THE IMPACT OF SOIL DISTURBANCE ON NITROGEN PARTITIONING AND CARBON SECUESTRATION IN MEDITERRANEAN SOILS

Memoria que presenta:

María López Martín para optar al título de
Doctor por la Universidad de Sevilla en:
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Memoria que presenta
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Doctor en Recursos Naturales y Medio Ambiente

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Certifica: Que la presente Memoria de Investigación titulada “The impact of soil disturbance on nitrogen partitioning and carbon sequestration potential in Mediterranean soils”, presentada por Dña. María López Martín para optar al grado de Doctor en Recursos Naturales y Medio Ambiente, ha sido realizada en el Departamento de Cristalografía, Mineralogía y Química Agrícola, bajo la dirección de la Dra. Dña. Heike Knicker, reuniendo todas las condiciones exigidas a los trabajos de Tesis Doctorales.

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“***Hay que perseverar y sobre todo, tener confianza en uno mismo***”

Marie Curie

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-Resumen-

Los incendios forestales pueden ser considerados como uno de los factores más influyentes en la degradación de los suelos en la cuenca Mediterránea. El fuego puede modificar las propiedades físicas y químicas del suelo, y, en particular, alterar la calidad y cantidad de la materia orgánica del suelo (MOS), a corto, medio y largo plazo.

La quema de la vegetación produce residuos altamente alterados químicamente conocidos genéricamente como material orgánico pirogenético (*PyOM*, acrónimo del término inglés *pirogenic organic matter*), y el nitrógeno (N) orgánico que contiene es llamado *Black Nitrogen* (*BN*). Químicamente, el *BN* está compuesto por compuestos aromáticos heterocíclicos, y su incorporación a la MOS va a afectar presumiblemente el ciclo del N en el suelo.

En una primera fase de este trabajo fueron muestreados Cambisoles localizados en la Sierra de Aznalcóllar (Sevilla) bajo un bosque típico Mediterráneo propenso a los incendios, que habían sido afectados por distinto número de incendios. Los suelos elegidos fueron afectados por el fuego por primera vez en 1996, y posteriormente una segunda vez en 2004.

Con objeto de evaluar la reproducibilidad de nuestros datos sobre el impacto del fuego en la calidad y cantidad de la MOS en las áreas estudiada, así como la influencia de la variabilidad espacial, principalmente en cuanto a la distribución del combustible antes del fuego se compararon dos métodos de muestreo diferentes En el primero de ellos (método 1), las esquinas y el centro de un cuadrado elegido al azar con una longitud de lado de 15 m fueron muestreados y analizados por separado. En el segundo, se utilizaron muestras compuestas (método 2) obtenidas de tres suelos. Los análisis efectuados permitieron describir las propiedades físicas y químicas de los suelos, así como sus características espectroscópicas mediante resonancia magnética nuclear (RMN), que fueron comparadas estadísticamente usando ANOVA. Ambos métodos de muestreo arrojaron resultados comparables con similares desviaciones estándar. No se observaron grandes diferencias con respecto a las propiedades físicas y químicas, contenido de carbono (C) y N entre los suelos no afectados y afectados por el fuego. Sin embargo los resultados de la RMN en estado sólido de ^{13}C mostraron que los suelos quemados presentaban un incremento en aromaticidad. En base a estos resultados se concluyó que el muestreo con número reducido de réplicas (método 1) puede ser adecuado para aportar datos representativos del comportamiento por RMN de los suelos. Los resultados indicaron que en el área de estudio

afectada por el fuego recuperó rápidamente las propiedades típicas del suelo y de su MOS, y que los cambios provocados por el fuego no se mantuvieron a largo del tiempo.

Para elucidar la estabilidad bioquímica del *Black Carbon* (BC) y del *BN* de los suelos estudiados, muestras seleccionadas de la campaña del 2011 fueron estudiadas por RMN en estado sólido de ^{13}C y ^{15}N, y sus espectros fueron comparados con aquellos que fueron adquiridos de las muestras muestreadas en la misma zona, 4 semanas después del fuego en 2004. El análisis confirmó una incorporación rápida del material quemado en la MOS, justo después del incendio. Como consecuencia, el N tipo pirrol dominó la fracción de N orgánico del suelo. Después de siete años de recuperación del suelo se produjo una considerable disminución del C aromático, aunque su contribución al C total fue mayor en las zonas quemadas que en el suelo control. El hecho de la pérdida de *PyOM* durante la recuperación del suelo cuestionó la longevidad de los residuos quemados en el suelo. El *BN* mostró incluso menor recalcitancia bioquímica. El fraccionamiento por densidad y tamaño de partícula de la materia orgánica (MO) de los suelos que se están recuperando del incendio indicaron que el material quemado remanente se acumuló principalmente en la fracción orgánica particulada. Sin embargo, la baja recalcitrancia bioquímica debido a la falta de interacción entre la fracción mineral, el *BN* y el *BC* requiere de futuras investigaciones.

Con el fin de aportar más luz sobre el impacto del *BN* en el ciclo del N en el suelo, se llevó a cabo un experimento en macetas durante 0.5, 1, 5, 8, 12 y 16 meses bajo condiciones controladas en invernadero. Para ello, *Lolium perenne* fue cultivado en suelos quemado y no quemado después de ser cubiertos con MO fresca enriquecida con ^{15}N (^{15}N-MO) o su *^{15}N-PyOM*. Paralelamente, los contenedores fueron fertilizados con KNO_3 no enriquecido (N_i), mientras que otros fueron cubiertos con *PyOM* y MO junto con $K^{15}NO_3$ ($^{15}N_i$) y otros con sólo $^{15}N_i$. La determinación de la recuperación del ^{15}N añadido ($^{15}N_{add}$) junto con un análisis estadístico de los resultados mostró que el impacto del fuego no produjo cambios en la distribución del $^{15}N_{add}$ entre plantas (hojas y raíces) y suelo. En las hojas de las plantas, las mayores recuperaciones de $^{15}N_{add}$ se observaron en las macetas tratadas con $^{15}N_i$ y ^{15}N-MO en comparación con *^{15}N-PyOM*. La adición del N_i no alteró el porcentaje de $^{15}N_{add}$ recuperado derivado de *^{15}N-PyOM* y ^{15}N-MO. Comparativamente, la adición de *PyOM* and OM no alteró la eficiencia de $^{15}N_i$ incorporado en los residuos vegetales.

Después de 16 meses de incubación, los suelos cubiertos con *^{15}N-PyOM* mostraron el mayor secuestro de $^{15}N_{add}$ mientras que la RMN de estado sólido de ^{15}N sugirió que parte del $^{15}N_{add}$

secuestrado en los suelos cubiertos con ^{15}*N-PyOM* se encontraba en forma de N amida, probablemente perteneciente a residuos de biomasa microbiana.

En resumen, nuestros resultados permiten concluir que la adición de *PyOM* rico en N y las cenizas incorporadas al suelo después de un incendio tienen un fuerte impacto a corto plazo en el ciclo del N. Por consiguiente, justo después de un incendio, el N_i de las cenizas reemplaza el N, que había sido suministrado por la degradación de la hojarasca. Esto prueba una rápida fertilización con N, necesaria para una rápida recuperación de la cobertura vegetal. Dado que el N_i no es usado directamente para la producción de biomasa, puede perderse rápidamente por volatilización o lixiviado. Por tanto el *PyOM* sirve como sumidero eficiente de N, que es liberado lentamente y así aporta una fertilización adicional a medio o largo plazo. A largo plazo, casi todo el N del *PyOM* será transformado en N orgánico del suelo (NOS), con propiedades y funciones comparables al NOS derivado de la enmienda no quemada.

Finalmente, investigamos la incorporación of ^{15}N derivado del ^{15}N-MO o de ^{15}*N-PyOM* en los aminoácidos extraíbles (AA_s) de la MOS, derivados de suelos quemado y no quemado enmendados con dichos residuos con el fin de revelar el papel del *BN* en el crecimiento de la biomasa del suelo. Los AA_s fueron extraídos con ácido clorhídrico de los suelos utilizados en el experimento con macetas. La cantidad de ^{15}N añadido recuperado en la fracción de los AA_s fue determinada mediante cromatografía de gases - espectrometría de masas.

Este experimento confirmó que el N de *PyOM* es incorporado en la fracción peptídica de la MOS y que la eficiencia de su incorporación no se altera debido a la presencia de N_i y viceversa, pero si es afectada cuando el suelo ha sido quemado.

-Abstract-

Wildfire can be seen as one of the major factors for soil degradation in the Mediterranean basin. The fire can modify the physical and chemical soil properties and particularly the quality and quantity of soil organic matter (SOM) at a short and long term scale.

Vegetation fires produce chemically altered residues known as pyrogenic organic matter (PyOM) and its organic nitrogen (N) is referred to as Black Nitrogen (BN). Chemically, the BN consists of N heterocyclic aromatic compounds and its input into SOM is likely to affect the N-cycling in soil. Therefore, the basic objective of this doctoral thesis was to gain quantitative knowledge of how and to what extent the input of PyOM alters the quality of soil organic carbon and nitrogen and which role BN plays for the development of biomass during the recuperation of a fire-affected soil.

In a first attempt, Cambisols with different fire history were sampled from the Sierra de Aznalcóllar, Southern Spain (Sevilla) in 2011, which is a characteristic fire-prone Mediterranean forest area. The selected soils were either burnt once in 1996 or twice in 1996 and 2004.

In order to evaluate the impact of spatial inhomogeneity and pre-fire fuel-distribution on the quantity and quality of SOM in the studied areas and thus on the reproducibility of our data, the results of two different sampling methods were compared. In a first approach (method 1), the corners and the center of a randomly chosen square with a side length of 15 m were sampled and analyzed separately. For comparison, composite samples (method 2) were obtained from three soils. Data describing the physical and chemical properties of the soils together with the NMR spectroscopic characterization were analyzed using ANOVA. Both sampling methods yielded comparable results with comparable standard errors. No major differences between the fire-affected and unaffected soils were observed with respect to physical and chemical properties and C and N contents, but solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy indicated a small but significant elevation of aromaticity in the soils with fire history. Based on the findings it was concluded that sampling with reduced replicates (method 1) can still lead to representative NMR data. The results indicated further that in the study area typical properties of the soil and its SOM induced by former burnings recover quickly and fire-induced changes will not persist on a long-term scale.

