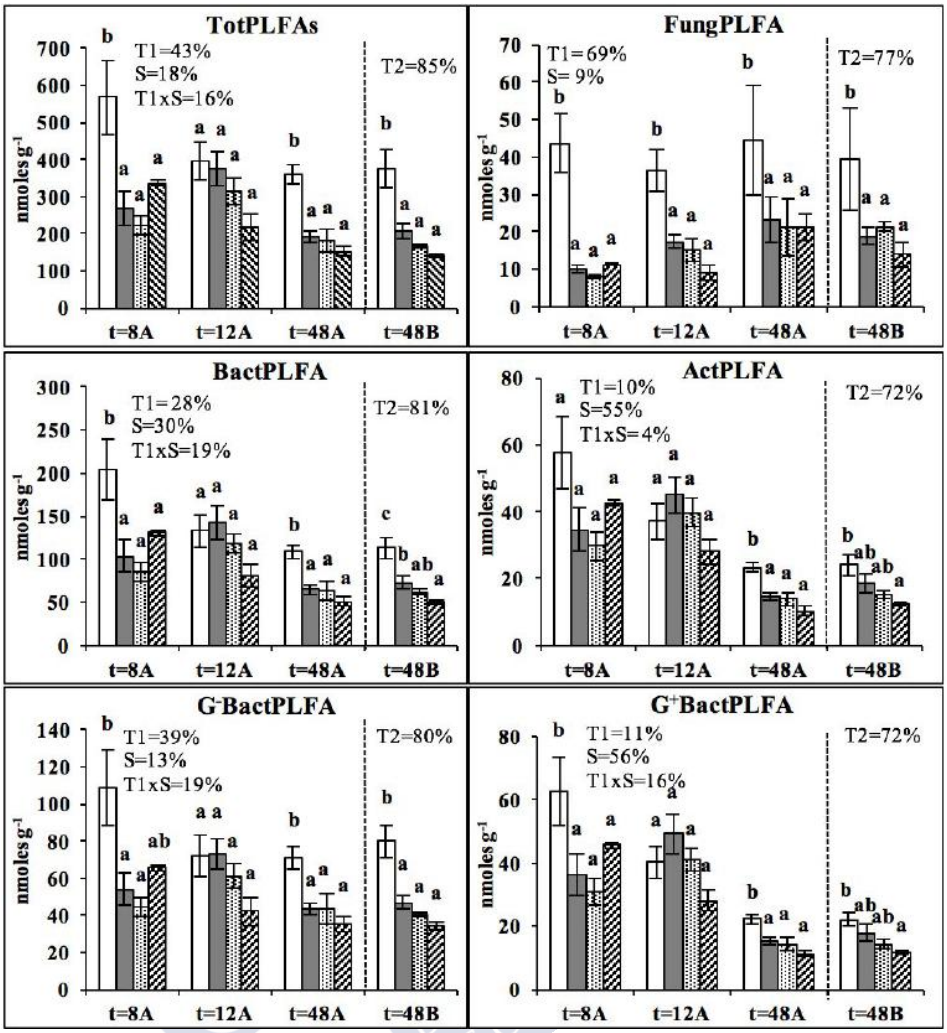

Figure 14. Total microbial biomass (TotPLFAs) and biomass of the specific groups: fungi (FungPLFA), bacteria (BactPLFA), actinobacteria (ActPLFA), G^+ bacteria (BactG^+PLFA), G− bacteria (BactG^-PLFA) of the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA2 (T2, treatment; D, depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p > 0.05$) between different depths.

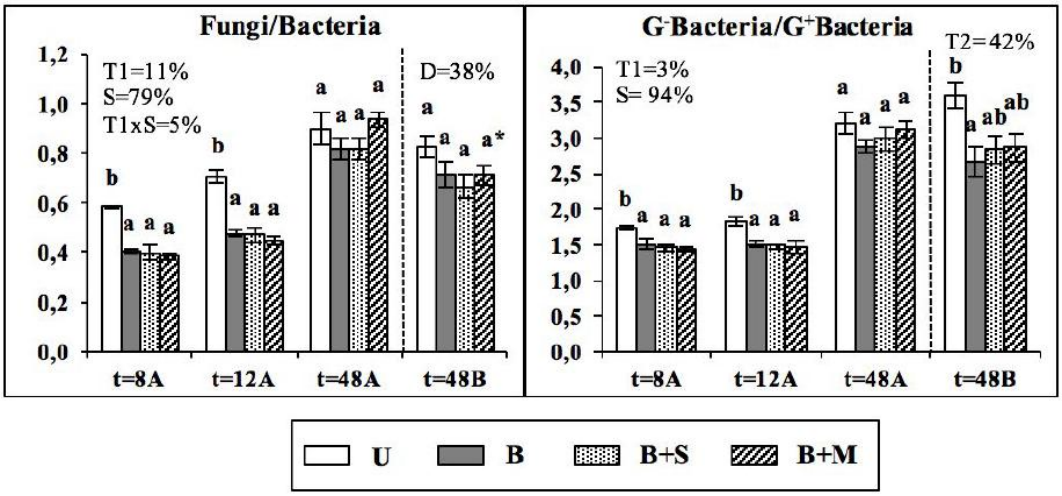

Figure 15. Fungi/Bacteria and G^- bacteria/ G^+ bacteria ratios of the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA2 (T2, treatment; D, depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p > 0.05$) between different depths.

The results of PLFA pattern analysis (Figure 16) showed that the time elapsed after the fire was the main factor determining the composition of the microbial communities at medium term (factor 1: 45% variance explained). Factor 2, that explained 18% of variance, was determined by burning and separated the unburned from burned samples. Factor 1 in its negative part was characterized by high concentration of PLFA of fungi 18:2ω6, 18:1ω9, and its part positive was determined by PLFA from actinobacteria 10Me18:0, 10Me:17:0. F2 in its parte negative was determined for saturated PLFA as 18:0. 16:0 y 17:0. These results confirm that bacteria and actinobacteria are favored by post-fire conditions (Pietikainen and Fritze, 1995; Bárcenas-Moreno and Bååth, 2009; Carballas *et al*., 2009; Bárcenas-Moreno *et al*., 2011) and bacteria recovered faster than other microbial groups after a fire (Ponder *et al*., 2009). Furtermore, they are coincident with data obtained as estimation of biomass by PLFA and fungi/bacteria and G^-/G^+ bacteria ratios. For each sample time, fire was the main factor determining the composition of microbial communities, explained 30%, 51%, and 37% of variance in 8, 12 and 48 month respectively. Previous studies showed a clear effect in the microbial community structure as consequence of prescribed fire and wildfire (Díaz-Raviña *et al*., 2006; Barreiro *et al*., 2010; 2016b).

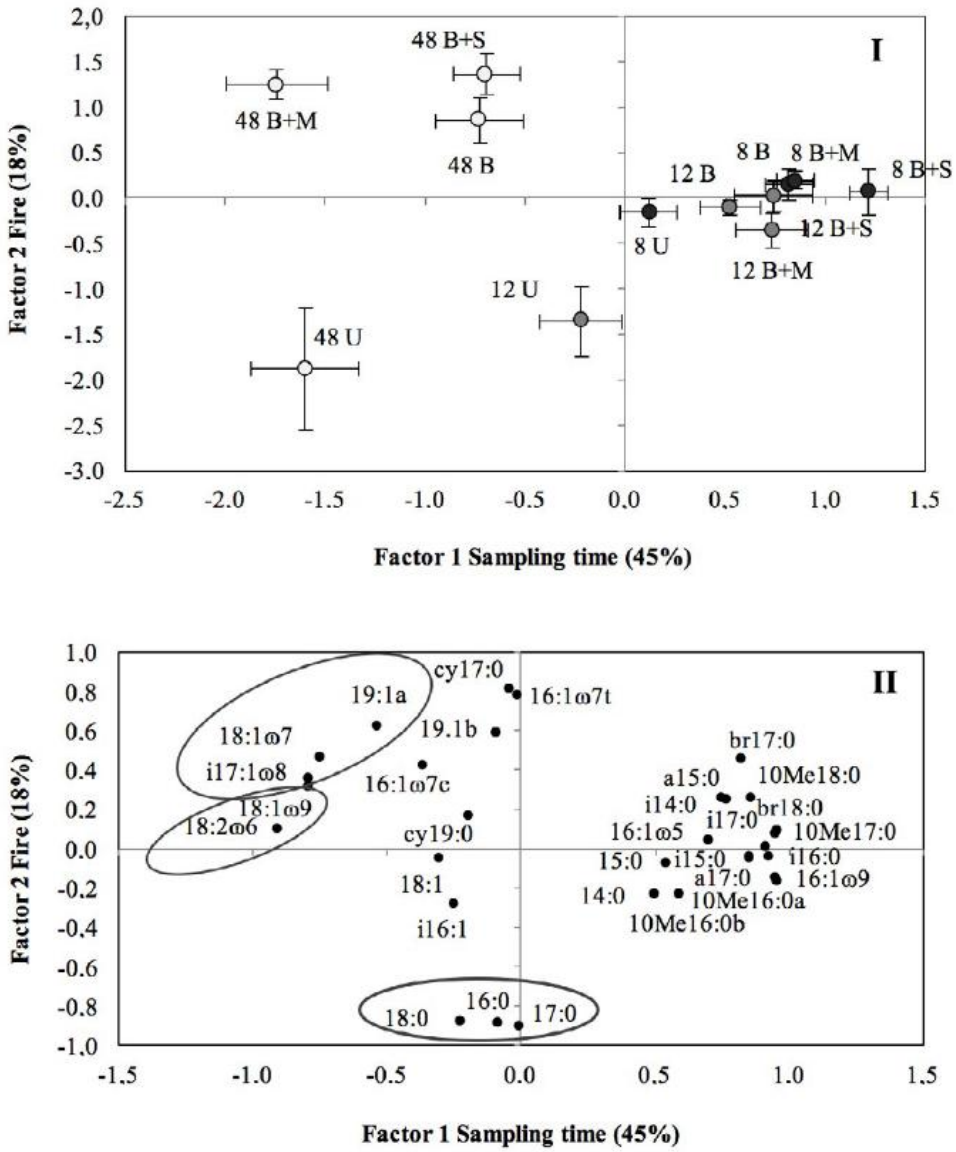

Figure 16. Results of the PCA performed for the whole PLFAs data set of the different soils collected 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques. Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Scores ± SE of three field replicates (I) and loading plots (II).

In order to evaluate the effect of fire and the soil recovery on depth, samples form 2-5 cm were collected at 48 m sample time. The negative effect caused by fire on soil properties were also detected at 2-5 cm depth, except for β-glucosidase, bacterial activity and qCO_2 which no differences were detected as consequence of the different treatments. In general, values of pshysicochemical and biochemical properties were lower at 2-5 cm depth. Soil properties most affected by depth were the water soluble carbon (14%), Microbial C (28%), qCO_2 (47%) and bacterial activity (34%). Regarding the total biomass and the biomass of specifics groups (PLFA), values at 2-5 cm depth were quite similar than those at surface. Nevertheless, the fungal/bacterial index was strongly affected by depth, as indicated a lower proportion of fungi to bacteria at higher depth.

The combined interpretation of chemical and biochemical properties showed that burned soils need more than 4 years to recovery after a wildfire. Data are coincident with previous studies that demonstrated that the recovery of soil microorganisms affected by wildfire (Prieto-Fernández *et al*., 1998) and experimental fire (Fritze *et al*., 1993) did not occur until 10-13 years after the fire.

5.1.3. Effectiveness of post-fire treatments (straw mulching and seeding) and effects on soil quality and cover regeneration. Laza experiment.

No significant differences were found among the burned plots assigned to the different burned treatments for any of the variables studied in all sampling times. This demonstrated that the experimental study area had an acceptable spatial homogeneity, and changes detected between samples were due to the different treatments instead of the terrestrial heterogeneity. The analysis of sediments in soils with different treatments allows evaluating the effectiveness of post-fire stabilization treatments used to control soil erosion. Rainfall in those 4 months (September 2010-January 2011) was 745 mm, a value similar to the average for that area, and the erosion factor was 170 MJ cm ha^{-1} h^{-1}. According to precipitation data, high erosion values were expected in September and low values in October. During the first four months, sediments were collected at three different sample times (Figure 17). Soil losses were much higher in burned soils (Q) than in those plots treated with seeding (128 g m^{-2}) or mulching (22 g m^{-2}).

The total sediments collected in the burned plots were 204 g m^{-2} with yields of 5%, 23% and 72% in the first, second and third sample time respectively. The data indicated that mulching proved to be the most effective treatment against erosion in the short term as it reduced by 73-94% soil sediment production, while seeding reduced sediment production by about 34- 42%. Although the sediment values obtained are within the range that other authors found for some burned soils (Díaz-Fierros *et al*., 1987; Vázquez *et al*., 1993; Fernández *et al*., 2011), these were much lower than expected considering the slope, the high erosivity of rainfall as well as soil characteristic such as severe water repellency. In addition, erosive processes took place later than expected according to rainfall data. This is probably due to the fact that in the first period water infiltration occurred in the soil without reaching the maximum water retention capacity due to the high value of organic matter (226 g C kg^{-1}), high aggregate stability (93%) and extremely high water retention capacity (600 g water kg^{-1}).

During the second period of sediment sampling, soil saturation was rapidly reached, resulting in erosion 4 times greater than that registered in the first period. Finally, in the third period, where the strongest rainfall events occurred, erosion was very high, accumulating more than 70% of the total of sediment production. The sediment values obtained showed a correlation with surface water runoff and not with total rainfall, indicating that the main mechanism that causes runoff is saturation excess overland flow (Dunne *et al*., 1975) instead of the infiltration excess overland flow. Other authors have already demonstrated that saturation excess overland flow (Hortonian flow) is more common in Galicia (Vega *et al*., 2012), while infiltration excess overland flow is more common in semi-arid and arid zones

(Dunne and Leopol, 1978). The physicochemical properties of the sediments did not differ from those found in soil samples, suggesting that their origin was the top layer of the soil.

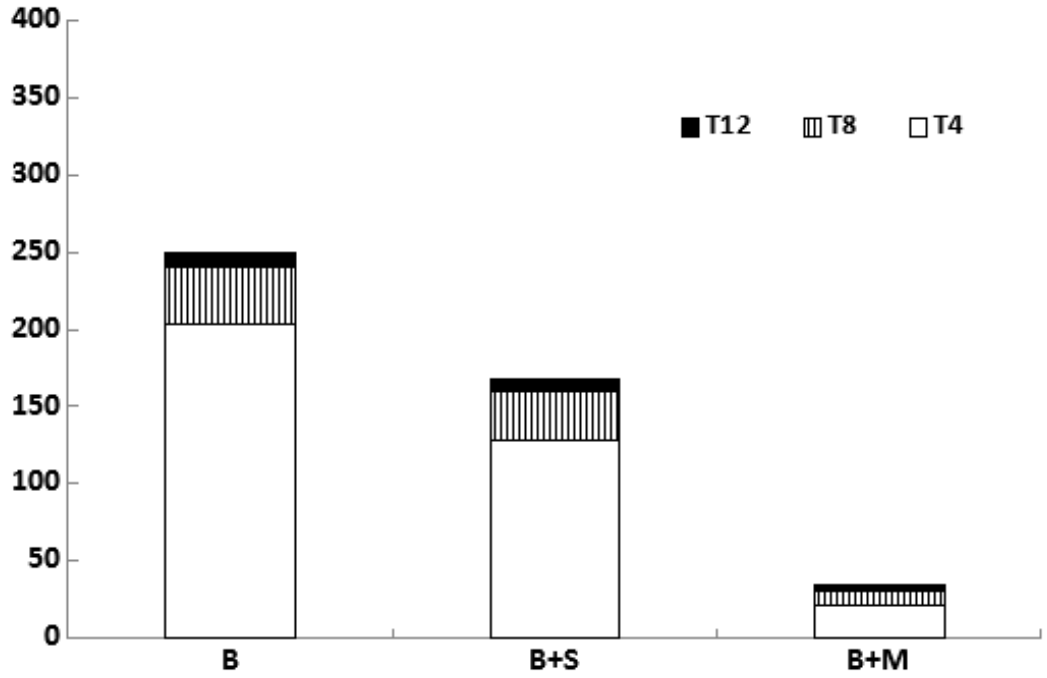

Figure 17. Accumulated sediment production for the burned soils collected in the first year after the fire and application of treatments. T4, 0-4 months; T8, 4-8 months; T12, 8-12 months. Mean values of three field replicates ± SE. Treatments: B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Different lowercase letters denote significant differences ($p < 0.05$) among treatments.

Subsequently, the sediments collected in the plots B, B+S and B+M in the 4 to 8 month and 8 to 12 month periods after the fire were 36 g m^{-2}, 32 g m^{-2} and 9 g m^{-2} respectively in the first period, and 9 g m^{-2}, 8 g m^{-2} and 4 g m^{-2} respectively in the second period. The efficiency of mulching and seeding reducing soil erosion was 75-56%% and 11% respectively. Efficiency decreased with regard to the values observed in the first four months, reaching similar values than those observed by other authors in different contexts such as areas affected by fire, agricultural land, pastures and anthropogenic zones (Badía and Martí, 2000; Fernández *et al*., 2012; 2016a; Prats *et al*., 2012; Fernández and Vega 2014; Vega *et al*., 2014; Prosdocimi *et al*., 2016). The effectiveness of mulching decreased over time, especially when the vegetation recovers reach the 40%. The ANOVA1 (soil treatment) analysis also confirmed the effectiveness of mulching versus seeding in reducing long-term erosion. The data obtained from erosion reduction are in agreement with studies by other authors who found reductions of up to 95% of erosion thanks to mulching (Bautista *et al*., 1996; Wagenbrenner *et al*., 2006; Groen and Woods, 2008; Vega *et al*., 2015) and variable efficiency of seeding (Badía and Martí 2000; Beyers, 2004; Wagenbrenner *et al*., 2006; Kim *et al*., 2008; Robichaud, 2009).

The seeding of herbaceous plants produces competition with native species (Beyers,

2004), and their effectiveness will depend on the intensity of rain. Seeding is most suitable in areas with low rainfall intensity and with adequate light and temperature conditions for seed germination to create a relatively fast protective vegetation cover (De La Fuente and Blonde, 2010). The low seeding efficiency in this study is due to the low degree of germination of the seeds given the unfavourable climatic conditions that occurred. The effectiveness of mulching is due to the adherence of the straw to the soil, which increases the surface water storage capacity (Smets *et al*., 2008), reduces splash erosion and increases resistance to overland flow.

The comparison of the values obtained for the physical, chemical and microbiological properties analysed in the burned plots with seeding (B+S) and mulching (B+M) treatments, with the values of the burned samples (B) allows evaluating the effect of post-fire stabilization treatments on soil quality. The data indicated that no significant differences were found between burned soil and burned soil with different post-fire treatments in any of the properties analysed in any of the sample times. Therefore, either mulching or seeding produced changes in the quality of burned soils at short or medium term. This same trend was observed by Gómez-Rey and González-Prieto (2014) in the content of macronutrients (N, P, Ca, Mg and K) and trace elements (Al, Fe, Mn, Cu, Zn, Co and Bo) although changes induced by mulching and seeding were found in K, Mg and extractable Ca and $\delta^{15}N$ (Gómez-Rey *et al*., 2013). Other studies carried out in Galicia, in scrubland soils affected by forest fires of different severity, also found no differences in the biochemical properties of burned soil after mulching or seeding (Fernández *et al*., 2011; Fontúrbel *et al*., 2012). In contrast, in a study conducted at the same site Gómez-Rey and González-Prieto (2015) found a significant effect of mulching on N mineralization.

In order to analyse in more detail the effect of the treatment, separate PCA of each sample time were performed (Figure 18). Regarding the phospholipid fatty acid profile, in general, microbial community was not affected by the treatment applied at short or medium term and the main differences were due to soil burning. Similar result was observed in 8 and 12 month. The Factor 1, (30% and 51% of variance explained at 8 and 12 month sample respectively) allow us to separate unburned samples from burned ones. Factor 2 which accounted 23% and 15% of variance respectively separated burned samples treated with mulching from the other burned samples. However, 4 years after the fire, the microbial community of the B+M sample was different from that of the B and B+S samples along Factor 1, however this effect was not observed in the 2-5 cm layer (Figure 18). Some authors have already indicated that mulching may affect soil microclimate (humidity and temperature) (Bautista *et al*., 2009; Ferreira *et al*., 2015; Fernández *et al*., 2016) and the availability of C and N (Huang *et al*., 2008), which may affect the structure of the microbial community. It is also known that the incorporation of material such as wheat straw with a high C/N ratio favors the growth of fungi against bacteria (Rousk and Bååth, 2007; Barreiro *et al*., 2016b) and produce change in microbial community structure (Marschner *et al*., 2011; Zhao *et al*., 2016).

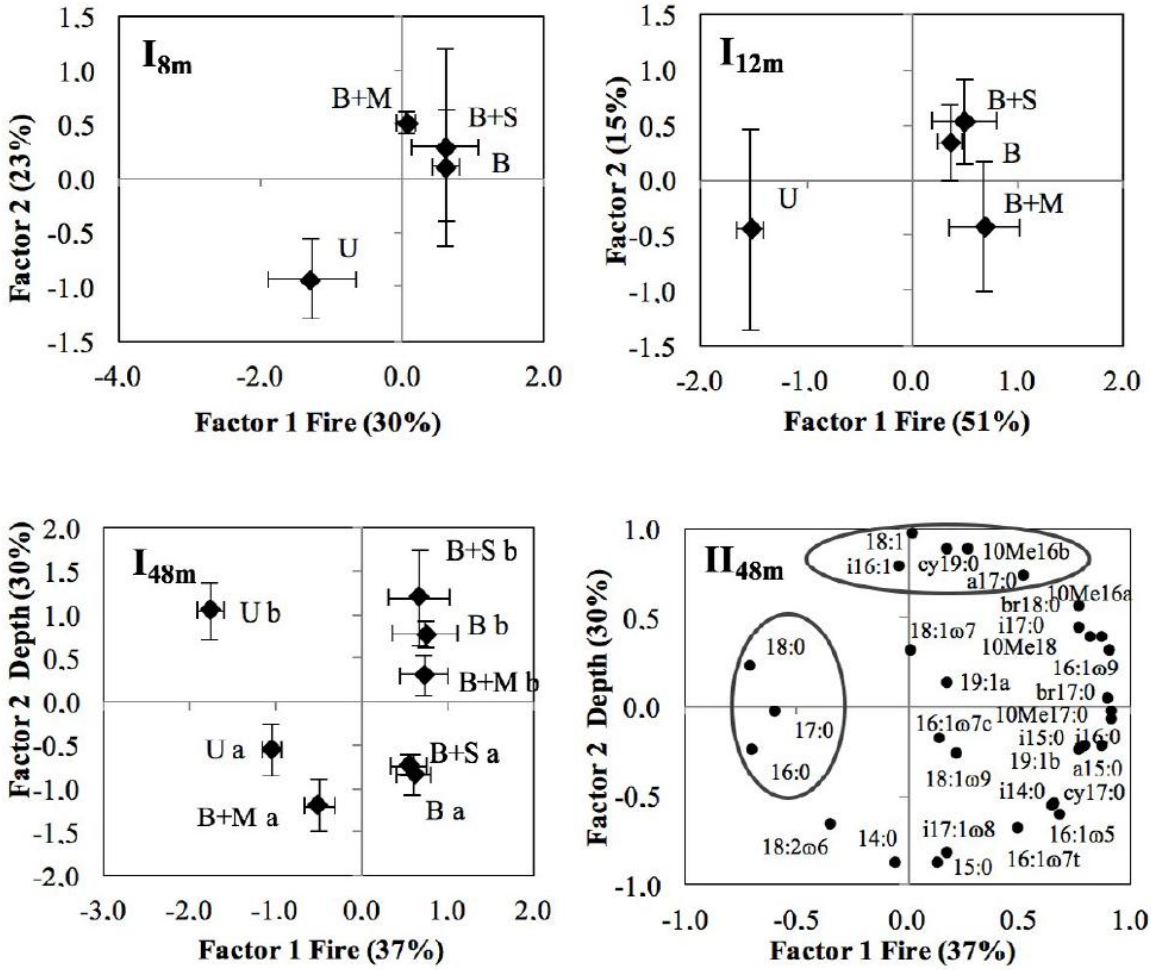

Figure 18. Results of the PCA performed for the PLFAs of the soils collected at different sampling times, 8, 12 and 48 months after the fire and application of the post-fire stabilization techniques. Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus straw mulching. Scores ± SE of three field replicates (I) and loading.

5.1.4. Effects of post-fire mulching treatments and way of application. Saviñao experiment

Table 5 showed the psychochemical properties of Saviñao samples. As with Laza samples, after the fire, the soil showed acid pH (4.5), low electrical conductivity (80 µS cm^{-1}), high organic matter content (151 g C kg^{-1}; 10.7 g N kg^{-1}) and high water retention capacity (595 g water kg^{-1}). These values were much higher than those obtained in Mediterranean burned soils (Caon *et al*., 2014) but in line with those obtained in burned Galician soil (Santín *et al*., 2008).

Table 5. Mean values ±SE of selected physicochemical properties of the studied soil at different sample times (3, 6, 9 and 12 month after fire). For each sampling time, different letters show significant differences (ANOVA1, p<0.05). For each parameter an ANOVA2 (T, time; M, mulching) was performed, but only the percentage of variance explained by significant factors (p<0.05) is indicated. (B, burned samples; B1S treatment with one straw trip per plot, bare soil; B1S+M, treatment with one straw trip per plot, soil with straw mulching; B2S, treatment with two straw trips per plot, bare soil; B2S+M, treatment with two straw trips per plot, soil with straw mulching.

	ANOVA2	time	B	B1S	B1S+M	B2S	B2S+M
pH_{water}		0			4.5 ± 0.1		
		3	5.3 ± 0.1 a	5.4 ± 0.1 a	5.5 ± 0.2 a	5.4 ± 0.0 a	5.2 ± 0.1 a
	T(80%)	6	4.5 ± 0.1 a	4.7 ± 0.0 a	4.6 ± 0.1 a	4.5 ± 0.1 a	4.5 ± 0.1 a
		9	4.6 ± 0.1 a	4.6 ± 0.1 a	4.5 ± 0.1 a	4.6 ± 0.1 a	4.7 ± 0.1 a
		12	4.5 ± 0.0 a	4.5 ± 0.1 a	4.7 ± 0.0 a	4.5 ± 0.1 a	4.6 ± 0.1 a
pH_{KCl}		0			3.3 ± 0.1		
		3	3.9 ± 0.1 a	3.9 ± 0.1 a	4.0 ± 0.2 a	3.9 ± 0.1 a	3.9 ± 0.1 a
	T(75%)	6	3.4 ± 0.0 a	3.5 ± 0.1 a	3.4 ± 0.1 a	3.4 ± 0.1 a	3.4 ± 0.1 a
		9	3.5 ± 0.0 a	3.5 ± 0.1 a	3.5 ± 0.1 a	3.5 ± 0.1 a	3.5 ± 0.1 a
		12	3.4 ± 0.0 a	3.5 ± 0.1 a	3.6 ± 0.0 a	3.4 ± 0.1 a	3.5 ± 0.1 a
Organic matter (%)		0			25.9 ± 0.6		
		3	24.9 ± 1.0 a	25.4 ± 0.5 a	25 ± 0.9 a	24.8 ± 0.5 a	25.9 ± 0.4 a
		6	23.7 ± 1.4 a	23.0 ± 1.4 a	23.2 ± 0.4 a	24.5 ± 1.0 a	24.8 ± 1.3 a
		9	23.0 ± 1.0 a	24.1 ± 0.9 a	23.2 ± 1.0 a	24.2 ± 1.0 a	25.0 ± 1.0 a
		12	23.9 ± 1.6 a	23.6 ± 1.5 a	25.6 ± 2.5 a	23.8 ± 0.5 a	25.0 ± 1.1 a
Moisture (%)		0			13.2 ± 1.1		
		3	31.6 ± 1.1 a	30.8 ± 1.0 a	34.0 ± 1.2 a	31.4 ± 0.5 a	32.3 ± 0.6 a
	T (91%)	6	15.4 ± 0.6 a	16.3 ± 1.2 a	16.5 ± 1.8 a	16.3 ± 1.1 a	18.4 ± 1.4 a
		9	25.0 ± 0.7 a	25.3 ± 1.8 a	22.3 ± 0.2 a	22.1 ± 2.0 a	26.7 ± 1.3 a
		12	30.3 ± 0.9 a	30.8 ± 1.4 a	33.8 ± 1.1 a	28.6 ± 2.3 a	33.3 ± 0.5 a
Electric conductivity ($\mu S\ cm^{-1}$)		0			80.2 ± 3.6		
		3	69.3 ± 1.6 a	82.6 ± 5.4 a	74.2 ± 1.4 a	73.7 ± 4.9 a	75.7 ± 4.0 a
	T (17%)	6	71.1 ± 8.9 ab	87.8 ± 4.9 b	49.1 ± 5.8 a	66.2 ± 9.3 ab	54.3 ± 3.0 a
		9	109 ± 19.1 a	150 ± 45.3 a	82.5 ± 21.0 a	103 ± 17.4 a	85.7 ± 10.8 a
		12	78.6 ± 10.7 a	90.9 ± 18.1 a	114 ± 34.2 a	66.2 ± 11.6 a	76.3 ± 8.9 a
Water retention (g water kg^{-1})		0			594.5 ± 8.3		
		3	586 ± 15.7 a	589 ± 17.4 a	617 ± 18.1 a	593 ± 9.6 a	583 ± 8.7 a
	T (56%)	6	495 ± 6.9 a	522 ± 17.3 a	509 ± 17.9 a	484 ± 34.1 a	504 ± 26.6 a
		9	549 ± 9.6 a	545 ± 11.9 a	535 ± 11.0 a	522 ± 9.1 a	579 ± 34.9 a
		12	588 ± 21.2 a	610 ± 19.6 a	626 ± 32.2 a	571 ± 20.8 a	640 ± 15.6 a
Total C (g/kg^{-1})		0			151 ± 3.1		
		3	149 ± 4.8 a	155 ± 4.7 a	147 ± 5.3 a	145 ± 4.2 a	155 ± 2.4 a
		6	145 ± 9.1 a	139 ± 7.9 a	137 ± 0.9 a	154 ± 3.2 a	149 ± 9.2 a
		9	138 ± 8.6 a	138 ± 1.8 a	131 ± 6.0 a	141 ± 7.0 a	142 ± 6.3 a
		12	142 ± 12.0 a	141 ± 9.2 a	160 ± 14.7 a	136 ± 3.9 a	152 ± 7.1 a
δ13C (‰)		0			-27.3 ± 0.1		
		3	-27.1 ± 0.1 a	-27.0 ± 0.2 a	-27.2 ± 0.2 a	-26.9 ± .01 a	-27.0 ± 0.1 a
	T (18%) M (14%)	6	-27.1 ±0.2 a	-26.8 ± 0.2 a	-27.3 ± 0.2 a	-27.1 ± 0.0 a	-27.0 ± 0.0 a
		9	-27.1 ± 0.2 a	-27.0 ± 0.1 a	-27.3 ± 0.1 a	-27.1 ± 0.1 a	-27.1 ± 0.0 a
		12	-27.0 ± 0.1 a	-26.8 ± 0.2 a	-27.2 ± 0.0 a	-26.9 ± 0.1 a	-26.9 ± 0.1 a
Total N ($g\ kg^{-1}$)		0			10.7 ± 0.4		
		3	10.4 ± 0.7 a	11.0 ± 0.3 a	10.8 ± 0.5 a	10.6 ± 0.4 a	10.7 ± 0.1 a
		6	10.0 ± 0.9 a	10.0 ± 0.7 a	9.5 ± 0.5 a	10.1 ± 0.2 a	9.8 ± 0.3 a
		9	9.8 ± 0.5 a	10.2 ± 0.2 a	9.3 ± 0.5 a	9.9 ± 0.1 a	10.0 ± 0.3 a
		12	10.1 ± 0.9 a	10.4 ± 0.6 a	11.4 ± 1.1 a	9.6 ± 0.6 a	10.3 ± 0.4 a
δ14N (‰)		0			2.6 ± 0.2		
		3	2.0 ± 0.1 a	1.8 ± 0.2 a	1.9 ± 0.2 a	1.8 ± 0.1 a	1.8 ± 0.1 a
	T (58%)	6	2.1 ± 0.1 a	1.9 ± 0.2 a	2.0 ± 0.2 a	1.9 ± 0.2 a	2.1 ± 0.1 a
		9	1.8 ± 0.1 a	1.8 ± 0.1 a	1.9 ± 0.1 a	1.5 ± 0.1 a	1.5 ± 0.1 a
		12	1.8 ± 0.2 a	1.9 ± 0.2 a	1.7 ± 0.1 a	2.1 ± 0.1 a	1.9 ± 0.0 a

No differences were detected between the values of the physical-chemical properties analysed between burned soils without treatment and soils treated with straw mulching, regardless of the dose applied. The ANOVA2 (T, time; M, mulching) analysis detected differences due to sample time in moisture (91%), water retention capacity (56%), pH in water and KCl (80% and 75%), $\delta^{15}N$ (58%) and electric conductivity (17%) while there were no differences in total N values (Table 5).

After fire, soluble carbon was 288 mg kg^{-1} (0.5% of total carbon) and carbon extractable in hot water was 2383 mg kg^{-1} (1.7% of total C), representing carbohydrates around 26-28%. The ANOVA2 (T, time; M, mulching) analysis found differences in the values of the most labile carbon fractions produced by the different sample time explaining 20-85% of the differences Differences were only found due to the treatment applied (14%) in the case of $\delta^{13}C$. These data indicate that carbohydrates and extractable carbon vary in size and composition according to the season and are considerably affected by forest fires (Díaz-Raviña *et al*., 1992; 1995; Prieto-Fernández *et al*., 1998; Martín *et al*., 2009; Almendros and González-Vila, 2012; Rovira *et al*., 2012). This labile fraction of organic matter is especially important in soil ecosystems because it controls productivity in the short term and is affected by disturbances such as forest fires or prescribed fires, which allows them to be used as an indicator of seasonal effect and fire, especially the fraction extractable in cold water (Table 6).

Table 6. Mean value ± SE of the C labile fractions (WSCH, water soluble carbohydrates, WSC, water soluble carbon) at 22°C (22) And 80°C (80) water at different sampling times (3, 6, 9 and 12 month after the fire). For each sampling time, different letters show significant differences (ANOVA1, p<0.05). For each parameter an ANOVA2 (T, time; M, mulching) was performed, but only the percentage of variance explained by significant factors (p<0.05) is indicated. (B, burned samples; B1S treatment with one straw trip per plot, bare soil; B1S+M, treatment with one straw trip per plot, soil with straw mulching; B2S, treatment with two straw trips per plot, bare soil; B2S+M, treatment with two straw trips per plot, soil with straw mulching.

	ANOVA2	time	B	B1S	B1S+M	B2S	B2S+M
$WSCh_{22}$		0			76.6±8		
(mg C-Glu kg^{-1})		3	33±3 a	32±1 a	32±3 a	38±5 a	34±4 a
	T (85%)	6	44±4 a	42±5 a	35±6 a	43±3 a	46±3 a
		9	30±3 a	37±5 a	32±5 a	35±5 a	40±6 a
		12	45±1 a	43±4 a	37±1 a	41±2 a	40±2 a
$WSCh_{80}$		0			665±46		
(mg C-Glu kg^{-1})		3	636±67 a	611±45 a	602±60 a	650±36 a	595±46 a
	T (20%)	6	763±58 a	742±41 a	666±89 a	769±67 a	784±81 a
		9	677±103 a	840±112 a	646±108 a	661±81 a	714±68 a
		12	678±59 a	533±77 a	575±56 a	493±36 a	633±42 a
WSC_{22}		0			288±47		
(mg C-Glu kg^{-1})		3	141±35 a	137±32 a	151±28 a	164±40 a	151±36 a
	T (60%)	6	169±37 a	178±29 a	121±40 a	175±41 a	168±29 a
		9	143±16 a	215±13 a	128±33 a	157±44 a	172±41 a
		12	97±12 a	108±26 a	82±10 a	113±14 a	101±12 a
WSC_{80}		0			2383±169		
(mg C-Glu kg^{-1})		3	2135±173 a	1983±180 a	1911±148 a	2316±190 a	1931±134 a
	T (34%)	6	2164±213 a	2000±144 a	1794±221 a	2205±219 a	2224±148 a
		9	1761±183 a	2129±224 a	1640±126 a	1657±211 a	1916±181 a
		12	2091±118 a	1671±199 a	1740±182 a	1468±58 a	1733±141 a

Soil biomass carbon was 346 mg C kg^{-1} and represented 0.235 % of the total C. Soil respiration was 74 µg CO_2 g^{-1} day and bacterial activity was 3.9 x10 $^{-15}$ mol leucine g^{-1} day^{-1}. Values for enzyme activity were 48 µg *p*-nitrophenol g^{-1} h^{-1} for β-glucosidase, 33 µg NH_4^+ g^{-1} h^{-1} for urease and 129 µg *p*-nitrophenol g^{-1} h^{-1} for phosphatase. These values are within the ranges observed in Galician soils affected by forest fires of medium or high severity. These soil properties were greatly affected by the sampling time, so that about 15-71% of the

differences detected are explained by the seasonal effect. Except for bacterial activity and respiration, which were affected by mulching (9% and 5% of the variance explained by mulching respectively), post-fire treatments did not affect the rest of the variables analysed. With respect to the sampling time, different patterns were seen: biomass carbon (62% variance explained by sampling time) increased 9 months after the fire. Bacterial activity (53% of variance explained by sampling time) increased after the fire, especially at 3 months. Respiration (71% variance explained by sampling time) decreased with respect to the samples collected immediately after the fire. The β-glucosidase activity (65% variance explained by sampling time) increased at 3 months and then decreased below the initial values. The urease and phosphatase activities presented the lowest percentages of variance explained by the sampling time (38% and 15% respectively) and showed an increase in their values (Table 7).

Table 7. Mean value ± SE of the biochemical properties analized at different sampling times (3, 6, 9 and 12 month after the fire). For each sampling time, different letters show significant differences (ANOVA1, p<0.05). For each parameter an ANOVA2 (T, time; M, mulching) was performed, but only the percentage of variance explained by significant factors (p<0.05) is indicated. (B, burned samples; B1S treatment with one straw trip per plot, bare soil; B1S+M, treatment with one straw trip per plot, soil with straw mulching; B2S, treatment with two straw trips per plot, bare soil; B2S+M, treatment with two straw trips per plot, soil with straw mulching.

	ANOVA2	time	B	B1S	B1S+M	B2S	B2S+M
Extractable C ($\mu g\ g^{-1}$)		t=0			19.9 ± 1.3		
		t=3	15.2 ± 2.9 a	15.4 ± 2.9 a	15.4 ± 2.9 a	14.7 ± 2.0 a	17.1 ± 3.0 a
	T (25%)	t=6	15.9 ± 1.7 a	13.9 ± 1.9 a	14.4 ± 0.8 a	16.1 ± 0.7 a	16.4 ± 1.1 a
		t=9	16.2 ± 1.9 a	17.1 ± 0.6 a	18.9 ± 2.7 a	16.1 ± 2.0 a	17.9 ± 2.6 a
		t=12	13.8 ± 1.3 a	14.5 ± 1.6 a	13.7 ± 3.3 a	16.8 ± 0.8 a	13.2 ± 1.6 a
Microbial C ($\mu g\ g^{-1}$)		t=0			346 ± 38		
		t=3	407 ± 33 a	344 ± 34 a	355 ± 23 a	371 ± 13 a	429 ± 41 a
	T (62%)	t=6	323 ± 23 a	241 ± 34 a	310 ± 50 a	380 ± 34 a	339 ± 26 a
		t=9	535 ± 45 a	402 ± 55 a	486 ± 25 a	471 ± 47 a	384 ± 67 a
		t=12	439 ± 65 a	377 ±36 a	379 ±63 a	368 ± 44 a	383 ± 59 a
Soil Respiration ($mg\ CO_2\ kg^{-1}\ day^{-1}$)		t=0			74.1 ± 8.3		
	T (71%)	t=3	38.9 ± 2.6 a	37.4 ± 2.2 a	44.4 ± 4.8 a	42.1 ± 4.1 a	44.5 ± 4.5 a
	M (5%)	t=6	54.7 ± 1.9 a	45.1 ± 4.1 a	61.7 ± 7.0 a	53.8 ± 4.0 a	62.1 ±3.4 a
		t=9	45.8 ± 4.1 a	39.0 ± 3.5 a	43.6 ± 5.3 a	42.3 ± 4.2 a	42.5 ± 5.3 a
		t=12	48.3 ± 3.8 ab	37.9 ± 2.6 a	57.3 ± 5.5 b	42.2 ± 4.2 ab	51.0 ± 1.7 ab
Bacterial activity ($x10^{-14}$ mol Leu $g^{-1}\ h^{-1}$)		t=0			390 ± 268		
	T (53%)	t=3	4126 ± 911 a	3310 ± 898 a	3594 ± 1277 a	4552 875 a	5639 ± 859 a
	M (9%)	t=6	880 ± 135 a	747 ± 130 a	1692 ± 398 ab	3353 ± 963 b	2246 ± 231 ab
		t=9	2249 ± 501 a	1597 ± 466 a	3555 ± 736 a	3320 ± 260 a	3157 ± 636 a
		t=12	1338 ± 322 a	1324 ± 102 a	1904 ± 456 ab	1427 ± 378 a	2947 ± 306 b
Glucosidase (μg p-nitrophenol $g^{-1}\ h^{-1}$)		t=0			47.8 ± 5.4		
		t=3	74.2 ± 12.8 a	64.1 ± 9.7 a	63.2 ± 14.5 a	56.2 ± 6.1 a	76.5 ± 10.1 a
	T (65%)	t=6	29.9 ± 8.2 a	26.8 ± 4.3 a	32.0 ± 6.0 a	25.6 ± 3.5 a	30.2 ± 4.9 a
		t=9	28.4 ± 4.4 a	24.6 ± 2.5 a	35.1 ± 8.1 a	27.8 ± 3.2 a	24.8 ± 3.0 a
		t=12	34.8±3.0 a	28.0±2.0 a	30.0±5.4 a	29.7±2.6 a	27.3±1.3 a
Urease ($\mu g\ NH_4^+\ g^{-1}\ h^{-1}$)		t=0			33.1 ± 3.9		
		t=3	31.3 ± 6.0 a	23.3 ± 5.6 a	26.5 ± 4.2 a	32.6 ± 6.4 a	28.4 ± 2.7 a
	T (38%)	t=6	45.1 ± 4.2 a	44.1 ± 6.2 a	62.3 ± 6.6 a	70 ± 10.4 a	55.8 ± 8.6 a
		t=9	35.0 ± 5.7 a	42.6 ± 4.7 a	53.2 ± 10.2 a	45.1 ± 9.4 a	44.4 ± 5.8 a
		t=12	37.4 ± 6.5 a	26.9 ± 3.2 a	45.6 ± 12.4 a	35.5 ± 3.9 a	39.4 ± 2.9 a
Phosphatase (μg p-nitrophenol $g^{-1}\ h^{-1}$)		t=0			0.9 ± 0.1		
		t=3	1.4 ± 0.2 a	1.2 ± 0.2 a	1.3 ± 0.1 a	1.2 ± 0.1 a	1.4 ± 0.0 a
	T (15%)	t=6	1.3 ± 0.1 a	1.0 ± 0.1 a	1.3 ± 0.1 a	1.1 ± 0.1 a	1.3 ± 0.2 a
		t=9	1.2 ± 0.2 a	1.0 ± 0.2 a	1.6 ± 0.2 a	1.1 ± 0.1 a	1.1 ± 0.2 a
		t=12	1.1 ± 0.1 a	1.1 ± 0.2 a	1.3 ± 0.2 a	0.9 ± 0.1 a	1.0 ± 0.0 a