In order to elucidate the biochemical stability of Black Carbon (BC) and BN in the soils of the studied area, selected samples from the sampling campaign in 2011 were subjected to solid-state ^{13}C and ^{15}N NMR spectroscopy and their spectra were compared to those acquired from samples

collected from the same soils but already 4 weeks after the fire in 2004. The analysis confirmed quick incorporation of charred residues into SOM, directly after the fire. Correspondingly, pyrrole-type N dominated their soil organic N fraction. Seven years of recuperation led to a considerable decrease of aryl C, although its contribution to the total organic C was still higher at the burnt sites than in the unburnt reference soil. The fast loss of PyOM during soil recuperation calls the claimed longevity of charred residues in soils into question. The BN showed even lower biochemical recalcitrance. Density and particle size fractionation of the OM of the recuperating soils indicated that the remaining char accumulated mainly in the particulate organic matter fractions (POM). However, to what extent, the lack of interaction between the mineral fraction and the BN and BC contributed to their low biochemical recalcitrance needs further research.

In order to bring some light on the impact of BN on the soil N-cycle, a pot experiment was carried out for 0.5, 1, 5, 8, 12 and 16 months under controlled condition in a greenhouse. Therefore, *Lolium perenne* was grown on soil material from unburnt and burnt forest after covering it with ^{15}N-enriched fresh grass litter (^{15}N-OM) or its ^{15}N-PyOM. In parallel, pots were additionally fertilized with non-enriched KNO_3 (N_i). Further pots contained unenriched burnt (PyOM) or unburnt plant (OM) residues together with $K^{15}NO_3$ ($^{15}N_i$) and only $^{15}N_i$. Determination of the recovered added ^{15}N ($^{15}N_{add}$) together with a statistical analysis of the results showed that fire history of the soil had no impact on the $^{15}N_{add}$ partitioning between plant leaves, roots and soils. In the plant leaves clearly higher $^{15}N_{add}$ recoveries were obtained for pots treated with $^{15}N_i$ and ^{15}N-OM than for those with ^{15}N-PyOM. The addition of N_i did not alter the percentage of $^{15}N_{add}$ derived from ^{15}N-PyOM and ^{15}N-OM found in the leaves. Comparably, addition of PyOM and OM did not alter the efficiency of $^{15}N_i$ incorporation into plant residues. After 16 months of incubation, the soils with ^{15}N-PyOM amendment showed the highest $^{15}N_{add}$-sequestration. Solid-state ^{15}N NMR spectroscopy revealed that some of the $^{15}N_{add}$ sequestered in the soils topped with ^{15}N-PyOM occurred as amide N, most likely of microbial biomass residues.

In summary, our results allow the conclusion that addition of N-rich PyOM and ash to soils after fires has a strong short-term impact on N-cycling in the affected soils. Accordingly, directly after the fire, N_i of the ash replaces the N which otherwise had been supplied by the decaying plant litter. It provides a fast N-fertilization that is necessary for a quick recovery of the plant cover. Since N_i which is not directly used for biomass production can be quickly lost by volatilization or leaching, PyOM serves as an efficient N sink which only slowly releases N and thus provides additional fertilization on a medium and long-term scale. On a long-term scale, almost all of the N in PyOM will be transformed into soil organic N with comparable properties and functions as SON derived from unburnt litter.

Finally, we investigated the incorporation of ^{15}N from ^{15}N-OM or their ^{15}N-PyOM into extractable amino acids (AA_s) of SOM from an unburnt and a burnt soil amended with those residues in order to reveal the role of BN for the built-up of soil biomass. Therefore, the AA_s were extracted with hot hydrochloric acid from the soils of the pot experiment. Their amount and the percentage of added ^{15}N, which was recovered with the AAs fraction was determined by gas chromatography mass spectrometry.

Our experiment confirmed that N from PyOM is incorporated into the peptidic fraction of SOM. The efficiency of this incorporation is not altered by the presence of Ni and vice versa, but it is affected by the fire history of the soil.

Capítulo 1.

Introducción

Chapter 1.

Introduction

INTRODUCCIÓN

1.1. Objetivo de la tesis

El fuego es responsable de una de las mayores perturbaciones de los ecosistemas. En particular, produce cambios en la cantidad y calidad de la materia orgánica del suelo (MOS), afectada notablemente por la incorporación de la materia orgánica (MO) pirogénica (*PyOM*). El nitrógeno orgánico (N_{org}) como parte de la MOS va a sufrir cambios estructurales por el impacto del fuego dando lugar a la formación de compuestos heterocíclicos aromáticos de nitrógeno (N) o "*Black Nitrogen*" (*BN*). La formación del *BN* afecta a la biodisponibilidad del N y establece competición entre plantas, microorganismos y MOS. El objetivo principal de la presente tesis es dilucidar el impacto del fuego en la inmovilización del N, la cual va a afectar su tasa de degradación y por consiguiente al tamaño y composición de los distintos compartimentos de N del suelo.

1.2. El sistema Mediterráneo

1.2.1. Características y distribución

El bosque mediterráneo se desarrolla en regiones con clima mediterráneo, que son: California, el centro de Chile, los países del mar Mediterráneo (Fig. 1.1), la ciudad de El Cabo y el sur de

Australia, ocupando un 1.5% de la superficie forestal de la tierra (M'Hirit 1999). El clima mediterráneo se caracteriza por veranos secos y prolongados con temperaturas diurnas superiores a los 30 ºC, que reducen la humedad de la materia vegetal a menos de un 5%.(Vélez R 1990), lo que favorece el desarrollo de incendios forestales. El viento también contribuye al traslado de pavesas a otros puntos cercanos del incendio, lo que ayuda a propagar los fuegos. Los montes mediterráneos se caracterizan por su elevada heterogeneidad, lo que se explica por la riqueza florística y la gran variedad climática, geológica, geomorfológica y edáfica de las zonas en las que se desarrollan. Son sistemas con baja resiliencia, es decir tienen una capacidad limitada de respuesta ante las perturbaciones.

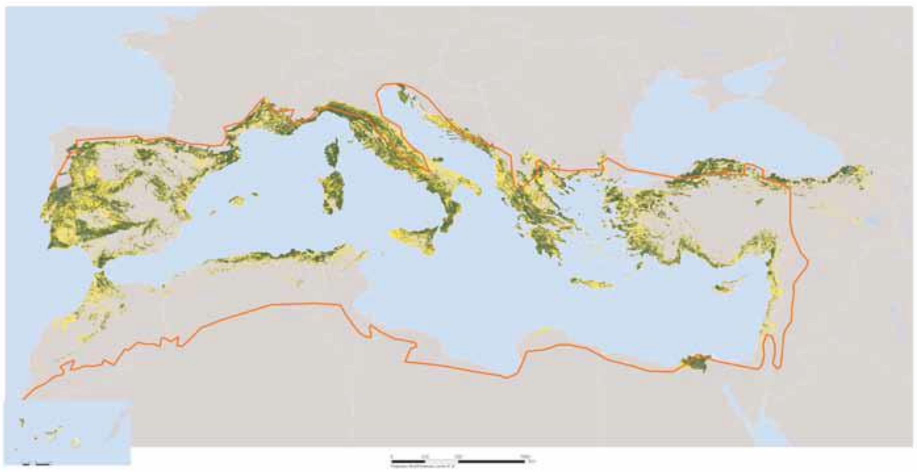

Fig. 1.1. Mapa de los ecosistemas forestales mediterráneos (verde, bosques mediterráneos; amarillo, otras superficies boscosas; en naranja, límite bioclimático del Mediterráneo).

1.2.2. Vegetación

Parte de la vegetación del bosque mediterráneo ha desarrollado adaptaciones a la incidencia del fuego durante su ciclo reproductivo, como puede ser la apertura de las piñas o el aumento de la tasa de germinación con el calor (Lloret & Vilà 1997). Los pinares constituyen las masas arboladas más extensas del bosque Mediterráneo. El pino carrasco (*Pinus halepensis*) es el más extendido en las costas de España, Francia, Italia, Grecia, Turquía, Marruecos, Argelia y Túnez. El pino piñonero (*P. pinea*), el pinaster (*P. pinaster*) y el laricio (*P. nigra*) abundan en el occidente de la cuenca, y el brutia (*P. brutia*) en el oriente. Dichas especies suelen tener un elevado contenido en resinas y aceites esenciales, extremadamente inflamables. Otras especies importantes son la encina (*Quercus ilex*) y el alcornoque (*Q. suber*).

1.2.3. Importancia del fuego

El fuego es el mayor factor de degradación natural y destrucción de los bosques y las zonas arboladas de la cuenca Mediterránea. Cada año cerca de 50000 incendios queman una superficie entre 700000 y 1000000 de hectáreas de monte (FAO 2006).

Los fuegos conllevan la desaparición o modificación de la cobertura vegetal (Mayor et al. 2016, Tessler et al. 2016), afectan al comportamiento hidrogeomorfológico (Mayor et al. 2007) y las propiedades físicas, químicas, mineralógicas y biológicas del suelo. El grado de dichos efectos dependen principalmente de la severidad del incendio, determinada por su duración y la temperatura que alcanzan (Certini 2005). Los principales efectos en las propiedades del suelo afectado por el fuego son el incremento del pH (Lombao et al. 2015) debido a la desnaturalización de los ácidos orgánicos, cambios en los contenidos del carbono (C) y N (Caon et al. 2014b) como consecuencia de la incorporación de cenizas y material parcialmente quemado (Nocentini et al. 2010), y alteraciones en la calidad de la MOS por el aporte del *PyOM*. El aporte de cenizas debido a la combustión de la vegetación y hojarasca incrementa los porcentajes de: calcio, magnesio, fósforo y potasio en los suelos quemados (Bodí et al. 2014), lo que favorece la regeneración de la vegetación debido a un aumento de la fertilidad tras un incendio.