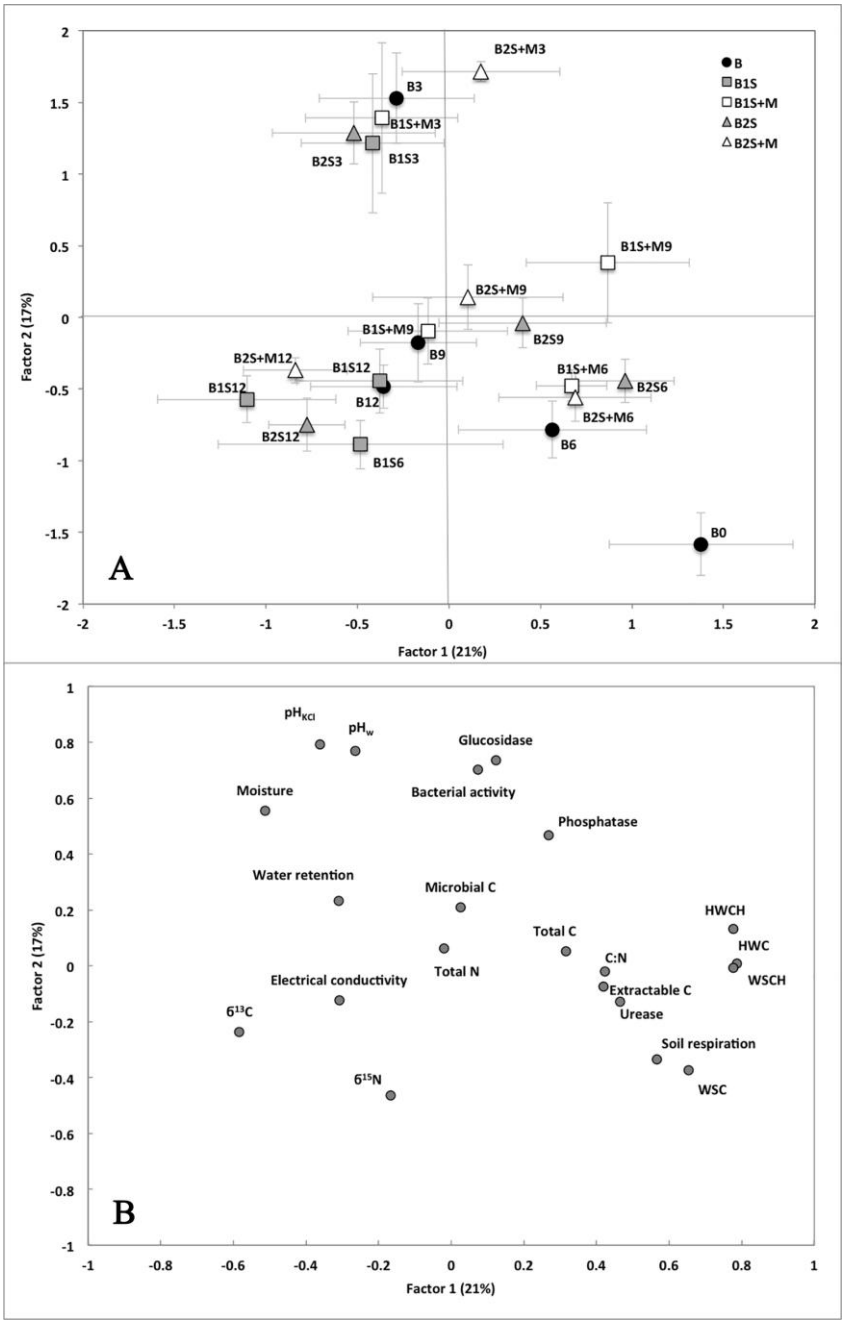

Figure 19. Score (mean value ± SE, n=4) (A) and loading plots (B) from a PCA performed with the total of soil properties analysed of the different soil treatments at different sampling time (0, 3,6,9,12 month after the fire and the application of the stabilization treatment). B, burned soil; B1S+M, treatment with 1 straw strip per plot, soil with straw mulching; B1S, treatment with 1 straw strip per plot, bare soil; B2S+M, treatment with 2 straw strips per plot, soil with straw mulching; B2S, treatment with 2 straw strips per plot, bare soil.

In order to facilitate the interpretation of the results with respect to the influence of time and treatment factors on soil properties, an analysis of main components was carried out with values of all properties analysed (Figure 19). Factor 1 and Factor 2 accounted for 38% of the total variance. The Factor 1 that explained 21% of the variance was determined by the organic matter content while the Factor 2, that accumulated 17% of the variance, was related to pH,

moisture, β-glucosidase and bacterial activity. The distribution of the samples in the two planes defined by Factor 1 and Factor 2 allowed separating the burned samples according to the moment of sampling. The samples collected after the fire show high contents in the labile fraction of C. Those collected after 3 months show high values of β-glucosidase and bacterial activity, both attributed to the increase in pH and moisture content. Samples collected after 12 months show low labile organic matter fraction content. The effect of the fire persists in such a way that the samples collected immediately after the fire are located at the opposite extreme than the samples collected after one year, demonstrating a great difference between them. The data indicate that seasonal effect (either by inter-annual variability or by changes produced by medium and long-term effects on soil quality) is so important that it masks the effect of mulching application. The important seasonal effect is due to the marked seasonal fluctuations observed in soil properties, associated with vegetation effects and variations in climatic conditions (Díaz-Raviña *et al*., 1993; 1995; Martín *et al*., 2011).

5.1.5. Effect of post-fire treatments on vegetation cover and species composition. Laza experiment

The percentages of soil cover and the percentage of each species in Laza samples were reported in Table 8. The fire completely destroyed the existing vegetation. During the first months there was no vegetation recovery due to unfavorable climatic conditions (temperatures around 0ºC-1ºC between the 3rd and 7th month and 6.7ºC-11.7ºC in the following months). Vegetation recovery at 4 months was less than 12%. Between the 4th and 8th months there was a slight increase in the percentage of soil with vegetation cover, reaching values of 18%. One year after the fire the vegetation cover was 36-40%. A similar value was found in previous studies conducted by Barreiro *et al*. (2015). Other studies performed in the humid temperate zone the recovery of vegetation cover reached values of 70% (Vega *et al*., 2014) but these differences were due to severity, since the fire in Vega *et al*. (2014) study was an experimental fire of low severity while the wildfire in Laza was of high severity.

The most abundant plant species and the first to appear were the pioneer species *Luzula lactea* L (21-73% of total vegetation), followed by other herbaceous species such as *Festuca* spp. (11-30%) and *Agrostis* spp (0.2-14%). Shrub species did not appear until 8 months after the fire, with a percentage of less than 5% of the total. One year after the fire, shrub species such as *Pterospartum tridentatum* Wilk, *Erica* spp. and *Cistus* spp., represented the 58-68% of the total species. This behavior has already been observed in other studies such as Vega *et al.* (2014) and Morgan *et al.* (2014) in which the vegetation cover is not dominated by scrub until one year and more than 6 years after the fire respectively. The presence of these 3 species is justified, as they were the dominant and most abundant species in the adjacent unburned areas. Studies by Duguy and Vallejo (2008) indicate that in the burned areas of the Mediterranean area, the vegetation formations are dominated by resprouting species, more resilient to fire than germinating species, such as *Pterospartum tridentatum* Wilk, which has a very high resprouting capacity (Reyes *et al*., 2009). At the end of the study, after 4 years of

the fire (data not shown), plant cover reached values of 60-80%. Researchers such as Fernández and Vega (2014) and Fernández *et al*. (2016) showed those two years after the fire the vegetation had reached values similar to the unburned scrub areas in NE Spain. This is probably due to the resprouting capacity of the shrub species and the more favourable post-fire conditions. In this study, given the high temperatures reached, there was a total destruction of the vegetation and the death of the present arboreal stratum. Shrub growth was slow and with a different species composition, suggesting that vegetation cover recovery requires more than four years to reach a development similar to that before a high severity fire (Robichaud *et al*., 2013; Morgan *et al*., 2014).

Table 8. Mean values ±SE of percentages of the principal plant species and the ground cover by plants for the principal plant species in Laza soil at different sampling times (4, 8 and 12 month after the fire and the application of the postfire stabilization techniques). Treatments:U, unburned soil; B, burned soil; B+S, burned soil with seeding treatment; B+M, burned soil with mulching treatment). For each parameter different lowercase letters denote significant differences (p<0.05) among treatments.

Plant Species	Time	B	B+S	B+M	B	B+S	B+M
		Ground Cover (%)			Species (%)		
Total vegetation	4	7.4±0.3 b	12.0±0.3 c	4.5±1.1 a			
Luzula lactea L.		4.7±0.4 a	4.4±0.4 a	2.6±0.7 a	66.5±8.1 b	32.8±2.6 a	56.5±4.8 b
Festuca spp.		1.6±0.7 a	1.5±0.3 a	1.2±0.1 a	19.2±7.9 a	12.7±2.0 a	30.0±3.9 a
Agrostis spp.		1.1±0.2 a	0.7±0.1 a	0.7±0.2 a	14.3±2.8 b	5.1±0.7 a	13.5±0.1 b
Total vegetation	8	12.4±1.8 ab	18.3±0.7 b	9.7±0.0 a			
Luzula lactea L.		8.7±1.1 a	7.4±1.1 a	6.4±1.6 a	72.8±5.2 b	38.7±5.6 a	65.1±3.7 b
Festuca spp.		2.1±0.5 a	2.4±0.3 a	1.9±0.1 a	17.2±3.8 a	13.7±1.8 a	19.2±2.8 a
Agrostis spp.		0.9±0.1 b		0.7±0.3 b	5.7±0.9 b		8.6±3.0 b
Pterospartum tridentatum Wilk.		0.1±0.1 a	0.5±0.2 a	0.1±0.1 a	0.4±0.4 a	2.7±0.9 a	0.6±0.6 a
Cistus spp.		0.5±0.2 a	0.7±0.3 a	0.5±0.0 a	3.5±1.1 a	3.5±1.3 a	5.0±0.7 a
Erica spp.		0.1±0.1 a	0.1±0.1 a	0.1±0.1 a	0.4±0.4 a	0.1±0.1 a	1.5±0.5 a
Vaccinium myrtillus L.			0.1±0.1 a			0.1±0.0 a	
Total vegetation	12	35.6±3.7 a	36.5±5.0 a	39.9±2.7 a			
Luzula lactea L.		10.0±1.1 a	9.3±1.9	6.5±1.7 a	31.1±2.1 a	27.2±8.8 a	20.7±1.6 a
Festuca spp.		3.6±0.7 a	4.1±1.4	4.3±1.2 a	10.6±1.6 a	11.5±3.2 a	11.4±3.6 a
Agrostis spp.		0.1±0.1 a			0.2±0.2 a		
Pterospartum tridentatum Wilk.		10.0±1.6 a	6.8±2.6 a	11.3±0.2 a	27.1±1.6 b	18.1±1.6 a	29.0±1.7 b
Cistus spp.		4.2±1.6 a	5.5±2.0 a	6.5±0.8 a	10.4±3.3 a	16.0±5.2 a	16.5±0.7 a
Erica spp.		7.7±0.5 a	8.9±4.1 a	11.3±3.3 a	20.6±1.3 a	21.5±7.1 a	22.4±4.0 a

The total coverage did not present important variations due to the different treatments, however, the results of ANOVA2 (T, soil treatment; S, sampling time) showed a significant effect of the sampling time, explaining 89% of the observed variance in the total coverage and 44-59% of the variance for herbaceous species and 67-88% for scrub species. This may be related to the availability of nutrients after the fire, especially N, which may produce a displacement of some species versus others (Morgan *et al*., 2014). The soil treatment effect (unburned, burned, burned plus mulching, burned plus seeding) was observed in the species *Luzula lactea* L. and *Agrostis* spp. (15 and 17% of variance explained by soil treatment) and *Vaccinium myrtillus* L. (40% variance explained) which only appears in burned soil treated with seeding 8 months after the fire. *Luzula lactea* L. and *Agrostis* spp. presented lower percentages compared to the total in burned soil with seeding than in burned soil without

treatment and soil burned with mulching. In general, mulching and seeding did not seem to affect plant diversity in burned soils, although other authors did seem to find other effects in the Mediterranean and humid zone of Spain (Badía and Martí, 2000).

5.1.6. Effect on mulching on vegetation rate and cover composition. Saviñao experiment

In order to study the mulching effect in the seed bank, a greenhouse experiment was conducted, and the recovery of the vegetation in the field was also analysed. Table 9 showed the data obtained from greenhouse and field experiments. In the greenhouse measurements, the number of seedling that emerged from the seed bank after mulching (633.7) was not different for the untreated ones (731.5). Species richness (22 species emerged in the greenhouse experiment) was different between untreated and treated plots, while species composition was similar in both cases, although herbaceous species were more abundant in the treated samples. Only two non-native species were found in mulching soils (*Hordeum vulgare* and *Avena sativa*).

In the field study, mulching favored the establishment of vegetation. The number of emerged seedling was higher in treated plots (167.5) than in untreated ones (114.5). In addition, the percentage of vegetation cover was also higher in mulching samples and the species richness was significantly different. One year after treatments, 24 species appeared and two of them, *Hordeum vulgare* and *Avena sativa,* were non-native. The most abundant species was *Cytisus striatus*, because the heat induces the germination of its seeds (Rivas *et al*., 2006) as well as *Halimium lasianthum* (Fernández *et al*., 2013). The high percentages of *Erica arborea* seedling emerged may reflect a high and persistent seed bank (Thompson *et al*., 1997). Previous studies showed that mulching can inhibit (Kruse *et al*., 2004) or favour plant regrowth (Peterson *et al*., 2009; Fernández *et al*., 2014), and this contradiction can be explained by factors such as soil moisture, temperature or mulching dose. Santana *et al*. (2014) detected an increase in soil moisture and a temperature buffering effect by wood chips mulch, which could explain the increased of seedling emerged in treated plots. The dose used in this study seems to favor the richness of species in both field and greenhouses conditions. Previous studies observed a maximum in species richness at an intermediate level of mulch cover (60-70%) (Dodson and Peterson, 2010). The similarity in species composition of emerged seedling and mature vegetation was comparable to those reported in previous studies (Enright *et al*., 2007). Mulching is considered to be related to the presence of vegetative cover with non-native species (Kruse *et al*., 2004; Dodson and Peterson, 2010; Dodson *et al*., 2010) that may compete with native species. Authors such Amaranthus *et al.* (1993) or Keeley *et al*. (2004) detected that the germination of grasses species and pines was affected by competition with rye seeds. However, only two non-native species were detected in this study that do not present a risk of being invasive (Sanz *et al*., 2004) because after an initial appearance, they disappear rapidly (Dodson and Peterson, 2010; Dodson *et al*., 2010).

Table 9. Complete list of species and mean density ± SE (seedling m^{-2}) appearing in greenhouse samples and in the field from each treatment type.

Species	Greenhouse		Field	
	Untreated	Mulching	Untreated	Mulching
Cytisus striatus	183±33	127±35	57±8	64±13
Rumex acetosella L.	179±47	150±38	9±2	18±4
Erica arborea L.	93±44	60±19	13±7	7.8±4.5
Ornithopus compressus L.	80±26	73±19	18±5	32±8
Halimium lasianthum ssp. alyssoides (Lam.) Greuter	59±21	16±8	4.2±1.4	3.4±1
Anthoxanthum odoratum L.	44±13	32±13	4.1±1.4	11±3
Lotus corniculatus L.	22±7	18±8	6.6±2.5	5.7±2.6
Jasione montana L.	19±10	30±10	1.9±0.8	10±3
Lysimachia nemorum L.	18±11	16±7	0	0
Lolium perenne L.	16±1	65±15	0.8±0.4	2±0.8
Sedum brevifolium DC.	7.8±4.9	1±0.4	0	0
Spergula arvensis L.	3.9±1.5	1±0.5	0	0
Bromus hordeaceus L.	3.1±1.0	9.4±3.2	0	0
Rubus sp. L.	2.9±2.2	7.8±5.2	0	0
Stellaria media (L.) Vill	1.0±0.5	5.9±2.5	0.7±0.2	0.7±0.2
Polygala vulgaris L.	2.0±0.5	1.0±0.2	0.3±0.1	0.1±0.1
Tesdalia nudicaulis (L) R.Br.	1.0±0.5	2.0±1.0	0.5±0.3	0.8±0.4
Avena satina L.	0	7.8±2.3	0	3.1±0.8
Hordeum vulgare L.	0	6.2±1.8	0	10±2
Erodium cicutarium L.	0	2.9±2.1	0.1±0.1	0.4±0.2
Senecio vulgaris L.	0	2.0±1.3	0	0
Pterospartum tridentatum (L.) Willk	0	1.0±0.3	0.1±0.1	0.1±0.1
Total number of emerged seedling	**732±77**	**634±69**	**114±12**	**168±16**

5.2 LABORATORY EXPERIMENTS

5.2.1. Physico-quemical properties and color

Laza and Estrada soils were selected to perform the laboratory experiment. Both soils showed acid pH (around 4 units) and a high organic matter content. Prescribed fire (low severity) produced light changes on psysicochemical properties while the effect of wildfire (high severity) was more marked (Table 10). No changes or minor changes were detected in soil pH and total C content due to the cumulative effect of successive temperature stress (muffle heating) (data not shown).

Table 10. Physicochemical properties on unburned (U) and burned (B) soil samples before the heating treatment L, Laza soil, affected by a high severity fire; E, Estrada soil affected by a low severity prescribed fire.

	LU	LB	EU	EB
Moisture (%)	35	29	40	36
pH H_2O	4.2	4.8	4.1	4.1
pH KCl	2.9	3.3	3.4	3.5
Total C (%)	23.8	14.6	17.7	16.2
Total N (%)	1.1	0.7	1.2	1.2

Although no changes were detected due to fire in Total C and Total N (Table 10), soluble C showed variations between the first and second heating treatments (Table 11). After the first heating event soluble C increased in both soil and a decreased was produced after the second heating event. These data are consistent with finding of several authors showing

increased in the labile fraction of C after soil heating below 200ºC (attributed to nutrients produces by death and lysis of microorganisms) and a further decrease after caused by the use of this labile pool as source of energy during the recovery of the microbial population (Díaz-Raviña *et al*., 1992; Prokushkin and Tokareva, 2007).

Table 11. Soluble C fraction of organic matter on unburned (U) and Burned (B) soil samples. WSC, Water soluble carbon (22ºC); HWSC, hot water soluble carbon (80ºC). L, Laza soil affected by a high severity fire; E, Estrada fire affected by a low severity presibed fire. Treatments: C, control; 1H, first heating; 2H, second heating.

		Total soluble C (mg C-Glu kg^{-1})	WSC (mg C-Glu kg^{-1})	HWSC (mg C-Glu kg^{-1})
LU	C	791	91	700
	1H	764	85	679
	2H	555	81	475
LB	C	446	50	397
	1H	483	88	395
	2H	297	48	248
EU	C	685	96	589
	1H	800	226	574
	2H	397	65	332
EB	C	372	74	298
	1H	412	168	409
	2H	226	59	168

Despite of the low values of Temperature reached by soil, the successive thermal shocks can provoke changes in the quality of the organic matter. This supports the fact that labile fractions of the soil organic matter rather than the total organic matter content are more adequate to detect the impact of fire (Prieto-Fernández *et al*., 1998; Villar *et al*., 2004; Díaz-Raviña *et al*., 2010; Martín *et al*., 2012; Almendros and González-Vila, 2012).

Change in color parameters were also detected as consequence of fire (Table 12). In Laza soil samples wildfire led to a significant decreased in lightness (L*) of 2.4 to 2.8 CIELAB units:; furthermore, the differences in the L* parameter between the burned and unburned samples in the area affected by a high severity wildfire were notably higher than those observed between the unburned and burned samples in the area affected by a low severity prescribed fire. Likewise a difference (higher than 3 CIELAB units) appears between the unburned and burned samples of Laza soil in the color tone (h_{ab}) and only slight differences were detected in Estrada soil. Regarding the heating in laboratory conditions changes in those parameters were observed at high heating temperatures (300ºC) (Table 12). These results indicating that fire caused change in color parameters at high temperatures as those reached in Laza wildfire. Changes in pH, organic matter and soil color clearly indicates that the burning temperatures had exceeded 400ºC (Fernández *et al*., 1997; Certini, 2005; Marcos *et al*., 2007).