En general, los fuegos que desarrollan temperaturas inferiores a 200 ºC no afectan a las características del suelo, entre 200 y 460 ºC causan la combustión de algunas sustancias orgánicas y por encima de 460 ºC y tiempo suficiente provocan la combustión del material orgánico y descomposición de los carbonatos (Certini 2005).

1.3. Causas de los incendios

El porcentaje de incendios de origen natural (fenómenos meteorológicos) en la cuenca Mediterránea oscila entre un 1 y 5%, mientras que el número y frecuencia de incendios provocados por actividades antrópicas es mucho más elevada (Alexandrian et al.1999). Así, el número de incendios causados por prácticas agrícolas y forestales tienen un peso importante en la zona Mediterránea, oscilando entre el 50 y el 77%. En otros países, Croacia, Grecia y Portugal esta tasa se encuentra entre el 25 y el 47%.

1.4. Situación de los incendios forestales en Europa y España

Los datos estadísticos del número de incendios y superficie afectada para el período comprendido entre 1980-2015 para los países del sur de Europa (Portugal, España, Francia, Italia y Grecia) se recogen en las Tablas 1.1 y 1.2. (“JRC Technical relports, Forest fire in Europe, Middle East and North Africa 2014”) (Comisión Europea) (http://effis.jrc.ec.europa.eu/media/cms_page_media/40/Forest_fires_in_Europe_Middle_east_and_North_Africa_2015_final_pdf_JkX4Yl3.pdf). La superficie afectada se estimó en 16000000 ha para dicho período, de la cual el 37% corresponde a España, el país que más superficie tiene quemada para dicho período y el segundo por número de fuegos (31%), después de Portugal (http://forest.jrc.ec.europa.eu/effis/ reports/annual-fire-reports).

Tabla 1.1. Número de fuegos en los cinco países miembro del sur de Europa en los últimos 36 años

Nº de fuegos	**Portugal**	**España**	**Francia**	**Italia**	**Grecia***	**Total**
2015	**15851**	**11928**	**4440**	**5442**	**510**	**38171**
% total 2015	42	31	12	14	1	100
1980-1989	7381	9515	4910	11575	1264	34645
1990-1999	22250	18152	5538	11164	1748	58851
2000-2009	24949	18369	4418	7259	1695	56690
2010-2015	18439	12994	3857	5492	1025	41536
1980-2015	18234	15953	4727	9248	1478	48641
Total	656437	538318	170177	332929	53206	1751067

* número de fuegos incompletos desde 2009

Para el caso de España los datos estadísticos han sido recogidos del Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente para el último decenio (2005-2016) y año (2016) (http://www.mapama.gob.es/es/desarrollorural/estadisticas/Incendios_default.aspx).

En España, el total de fuegos forestales ha descendido un 32% en 2016 con respecto a la media del último decenio (2005-2015). En cuanto a la superficie afectada se produjo un descenso con respecto a la media del último decenio del 37% y 34% en la superficie arbolada y forestal, respectivamente. El caso particular de Andalucía para el período (2001-2010), ocupó el quinto y segundo puesto con un 6 y 30% con respecto al número de fuegos y superficie afectada por el mismo para los 10 años.

Tabla 1.2. Superficie quemada en los cinco países miembro del sur de Europa en los últimos 36 años

Superficie quemada (ha)	**Portugal**	**España**	**Francia**	**Italia**	**Grecia***	**Total**
2015	64443	103200	11160	41511	7096	227410
% total 2015	28	45	5	18	3	100
1980-1989	73484	244788	39157	147150	52417	556995
1990-1999	102203	161319	22735	118573	44108	448938
2000-2009	150101	127229	22362	83878	49238	432809
2010-2015	92377	98660	8947	59345	29609	288937
1980-2015	105893	164592	24895	107002	45424	447807
TOTAL	3812148	5925323	896216	3852072	1635277	16121036

* número de fuegos incompletos desde 2009

1.5. Zona de estudio

El área de estudio se localiza en la sierra de Aznalcóllar (Sevilla), situada entre el Aljarafe sevillano y la Sierra Norte. Parte de esta sierra pertenece a las primeras estribaciones de Sierra Morena.

La selección de la zona de estudio se fundamentó en diversos criterios, tales como: ***i***) la historia reciente de incendios en la Sierra de Aznalcóllar, la cual fue afectada por el fuego en los años 1996 y 2004, lo que permitió la selección de puntos de muestreo, no quemados, quemados una vez y quemados dos veces ***ii***) la existencia de una amplia documentación sobre estudios previos en dicha zona (Knicker et al. 2013), lo cual nos facilitaría comparar los resultados obtenidos con la información ya recopilada, transcurridos 7 años después del último incendio ***iii***) por ser un área con diversas zonas protegidas principalmente, El Corredor Verde del Guadiamar, que es espacio natural de interés comunitario) y ***iv***) representar al bosque mediterráneo, compuesto principalmente por encinas (*Quercues ilex*), alcornoques (*Q. suber*) y jaras (*Cistus*).

Las muestras utilizadas en el presente estudio proceden de suelos que han sido quemados una vez (1996), dos veces (1996 y 2004) o ninguna (suelo control) bajo un bosque de pinos o *Quercus*. Posteriormente, se seleccionaron dos suelos (suelo control y quemado una vez bajo

Quercus) para un experimento de incubación durante 0.5, 1, 5, 8 12 y 16 meses llevado a cabo en invernadero. Este experimento se inició, con la producción de *Lolium perenne* enriquecido en ^{15}N y no enriquecido para obtener los materiales de partida: MO fresca y *PyOM* enriquecida con ^{15}N y sin enriquecer. El *PyOM* se produjo a 350ºC durante 8 minutos en presencia de oxígeno (O). El experimento de incubación consistió en enmendar el suelo control y quemado una vez con diferentes enmiendas: i) ^{15}N inorgánico (N_i), ii) MO enriquecido en ^{15}N (^{15}N-MO), iii) MO más $^{15}N_i$, iv) ^{15}N-MO más N_i, v) *PyOM* enriquecido ^{15}N (*^{15}N-PyOM*), vi) *PyOM* más $^{15}N_i$ y vii) *^{15}N-PyOM* más N_i.

1.6. Impacto del fuego en el Ciclo del Nitrógeno

El N es un macronutriente que se encuentra en bajas concentraciones en el suelo Mediterráneo variando desde 0.1 hasta 0.7% (Aponte et al. 2010), López-Serrano et al. (2016) encontró valores medios de 0.3%, lo que convierte al N en un nutriente limitante para la fertilidad del suelo y, el crecimiento de biomasa. La cantidad de N que está disponible para los microorganismos es uno de los importantes factores que controlan la biodegradación de la materia orgánica en el suelo de los bosques. Es el tercer elemento más abundante en plantas (10-30 g kg^{-1}) (Rengel & Marschner 2007), y un componente clave de los amino ácidos, ácidos nucleicos y clorofila. El N es absorbido desde la solución del suelo en mayores cantidades que otros nutrientes y es asimilado por plantas y microorganimos en forma de NO_3^- y NH_4^+, urea y aminoácidos (Bacon 1995, Hodge et al. 2000). Nannipieri y Eldor (2009) afirman que los microorganismos suelen ser mejores competidores que las plantas por el N.

El ciclo del N terrestre comprende al N biológicamente activo presente en el suelo, plantas y animales y al N inerte que se encuentra en la litosfera y atmósfera. Por lo tanto, la entrada de N y su pérdida del sistema suelo-planta-animal determina la dinámica de su ciclo, que se ve afectada, entre otras perturbaciones, por el fuego.

Durante los incendios forestales, el combustible (restos vegetales, residuos animales, exudados de raíces, rizodepósitos, necromasa, MOS y las excretas animales) sufre un proceso de combustión por ello que parte del C y N_{org} son convertidos en dióxido de C (CO_2), monóxido de C (CO), metano (CH_4), óxidos de N (NOx) y amoniaco (NH_3) que son liberados a la atmósfera. El CH_4, CO_2, óxido nitroso (N_2O) figuran entre los principales gases de efecto invernadero (Gärdenäs et al. 2011) que contribuyen al calentamiento global. Por lo tanto la primera respuesta del N ante un incendio es la disminución debido a su volatilización (Johnson & Turner 2014).

Ésta pérdida de N del suelo por volatilización se puede enmascarar por la fijación atmosférica del nitrógeno (N_2) a NH_3 llevado a cabo mediante procesos biológicos (microorganimos especializados) (Johnson et al. 2008) o industriales (ciclo de Haber-Bosch, donde el H_2 se combina con el N_2 para obtener NH_3 a 500 ºC y 200 bares), rayos (el monóxido de N, NO es convertido fotoquímicamente en NO_2, ácido nitroso (HNO_2), ácido nítrico (HNO_3) y otros subproductos y mediante el proceso de deposición (seca o húmeda) del amonio (NH_4^+), NH_3 y nitrato (NO_3^-) procedentes de partículas de la atmósfera en la superficie terrestre.

Ante un fuego de intensidad moderada a alta la MOS puede ser consumida por volatilización y el contenido de C y N disminuir (Heydari et al. 2016). Sin embargo, en un incendio de baja intensidad, el contenido de NH_4^+, NO_3^- y N total aumenta (Alexis et al. 2010, Augustine et al. 2014), tanto por el aporte de formas de N inorgánicas como por la incorporación del *PyOM* (Knicker 2004). El NH_4^+ es un producto directo de la combustión y es absorbido en los sitios de intercambio catiónico del suelo (minerales de arcilla, MOS), mientras que el NO_3^- formado en la nitrificación después del incendio puede ser lixiviado (Certini 2005). Durante un incendio la mineralización aumenta (Johnson et al. 2008), aunque también se ha descrito la tendencia contraria debido a la disminución de la actividad microbiana por la disminución de nutrientes y disponibilidad del C (Wang et al. 2012). En la misma línea Rivas et al. (2012) afirman que tanto la amonificación, como la nitrificación y la mineralización del N fueron negativos indicando inmovilización del N. Sin embargo (Fultz et al. 2016) justifican la disminución de la amonificación debido a la pérdida de N_{org} de la fracción libre, mientras que la nitrificación no se alteró debido a un cambio en la comunidad microbiana desde hongos heterotróficos hacia bacterias gran negativas autotróficas.