Table 12. Color parameters on unburned (U) and burned (B) samples before heating (C), and after heating the first (1H) and second (2H) heating treatments at 300 ºC. L*, Lightness; h_{ab} , color tone. L, Laza soil affected by a high severity fire; E, Estrada soil affected by a low severity prescribed fire

	L*			h_{ab}		
	C	$1H_{300C°}$	$2H_{300C°}$	C	$1H_{300C°}$	$2H_{300C°}$
LU	14.9	17.2	17.5	59.2	62.4	63.3
LB	17.7	20.2	20.4	64.3	65.5	65.9
EU	12.5	14.8	15.4	61.4	64.7	64.8
EB	12.1	13.9	17.7	61.8	65.2	66.7

5.2.2.Temperature-time curves and degrees-hour methodology

The values of the degrees-hour (DH) reached by soil during the heating treatment were different depending on the temperature range (<10 DH for treatments below 100ºC, 10-30 DH for treatments between 100 and 200ºC and >30 DH for treatments of 300ºC). These values are very low compared to those from other studies such as Cancelo-González *et al*., (2012; 2015) who apply higher temperatures for a longer time. The real temperature values obtained during the heating of the samples (Tmax) were very different from the theoretical values that were applied with the muffle (e.g. applying a temperature of 300ºC, the maximum surface temperature obtained was between 187 and 249ºC and 178 to 243ºC at 1 cm depth depending on the sample and the heating cycle). This shows the importance of the measurement of the temperature reached in the samples during the laboratory heating. The Table 13 shows the DH reached by soil samples and the parameter obtained by exponential model for adjusting the temperature-time curves. The Table 13 shows the DH reached by soil samples and the parameter obtained by exponential model for adjusting the temperature-time curves. The results showed that the response of the soil depends on the temperature applied. Samples heated to temperatures of 50-75ºC were adjusted to the linear model with R^2 = 0.82 to 0.98 ($p<0.001$). Samples heated to temperatures above 100ºC are best suited to an exponential model R^2 = 0.741-0.987 ($p<0.001$). The data shows that, due to the good fit to the model, the estimated values of the maximum temperature reached according to the exponential model, were practically identical to those obtained with thermocouples (data not shown). Most studies concerning thermal shock impact on soil properties are related to temperature of the heating muffle and not the actual temperature achieved by soil and the duration of the heating treatment. Using the actual temperature reached by soil on heating treatments avoid to overestimate the temperature reached by soil in laboratory experiments and allow to compare between different studies.

Table 13. Degrees-hour values reached by soil and parameters obtained by the exponential and linear model ([1]) in soil from unburned (U) and burned (B) Laza (L) and Estrada (E) soil. (T (b), maximum temperature reached by soil: a, slope).

	1st HEATING								2nd HEATING							
	Surface				1cm depth				Surface				1cm depth			
	DH	R^2	T (b)	a	DH	R^2	T (b)	a	DH	R^2	T (b)	a	DH	R^2	T (b)	a
LU50	3.5	0.8	33	0.15	3.1	10.93	[1]30	10.97	4.2	10.99	[1]34	11.24	2.1	10.86	[1]27	10.68
LU75	8.9	0.91	55	0.33	4.1	10.93	[1]36	10.97	11.7	0.84	54	0.4	8.9	0.96	50	0.16
LU100	13.3	0.98	79	0.33	11.1	0.98	72	0.3	11.8	0.95	70	0.5	9	0.97	62	0.32
LU125	14.4	0.93	83	0.44	13.7	0.92	79	0.32	21.3	0.94	90	0.43	13.1	0.96	68	0.22
LU150	18.7	0.91	96	0.18	14.5	0.95	84	0.11	27.8	0.96	116	0.54	24.4	0.95	108	0.38
LU175	23.5	0.9	129	0.45	16.3	0.95	95	0.3	31.1	0.98	135	0.33	27.2	0.98	127	0.25
LU200	31.1	0.95	144	0.45	26.4	0.97	130	0.34	38.6	0.98	144	0.28	38.4	0.98	143	0.27
LU300	42.6	0.98	187	0.2	37.9	0.97	178	0.17	59.5	0.96	224	0.29	57.8	0.96	216	0.22
LB50	3.2	10.84	[1]33	11.00	3.1	10.82	[1]33	11.07	5.2	0.57	35	0.84	3.6	10.95	[1]33	10.67
LB75	11.6	0.88	66	0.54	7.2	0.74	50	0.65	10.1	0.86	62	0.56	5.6	0.61	44	0.44
LB100	11.8	0.91	72	0.44	10.8	0.92	70	0.28	14.8	0.94	79	0.63	11.6	0.91	71	0.37
LB125	15.3	0.97	83	0.49	13.9	0.97	81	0.37	20	0.96	94	0.3	16.1	0.98	81	0.23
LB150	22.5	0.95	105	0.31	20.3	0.95	102	0.21	28.2	0.95	113	0.53	20.8	0.95	101	0.22
LB175	31.4	0.98	128	0.37	27.2	0.96	117	0.32	28.6	0.98	123	0.33	29.1	0.98	130	0.27
LB200	39.3	0.91	152	0.38	30.2	0.72	97	0.54	29.9	0.97	125	0.27	21.6	0.93	94	0.26
LB300	58.2	0.97	212	0.25	58.4	0.95	209	0.25	65.3	0.94	254	0.22	60.2	0.93	240	0.2
EU50	5.6	0.71	35	1.12	2.4	0.82	23	0.71	4.1	0.91	34	0.77	2.5	0.93	29	0.51
EU75	7.7	0.83	53	0.43	5	10.96	[1]46	11.84	8.9	0.83	50	0.56	6	0.69	50	0.38
EU100	17.1	0.91	76	0.43	15.2	0.94	71	0.35	17.8	0.96	84	0.41	14.3	0.94	78	0.21
EU125	16	0.91	88	0.49	13	0.91	77	0.37	21.7	0.57	95	0.8	13.8	0.96	77	0.28
EU150	21.7	0.97	102	0.4	9.6	0.96	64	0.22	25.8	0.97	117	0.33	22.5	0.96	112	0.2
EU175	25.5	0.92	115	0.31	22.5	0.92	108	0.29	34.4	0.97	135	0.41	30.5	0.98	123	0.34
EU200	31.3	0.92	142	0.36	20.7	0.86	97	0.56	41	0.97	145	0.42	34	0.95	130	0.27
EU300	74.6	0.98	248	0.45	71.6	0.98	243	0.32	64.2	0.91	238	0.44	59.9	0.93	224	0.41
EB50	4.4	0.91	37	0.38	2.1	0.54	28	0.49	4.3	0.32	37	0.7	4	0.3	36	0.68
EB75	6.3	0.73	48	0.39	4.9	0.66	42	0.35	10.4	0.85	57	0.46	7.6	0.74	47	0.45
EB100	13	0.9	72	0.78	9.2	0.88	64	0.55	18.3	0.95	85	0.58	13.6	0.93	74	0.31
EB125	16.7	0.89	92	0.47	9.4	0.78	52	0.45	20	0.94	95	0.41	15.9	0.89	85	0.36
EB150	20.3	0.93	98	0.37	16.7	0.91	88	0.44	24.8	0.93	109	0.63	15.3	0.95	82	0.23
EB175	28.8	0.8	130	0.26	23	0.74	107	0.27	33.1	0.95	132	0.46	28.2	0.98	122	0.27
EB200	39.8	0.95	150	0.43	31.9	0.98	129	0.3	34.9	0.98	148	0.36	30.5	0.98	134	0.32
EB300	56.6	0.98	213	0.22	50	0.96	194	0.21	72.4	0.97	249	0.4	66.5	0.97	232	0.4

Figure 20 showed the relationship between the furnace temperature and the degrees-hour reached by soil in Laza samples. The slope indicated that unburned samples reached lower values of DH at the same temperature as the burned ones both under field or laboratory conditions. These data assessed that the susceptibility of samples to a new heating treatment depending on the fire recurrence (previous heating treatment or fire history in field conditions).

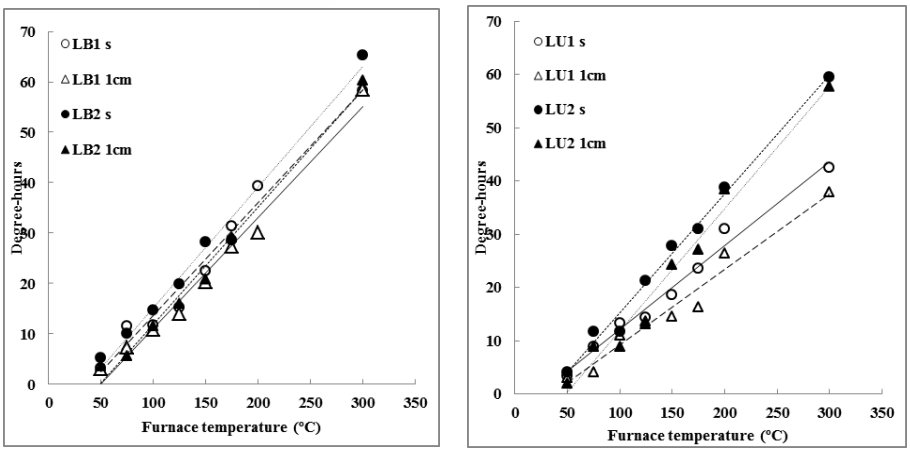

Figure 20. Relationship between the temperature applied to soil and the accumulated heat in the unburned (U) and burned (B) samples from Laza (L) soil subjected to thermal shock at different temperatures (50, 75, 100,125,150,175,200,300ºC) under laboratory conditions (1, first heating;2, second heating; a, surface; b, 1cm depth).

A total of 128 temperature-time curves were built, but an example of a temperature-time curve is shown in Figure 21. The surface temperature was higher than that reached at 1 cm depth due to the low heat transmission capacity of the soils (Terefe *et al*., 2008; Badía-Villas *et al*., 2014). For both soils, the parameters obtained from the exponential model (Tmax and ascending slope) and the degrees-hour reached vary depending on the previous history of soil. There are notable differences between the unburned and burned samples in field conditions and between samples heated unheated after a thermal shock (first and second heating in laboratory conditions). In most cases the Tmax and ascending slope were higher in burned than in unburned samples (p.e. Figure 21 showed that the ascending slope in samples heating at 300ºC were high in burned samples that unburned ones, as well as the maximum temperature reached by soil). Samples heated a second time also achieved higher temperature than those that were heated only once, (p.e. Figures 22 and 23 showed the temperatures reached and the ascending slope in unburned (Figure 22) and burned (Figure 23) samples heating once and twice). In other words, previously burned or heating samples reached higher temperatures and in a shorter time than those unburned. This indicates a cumulative effect of successive fires, given that the previous history of burning and/or heating alters the soil making it more vulnerable to subsequent heating or burning.

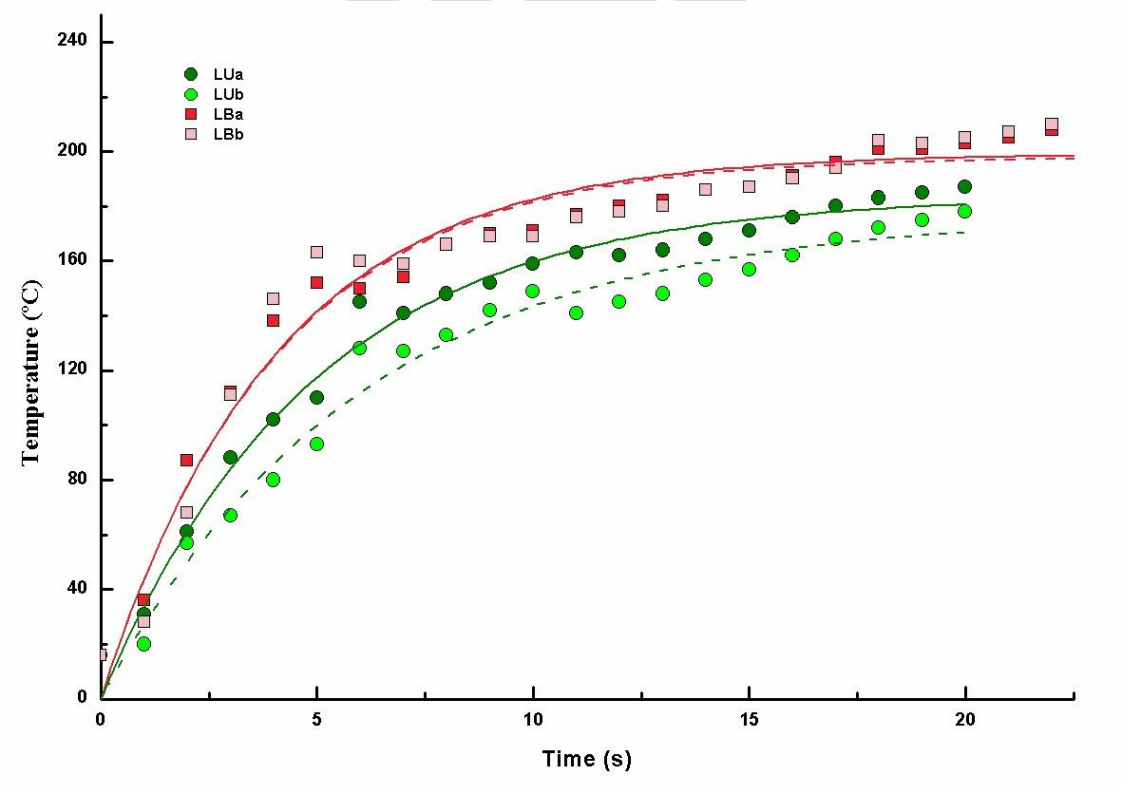

Figure 21. Temperature-time curves for the surface (a) and 1 cm depth (b) of samples from L soil subjected to thermal shock at 300°(U, unburned; B, burned; 1, first heating; 2, second heating). Effect of heating recurrence in field conditions.

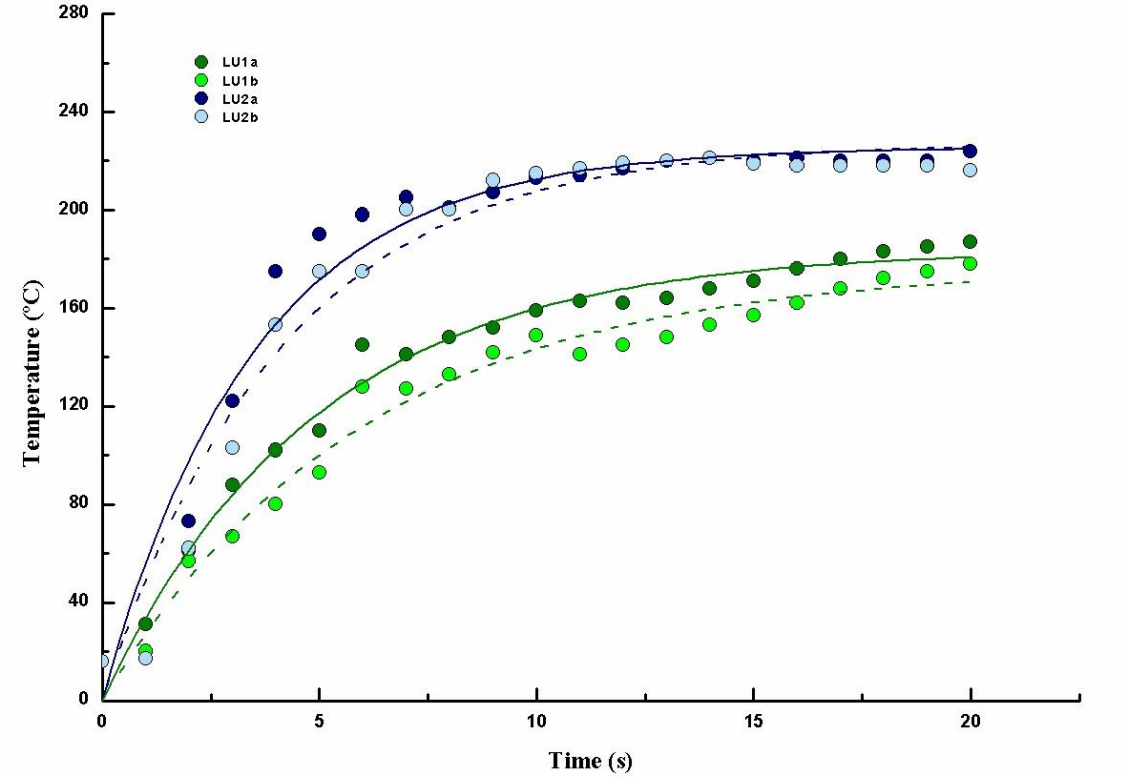

Figure 22. Temperature-time curves for the surface (a) and 1 cm depth (b) of unburned samples from Laza soil subjected to thermal shock at 300ºC. Effect of heating recurrence in laboratory conditions.

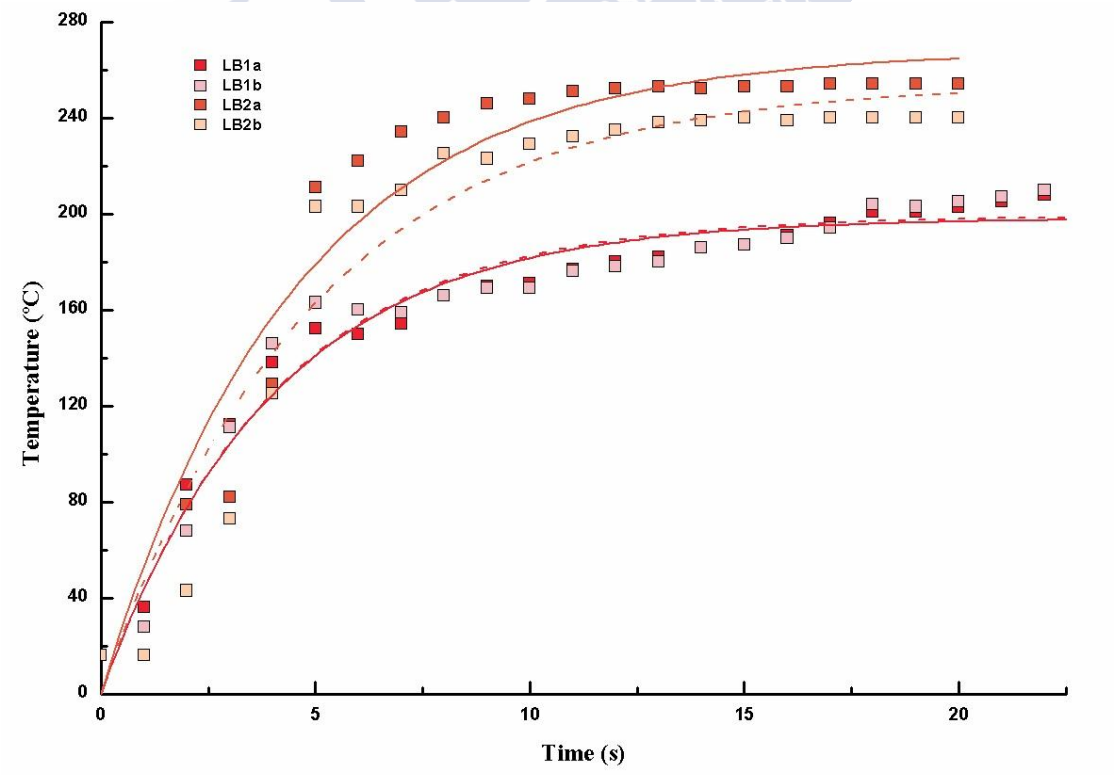

Figure 23. Temperature-time curves for the surface (a) and 1 cm depth (b) of burned samples from Laza soil subjected to thermal shock at 300ºC. Effect of heating recurrence in laboratory conditions.

Although information on how fire recurrence affects soil response to a new fire is very scarce, our data coincide with previous research indicating that the thermal properties of soil vary as a result of fire (Massman and Frank 2004; Verdes and Salgado, 2011; Rubio *et al*., 2012). Iverson and Hutchinson, (2002) and Massman *et al*., (2008) found that the surface temperature reached due to daily sun cycles is higher in the burned area than in the adjacent unburned area. Previous studies indicated that the properties that most influence the dynamics of soil heating are moisture and bulk density (Campbell *et al*., 1995; Busse *et al*., 2005; Rein *et al*., 2008; Cancelo-González *et al*., 2012; Rubio *et al*., 2012; Badia *et al*., 2017). However, in our study moisture remained constant indicating that there are other parameters involved. According to these data, the previous history of fires should be taken into account in decision making in the management of forest ecosystems to assess risks and reduce damage to the environment, especially in our region where the frequency of forest fires increases every year and where experimental fires are used in rural and forest areas as a tool for fire management and prevention. Previous studies show a negative effect of the recurrence of prescribed fires evidencing the loss of plant productivity and the reduction of the availability of soil nutrients (Eugenio *et al*., 2006; Ferrán *et al*., 2006).

5.2.3. Effect of fire regimen (severity and recurrence) on biochemical properties

The values of the enzymatic activities are represented by gram of soil organic matter.The minimum and maximum values of the biochemical properties analyzed of the unburned and burned soil of Laza and Estrada, heated in laboratory conditions, as well as those of control soil (unheated samples) are summarized in Table 14.

In Laza soil the β-glucosidase activity decreased as a consequence of the wildfire from 74.9 µg p-nitrophenol g^{-1} OM h^{-1} to 41.0 µg p-nitrophenol g^{-1} OM h^{-1}, the urease activity from 391 µg NH_4^+ g^{-1} OM h^{-1} to 287 µg NH_4^+ g^{-1} OM h^{-1} and the phosphatase activity from 2.654 µg *p*-nitrophenol g^{-1} OM h^{-1} to 2409 µg *p*-nitrophenol g^{-1} OM h^{-1}. In the Estrada soil, only urease activity showed a fire-induced decrease from 86.0 µg NH_4^+ g^{-1} OM h^{-1} to 42.4 µgNH_4^+ g^{-1} OM h^{-1}, while β-glucosidase and phosphatase activities increased from 53.4 *p*-nitrophenol g^{-1} OM h^{-1} to 66,7 µg *p*-nitrophenol g^{-1} OM h^{-1} and from 2279 µg *p*-nitrophenol g^{-1} OM h^{-1} to 2937 µg p-nitrophenol g^{-1} OM h^{-1}, respectively. Before the wildfire the values of the three selected enzymes were higher in the soil of Laza than Estrada, but both representative of the forest soils developed on acid rocks in the temperate-humid zone of NW Spain (Trasar-Cepeda *et al*., 2000; Álvarez *et al*., 2009; Díaz-Raviña *et al*., 2012; Fontúrbel *et al*., 2012; Barreiro *et al*., 2015). Field experiments have already demonstrated the effect of fire on enzymatic activities and their different sensitivity to fire. The fact that in low severity fire (Estrada) only urease is affected by fire confirms that it is one of the enzymatic activities most affected by fire, even low severity ones (Hernández *et al*., 1997; San Emeterio *et al*., 2016; Huffman and Madritch, 2018; Rodríguez *et al*., 2018).

Table 14. Maximum and minimum values of the biochemical properties analyzed in the different unburned (U) and burned (B) Laza (L) and Estrada (E) samples after the heating treatments and incubation period. C, control value; 1H, first heating; 1I, first incubation; 2H second heating; 2I, second incubation.

		Glucosidase (µg p-nitrophenol g^{-1} OM h^{-1})	Urease (µg NH_4^+ g^{-1} OM h^{-1})	Phosphatase (µg p-nitrophenol g^{-1} OM h^{-1})	Bacterial activity (10^{-14} mol Leu g^{-1} OM h^{-1})
Laza soil (unburnt)	C	74.9	391	2654	4049
	1H	67.6 - 99.7	38.3 - 365	244 - 2434	3284 - 7692
	1Hi	51.8 - 87.1	146 - 467	599 - 2930	8285 - 49775
	2H	20.1 - 65.4	0.0 - 318	161 - 1047	2895 - 17702
	2Hi	51.6 - 106	59.2 - 395	130 - 1095	2247 - 17660
Laza soil (burnt)	C	41.0	287	2409	6280
	1H	20.3 - 36.4	53 - 307	161 - 2973	987 - 10375
	1Hi	71.8 - 110	98.7 - 185	366 - 1966	7744 - 37047
	2H	36.0 - 92.2	94 - 441	14 - 720	5268 - 42367
	2Hi	41.9 - 77.6	0.0 150	37 - 778	3665 - 17718
A Estrada soil (unburnt)	C	53.4	86.0	2279	825
	1H	44.8 - 64.2	67.6 - 30.5	1338 - 2655	825 - 2197
	1Hi	56.4 - 99.4	14.9 - 33.9	601 - 2919	400 - 10705
	2H	63.9 - 139.3	0.0 - 27.9	566 - 1212	2444 - 31858
	2Hi	16.1 - 69.2	8.7 - 47.6	289 - 1429	1293 - 22590
A Estrada soil (burnt)	C	66.7	42.4	2937	5975
	1H	32 - 73.9	14.9 - 57.8	523 - 2671	486 - 10473
	1Hi	32.9 - 168.4	18.0 - 43.9	422 - 2359	2881 - 11550
	2H	198.2 - 59.7	0.0 - 28.7	189 - 1090	2645 - 16567
	2Hi	24.1 - 63.2	24 - 38.5	108 - 1040	3476 - 9989

The bacterial activity showed an opposite behavior to enzymatic activities. The bacterial activity suffered in both soil a marked increase as consequence of fire, especially in Estrada soil. In Laza soil samples increased from $4049x10^{-14}$ mol Leu g^{-1} OM h^{-1} to $6280x10^{-14}$ mol Leu g^{-1} OM h^{-1} and in Estrada soil samples from $825x10^{-14}$ mol Leu g^{-1} OM h^{-1} to $5975x10^{-14}$ mol Leu g^{-1} OM h^{-1}. This result is coincident with previous studies of Guerrero *et al*. (2005) on which heating the soil at medium temperatures produces a stimulation of bacteria.

In order to analyses and compare the effect of the different heating treatments in the two soils (Laza and Estrada) and samples (unburned, burned) the data were represented as the relative value respect to their control value (18ºC). Figures 24, 25, 26 and 27 represents the effect of each heating event (first heating, second heating), in different soil samples (Laza and Estrada, unburned and burned) at each heating event (first heating, second heating) respect to the DH reached by soil. No additional information is providing for the data of the incubation periods samples, so to facilitate the display, they are not showed. The vertical lines indicate the DH values, which the effect of heating started to be negative for each sample.

In general, the β-glucosidase activity showed a decreased in both soils and both unburned and burned samples as consequence of heating under laboratory conditions. The magnitude of the decreased depended of the degrees-hour reached and differed between different soils and the previous fire history of soils. In the unburned Laza samples and after the first heating event (1H) values were quite similar to the control (C) (67.6-99.7 µg *p*-nitrophenol g^{-1} OM h^{-1}, C:

74.9 µg *p*-nitrophenol g^{-1} OM h^{-1}) but the activity decreased after the first incubation period (1I) (51.8-87.1 µg *p*-nitrophenol g^{-1} OM h^{-1}). After the second heating treatment (2H) values were lower than the first heating (20.1-65.4 µg *p*-nitrophenol g^{-1} OM h^{-1}) and increased after the second incubation (2I) (51.6-105.7 µg *p*-nitrophenol g^{-1} OM h^{-1}) (Table 14). In burned Laza samples values decreased after the first heating event (20.3-36.4 µg *p*-nitrophenol g^{-1} OM h^{-1}; C: 41.0 µg *p*-nitrophenol g^{-1} OM h^{-1}) but a recovering was produced as consequence of the incubation period (71.8-110 µg *p*-nitrophenol g^{-1} OM h^{-1}). Nevertheless, after the second heating and incubation treatment, values showed a decreased (36.0-92.2 µg *p*-nitrophenol g^{-1} OM h^{-1}). In Estrada soil, both unburned and burned samples showed a decreased as consequence of the first heating (U1H: 44.8-64.2 µg *p*-nitrophenol g^{-1} OM h^{-1}, C: 53.4 µg *p*-nitrophenol g^{-1} OM h^{-1}), (B1H: 32.0-73.9 µg *p*-nitrophenol g^{-1} OM h^{-1}, C: 66.7 µg *p*-nitrophenol g^{-1} OM h^{-1}) followed by a slight recovery of the values. After the second heating event values were higher than the control ones (U2H: 63.9-139.3 µg *p*-nitrophenol g^{-1} OM h^{-1}; B2H: 59.7- 198 µg *p*-nitrophenol g^{-1} OM h^{-1}) but a marked decrease of values were detected after the second incubation (U2I: 16.1-69.2 µg *p*-nitrophenol g^{-1} OM h^{-1}; B2I: 24.1-63.2 µg *p*-nitrophenol g^{-1} OM h^{-1}).

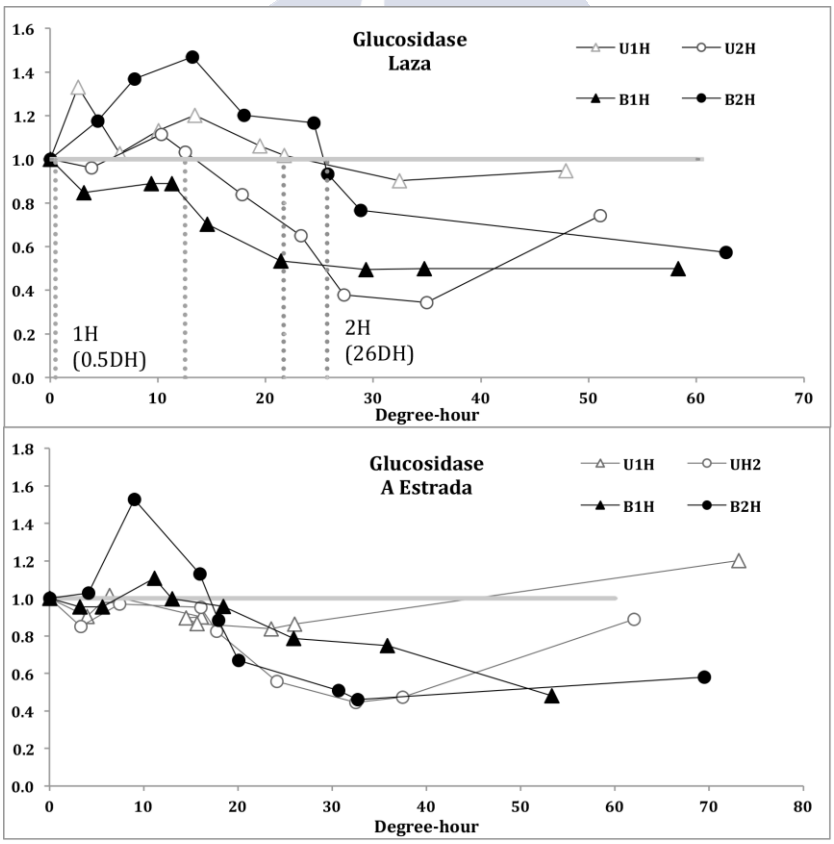

Figure 24. Effect of heating treatment under laboratory conditions on ß-glucosidase activity for the soil samples analyzed represented according the degrees-hour value reached by Laza and Estrada soil samples. U, unburned; B, burned; 1H, first heating; 2H second heating. The reference values in the control soil (18ºC) are indicated by horizontal lines. Data are expressed as relative values, heated soil/control soil: values higher than 1 denote a positive effect, values lower than 1 denote a negative effect of heating treatment.

Regarding the effect of the different heating treatments in burned and unburned samples, Figure 24 showed that in Laza unburned samples at low degrees-hour, a slight increase was produced. No appreciable negative effect of heating was shown until high temperatures (>22DH, >175 ºC). Nevertheless, after the second heating event, a negative effect happened at lower degrees-hour values (12.5 DH, 100ºC) than after the first heating event. The Laza burned samples showed a different behavior. After the first heating a negative effect was produced at all the treatments applied in a magnitude proportional to the heat applied to the samples. After the second heating event a positive effect was detected until 26 DH. The same tendency was observed for Estrada soil samples; no effect after the first heating in unburned samples and negative effect between 10-15 DH (75ºC) in burned ones. However, differences between unburned and burned samples were lighter than those observed in Laza samples, coinciding with the studies of Ajwa *et al*. (1999); Boerner *et al*. (2000); Fontúrbel *et al*., (2012), while when fire severity increases the β-glucosidase activity is more affected (Fultz *et al*., 2016), and activity values tended to recover with time in low severity fires (Moya *et al*., 2019; San Emeterio *et al*., 2016). Author as Fontúrbel *et al*., 2016; Fultz *et al*., 2016 related the values of β-glucosidase activity with moisture. Moisture decreased with heating and before incubation samples were reweeted, so that, the moisture could explain the recovery of the activity. Nevertheless, a third heating event (second heating in burned samples) produced a decreased after the incubation with values lower than the control ones indicating a negative effect of heating recurrence. The negative and cumulative effect on β-glucosidase activity coincides with previous studies by Eivazi and Bayan (1996), Boerner and Brinkman (2003), Gutknecht *et al*. (2010) and Rodríguez *et al*. (2018). In these studies, significant decreases in β-glucosidase activity are detected after several prescribed fires applied periodically on the same soil.

For urease activity data showed that in the four soils analyzed the heating treatment caused a decreased of 50-100% on urease activity values in temperatures above 75ºC, showing the great sensitivity of this enzyme to soil heating (Table 14). In Laza soil, after the first heating, the values decreased from 391 µg NH_4^+ g^{-1} OM h^{-1} to 38.3-365 µg NH_4^+ g^{-1} OM h^{-1} in unburned samples and from 287 µg NH_4^+ g^{-1} OM h^{-1} to 53-267 µg NH_4^+ g^{-1} OM h^{-1} in burned samples. After incubation, the values increased (U: 146-467 µg NH_4^+ g^{-1} OM $h^{-1,}$ B: 98.7-185 µg NH_4^+ g^{-1} OM h^{-1}). After the second heat treatment both Laza soils showed a decrease and even an inhibition of the activity at the highest temperatures (U: 0.0-318 µg NH_4^+ g^{-1} OM h^{-1}, B: 94-441 µg NH_4^+ g^{-1} OM h^{-1}). After the second incubation, unburned samples recovered activity (59.7-395 µg NH_4^+ g^{-1} OM h^{-1}) while burned samples remained below the control (0-150 µg NH_4^+ g^{-1} OM h^{-1}). Regarding the effect of the different thermal treatments, Figure 25 shows that in the unburned samples of Laza, a negative effect was observed from 10 DH and was maintained during incubation. After the second treatment the negative effect was stronger, reaching the total inhibition when > 20 DH was reached. In the burned Laza samples the same tendency was observed and negative effect occurred at lower DH (3-5 DH) although there is no total inhibition of the activity. Estrada soil showed the same tendency as those in Laza, however, there was a positive effect at low temperatures in the burned samples.

Previous studies have shown a decrease in urease activity as a result of fire (Saa *et al*., 1993; Staddon *et al*., 1997; Boerner *et al*., 2000; Miesel *et al*., 2007; Hamman *et al*., 2008; Fontúrbel *et al*., 2012; Xue *et al*., 2014; Rodríguez *et al*., 2018), even at low temperatures (Hernández *et al*., 1997; San Emeterio *et al*., 2016; Huffman and Madritch, 2018).

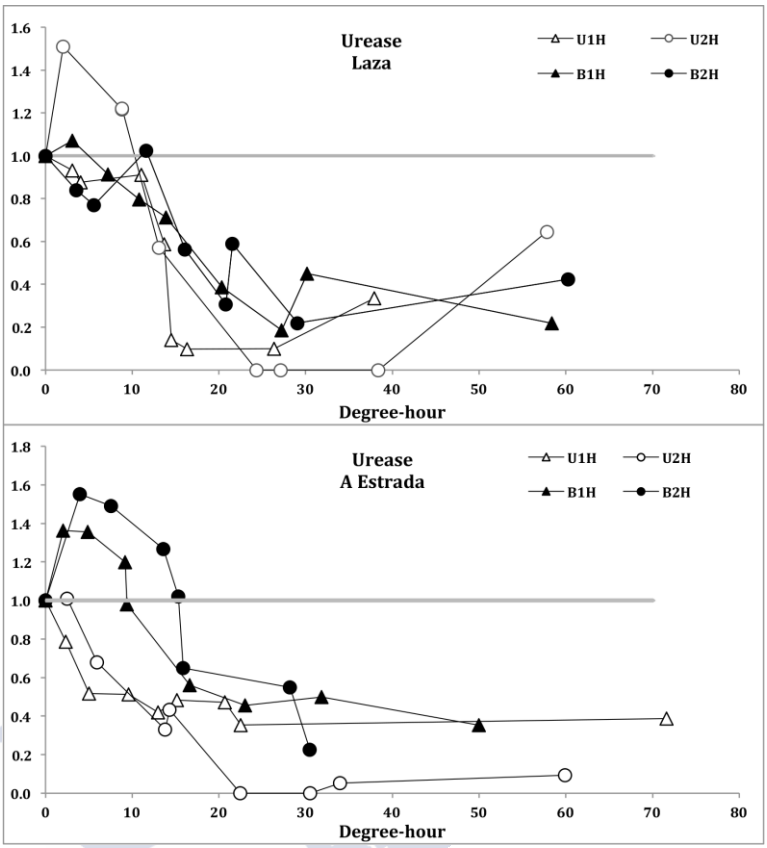

Figure 25. Effect of heating treatment under laboratory conditions (50ºC-300ºC) on urease activity for the soil samples analyzed represented according the degrees-hour value reached by Laza and Estrada soil samples. U, unburned; B, burned; 1H, first heating; 2H second heating. The reference values in the control soil (18ºC) are indicated by horizontal lines. Data are expressed as relative values, heated soil/control soil: values higher than 1 denote a positive effect, values lower than 1 denote a negative effect of heating treatment.

The reduction of urease activity could be due to a progressive decrease in activity at high substrate concentrations. Increases of NH_4^+ have been reported after soil heating (Certini, 2005; Prokushkin and Tokareva, 2007) due to release from protein-like components of organomineral complex and organic matter when temperatures above 100ºC are reached or thermal decomposition of nitrates at temperatures up to 150ºC (Prieto-Fernández *et al*., 1998). The increased of the urease activity reported at low temperatures (< 125 ºC) probably was due to those are the optimal temperatures for these activities (Karaca *et al*., 2011) since the actual temperatures reached by soil were lower than the muffle one.

Regarding the phosphatase activity, after heating the activity values tended to decreased in unburned and burned samples of Laza and Estrada. For Laza soils, values decreased from 2654 µg *p*-nitrophenol g^{-1} OM h^{-1} to 244-2434 µg *p*-nitrophenol g^{-1} OM h^{-1}, in unburned samples and in burned ones from 2409 µg *p*-nitrophenol g^{-1} OM h^{-1} to 161-2873 µg *p*-

nitrophenol g^{-1} OM h^{-1}. After the first incubation, the values showed an increase. The values were lower after the second heat treatment (Laza; U: 161-1047 µg p-nitrophenol g^{-1} OM h^{-1}, B: 14-720 and Estrada; U: 566-1212, B: 189-1090 µg *p*-nitrophenol g^{-1} OM h^{-1}). These values were maintained after the second incubation indicating the cumulative negative effect of heat on microbial activity. In the first heat treatment, the negative effect appeared at temperatures higher than 15 DH in unburned samples and in temperatures higher than 10 DH for burned ones. However, after the second heating the negative effect appeared earlier, at 10 DH in unburned samples, and in all treatments in burned samples (Figure 26).

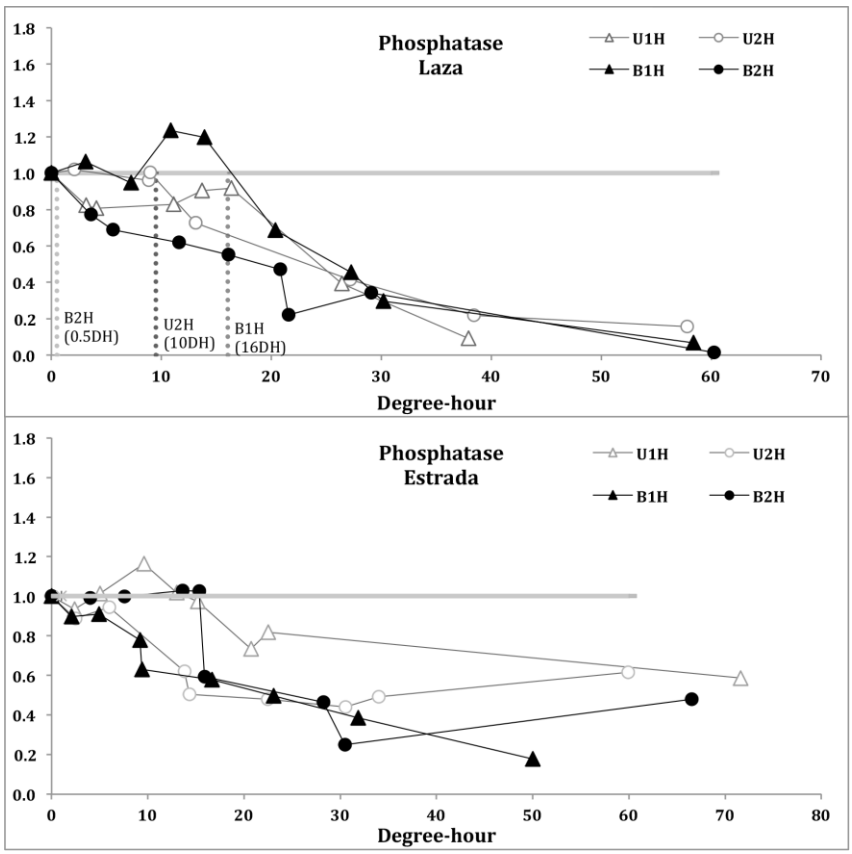

Figure 26. Effect of heating treatment under laboratory conditions (50°C-300°C) on phosphatase activity for the soil samples analyzed represented according the degrees-hour value reached by Laza and Estrada soil samples. U, unburned; B, burned; 1H, first heating; 2H second heating. The reference values in the control soil (18°C) are indicated by horizontal lines. Data are expressed as relative values, heated soil/control soil: values higher than 1 denote a positive effect, values lower than 1 denote a negative effect of heating treatment.

The results obtained agree with studies where phosphatase activity is strongly affected by wildfires (Saa *et al*., 1993; Staddon *et al*., 1997; Boerner *et al*., 2000; Miesel *et al*., 2007; Hamman *et al*., 2008; Xue *et al*., 2014). This decrease may have been due to a high assimilable P content that decreases the need for microorganisms to use this enzyme (Hamman *et al*., 2008).

The reduction of phosphatase activity was proportional to the degrees-hour applied to the soil. Pourreza *et al*. (2014) demonstrated that the degree of fire impact could efficiently represent by soil phosphatase activity. The negative effect on the phosphatase activity after the second heat treatment was more marked, indicating the influence of previous history on soil response. Authors as Eivazi and Bayan (1996) and Boerner and Brinkman (2003) also detected that recurrence produced a decreased in this activity. The reduction in phosphatase activity could be due to elevated available soil P that inhibits the microbial production of the enzyme (Hamman *et al*., 2008; López-Poma and Bautista, 2014). Also, the reductions of soil moisture after fire produced a marked negative impact on phosphatase activity (López-Poma and Bautista, 2014; Fontúrbel *et al*., 2016).