El *PyOM* contiene una porción de nitrógeno N_{org} cuyo origen es peptídico (Knicker 2007; Nannipieri & Eldor 2009) y se encuentra en forma de heterociclos aromáticos, *BN* (Knicker 2010), el cual podría representar el principal mecanismo de secuestro del N en el suelo (Knicker 2011).

Estos nuevos compuestos formados durante el fuego se esperaba que fuesen estables frente a la degradación química, biológica y térmica (Almendros et al. 1990, Knicker et al. 1996) y de ésta manera contribuyesen a la materia orgánica refractaria del suelo (Knicker 2004) y por lo tanto afectando al ciclo del N. Esta teoría es apoyada por Prieto-Fernández et al. (2004) quienes encontraron que después de un incendio forestal el porcentaje de N residual obtenido por hidrolisis aumentó un 45%, debido a un incremento en la aromaticidad de la MO, como consecuencia de la pérdida de los grupos oxigenados (OH and COOH), lo que hace que los compuestos sean resistentes a la hidrólisis ácida.

La inaccesibilidad del BN para los microorganismos va a establecer una competición por el N entre, microorganismos, plantas y MOS, por lo que la productividad de las plantas a corto plazo estaría influenciada por la disponibilidad del N para ser absorbido por estas.

Estudios recientes han puesto de manifiesto que la biodisponibilidad del *BN* es mayor de lo que se creía (de la Rosa & Knicker 2011, López-Martín et al. 2016), aunque, el uso de este N por las plantas sigue siendo objeto de estudio.

1.7. Cambios de la materia orgánica inducidos por el fuego

La combustión completa del combustible orgánico del suelo resulta en la producción y oxidación de gases como CO_2, CO, CH_4 o NO_2 y la formación del residuo mineral (ceniza). Sin embargo durante los incendios forestales, la disponibilidad de O es limitada y por tanto la combustión es incompleta,por lo que parte de la MOS es parcialmente quemada, dando lugar a necromasa (*PyOM*). Knicker et al. (2008a) definen el *PyOM* como una estructura derivada de lignina, furanos, anhidroazúcares y principalmente piranonas derivadas de la celulosa. En el caso de residuos quemados con alto contenido en N, el *PyOM* contendrá N heteroaromático (*BN*) derivado de péptidos. Durante un incendio en ausencia de O los polipéptidos pueden descarboxilarse, desaminarse y deshidratarse hasta dipéptidos, los cuales pueden volverse a deshidratar para formar la 2,5 dicetopiperazine.

A temperaturas superiores a 200 ºC, de acuerdo con Baldock and Smernik (2002), la MOS sufre deshidratación y formación de estructuras cíclicas insaturadas a la vez que las macromoléculas son fragmentadas en compuestos de más bajo peso molecular (de la Rosa et al. 2012). Al aumentar el tiempo de quema se producen reacciones de descarboxilación y desmetilación (Almendros et al. 2003). Con el aumento de la temperatura las estructuras alquílicas con O (derivadas principalmente de carbohidratos, como celulosa) son convertidas en estructuras aromáticas o de tipo furano (Baldock & Smernik 2002).

Durante un fuego se crean nuevas estructuras como resultado de las reacciones de deshidratación y ciclación de cadenas alifáticas con amino grupos o NH_3 (Almendros et al. 2003) que dan lugar al *BN*. Estas estructuras son de tipo pirrol, piridina o indol (Fig. 1.2) (Knicker et al. 1996, Knicker et al. 2005). El *BN* también se propone como un indicador de la existencia de un fuego reciente ya que sus estructuras son encontradas en suelos afectados por incendios mientras que en suelos donde los microorganimos degradaron la MOS no fueron observadas (Knicker & Lüdemann 1995a).

Estos cambios químicos en la MOS asociados al incremento de temperatura reducen la disponibilidad microbiana para descomponerla. Sin embargo el modelo de *PyOM* propuesto por Knicker (2007) define el *PyOM* como una mezcla heterogénea de biomacromoléculas alteradas por el fuego que contiene sustituciones de N, O y S, hecho que hace que la estructura del *PyOM* presente zonas de ataque biótico (ej: oxidación de la lignina por hongos) y abiótico, en lugar de una estructura grafítica compuesta principalmente por pequeños anillos poliaromáticos condensados. Recientes estudios (de la Rosa & Knicker 2011) encontraron incorporación de N procedente de material quemado enriquecido (^{15}*N-PyOM*) en un experimento en el que se hizo crecer césped en suelo mezclado con el material quemado enriquecido en ^{15}N. Este resultado se confirmó con la detección de estructuras tipo pirrol en el espectro de resonancia magnética nuclear de ^{15}N de dichas plantas, confirmándose que el *BN* presenta baja recalcitancia a la degradación biológica. Este hallazgo es apoyado por López-Martín et al. (2016) quienes evidenciaron la incorporación del N derivado del *BN* de material quemado enriquecido con ^{15}N en la fracción peptídica de la MOS.

De acuerdo con (González-Pérez et al. 2004) los cambios más importantes que sufre la MOS durante un incendios son: ***i***) reducción de la solubilidad debido a la perdida de los grupos oxigenados externos, ***ii***) reducción de la longitud de cadena de los ácidos graso, alcoholes y otros

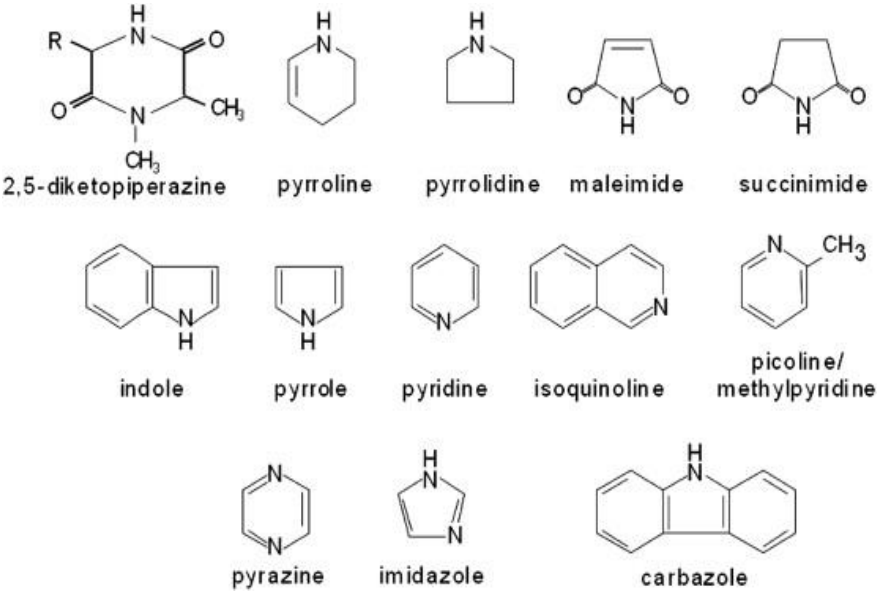

Fig. 1.2. Estructuras químicas de los compuestos del "*Black Nitrogen*" formados a partir de péptidos y proteínas formados durante el proceso de quema (Gärdenäs et al. 2011).

compuestos alquílicos, ***iii***) aumento de la aromaticidad de azúcares y lípidos, ***iv***) producción de *PyOM*, ***v***) formación de compuestos nitrogenados heterocíclicos y ***vi***) condensación de las sustancias húmicas.

1.8. Técnicas de estudio de la materia orgánica del suelo

1.8.1. Espectroscopía de resonancia magnética nuclear de ^{13}C y ^{15}N

La espectroscopía de resonancia magnética nuclear (RMN) en estado sólido de ^{13}C y ^{15}N es una técnica no destructiva válida para el estudio de la estructura química y los grupos funcionales que constituyen la MOS, así como para su cuantificación.

En el presente trabajo la espectroscopía de ^{13}C y ^{15}N RMN se empleó para la caracterización de la MO de muestras de suelo completo y las distintas fracciones obtenidas por fraccionamiento físico de muestras seleccionadas procedentes de suelo quemado y no quemado de la Sierra de Aznalcóllar. También se caracterizaron las muestras de suelo (quemado o no) tras ser enmendadas con fertilizante inorgánico, material enriquecido con ^{15}N ya sea MO o *PyOM* después de un tiempo de incubación de 0.5, 1, 5, 8, 12 y 16 meses.

1.8.2. Espectrometría de masas de relaciones isotópicas acoplado a un cromatógrafo de gases

La medida de isotopos estables, en particular es la determinación de la abundancia relativa del isótopo pesado con respecto al ligero recibe el nombre de traza o firma isotópica (δ), expresada en tanto por mil (‰) relativo a un estándar. En nuestro caso, medimos la δ^{15}N, y el estándar empleado fue el N_2 atmosférico utilizando la siguiente ecuación:

$$\delta = \left[\frac{R_{muestra} - R_{STD}}{R_{STD}}\right] \times 10^3$$

donde $R_{muestra}$ es el ratio ^{15}N/^{14}N para cada muestra estudiada y R_{STD} es la relación ^{15}N/^{14}N para el N_2 atmosférico.

Ésta técnica se aplicó para trazar y cuantificar la cantidad de ^{15}N incorporado en el sistema hojas-raíz-suelo del experimento de incubación tras 0.5, 1, 5, 8, 12 y 16 meses y de sus materiales de partida, MO y *PyOM* enriquecido o no en ^{15}M, así, como para cuantificar el ^{15}N incorporado en los aminoácidos de la MOS de dicho experimento.