Fernández-García *et al*. (2018) found that urease, β-glucosidase and phosphatase activity were affected by fire and the magnitude of change were related with fire severity. The influence of severity on the response of enzymatic activities was confirmed by the correlation between the activity values and the DH reached by soil. β-Glucosidase activity showed a negative correlation with DH values on both soils (Laza, R: -0.640; Estrada, R: -0.710, $p<0.001$). as well as the phosphatase activity (Laza, R:-0.673, Estrada, R:-0.679, $p<0.001$). For urease activity only the Laza samples showed a negative and significant correlation with the DH, considering separately the burned and unburned samples (Laza, U:-0.880, B:-0.762).

In general, heating under laboratory conditions produced an increase in bacterial activity, especially after incubation period. In general, heating under laboratory conditions produced an increase of the bacterial activity, especially after the incubation period. Values at the end of the experiment were up to 27 times respect the control ones (LU: from 4049 to 2247-17660x10^{-14} mol Leu g^{-1} OM h^{-1}, LB: from 6280 to 3665-17718x10^{-14} mol Leu g^{-1} OM h^{-1}, EU: from 825 to1293-22590x10^{-14} mol Leu g^{-1} OM h^{-1}, EB: from 5975 to 3476-9989x10^{-14} mol Leu g^{-1} OM h^{-1}) (Table 14). Figure 27 shows the effect of the different heating treatments on bacterial activity. The first and the second heating were represented separated in order to facilitate the display. During the first heating event, soil samples showed a positive effect of heating until 20 DH for Laza soil and 15 DH for Estrada soil. At higher temperatures, the effect was negative, with bacterial activity reductions of 80%, which differed between unburned and burned samples of both soils, being the burned ones negatively affected at temperatures lower than the unburned ones. After incubation, these values tended to recover and a positive effect occurred. After the second heating treatments no effect was found until 10 DH, where the effect became positive even at the highest temperatures. As happened after the first incubation period the activity tended to recover. No significant correlation between the degrees-hour reached by soil and bacterial activity values was found. This decreased of bacterial activity at higher temperatures is coincident with finding of authors as Díaz-Raviña *et al*. (1996) and Bárcenas-Moreno and Bååth (2009). The increased at low and medium temperatures and after the incubation periods, could be partly attributed to the increased of carbon due to death of microorganism as consequence of heating. Some studies showed a stimulation of respiration, microbial activity and biomass at low and medium temperatures. (Saa *et al.*, 1993; Guerrero *et al*., 2005). Studies on the effects of heating on bacterial responses to fire by means of abundance of cultivable microorganisms (Bollen, 1969;

Vázquez *et al*., 1993; Badía and Martí, 2003; Guerrero *et al*., 2005; Mabuhay *et al*., 2006) or PLFA composition (Bååth *et al*., 1995; Ponder *et al*., 2009) have also indicated that bacteria recover fast and reached high levels after a heating event.

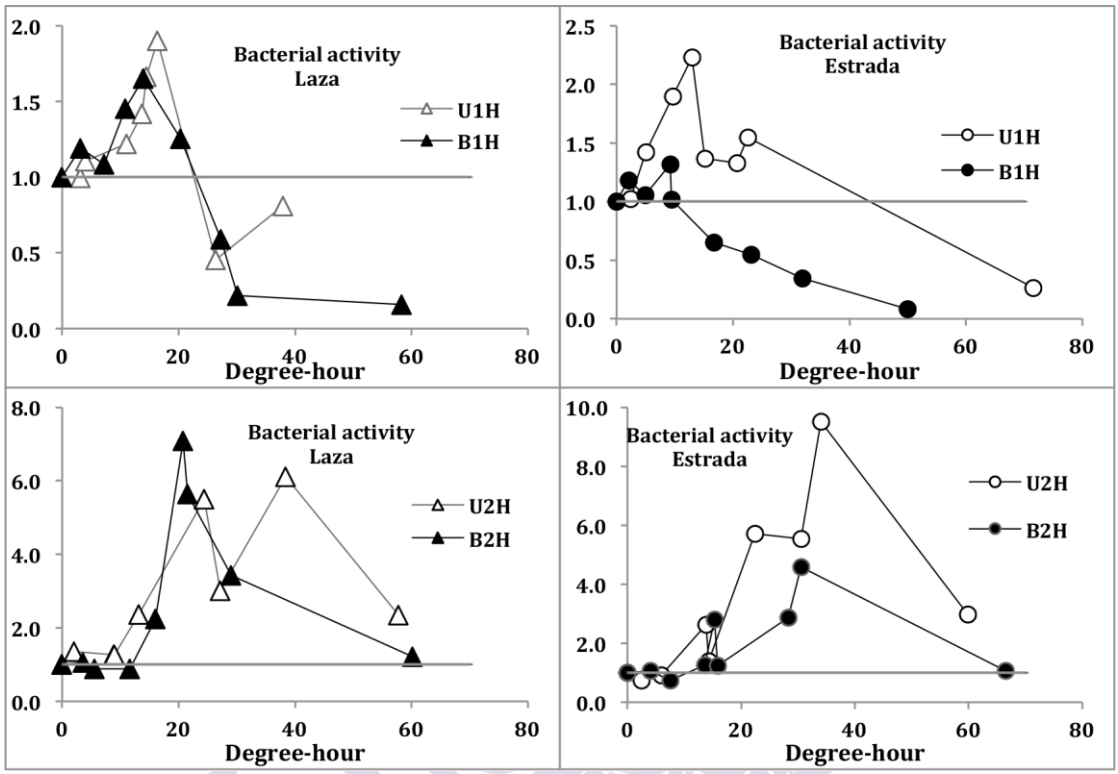

Figure 27. Effect of heating treatment under laboratory conditions (50ºC-300ºC) on bacterial activity for the soil samples analyzed represented according the degrees-hour value reached by Laza and Estrada soil samples. U, unburned; B, burned; 1H, first heating; 2H second heating. The reference values in the control soil (18ºC) are indicated by horizontal lines. Data are expressed as relative values, heated soil/control soil: values higher than 1 denote a positive effect, values lower than 1 denote a negative effect of heating treatment.

The different behavior observed between microbial and bacterial activity can be explained on the basis of the different information obtained from those microbial indexes. The higher microbial growth values estimated by the incorporation leucine technique, detected after the heating were due to the fact that only bacteria are able to incorporate this substrate. In contrast, enzyme determinations quantify both bacteria and fungi activity. Several studies found that fungi and bacteria differ in their sensitivity to temperature (Bollen, 1969; Bååth *et al*., 1995; Mataix-Solera *et al.*, 2009), being fungi considered more sensitive than bacteria (Dunn et al., 1985; D'Ascoli *et al.*, 2005). Enzyme activity showed a negative response to fire because fungi, the main group contributing to both biomass and metabolic activity, decreased notably after the heating. This probably masked the positive fire effect onthe enzymatic activities of soil bacteria. The results also support that bacteria rather than fungi are favored by post-fire conditions (Vázquez *et al*., 1993; Mataix-Solera *et al*., 2009; Bárcenas-Moreno *et al*., 2011; Lie *et al*., 2014; Pourreza *et al*., 2014; Muñoz-Rojas *et al*., 2016).

5.2.4. Effect of fire regime (severity, recurrence) on phospholipid fatty acid analysis

Table 15. Range of values for total biomass (Total PLFA), fungal biomass (Fung PLFA), bacterial biomass (Bact PLFA), Gram negative bacteria biomass (G- PLFA), Gram positive bacteria biomass (G+ PLFA), actinobacteria biomass (Act PLFA) and fatty acid biomass 16:1ω5 characteristic of mycorrhizae; and ratio between fungi and bacteria (Fung/Bact) and ratio of gram-negative and Gram-positive bacteria (G-/G+) in the different heated samples.(LU, unburned Laza; LB, burned Laza; EU, unburned Estrada; EB, burned Estrada).

	First cycle				Second cycle			
	Heating		Incubation		Heating		Incubation	
	LU	LB	LU	LB	LU	LB	LU	LB
TotPLFA (nmol g^{-1}OM)	374-758	210-416	344-803	193-424	358-760	174-504	524-826	162-379
FungPLFA (nmol g^{-1}OM)	77-158	28-59	53-163	26-69	46-158	28-80	68-196	25-57
BactPLFA (nmol g^{-1}OM)	147-296	87-166	144-330	75-165	159-292	70-212	189-298	64-172
G-PLFA (nmol g^{-1}OM)	76-140	38-78	67-135	31-68	70-169	34-95	84-155	30-77
G+PLFA (nmol g^{-1}OM)	47-93	34-60	49-132	30-71	64-94	26-95	68-101	22-72
ActPLFA (nmol g^{-1}OM)	46-90	34-65	48-124	31-77	69-95	38-91	66-95	26-81
16:1ω5 (nmol g^{-1}OM)	6.3-13.7	3.6-7.0	5.3-14.4	3.4-8.6	4.6-14.3	3.0-10.2	7.0-14.5	3.7-6.8
Fungi/Bacteria	0.45-0.56	0.32-0.42	0.36-0.6	0.27-0.42	0.25-0.56	0.21-0.39	0.27-0.52	0.23-0.39
G^-Bacteria/G^+Bacteria	1.37-1.79	1.07-1.39	1.0-1.45	0.9-1.05	0.93-1.76	0.86-1.33	1-1.79	1.4-1.37
	EU	EB	EU	EB	EU	EB	EU	EB
TotPLFA (nmol g^{-1}OM)	274-449	331-628	292-693	283-731	299-518	305-482	277-693	288-625
FungPLFA (nmol g^{-1}OM)	24-39	25-57	27-48	24-58	23-40	22-33	18-56	25-56
BactPLFA (nmol g^{-1}OM)	115-193	150-277	125-298	125-322	159-246	136-232	126-306	129-275
G-PLFA (nmol g^{-1}OM)	46-70	58-109	46-108	49-121	52-72	52-80	43-111	46-110
G+PLFA (nmol g^{-1}OM)	39-75	52.94	49-112	44-123	52-97	49-89	51-116	44-96
ActPLFA (nmol g^{-1}OM)	42-66	53-100	49-111	47-128	53-92	52-82	50-116	44-99
16:1ω5 (nmol g^{-1}OM)	4.1-6.0	5.9-11.0	4.1-10.4	4.9-11.3	4-6.1	4.4-6.7	3.2-8.7	3.5-10.4
Fungi/Bacteria	0.18-0.24	0.17-0.21	0.15-0.22	0.17-0.20	0.11-0.22	0.13-0.19	0.13-0.22	0.15-0.21
G^-Bacteria/G^+Bacteria	0.93-1.18	1.06-1.16	0.89-1.05	0.97-1.12	0.68-1.06	0.88-1.13	0.85-1.03	0.97-1.15

The estimated values of microbial biomass and biomass of the specific groups expressed per gram of soil organic matter can be seen in Table 15. Total biomass was higher in unburned soils than in burned soils, especially in Laza soil (Laza unburned: 2010 nmolg^{-1} OM, Laza burned: 1210 nmol g^{-1} OM; Estrada unburned: 1170 nmol g^{-1} OM; Estrada burned: 1082 nmol g^{-1} OM). The biomass of the specific groups shows the same trend. The biomass of the specific groups decreased in the Laza samples by 17-54% while in Estrada the decrease was of 2-18% confirming what we observed in other experiences: samples affected by a fire of high severity was strongly affected while a minor impact is observed in those burned in a fire of low severity (Barreiro *et al*., 2010; Díaz-Raviña *et al*., 2012; Fontúrbel *et al*., 2012; Vega *et al*., 2013). The percentage of PLFA characteristic of bacteria, actinobacteria and $Gram^+$ bacteria increased in both soils (3-4% for Laza and 1% in Estrada) due to fire, while PLFA characteristic of fungi and $Gram^-$ bacteria decreased (3-5% in Laza and 1% in Estrada) indicating that the relative abundance of the different groups differs between burned and unburned samples. This is confirmed by the fungi/bacteria and $Gram^-/Gram^+$ indices which in

the burned samples (LB: 0.39 and 1.27; EB: 0.19 and 1.05) were much lower than in the unburned samples (LU: 0.54 and 1.65; EU: 0.39 and 1.27) confirming the effect of fire on the composition of the microbial community.

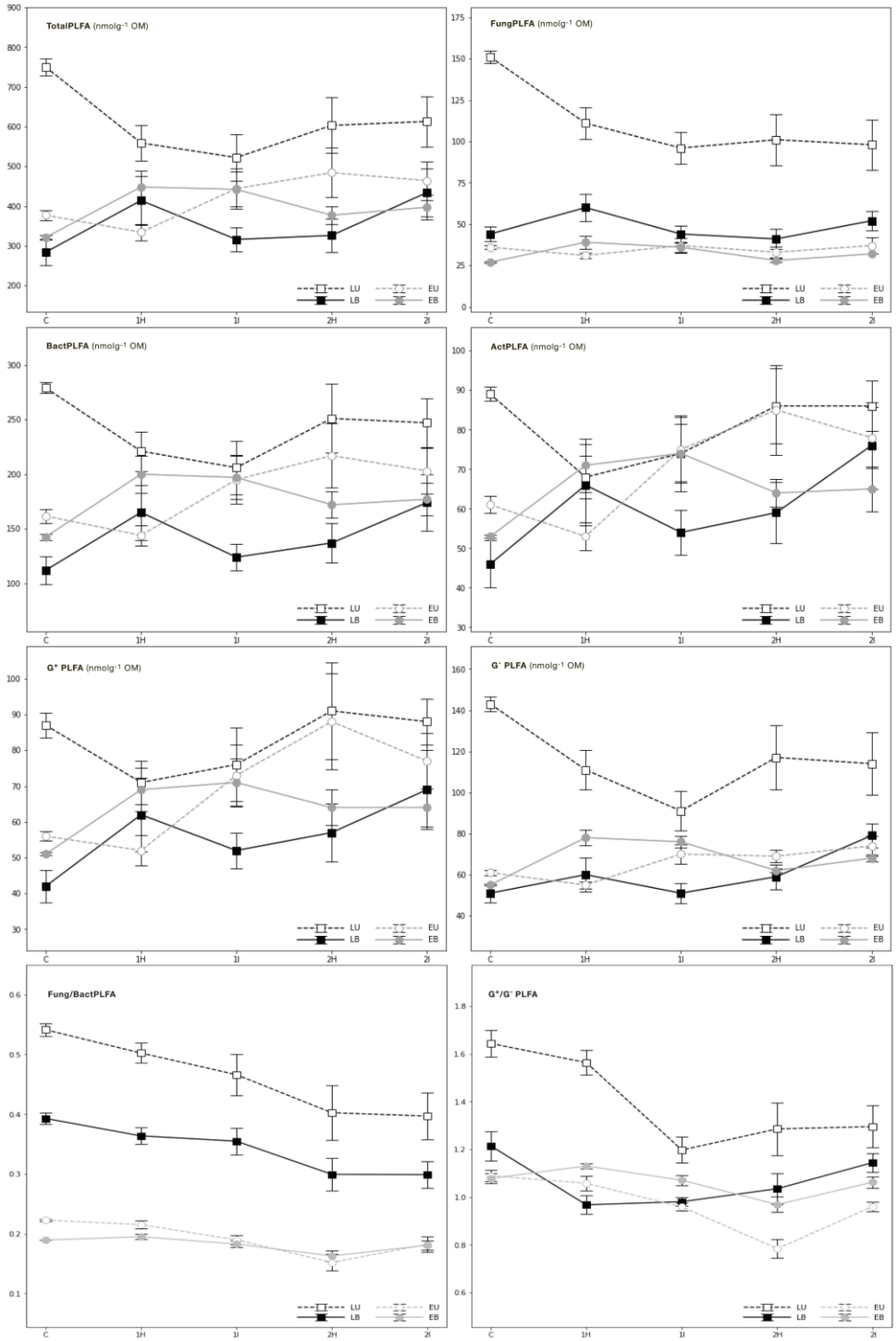

Figure 28. Total biomass and biomass of the specific groups and Fungi/bacteria PLFA and G-/G+ PLFA index of the different unburned (U) and burned (B) samples in Laza (L) and Estrada (E) (mean value ± SE, n= 8). C, Control; 1H, first heating; 1I, first incubation; 2H, second heating; 2I, second incubation.

Figure 28 shows the evolution of the values of total biomass and biomass of specific groups of microorganisms and the fungal/bacterial and $Gram^{-}/Gram^{+}$ ratios in the samples of Laza and Estrada unburned and burned soils during the two heating-incubation cycles. The results showed that some tendencies were found as consequence of recurrence, and a different behavior was observed for unburned and burned samples. Biomass values in burned samples were slightly higher after the first heating treatment, whereas values decreased in the unburned ones, suggesting different susceptibility of the unburned and burned soil to fire recurrence. Thereafter, for each soil values changed during the following heating incubation cycle and, thus, biomass values were quite similar among the different soil treatments. The microbial index FungPLFA/BactPLFA and $Gram^{-}$PLFA/$Gram^{+}$PLFA showed a continuous decreased due to the heating treatments. The magnitude of the decreased of fungi and G^{-} bacteria were proportional to the temperature applied to the soil since a negative and significant relationship between the degrees-hour and the biomass index (FungPLFA/BactPLFA: -0.564, $p<0.001$; $Gram^{-}$PLFA/$Gram^{+}$PLFA: -0.3578, $p<0.01$) were found. Our findings indicated that even though no changes were detected in the absolute values of biomass due to different severities of heating, the proportion between groups varied. These data confirm the greater sensitivity to the heating of fungi and Gram- bacteria against other microbial groups (Barreiro *et al*., 2015; 2016b; Bárcenas-Moreno *et al*., 2011; Guerrero *et al*., 2005; Holden *et al*., 2013; Ponder *et al*., 2009). As we observed in Fragas do Eume samples, although the estimation of biomass with the PLFA method is not appropriate to detect the changes produced in the soil due to fire, the proportion between the groups does seem useful to this purpose.

The principal component analysis (PCA) performed with the whole PLFA data (Figure 29) allowed to separate the samples according to the type of soil (Laza, Estrada) and the recurrence of fire, especially in the case of Laza. Factors 1 and Factor 2 accounted for 51% of the variance. PLFA pattern could discriminate between microbial communities from different soil types (Bossio *et al*., 1998; Stone *et al*., 2015). This differences could be as consequence of the type of parent material as suggested Yarwood *et al*. (2015) or due to the different plant community (Mahía *et al*., 2011) or both (Garbeva *et al*., 2004). Díaz-Raviña *et al*. (2006) observed in soils from the same area as this study, that those developed over granite (as Estrada soil) had higher abundance of branched fatty acid and smaller fraction of fungi than soils developed over schist (as Laza soil, developed over Phillites). Bárcenas-Moreno *et al*. (2016) also observed that the response of microbial community to fire was different according the plant community. Estrada samples are characterized by high concentrations of PLFA 10Me16:0a, 10Me17:0 and 10Me18:0 indicative of actinobacteria and br17:0, br18:0, i16:0, i17:0 indicative of G^{+} bacteria. Laza samples showed high concentrations of PLFA 16:1ω7t, 16:1ω9, 17:1ω8, 18:1ω7 and PLFA 18:2ω6, 18:1ω9, 16:1ω5, indicative of fungi (Figure 29). The samples of Estrada varied less than those of Laza as a consequence of the fire as indicated by the greater separation along F1 in the samples of Laza and the homogeneity and grouping of those of Estrada. This result indicates that the effect of the experimental fire on the soil microbial community of Estrada was masked by the effect of the soil type due to the lower temperatures reached.

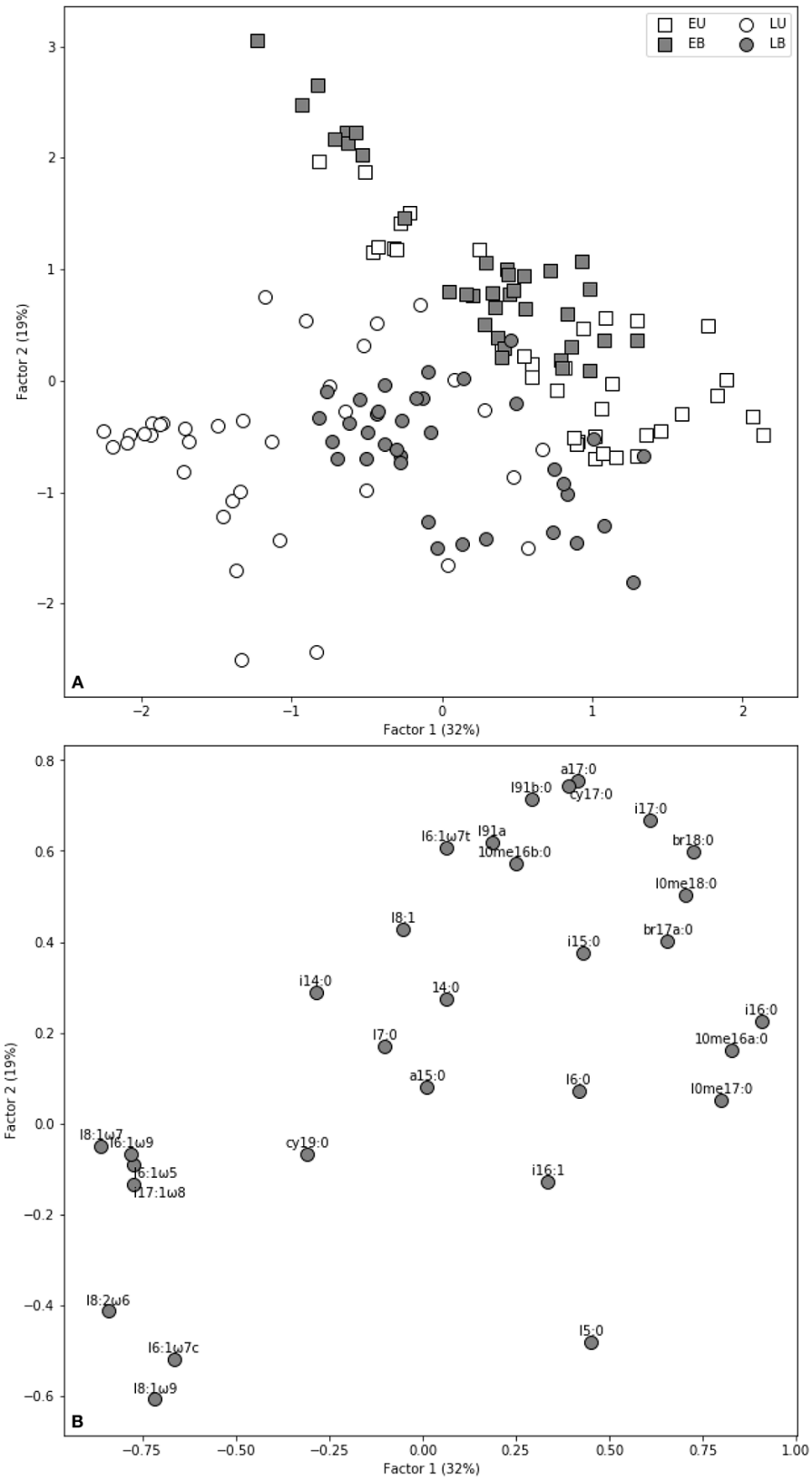

Figure 29. Sample distribution (A) and variable distribution (B) of the PCA results of the whole PLFA data set from Laza (L) and Estrada (E) unburned (U) and burned (B) samples heated at different temperatures in each heating and incubation time. L, soil affected by a wildfire; E, soil affected by an experimental fire.