1.8.3. Fraccionamiento de la materia orgánica del suelo

La MOS se fraccionó físicamente mediante densimetría usando politungstato de sodio, para obtener la materia orgánica particulada libre, ocluida y la asociada a la fracción mineral. La fracción mineral asociada a la MO fue fraccionada por tamaño de partícula mediante tamizado húmedo, obteniendo principalmente la MO asociada a arena, limo grueso y limo fino más arcilla. Cada una de estas subfracciones se caracterizaron para tener distintos tiempos de intercambio, diferentes edades, estado de degradación, función y forma de reaccionar ante un aporte de C. Este enfoque permite discernir sobre la estabilidad del C y el N en el suelo después de siete años desde el último incendio.

1.9. Justificación del trabajo

Para la consecución de los objetivos de la tesis, se llevaron a cabo cuatros estudios que se describen a continuación.

1.9.1. Estudio 1: variabilidad de la MOS y su cambio físico y químico después de múltiples incendios y tras siete años de recuperación

En primer lugar se estudió la variabilidad espacial de la MOS debida a la heterogeneidad de la zona de muestreo y la variable distribución del combustible disponible antes de un incendio forestal.

Este estudio permitió evaluar la fiabilidad de la espectroscopía de RMN de estado sólido de ^{13}C aplicada a un caso de impacto medio del fuego sobre la SOM. Para ello evaluamos la variabilidad estadística de las características del suelo y de los datos de RMN obtenidos mediante dos estrategias de muestro de los suelos, que habían sido afectados por un intenso fuego 7 años antes del muestreo. El primer muestreo (método 1) fue realizado en las cuatro esquinas y el centro de un cuadrado elegido al azar con una longitud de lado de 15 m. Las muestras fueron analizadas por separado. Para su comparación, muestras compuestas (método 2) fueron obtenidas de tres suelos. Se caracterizaron suelos procedentes de zonas quemadas una y dos veces y suelos no quemados. Las propiedades físicas y químicas de los suelos junto con la caracterización espectroscópica fueron analizadas mediante el estadístico ANOVA para ver los cambios en un período medio de tiempo.

1.9.2. Estudio 2: Alteración de la calidad del carbono y nitrógeno orgánico del suelo después de un fuego intenso y tras 7 años de recuperación

El objetivo del segundo estudio fue estudiar la estabilidad del *BN* en suelos de Sierra de Aznalcóllar afectados por el fuego. Para ello, caracterizamos la composición química de la MOS tanto para suelos muestreados 7 años como 4 semanas después del fuego mediante espectroscopia de resonancia magnética nuclear en estado sólido de ^{13}C y ^{15}N. Para dilucidar el impacto de la interacción entre la fase mineral y MOS en la estabilización del *PyOM*, evaluemos la distribución del *PyOM* entre las subfracciones obtenidas por densidad y tamaño de partícula, y si la interacción con la fase mineral puede explicar la supervivencia del *PyOM*, en particular el *BN*, siete años después del fuego.

1.9.3. Estudio 3: incorporación de nitrógeno en la biomasa procedente del *PyOM* tanto en suelos afectados como no por el fuego

El objetivo del tercer experimento se centró en estudiar el biodisponibilidad e incorporación del ^{15}N procedente de *PyOM* en el sistema hojas-raíces-suelo en un experimento de incubación. Se utilizó, *Lolium perenne* crecido en suelos quemados y no quemados procedentes de la Sierra de Aznalcóllar, que posteriormente fueron cubiertos con material enriquecido en ^{15}N, césped fresco (^{15}N-MO) o su *PyOM* (^{15}N-*PyOM*). Paralelamente, las macetas fueron fertilizados adicionalmente con KNO_3 (N_i), mientras otras fueron enmendados con residuos de material quemado (*PyOM*) o fresco (MO) junto con fertilizante enriquecido $K^{15}NO_3$ ($^{15}N_i$) y sólo $^{15}N_i$.

1.9.4. Estudio 4: biodisponibilidad de *BN* por las plantas usando como enmienda el *PyOM* tanto en suelos quemados como no quemados

En el cuarto trabajo se evaluó si la entrada de *PyOM* afecta al ciclo del N en el suelo y si el *BN* puede ser utilizado para la síntesis de biomasa microbiana. Con este fin se llevó a cabo un experimento en macetas con *PyOM* altamente enriquecido en ^{15}N procedente de *Lolium perenne*, utilizando suelo afectado por un incendio en la Sierra de Aznalcóllar y un control durante períodos de incubación de 0.5, 1, 8 y 16 meses. Transcurrido el tiempo de incubación se determinó la incorporación del ^{15}N del *PyOM* en los aminoácidos (AA_s) extraíbles de la MOS. Se utilizaron para los experimentos enmiendas de residuos de plantas enriquecidas en ^{15}N, *PyOM* no enriquecido o N inorgánico y viceversa, con la idea de identificar la fuente de N preferencial.

1.10. Bibliografía

Alexandrian D, Esnault F, Calabri G (1999) Incendios forestales en el área Mediterránea. Unysilva, 50, (197). http://www.fao.org/3/a-x1880s/x1880s00.htm

Alexis MA, Rumpel C, Knicker H, Leifeld J, Rasse D, Péchot N, Bardoux G, Mariotti A (2010) Thermal alteration of organic matter during a shrubland fire: A field study. Organic Geochemestry 41:690-697

Almendros G, Gonzalez-Vila FJ, Martin F (1990) Fire-induced transformation of soil organic matter from an oak forest: experimental approach to the effects of fire on humic substances. Soil Science 149:158-168

Almendros G, Knicker H, González-Vila FJ (2003) Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy. Organic Geochememestry 34:1559-1568

Aponte C, Marañón T, García LV (2010) Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry 101:77-92 doi:10.1007/s10533-010-9418-5

Augustine DJ, Brewer P, Blumenthal DM, Derner JD, von Fischer JC (2014) Prescribed fire, soil inorganic nitrogen dynamics, and plant responses in a semiarid grassland. Journal of Arid Environments 104:59-66

Bacon P (1995) Nitrogen Fertilization in the Environment. Taylor & Francis,

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally altered Pinus resinosa (Red pine) wood. Organic Geochemestry 33:1093-1109

Bodí MB et al. (2014) Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. Earth-Science Reviews 130:103-127

Caon L, Vallejo VR, Ritsema CJ, Geissen V (2014) Effects of wildfire on soil nutrients in Mediterranean ecosystems. Earth-Science Reviews 139:47-58

Certini G (2005) Effects of fire on properties of forest soils: a review. Oecologia 143:1-10 doi:10.1007/s00442-004-1788-8

de la Rosa JM, Faria SR, Varela ME, Knicker H, González-Vila FJ, González-Pérez JA, Keizer J (2012) Characterization of wildfire effects on soil organic matter using analytical pyrolysis. Geoderma 191:24-30

de la Rosa JM, Knicker H (2011) Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43:2368-2373

FAO (2006). Global Forest Resources Assessment 2005 – Report on fires in the Mediterranean Region. Fire Management Working Paper 8. http://www.fao.org/forestry/site/fire-alerts/en

Fultz LM et al. (2016) Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology 99:118-128

Gärdenäs AI et al. (2011) Knowledge gaps in soil carbon and nitrogen interactions – From molecular to global scale. Soil Biology and Biochemistry 43:702-717

González-Pérez JA, González-Vila FJ, Almendros G, Knicker H (2004) The effect of fire on soil organic matter—a review. Environment International 30:855-870

Heydari M, Faramarzi M, Pothier D (2016) Post-fire recovery of herbaceous species composition and diversity, and soil quality indicators one year after wildfire in a semi-arid oak woodland. Ecological Engineering 94:688-697

Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen?. Trends in Plant Science 5:304-308

Johnson DW, Fenn ME, Miller WW, Hunsaker CF (2008) Chapter 18 Fire Effects on Carbon and Nitrogen Cycling in Forests of The Sierra Nevada. In: Andrzej Bytnerowicz MJAARR, Christian A (eds) Developments in Environmental Science, vol Volume 8.

Johnson DW, Turner J (2014) Nitrogen budgets of forest ecosystems: A review. Forest Ecology and Management 318:370-379

Knicker H, Lüdemann H-D (1995): N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemestry. 23, 329-341

Knicker H (2004) Stabilization of N-compounds in soil and organic-matter-rich sediments—what is the difference?. Marine Chemistry 92:167-195

Knicker H (2007) How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry

Knicker H (2010) "Black nitrogen" – an important fraction in determining the recalcitrance of charcoal. Organic Geochemistry 41:947-950

Knicker H (2011) Soil organic N - An under-rated player for C sequestration in soils?. Soil Biology and Biochemistry 43:1118-1129

Knicker H, Almendros G, González-Vila FJ, Martin F, Lüdemann HD (1996) ^{13}C- and ^{15}N-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology and Biochemistry 28:1053-1060

Knicker H, González-Vila FJ, González-Vázquez R (2013) Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biology and Biochemistry 56:31-39

Knicker H, González-Vila FJ, Polvillo O, González JA, Almendros G (2005) Fire-induced transformation of C- and N- forms in different organic soil fractions from a Dystric

Cambisol under a Mediterranean pine forest (Pinus pinaster). Soil Biology and Biochemistry 37:701-718

Knicker H, Hilscher A, González-Vila FJ, Almendros G (2008) A new conceptual model for the structural properties of char produced during vegetation fires. Organic Geochemistry 39:935-939

Lombao A et al. (2015) Changes in soil properties after a wildfire in Fragas do Eume Natural Park (Galicia, NW Spain). CATENA 135:409-418

López-Martín M, Nowak KM, Milter A, Knicker H (2016) Incorporation of N from burnt and unburnt ^{15}N grass residues into the peptidic fraction of fire affected and unaffected soils. Journal of Soils and Sediments:1-11

López-Serrano FR et al. (2016) Influences of recovery from wildfire and thinning on soil respiration of a Mediterranean mixed forest. Science of The Total Environment 573:1217-1231

Lloret F, Vilà M (1997) Clearing of vegetation in Mediterranean garrigue: response after a wildfire. Forest Ecology and Management 93:227-234

Mayor AG, Bautista S, Llovet J, Bellot J (2007) Post-fire hydrological and erosional responses of a Mediterranean landscpe: Seven years of catchment-scale dynamics. CATENA 71:68-75

Mayor ÁG, Goirán SB, Vallejo VR, Bautista S (2016) Variation in soil enzyme activity as a function of vegetation amount, type, and spatial structure in fire-prone Mediterranean shrublands. Science of The Total Environment 573:1209-1216

M'Hirit O, (1999) El bosque mediterráneo: espacio ecológico, riqueza económica y bien social, Unasylva,50, (197). http://www.fao.org/docrep/x1880s/x1880s00.htm#Contents

Nannipieri P, Eldor P (2009) The chemical and functional characterization of soil N and its biotic components. Soil Biology and Biochemistry 41:2357-2369

Nocentini C, Certini G, Knicker H, Francioso O, Rumpel C (2010) Nature and reactivity of charcoal produced and added to soil during wildfire are particle-size dependent. Organic Geochemistry 41:682-689

Prieto-Fernández Á, Carballas M, Carballas T (2004) Inorganic and organic N pools in soils burned or heated: immediate alterations and evolution after forest wildfires. Geoderma 121:291-306

Rengel Z, Marschner P (2007) Nutrient cycling in terrestrial ecosystems. Springer,

Rivas Y, Huygens D, Knicker H, Godoy R, Matus F, Boeckx P (2012) Soil nitrogen dynamics three years after a severe Araucaria–Nothofagus forest fire Austral Ecology 37:153-163

Tessler N, Wittenberg L, Greenbaum N (2016) Vegetation cover and species richness after recurrent forest fires in the Eastern Mediterranean ecosystem of Mount Carmel, Israel. Science of The Total Environment 572:1395-1402

Velez R (1990) Los incendios forestales en el mediterráneo: Perspectiva regional, Unysilva, 41, (162). http://www.fao.org/docrep/t9500s/t9500s00.htm

Wang Q, Zhong M, Wang S (2012) A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. Forest Ecology and Management 271:91-97

Capítulo 2.

Objetivos específicos de la Tesis

Chapter 2.

Specific objectives of the Thesis

OBJETIVOS ESPECIFICOS DEL TRABAJO

Después de un incendio la composición y el tamaño de las reservas de materia orgánica del suelo se ven afectadas, alterando consecuentemente el ciclo del nitrógeno (N). En términos generales, los mecanismos de estabilización del nitrógeno y su relación con el ciclo del carbono son poco conocidos, lo cual dificulta la predicción del impacto a largo plazo de la inmovilización del nitrógeno en el potencial secuestro de carbono.

En base a estas consideraciones, las hipótesis de partida de la presente tesis fueron:

1. La estabilización del nitrógeno a largo plazo en el suelo, está determinado por los mecanismos de control del reparto de nitrógeno entre la biomasa, asociaciones órgano-minerales y las formas orgánicas protegidas, jugando un papel menos importante la inmovilización abiótica del nitrógeno.

2. La alteración del nitrógeno disponible en el suelo por incendios disminuye la entrada de materia orgánica o el nitrógeno añadido procedente de fertilizantes, modificando el reparto de nitrógeno entre la biomasa viva y las fracciones de materia orgánica del suelo. Este hecho afectará al tamaño y composición de los diferentes compartimentos de la materia orgánica del suelo y al potencial de secuestro de carbono y nitrógeno. Como consecuencia, se verá alterada la capacidad del suelo para actuar como sumidero de gases de efecto invernadero.

Teniendo en cuenta estas hipótesis, el objetivo principal de este estudio es mejorar el conocimiento cuantitativo del papel que tiene el fuego como perturbación del suelo en los procesos competitivos de secuestro del N a corto y largo plazo, así como el uso del nitrógeno orgánico e inorgánico por las plantas y los microorganismos frente a la estabilización del N a largo plazo en la materia orgánica del suelo.

Los objetivos secundarios son:

1. Muchas de las áreas en la region Mediterránea afectadas por el fuego son montañosas, por lo que la influencia del relieve en las propiedades de suelos y en la composición de la materia orgánica del suelo (MOS) debe ser estudiado. Además, teniendo en cuenta que, la distribución del combustible puede no ser homogéneo, se pueden producir variaciones considerables en la calidad y cantidad del material pirogénico (*PyOM*). Por lo tanto, los enfoques estadísticos (Li 2010) deben tener en cuenta dicha inohomegeidad. Por otro lado, muchos de estos análisis requieren un número elevado de réplicas, lo cual raramente es factible debido a la intensa labor o el alto coste de las técnicas analíticas avanzadas, aunque podrían aportar información necesaria para el mejor conocimiento de la dinámica ecológica y de las medidas a largo plazo para la recuperación de zonas alteradas. Una de estas técnicas es la espectroscopía de RMN en estado sólido de ^{13}C. Para estudiar las propiedades de MOS de nuestra área de estudio y evaluar la fiabilidad de nuestros datos, el primer paso de nuestro trabajo fue evaluar el efecto del relieve y la distribución previa del combustible en las propiedades y composición de la MOS en suelos afectados y no por el fuego en una zona montañosa de Sierra de Aznalcóllar, Andalucía (España). (**Capítulo 3**).

2. Los estudios de los últimos años indicaron que las condiciones medioambientales incuestionablemente determinan si el *PyOM* permanece en el suelo o es transformado rápidamente en residuos orgánicos, los cuales no pueden ser diferenciados de la MOS formada sin el impacto del fuego (Knicker 2011). Sin embargo, los mecanismos implicados aún están lejos de ser comprendidos. E incluso menor es el conocimiento con respecto al *Black Nitrogen* (*BN*). A parte de las propiedades protectoras de la estructura molecular y los mecanismos químicos, los compartimentos de MOS están protegidos de la descomposición vía mecanismos físicos. Esto último limita la accesibilidad de la materia orgánica (MO) debido a su interacción con la fase mineral. Por lo tanto, el fraccionamiento por densidad y de tamaño de partícula de la MOS fue usado para evaluar el papel de la asociación MOS-mineral en la supervivencia del *PyOM* en los suelos. (**Capítulo 4**).

3. Experimentos previos de corto período de tiempo usando *PyOM* enriquecido en ^{15}N como enmienda del suelo, mostraron que el ^{15}N derivó del *BN* (Hilscher & Knicker 2011) y por tanto que puede ser usado para el crecimiento de la planta (de la Rosa & Knicker 2011). Para obtener más información de la biodisponibilidad, fue llevado a cabo un experimento con macetas con suelos de zonas afectadas y no por el fuego en Aznalcóllar, que fueron enmendadas con *PyOM* u MO enriquecida en ^{15}N tras plantar semillas de *Lolium perenne*. Se controló la incorporación de ^{15}N en el suelo y en material de planta, con el objetivo de obtener más información acerca el papel del *BN* dentro del ciclo del N. (**Capítulo 5**).

4. Es ampliamente conocido que el N es un nutriente limitante para el crecimiento de las plantas y los microorganismos, de ahí que un cambio en la disponibilidad del N para los organismos del suelo debido a la alteración de la calidad del material que se incorpora puede tener un gran impacto en la productividad biológica de un suelo. Sin embargo, a pesar de la importancia del N en el ecosistema del suelo poco se sabe sobre como la transformación de las proteínas en *BN* afecta el ciclo del N, en suelos afectados por fuego. Por ello, se intentó incrementar nuestro conocimiento sobre el papel del *BN* en la producción de la biomasa microbiana, analizando la calidad y cantidad de los aminoácidos extraíbles procedentes del experimento en macetas enmendados con restos de plantas enriquecida en ^{15}N y su PyOM correspondiente. El control del ^{15}N incorporado en los aminoácidos en presencia y ausencia de N inorgánico fue diseñado para revelar los posibles efectos negativos o positivos relacionados con el uso del N para la producción de la biomasa (**Capitulo 6**).

Referencias

de la Rosa JM, Knicker H (2011): Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373

Hilscher A, Knicker H (2011): Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology and Biochemistry 43, 261-270

Knicker H (2011): Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. Quaternary International 243, 251-263

Li Y (2010): Can the spatial prediction of soil organic matter contents at various sampling scales be improved by using regression kriging with auxiliary information?, 159. Elsevier, Amsterdam, PAYS-BAS, 13 pp

Publicaciones científicas

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Capítulo 3.

Variabilidad de la calidad y la cantidad de la materia orgánica en suelo afectado por múltiples incendios

Chapter 3.

Variability of the quality and quantity of organic matter in soil affected by multiple wildfires

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Variability of the quality and quantity of organic matter in soil affected by multiple wildfires

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Abstract

Purpose. Fire in mountainous areas can lead to increased variability of their soil organic matter (SOM) due to spatial inhomogeneity and pre-fire fuel distribution. Here, we elucidated if this was the case in our study area and how this affected the reliability of solid-state ^{13}C NMR spectroscopy applied for the study of the medium-term impact of fire on SOM.

Materials and methods. The study occurred in the Sierra de Aznalcóllar, Southern Spain, which experienced their last intense fire 7 years before sampling. In a first approach (method 1), the corners and the center of a randomly chosen square with a side length of 15 m were sampled and analyzed separately. For comparison, composite samples (method 2) were obtained from three soils. We characterized material from unburnt, burnt and double burnt regions. Data describing the physical and chemical properties of the soils together with the NMR spectroscopic characterization were analyzed using ANOVA.

Results and discussion. Both sampling methods yielded comparable results with comparable standard errors. No major differences between the fire-affected and unburnt soils were observed with respect to physical and chemical properties and C and N contents, but solid-state ^{13}C NMR spectroscopy indicated a small but significant elevation of aromaticity in the soils with fire history.

Conclusions. The analysis showed that sampling with reduced replicates (method 1) can still lead to representative NMR data. The more complex sampling of comparing three composite samples (method 2) did not decrease the standard error. Our results indicate that in the study area typical properties of the soil and its SOM induced by former burnings will not persist beyond a few decades.