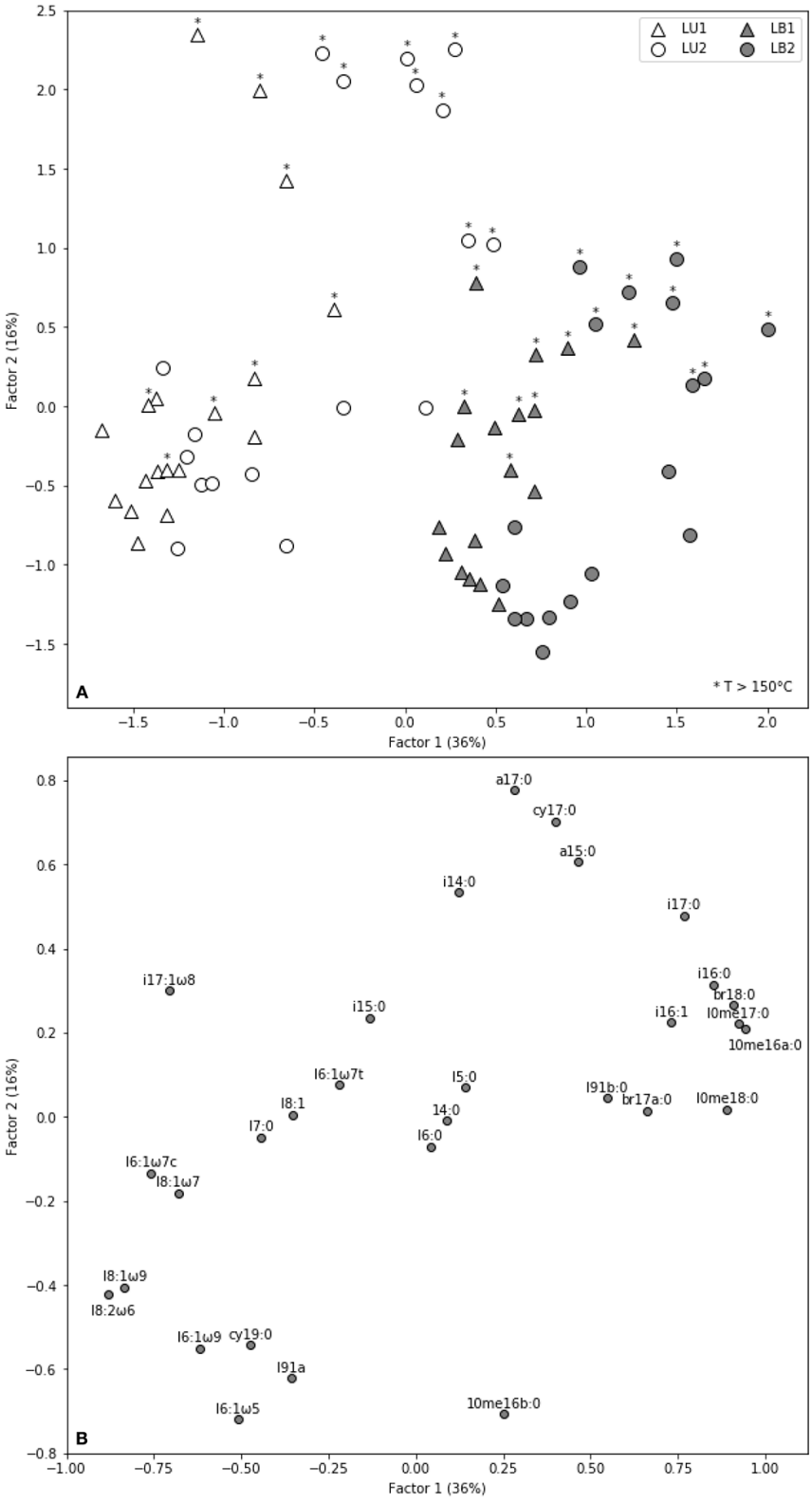

Figure 30. Distribution of samples (A) and variables (B) of the PCA result obtained with PLFA data from unburned (U) and burned (Q) Laza samples heated to different temperatures in each heating and incubation treatment. 1, first heating; 2, second heating; I, incubation. * indicates that the applied temperature is above 150°C.

To analyse the influence of the fire history and severity in the response of the microbial community to heating without the masking effect of soil type, the individual PCAs of each soil were made. In Laza soil (Figure 30), the samples were separated according to fire severity and recurrence (both in the field and in the laboratory). The first factor (F1), which explained 36% and separated the samples depending on the fire history, separated the burned samples from the unburned ones and within each of these groups, allowed us to separate to a lesser extent the samples according to the number of heat treatments applied. Samples that suffered more heat treatments presented higher values of factor 1 following the order; LU1 (one heat treatment) < LU2 (two heat treatments) < LB1 (two heat treatments, one of high severity) < LB2 (three heat treatments, one of high severity). LU samples with negative values of Factor 1, showed relatively high concentrations of PLFA characteristic of fungi (18:2ω6, 18:1ω9) and G- bacteria (16:1ω7c, 18:1ω7). While the LB samples with positive values of Factor 1, presented PLFAs characteristic of actinobacteria 10Me16:0a, 10Me17:0, 10Me18:0 and PLFA of bacterial origin (i16:0, i16:1, i17:0, br17:0, br18:0). The Factor 2 separated the samples according to the severity of the heating treatment applied and explained 16%. Samples heated to temperatures ≥ 150ºC (positive Factor 2 values) of samples heated to < 150ºC (negative Factor 2 values). Factor 2 showed a significant positive correlation with the degrees-hour values obtained ($r = 0.74$, $n = 72$, $P < 0.001$), indicating the effect of the heat applied on the samples (Figure 30). Samples heated to temperatures above 150ºC showed higher concentrations of a17:0, cy17:0, a15:0 and i14:0, while samples heated to temperatures below 150ºC had higher concentrations of 16:1ω5, 16:1ω9, 19:1a, 10Me16b:0 and cy19:0.

The PCA performed with the Estrada samples indicated that the main differences were due to the recurrence of the fire and the intensity of the heat treatment (Figure 31). Factor 1 explained 24% of the variance and separated burned samples (positive values of Factor 1) from unburned samples (negative values of Factor 1). The unburned Estrada samples presented high concentration of i16:1, br17:0a, i16, 16:1ω7t and the burned ones were richer in concentration of cy17:0, i17:0, 16:1ω7c, 16:1ω5, 10Me18:0. The Factor 2, that explained 21% of the variance, separated the samples according to the intensity of the heat treatment in laboratory conditions. As happened for Laza samples, Factor 2 showed a significant correlation, in this case negative with DH values ($r = -0.73$, $n:72$, $P < 0.001$). Samples heated to low temperatures (< 150ºC) showed higher concentrations of PLFAs 18:2ω6, 18:1ω9, 16:1ω5 characteristic of fungi and 18:1ω7, 19:1a, cy19:0, 17:0, 16:0. Samples heated to temperatures ≥ 150 ºC, located in the negative part of the Factor 2 axis showed an increase of PLFAs, 10Me16:0a, i15:0, a15:0, a17:0 (Figure 31). In the case of Estrada, it is Factor 3, which explained 20% of the differences, which allows differentiating between the first and second heating in the laboratory. The samples that suffered a heat treatment were placed in the positive part of the axis while those that suffered two treatments did it in the negative part of the Factor 3 axis. The effect of severity and recurrence in the laboratory is more intense in Estrada (fire severity and recurrence under laboratory conditions explained 41% of the differences), since in Laza the effect of the two heating is of less importance than that produced by the previous high severity fire. The fire under field conditions produced such a large change in the microbial community that it masked the effect of laboratory heating on

microorganisms.

Our findings are coincident with previous studies where the magnitude of changes in the microbial community was related with the severity of fire (Brown *et al*., 2019; Reazin *et al*., 2016; Weber *et al*., 2014). Thus, microbial community structure was strongly affected by wildfire (Vázquez *et al*., 1993; Bååth *et al*., 1995; Díaz-Raviña *et al*., 2006; 2012; Fultz *et al*., 2016) and moderate changes to the microbial community structure were detected as consequence of low intensity prescribed fire (Ponder *et al*., 2009; Fontúrbel *et al*., 2012; Williams *et al*., 2012; Barreiro *et al*., 2016b; 2016a; Fultz *et al*., 2016). The significant correlation observed between Factor 2 of both Laza (0.74, $p<0.001$) and Estrada (-0.73, $p<0.001$) PCA and degrees-hour reached by soils (Figure 32) indicates that both temperature and time of heat retention are important in determining the impact of fire on edaphic microbial communities.

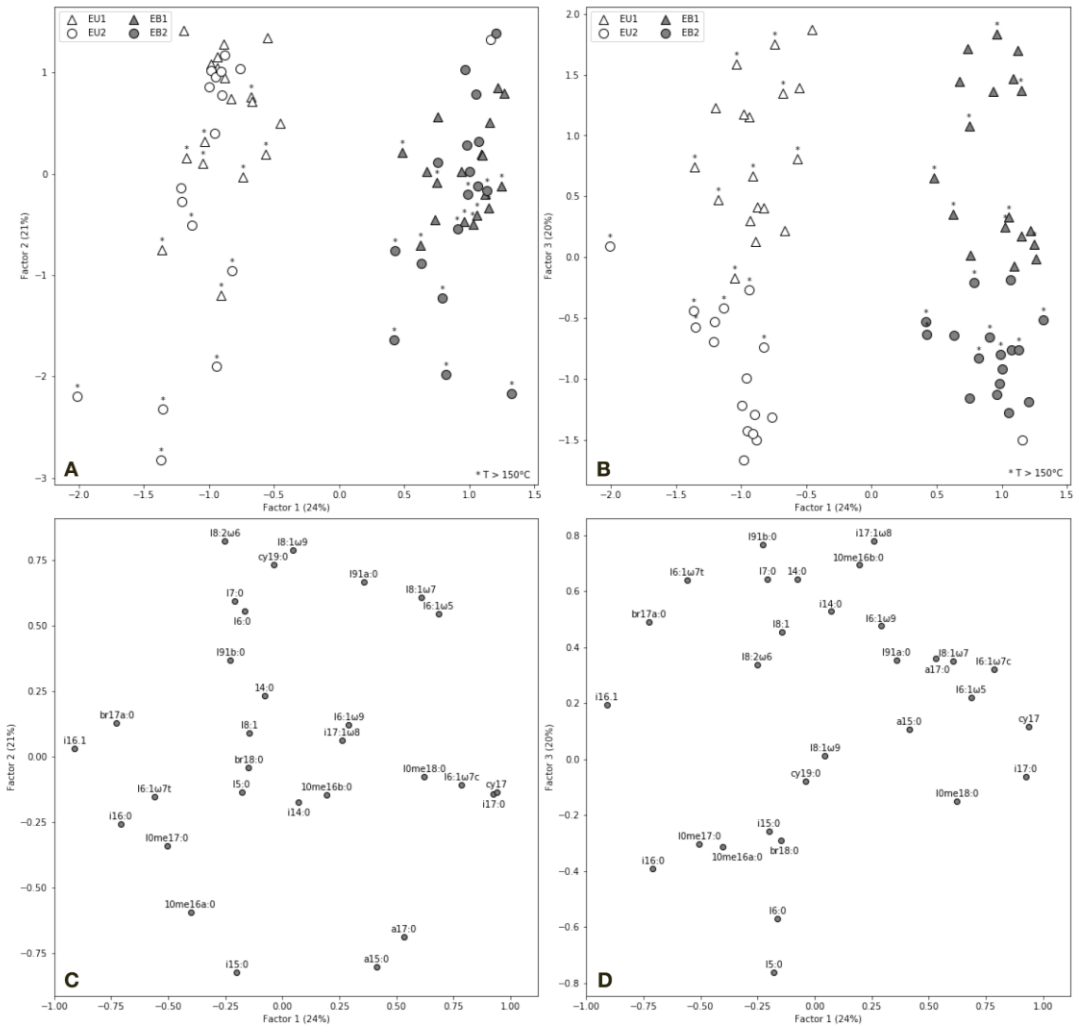

Figure 31. Sample distribution (A, B) and variable distribution (C, D) of the PCA results of the whole PLFA data set from Estrada unburned (EU) and burned (EB) samples heated at different temperatures in each heating and incubation time. 1, first heating; 2, second heating. * Indicates samples heated at temperature up to 150ºC.

The results also confirmed the different sensibility of the soil microbial groups (Fultz *et al*., 2016; Muñoz-Rojas *et al*., 2016; Pietikäinen and Fritze, 1995; Whitman *et al*., 2019), which is consistent with the biomass estimates, obtained using the PLFAs and the microbial index.

Fungal diversity (Buscardos *et al*., 2015) and recovery of fungi after a fire were influenced by severity (Owen *et al*., 2019). Previous studies found that the microbial community structure was altered also by repetitive prescribed burning (Campbell *et al*., 2008) and wildfire frequency (Shen *et al*., 2015). Other authors demonstrated that fire recurrence produced changes in fungal community (Bastias *et al*., 2006a; Bastias *et al*., 2006b; Anderson *et al*., 2007; Artz *et al*., 2009; Oliver *et al*., 2015). Ranneklev and Bååth (2003) concluded that some PLFAs appeared to be good indicators of a previous heating in peat. Saa *et al.* (1993) and Fioretto *et al.* (2005) found that recovery time for microbial communities after fire may depend on fire severity in relation to the historical fire regimen of the forest ecosystem.

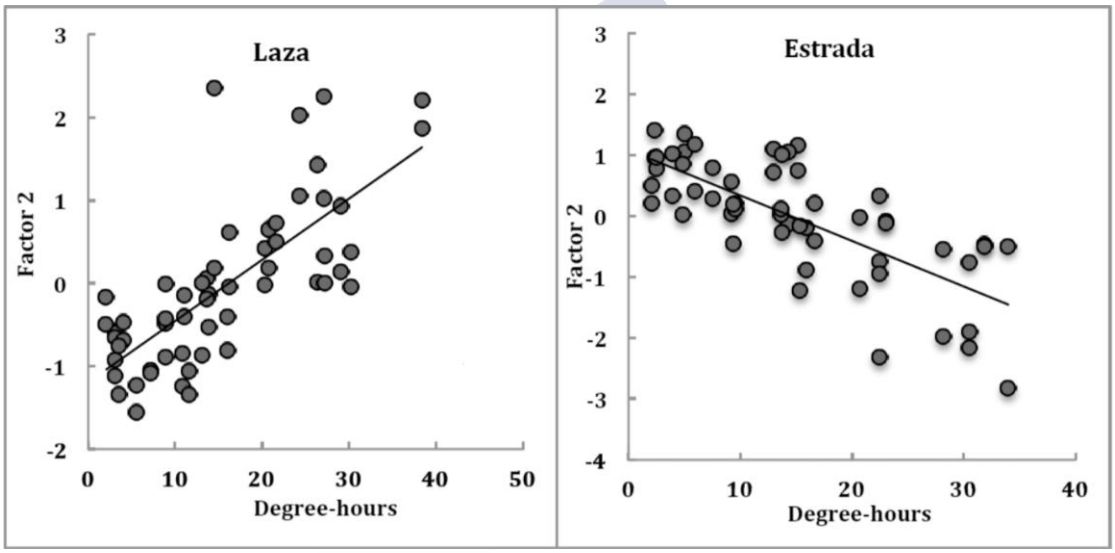

Figure 32. Relationship between factor 2 of PCA and the fire severity (degrees-hour) obtained for the set samples heated at different temperatures (50ºC-300ºC) under laboratory conditions (n, 72; p<0.001) in Laza (R:0.74) and A Estrada (R:-0.73) samples. L, soil affected by a wildfire; E, soil affected by an experimental fire.

Differences in the soil pH which is one of the most determinant factors for the microbial community structure (Rousk *et al*., 2009; 2010) as well as other variations in soil environment conditions induced directly or indirectly by the fire can partly explain that burned samples communities different notably from the unburned ones (Pietikäinen *et al*., 2000). Under laboratory conditions, changes in microbial community cannot be due to these factors, as no changes were detected on the physicochemical properties of soils evidencing that the microbial communities may differentiate due to variables not measured in this study.

5.2.5. Effects of fire regimen (severity and recurrence) on the community level physiological profile

The average well-color development (AWCD) values varied in Laza samples from 0.43 to 0.67 whereas Estrada samples values varied from 0.10 to 0.52 (Figure 33). Although no significant differences were detected in mean AWCD values between unburned and burned samples, some changes were detected. The results showed that the wildfire (Laza) caused an increase in AWCD values (from 0.54 to 0.63), while in Estrada the values of burned and unburned samples showed no variation.

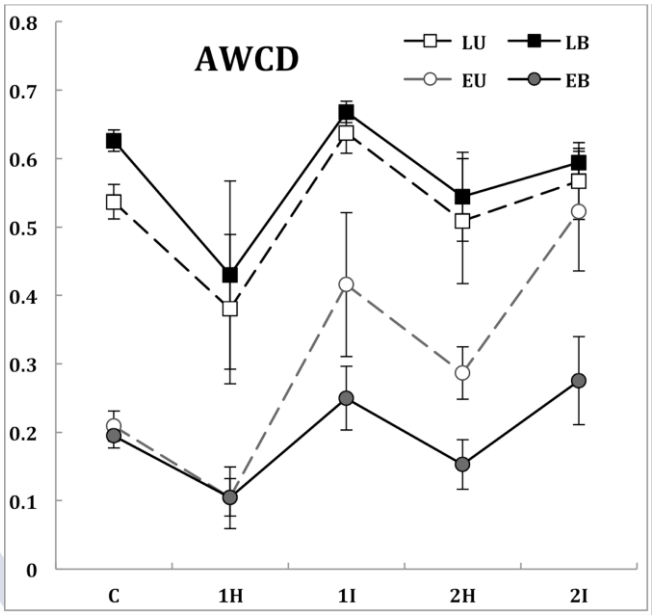

Figure 33. Average well colour development (AWCD) of the different unburned (U) and burned (B) Laza (L) and Estrada (E) samples (mean value of the different heating treatments under laboratory conditions ± SE, n= 5). C, unheated control; 1H, first heating; 1I, incubation following first heating; 2H, second heating; 2I, incubation following second heating.

After both heating treatments under laboratory conditions, values tend to persist at the same magnitude of the control samples. The first and second laboratory heating treatments reduced the AWCD in burned and unburned Laza and Estrada soils, whereas the first and second incubation of the heated soils produced an increase in AWCD, less marked in the last incubation of Laza soils. After both incubation periods, the AWCD values tended to increase to values similar to those with the sharpest increase in the Estrada unburnt soils. Nevertheless, a decrease was produced in AWCD at the highest temperature treatments, confirming that the magnitude of the decrease in AWCD was related with the degrees-hour applied to soil as shown the Figure 33. After both incubation period values tended to recover to the same that control ones. The temperature necessary to produce that decrease of the AWCD values varied between unburned and burned samples and between the first and the second heating treatment. Samples suffering more heating events were more tolerant to high temperatures and needed higher DH values to show a decrease in the AWCD values. As it is shown in

Figure 33 after first heating AWCD values in Laza decreased for temperatures around 20 DH and after second heating AWCD values decreased above 25 DH. In contrast, in Estrada samples a different behavior was observed and a clear tendency was not observed (data not showed).

The lack of response coincides with previous studies that indicate that changes in functional diversity after fires are scarce and short in time (Staddon *et al*., 1997; Cookson *et al*., 2008; Fontúrbel *et al*., 2011; 2016). D'Ascoli *et al.* (2005) found that the microbial community quickly recovers its functional diversity after a low severity fire. However, high severity fires or repetitive fires induce large changes in the functionality of soil microorganisms, related to changes in organic matter and nutrient availability (Guénon *et al*., 2011; Overby *et al.*, 2006; Wang *et al*., 2016). Sun et al. (2016) analyzing the genes involved in the nutrient cycles, detected that the functionality of the bacterial community change as consequence of fire according with the fire history.