Keywords

Medium-term fire effects; Pyrogenic organic matter; Sampling reliability; Solid-state ^{13}C NMR spectroscopy; SOM characterization.

3.1. Introduction

Wildfires represent perturbations that affect a big variety of ecosystems. During such events not only the vegetation cover and the litter layer is attacked but also the physical and chemical soil properties are affected. For instance, pH and electrical conductivity increase due to the accumulation of ash on the soil surface (Badía et al. 2014, Martín et al. 2012). The nutrients content changes, depending on the vegetation type, fire recurrence and fire intensity (Caon et al. 2014a). High intensity fires combust most of the vegetation and much of the litter layer. As a consequence, the soil organic carbon (SOC) content decreases, whereas low or moderate intensity fires result in its increase (Knicker et al. 2006). Comparable tendencies have been observed for organic N (Prieto-Fernández et al. 2004). However, beside N losses to the atmosphere and incorporation of N into the char network, biological and non-biological processes during and after low-intensity burning transform organic N forms into more available ammonium and nitrate N and dissolved organic matter which can be lost easily in the post–fire system (Certini 2005).

Fire transforms the vegetation and the litter layer, which are mostly composed of cellulose, lignin, but also peptides and amino sugars into pyrogenic organic matter (PyOM). The latter comprises a heterogeneous mixture of heat-altered biopolymers with domains of relatively small polyaromatic clusters, but considerable substitution with N, O and S functional groups (Knicker et al. 2008a). Its structure depends both on the source material but also on the charring conditions. This transformation leads to an increase of the aromaticity of post-fire SOM (Almendros & González-Vila 2012, Knicker et al. 2005a).Commonly, charcoal is considered to be highly recalcitrant, but newer results indicate that post-depositional processes (biological, chemical or physical) affect the preservation potential of charred residues. It was found that under alkaline conditions, which occur in calcareous soils or pyroclastic layers of volcanic ash, chemically mediated physical interactions can occur which result in fragmentation of the charcoal (Braadbaart et al. 2009). Nowadays, more and more evidence for the biochemical degradability of PyOM in soils has been reported, although not much is known with respect to the participating organisms. Possibly, lignin degraders are involved as it has been suggested for lignite coals (Fakoussa & Hofrichter 1999). Subjecting SOM of fire-affected and unaffected soils from the Sierra de Aznalcóllar, Southern Spain, to biochemical degradation under laboratory conditions over a period of 7 months revealed mean residence times (MRTs) of PyOM which were only five to six times longer than those determined for unburnt SOM (Knicker et al. 2013). Considering further that without additional burnings, degraded SOM and PyOM is replaced by constant input of fresh litter, such short MRTs should lead to a decrease of the aromaticity of SOM with increasing time elapsed since the last fire.

On the other hand, loss of PyOM may also occur due to erosion, as was recently shown in catchments of mountainous areas (Kloss et al. 2012), or due to vertical leaching as indicated after characterizing the SOM in soil profiles under a Brazilian Cerrado (Velasco-Molina et al. 2016). However, irrespective of the involved mechanism, the observed alterations of the post-fire SOM suggests a steady recuperation of a fire-affected ecosystem, although it is unclear to what extent such processes proceed. This is mainly due to the fact that most of the studies on the impact of vegetation fires on soil ecosystems have compared unburnt soils by fire with soil having been burnt just recently before sampling (Certini et al. 2011, Kara & Bolat 2009, Knicker et al. 2006). Only a few investigations have monitored medium-term effects (De la Rosa et al. 2013, Prieto-Fernández et al. 2004, Vergnoux et al. 2011), reporting decreasing microbial activity, pH, C- and nitrate contents, with prolonged post-fire recovery time. However, in order to elucidate lasting impact of fire on SOM and related biochemical cycles, more studies following the changes of post-fire SOM as a function of recuperation time on a medium and long term-scale are needed.

Many of the fire-affected areas in the Mediterranean region affected by fire are mountainous. This implies that the impact of relief on the spatial distribution of soil properties and SOM composition has to be taken into account. In addition, one has to bear in mind that, prior to the burning, fuel distribution may not have been homogeneous. This could cause considerable variation of the quantity and quality of produced PyOM. Certainly, well accepted statistical approaches available (Li 2010a) accounting for such inhomogeneities are available. However, many of those require sample replication numbers which are rarely feasible for many labor-intense or expensive analytical techniques, although the latter could provide information that is urgently needed for a better understanding of the ecological dynamics and the long term measures for recuperation of such areas.

One of those is solid-state ^{13}C NMR spectroscopy. As a non-degradative approach, it represents the only method which allows the quantitative determination of the chemical composition of SOC without a prior extraction (Knicker 2011b). Unfortunately, only a few laboratories offer access to such instruments and the usable instrument time is often extremely limited. As a consequence, in many cases, it is simply not possible to measure the amount of replicates commonly required for an appropriate statistical approach.

Thus, if one does not want to abstain from the valuable information this technique can deliver, sampling approaches which allow the researcher to extract meaningful results from a manageable amount of samples are critically important. In the present study, we elucidated the extent to which relief and pre-fire fuel distribution affects soil properties and composition of SOM in fire-affected and unburnt soils of the hilly area of the Sierra de Aznalcóllar, Andalusia, Spain. Therefore, samples from several spots of various sites with different relief, fire history and vegetation were compared with respect to their C and N contents, their acidity and the composition of their SOM as revealed by means of solid-state ^{13}C NMR spectroscopy. Within this study two sampling methods with reduced numbers of replicates were tested. For the first, at each site, five spots from the corners and the center of a square with a side length of approximately 15 m were probed and each sample was analyzed. The respective results were obtained by the means of those five measurements. For the second approach, for each site, three composite samples of five spots were obtained with the same scheme. Here, the results derived from the average of the analysis of the three replicate plots. With the help of standard errors, we sought to test if this approach can be used to determine differences in soil parameters and SOM composition of unburnt soils and soils which were burnt once or twice.

3.2. Material and methods

3.2.1. Site description

The samples derived from the Sierra de Anzálcollar which is close to Seville in Southern Spain. This mountain area is part of the Sierra Morena and covers the Northwest of Andalusia. The sampled soils are classified as Cambisols (IUSS Working Group WRB 2014) and developed on slate, sandstones, and quartzite with occasional carbonates outcrop. The Sierra de Aználcollar represents a region with a high-to-moderate fire risk during the summer months.

In 2004, this area suffered a very intense fire which destroyed approximately 30 000 ha of forest. Some sampling locations were also affected by an intense fire in 1996. A more detailed description of the sites is given in Table 3.1.

For our study, we used two different sampling methods. For the first, four corners and the center of a randomly chosen square with a side length of 15 m (Fig. 3.1). For the second approach, composite samples were obtained by mixing material from the corners and center of a square with a side length of 15 m. From each site, three different soils probed. The first sampling method was applied to three sites approached in June 2011. Since the whole study area was affected by the intense fire in 2004, we were not able to collect an unburnt reference soil. Two of the chosen sites were only burnt once in 2004, and were covered either by *Pinus pinaster* (site AZPB1) or *Quercus suber* grew (AZQB1) before the burning. The 2004 fire largely destroyed the vegetation. At site AZPB1 the remaining tree trunks and their roots were removed after the fire and the area was terraced for restoration. The terrace was included in the sampling approach. Presently, it is used as pasture. Here, charcoal particles were visually detected during sampling. At site AZQB1, the trees which survived the fire recovered together with a dense shrub cover. Site 3 represents a double burnt area (AZPDB1) which prior to the first fire in 1996, was covered with *P. pinaster*. After the first fire, most of the burnt trees were removed and a maquia (*Cistus ladanifer*, *Laurus nobilis*, *Retama sphaerocarpa*) developed.

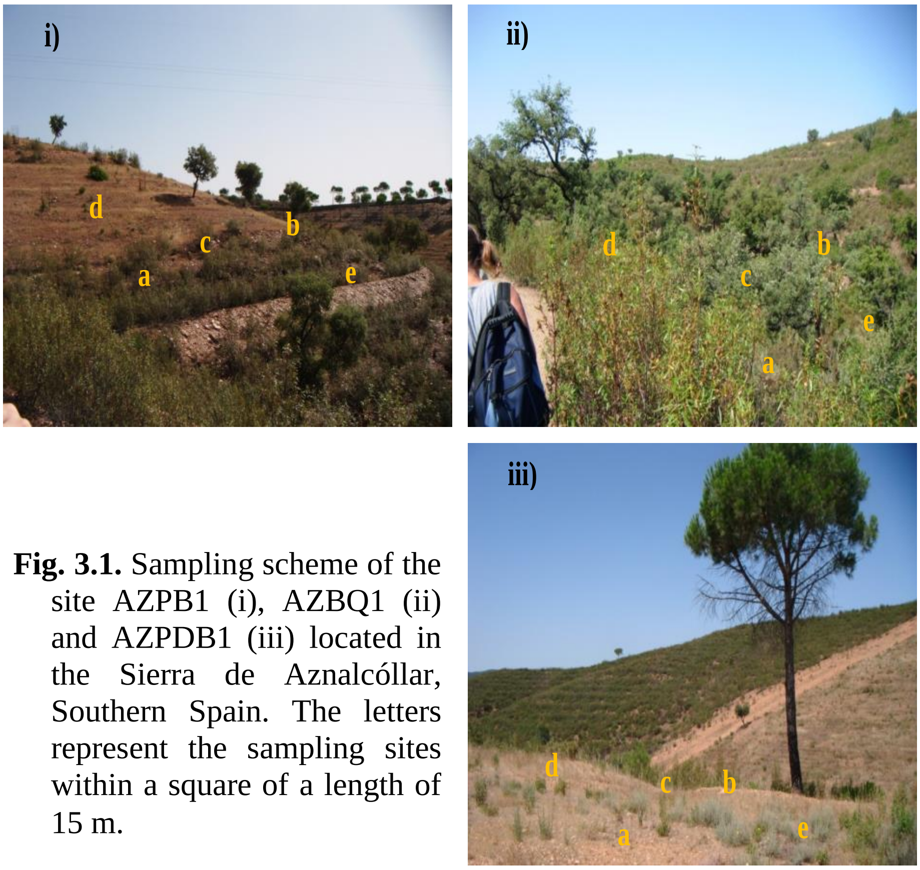

Fig. 3.1. Sampling scheme of the site AZPB1 (i), AZBQ1 (ii) and AZPDB1 (iii) located in the Sierra de Aznalcóllar, Southern Spain. The letters represent the sampling sites within a square of a length of 15 m.