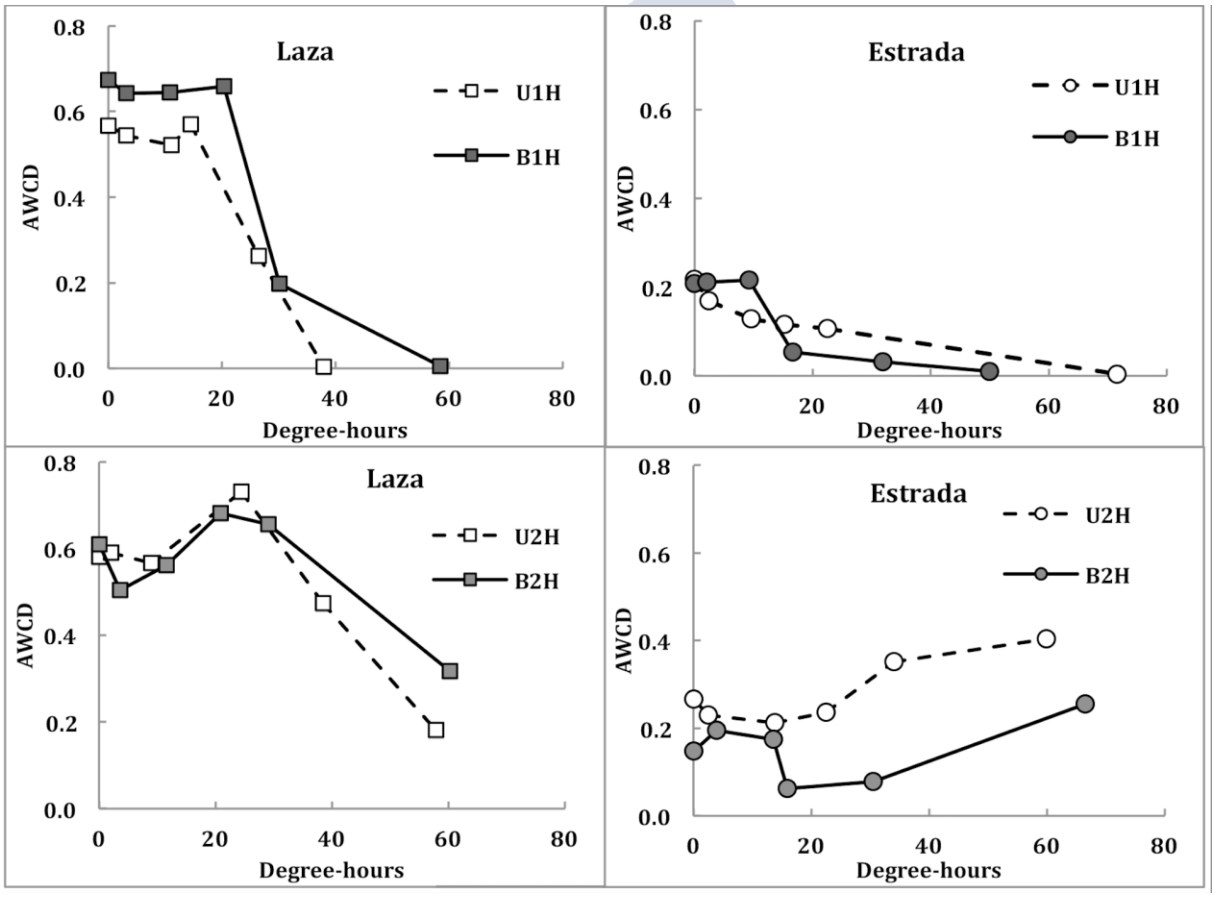

Figure 34. Average well colour development (AWCD) in the different unburned (U) and burned (B) Laza (L) and Estrada (E) samples at different heating temperatures. H, first heating; 2H, second heating.

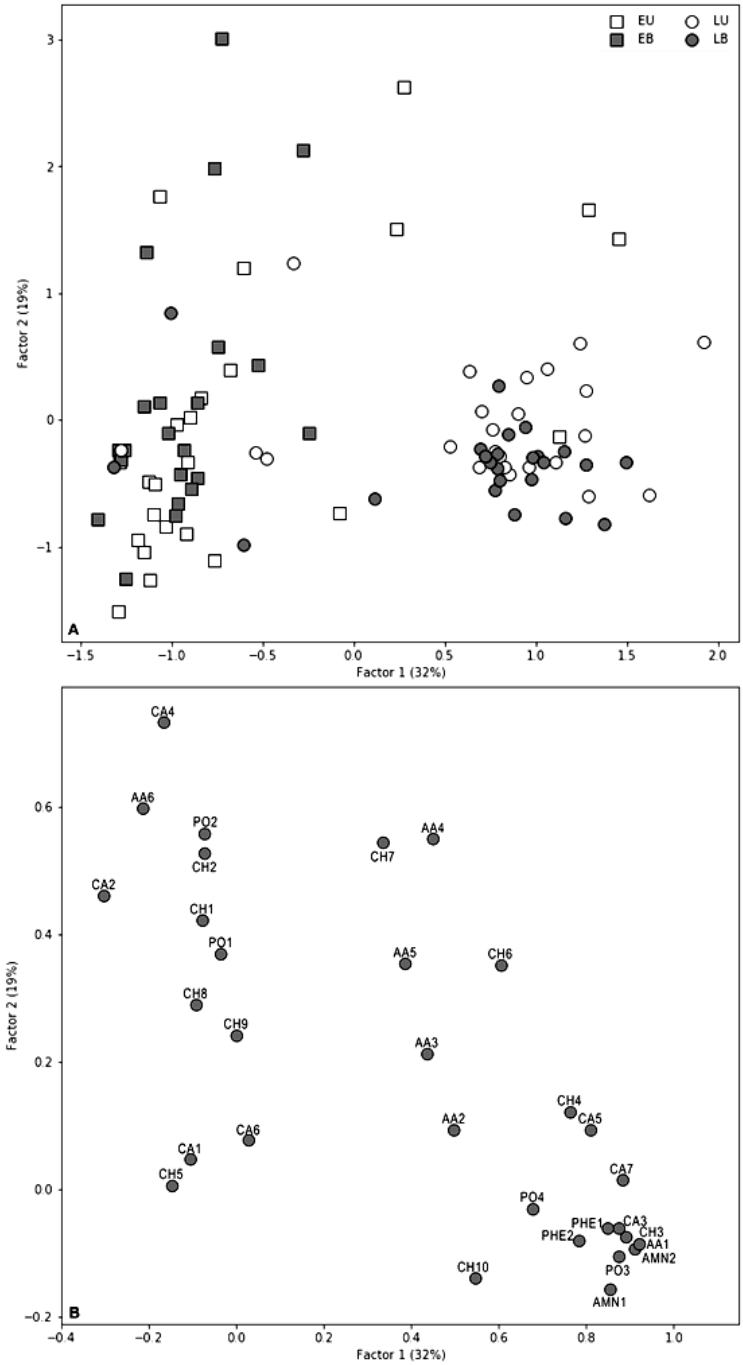

Figure 35. Sample distribution (A) and variable distribution (B) of the PCA results of the whole pattern use of substrates by the microbial communities from Laza (L) and Estrada (E) unburned (U) and burned (B) samples heated at different temperatures in each heating and incubation time. AA1, Glycil-L-Glutamic acid; AA2, L-Arginine; AA3, L-Asparagine; AA4, L-Phenylalanine; AA5, L-Serine; AA6, L-Threonine; AMN1, Phenylethylamine; AMN2, Putrescine; CA1, D-Galacturonic acid; CA2, D-Glucosaminic acid; CA3, γ-Hydroxybutyric acid; CA4, Itanonic acid; CA5, α-Ketobutyric acid; CA6, D-Malic acid; CA7, Pyruvic acid Methyl Ester; CH1, N-Acetyl-D-Glucosamine; CH2, D-Galactonic acid-γ-lactone; CH3, D-Cellobiose; CH4, i-Erythitol; CH5, D,L-α-Glycerol Phosphate; CH7, α-D-Lactose; CH8, D-Mannitol; CH9, ß-Methyl-D-Glucoside; CH10, D-Xylose; PHE1, 2-hydroxybenzoic acid; PHE2, 4-Hydroxybenzoic acid; PO1, α-Cyclodextrin; PO2, Glycogen; PO3, Tween 40; PO4, Tween 80.

The principal component analysis (PCA) for the microbial use of the substrates (Figure 35) revealed that the main differences were due to the soil type. Factor 1 explained 32% of variance and tended to separate Laza soil (positive values of Factor 1) from Estrada soil (negative values of Factor 1). No clear trend between unburned and burned samples of the two soils studied or in the degradation of different C substrate groups were observed. Likewise, no significant correlations were found between the DH values and the PCA factors. Previous studies found that the addition of charred organic matter from wildfire (Guénon *et al*., 2011) and wood bottom ashes (Merino *et al*., 2016) produced shifts in the bacterial substrate utilization pattern. So that, the pool of organic matter has been indicated as the most important factor controlling the functional diversity (Artz *et al*., 2006; Huang *et al*., 2008) and no changes were detected on these parameters as consequences of low severity fire and heating treatments.

Differences in functional diversity are given by soil type, which coincides with previous studies by Grayston *et al.* (1998); Myers *et al*. (2001) Priha *et al.* (2001) where differences were observed in PLFA and CLPP patterns caused by different vegetation.

The differences between PCAs performed with PLFA data and CLPP data were evident. Although both, the PLFA and CLPP profile analysis showed that there were marked differences between soils, only PCAs performed with the PLFA data set allows us to detect differences caused by fire under field conditions or laboratory heating and by the fire recurrence, suggesting that soil type had a greater impact on functional diversity than fire regime. These results seem to indicate that factors regulating the metabolic activity are different from those controlling the community structure and functional diversity and microbial community structure may not be linked. This is coincident with results of San Emeterio *et al*. (2016) where no changes were detected in AWCD values due to prescribed fire, and studies from Priha *et al*. (2001) and Bergner *et al.* (2004) where the microbial community structure (PLFA) varied due to fire meanwhile CLPP profile was not affected. Söderberg et al. (2004) also found CLPP method less suitable than PLFA method for rhizosphere studies and Waldrop *et al*. (2000) did not find correlation between BIOLOG and microbial community composition or activity. Metabolic profiles obtained using the Biolog® techniques reflects only the potential response to a different C substrates of the culturable portion of the soil microbial community and may not reflect the natural environment (Preston-Mafham *et al*., 2002) whereas PLFA includes the total community (Torsvik *et al*., 2002). Another possible explanation for the lower response of CLPP than PLFA can be that the microbial communities are functionally redundant that means that many taxonomically distinct members of the microbial communities can utilize the same carbon source, and consequently the taxonomic diversity could be underestimate with the CLPP technique (Staddon *et al*., 1997). In contrast, the PLFAs method analyzes the microbial community "in situ" and takes into account fungi and slow-growing bacteria (Frostegård *et al*., 2011). However, other studies found consistent changes in the size of the microbial biomass and its functional and structural composition, assessed by PLFA and CLPP methods, after fire (Overby *et al*., 2006; Campbell *et al*., 2008; Wang *et al*., 2016). However, microbial functional diversity has shown a greater response to prescribed fire and mechanical treatment

in scrubland than other microbial properties (enzymatic activities, microbial biomass C) considered good indicators of soil quality (Fontúrbel *et al*., 2012; 2016). In all cases, it is clear that the study of several microbial parameters taken together can best reflect the status of soil microorganisms and its role in soil functioning.

The DH methodology is a promising tool for examining the fire severity (temperature and residence time of soil heating). This technique for evaluating the effect of heating resulted a realistic way to measure the temperature reached by the soil as it take into account the temperature applied to the soil and the time that this soil suffers that thermal impact (Cancelo-González *et al*., 2012). This aspect is of great interest for evaluating, under both laboratory and field conditions, the thermal shock effects on soils and to compare the results obtained in contrasting soils showing different fire regime (severity, recurrence) as well as those obtained from the same soil in different conditions of heating and repetitions of the treatment (cumulative effects). Data also suggest that soil microorganisms, even in this scrubland ecosystem adapted to fire, could be affected by fire of low and medium severity if they are applied in a repetitive way. Thus, we should be cautions in applying prescribed fires to reduce fire occurrence, especially in natural areas with important ecological and biodiversity resources and/or when they are used periodically in the same burnt forest zones as a management tool to fire control. Although several studies show a recovery of microbiological properties at medium term after a fire (Fritze *et al*., 1993; Hedo *et al*., 2015), they are differences in the microbial response in the soil samples when the scenario changes; i.e. before and after the heating/burning of soil. Therefore, in previously burned or heated soil samples the microbial activity values showed a decrease in lower temperatures than unburned soil and the effect was more marked in a new heating event, demonstrating the negative effect of recurrence. The effect of a single fire differs from those of repeated fires (Vance and Henderson, 1984). This is supported by previous studies that showed that fire recurrence had a negative effect on physicochemical properties as change in N dynamics (DeLuca and Sala, 2006), and nutrient availability (Eugenio *et al*., 2006). Repeated fires have also a negative effect on microbial activity (Banning and Murphy, 2008; Guénon *et al*., 2011; Rodríguez *et al*., 2017), on the composition of microbial communities (Fultz *et al*., 2016) and vertebral fauna (Darraqc *et al*., 2016), as well as on plant productivity (Ferrán *et al*., 2006). These negative effects may require more than a decade after fire to disappear (Goulden *et al*., 2011; Oliver *et al*., 2015).

Variations on the effect of fire on soils suggest that fire response may be site specific (Mikita-Barbato *et al*., 2015) as the impact of prescribed fires differ greatly depending on the initial soil characteristic and vegetation type (Alcañiz *et al*., 2018). Therefore, previous site management and disturbance history must be considered to effectively predict the potential influence of fire on soil nutrient concentrations and biochemical properties (Choromanska and DeLuca, 2002). This supports previous studies showing that the response of soil microbial communities to a stress agent derived from different or land use management and other human activities (heavy metals, antibiotic, herbicides, heat, salinity…) differed depending on the previous exposition to this specific agent (Díaz-Raviña *et al*., 1994; Díaz-Raviña and Bååth, 2001; Mahía *et al*., 2011; Santás-Miguel *et al*., 2020). This fact, in somehow, allows us

to think that the microorganisms, as well as all living organisms of different sizes, have "historical memory". The fact that the fire regime caused accumulative negative effects on soil microorganisms, which are the main responsible for soil functioning, has important consequences on the acceleration of soil degradation, which is associated to the loss of quality and stability of soil, as well as also on the speed of recovery (resilience) towards its pre-disturbance state or a new state (Griffiths and Philippot, 2013).

There are several studies using heating laboratory experiments to evaluate the effect of fire severity in physical (Fernández *et al*., 1997; Terefe *et al*., 2008; Varela *et al*., 2010) and biological (Díaz-Raviña *et al*., 1992; 1996; 2006; Bárcenas and Bååth, 2009; Bárcenas-Moreno *et al*., 2011) properties. In most of these studies the fire severity is estimated using the oven temperature, which did not reflect the actual temperature and, in addition, its duration is often not taken into account. This makes difficult the comparison and interpretation of data obtained in all the investigations mentioned above. Our data showed that the oven temperature was higher than that reached by soil during the heating treatment, which is recorded with thermocouples; therefore, this last procedure allows us to have a more reliable approach about the real impact of soil heating. Therefore, since the degrees-hour methodology takes into account both the soil temperature reached by soil and its duration resulted a more realistic way than the maximum temperature for evaluating fire severity (Cancelo-González *et al*., 2012; Barreiro *et al*., 2015). Cancelo-González *et al*. (2015) successfully applied this methodology to examine the impact of temperatures in the range of 200-400ºC on physicochemical properties. Data obtained in the present work showed that the DH methodology seems to have a useful tool to determine the impact of the severity in laboratory experiments in a lower temperature range (50-300ºC). This aspect is of great interest for evaluating, under both laboratory and field conditions, the thermal shock effects on soils and to compare the results obtained in contrasting soils showing different fire regime (severity, recurrence) as well as those obtained from the same soil in different conditions of heating and repetitions of the treatment (cumulative effects).

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CONCLUSIONS

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6. CONCLUSIONS

Field experiments conducted showed that the quantity and quality of the soil organic matter derived of vegetation remains are the key factor of soil quality that determines the main source of variation among the different soil samples analyzed; thus soil developed under *Quercus* have a higher quality and different microbial communities structure than those under *Eucalyptus*. With respect to the soil depth influence, as expected, soil quality diminished with depth.

Wildfires induced important changes in most of soil physical, chemical and microbiological properties analysed. However, the biochemical and microbiological properties exhibited a higher sensitivity to the fire impact than physical and chemical properties. The labile fractions of organic matter such as soluble C and carbohydrates can be good indicators of fire effects on short term. The analysis of enzymatic activities and biomass of soil microorganisms also resulted a useful index to estimate the effect of fire, especially at short- and -medium term. The analysis of the microbial community structure by means of the PLFA technique was the most effective tool to detect the impact of disturbances such as wildfires or prescribed fires, and allows us to determine the importance of other factors such as vegetation or depth as a source of soil samples variation. The PLFA presents limitations for estimating the effect of fire on total microbial biomass and biomass of different groups, however, in some cases fire can induce variations in the values of ratios fungal biomass/bacteria biomass and $Gram^-$ bacterial biomass/$Gram^+$ bacterial biomass ratios.

Changes in soil properties were related to severity of fire and the negative effects could even persist up to 4 years after a high severity burning. The soil biochemical and microbiological properties analysed in burned soil showed fluctuations among the different samples collected at different sampling times, which are associated with the variation of climatic conditions and C and nutrient availability within the season. This showed the importance of sampling time in the evaluation of soil quality status.

The two postfire soil stabilization techniques applied, mulching and seeding, resulted to be

effective in reducing soil erosion, especially the mulching. No significant changes in the physical-chemical, biochemical properties or in the structure of soil microbial communities were produced by seeding or mulching treatments, independent of the applied dose and way of application. Likewise, these two rehabilitation burnt soil treatments had no effect on the vegetation recovery. Therefore, taking into account their efficiency and the absence of negative effects on the recovery of soil quality and the regeneration of vegetation, the mulching technique is recommended to be implanted in this temperate humid zone (NW Spain) by its high potential to reduce erosion (70-90% reduction).

The results obtained in the experiments performed under laboratory conditions demonstrated that the actual temperature reached by the soil during the heating treatments was lower that the theoretical temperature indicated by the muffle, and that the use of degrees-hours methodology (based on the temperature-time curves) instead of Tmax temperature is a better approximation to estimate the fire severity. This makes difficult the comparison of studies on this topic performed by different authors even under laboratory experiments.

The thermal treatments produced changes in all the activity of soil microorganisms (enzymes and bacteria) and the intensity of those changes were determined by the severity of the previous fire under field conditions and further soil heating at the laboratory. These activity parameters presented different sensitivity to heat, following the order: bacterial activity < β-glucosidase < phosphatase < urease. The negative effect on enzymatic activities was greater in samples burned or previously heated, showing the importance of the previous fire/heating history of the samples in the further response of soil microorganisms to a new heating event.

The PLFA analysis showed that the most important factor determining the variation among the samples regarding to the microbial community structure was the soil type, followed by the wildfire (severity under field conditions) and in a lesser degree by laboratory heating. The PLFA analysis allows us to detect changes of microbial community structure due to the fire severity and recurrence. The data showed that the response of soil microbial communities to fire depended on the severity in the field and the amount of heat supplied to the samples and the previous fire/heating history of the samples. The magnitude of the changes induced by fire on microbial community structure was closely related to the fire severity measured by the degrees-hour method. The results clearly confirmed that PLFA analysis is a very useful an extremely sensitive tool to evaluate the impact of fire disturbance on soil quality by means of changes induced by fire on microbial community structure (taxonomic biodiversity).

These fires induced changes in the microbial structure of microbial community or taxonomic diversity (PLFA) are not necessarily related to changes observed in functional diversity as indicated by the results obtained from community level physiological profile (CLPP). The CLPP technique allows differentiating soil communities from different soils, but is not able to detect changes due to fire or heating probably due to its sensitivity. However, the results may indicate that these methods (PLFA and CLPP) give us complementary information and/or the presence of redundant species in these burned treated soils.

The knowledge about different aspects of soil microorganisms such as biomass, activity and diversity, particularly the later, as well as the soil biological processes may improve the scientific basis for understanding their responses to different soil perturbations such as fire regimen (severity, recurrence, etc.). In this study, the data indicated that soil heating had negative effects on microbial activity and provoked changes in the taxonomic diversity (PLFA) of microbial communities (death of most fire sensitive microorganisms and selection of the most tolerant ones) which are closely related to the fire severity. The data also indicate that the previous history of soil fire regime (severity, recurrence) is a determinant factor in the response of microorganisms to a further fire event, in other words, that the fire effects on soil are cumulative. Thus, the use of repetitive prescribed fires as a forest management tool should be performed with caution since, even if they are of low severity, they can provoke negative effects on soil microbial communities and, hence, on soil quality. Therefore, these aspects should be taken into account when making decisions to evaluate both the benefits and the risk assessment of the implementation of these forest management practices.

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APPENDIX

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1- Lombao, A., Barreiro, A., Carballas, T., Fontúrbel, M.T., Martín, A., Vega, J.A., Fernández, C., Díaz-Raviña, M. 2015. ***Changes in soil properties after a wildfire in Fragas do Eume Natural Park (Galicia, NW Spain).*** Catena. 135, 409-418.

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