Table 3.1. Localization and vegetation cover of the sampled Cambisols in fire-affected areas of the Sierra de Aznalcóllar, Southern Spain with the indication of the respective sampling method. For the first, five spots within a square of 15 m side length were probed and compared. For the second approach composite samples were obtained for each site

Sample	**Pre-fire cover**	**Post-fire cover**	**Nº fires**	**Coordinates**	**Altitude (m)**	**Slope (º)**	**Sampling method**
AZPB1	*Pinus Pinaster*	*Pinus Pinaster*	1	N 37º 34´ - W 06º 19´	267	30	1
AZQB1	*Quercus suber*	*Quercus suber*	1	N 37º 36´- W 06º 20´	330	30	1
AZPDB1	*Pinus Pinaster*	*Pinus Pinaster*	2			30	1
AZQU2-1	Quercus suber	-	0	N 37º 30´ - W 6º 19´	158	30	2
AZQU2-2	*Quercus ilex*	-	0	N 37º 29´ - W 6º 20´	104	30	2
AZQU2-3	*Q. suber – Q. ilex*	-	0	N 37º 32´ - W 6º 15´	184	30	2
AZQB2-1	*Quercus ilex*	*Q. ilex - P. Pinaster*	1	N 37º 30´ - W 6º 19´	308	30	2
AZQB2-2	*Pinus Pinaster*	*Pinus Pinaster*	1	N 37º 34´- W 6º 20´	258	30	2
AZQB2-3	*Quercus suber*	*Quercus suber*	1	N 37º 33´ - W 6º 19´	260	30	2
AZQDB2-1	*Q. suber – P. Pinaster*	*Cistaceas*	2	N 37º 36´ - W 6º 20´	396	30	2
AZQDB2-2	*Quercus suber*	*Quercus suber*	2	N 37º 35´ - W 6º 22´	300	30	2
AZQDB2-3	*Quercus suber*	*Quercus suber*	2	N 37º 35´ - W 6º 23´	357	30	2

The second sampling approach was used during a sampling campaign in December 2011.Here, two sites under *Quercus suber* and one under *Pinus pinaster* and maquia (*Cistaceae*, *Genista scorpius*, *Arbutus unedo*), which were burnt only in 2004 (AZQB2-1, AZPB2-2, AZQB2-3) and three under the same vegetation but burnt both in 1996 and 2004 (AZQDB2-1, AZQDB2-2, AZQDB2-3) were probed. Unburnt soils under *Quercus* (AZQU2-1, AZQU2-2, AZQU2-3) were chosen as reference.

At all sites, the litter layer was removed manually and soil material was taken from the first 2 cm of the A horizon. After drying at 40 ºC and removal of the fine roots by hand-picking, the soils were sieved through a 2 mm mesh and stored for further analysis.

3.2.2. Determination of chemical and physical properties of the soils

The pH of the soils was determined in the aqueous phase of a soil suspension (2.5 g of dry fine earth with 10 ml of deionized water) after the solid phase had settled for 30 min. The electrical conductivity was measured in the filtered supernatant.

Total C and total N contents were measured in duplicate via dry combustion using an elemental analyzer (Carlo-Erba EA-1108-CHNS). Due to the acidity of the soils, the presence of inorganic C can be excluded.

Inorganic nitrogen form (NO_3^-) was extracted with 2M of KCl and determined calorimetrically (Keeney & Nelson 1982) using an Jenway 6315 Spectrophotometer at 410 nm. During the determination of the ammonium contents, volatilization of the ammonia was indicated. Therefore, the obtained values were not uses for further analysis.

3.2.3. Demineralization of soil

In order to remove paramagnetic ions and concentrate the SOM prior to solid-state NMR analysis, samples were treated with 10% (*v/v*) hydrofluoric acid (HF) according to Gonçalves et al. (2003). Earlier analysis of the impact of this treatment on the SOM composition in soils of the same area clearly evidenced that no preferential extraction is induced by this demineralization technique (Knicker 2011b). Differences between the intensity distribution of the solid-state ^{13}C NMR spectra before and after the demineralization were shown to be caused by the selective impact of paramagnetics on the cross-polarization efficiency of certain C groups in the untreated samples.

For the demineralization, approximately 10 g of the dried soil were weighed into polyethylene bottles to which 40 ml of the HF solution was added consecutively. Then, the closed bottles were shaken for 2 h at 250 rpm and centrifuged for at 3000 rpm for 10 min. Subsequently, the supernatant was carefully removed and discarded. This procedure was repeated four times. After that, the sediments were cleaned by rinsing with 50 ml of deionized water until a pH > 5 was yielded. Finally, the samples were freeze-dried. Based on the fact that, on average, only 10% of the organic C (C_{org}) was lost during the treatment (Table 3.2), significant alteration of the organic matter composition due to selective removal of specific C groups can be excluded.

Table 3.2. Carbon loss after 10% HF treatment of fire-affected and unaffected Cambisols obtained from the Sierra de Aznalcóllar

Sampling site	C loss (%)
AZPB1	6.7 ± 3.1
AZQB1	4.9 ± 2.4
AZPDB1	8.8 ± 3.5
AZQU2-1/2/3	25.6 ± 2.7
AZQB2-1/2/3	8.9 ± 2.3
AZQDB2-1/2/3	5.5 ± 3.7

3.2.4. Chemical oxidation with acid potassium dichromate

To estimate the PyOM contents of the samples, approximately 0.6 mg of demineralized samples was oxidized according to Knicker et al. (2007) with 20 ml of 0.1 M $K_2Cr_2O_7$ and 2 M H_2SO_4 solution but in an oven during 6 h at 60ºC. The dichromate solution was changed as soon as the color of the solution turned to greenish, which indicated its complete reduction. After 6 h, the samples were centrifuged, the supernatant was discarded, and the residues were rinsed until the solution reached a pH value between 5 and 6. The organic carbon remaining in the oxidation residue (chemical oxidation resistant carbon, COREC) was quantified by elemental analysis and further characterized by solid-state ^{13}C NMR spectroscopy. The aromatic C content of COREC ($COREC_{arom}$) was assigned as PyOM. Its contribution to the total organic C was determined by multiplying the relative intensity of the chemical shift region of aryl C of the solid-state ^{13}C NMR spectra of the COREC samples with the percentage of C surviving this treatment. Earlier studies demonstrated that not all PyOM constituents resist this harsh oxidation method and that the chemical recalcitrance of PyOM constituents resist this hard oxidation method and that the chemical recalcitrance of PyOM depends on its humification state. Bearing in mind that, in the study area, the last fires were within the last 20 years before sampling, we considered the present PyOM as young. Accordingly, we multiplied the amount of $COREC_{arom}$ with the correction factor f_{young} = 2.4 which was suggested by for weakly humified PyOM (Knicker et al. 2008b).

3.2.5. Solid-state ^{13}C NMR spectroscopy

The solid-state ^{13}C NMR spectra of HF-treated soil samples and the chemically oxidized residues were acquired with the cross-polarization (CP) magic-angle spinning (MAS) technique on a Varian 7.05 T Unity Inova NMR Spectrometer (300 MHz) and a Bruker Avance HD III (400 MHz) using spinning speeds of 8 kHz and 14 kHz, respectively. All spectra were obtained with a ramped ^{1}H pulse during the Hartmann-Hahn contact of 1 ms, and delay times between 0.3 and 0.5 s. The chemical shift scale was referenced to tetramethylsilane using glycine (COOH:176.04 ppm). Depending on C content of the sample, between 2300 and 55500 scans were accumulated for material treated with HF and between 46100 and 118500 scans for the COREC samples. After Fourier transformation, the quantification of the spectra was performed by integration of signal intensity in the following chemical shift regions: alkyl C (45 to 0 ppm), O/N-alkyl C (110 to 45 ppm), aryl C (160 to 110 ppm), carbonyl C (245 to 160 ppm) (Knicker 2011b). For the determination of $COREC_{arom}$, the aryl C region was expanded to 160 to 90 ppm since signals of anomeric C commonly attributed to the region between 110 and 90 ppm should be absent after efficient oxidation of labile C with acid dichromate solution.

Owing to insufficient averaging of the chemical shift anisotropy at a spinning speed of 8 kHz and 14 kHz at magnetic fields of 7.05 T and 9.40 T, spinning side bands of the aromatic C signal (140 to 110 ppm) occurred at a distance corresponding to the frequency of the spinning speed at both sides of the parent signal (300 to 275 ppm and 0 to -50 ppm). They were considered by adding their intensities to that of the parent signal as described in Knicker et al. (2005b). One spinning side band of the carboxyl C signal is found in the region 325 to 300 ppm. Assuming that the second side band for carboxyl C between 0 and 45 ppm is equal in size, the integral of the side band between 325 and 275 ppm was doubled and added to the carboxyl signal (160 to 220 ppm), but subtracted from the intensity of the alkyl C region (0 to 45 ppm).

3.2.6. Statistical analysis

All statistical analyses were performed using STATGRAPHICS plus 5.1. For the comparison of the chemical soil properties, the SOM quality and the PyOM contents of the soils probed with method 1 and 2 one-way analysis of variance (ANOVA) were used .Only the N and NO_3^- contents of the composite samples were treated with the Kruskal Wallis test since they did not have normal distribution. The differences between means were tested by applying the Tukey´s honestly significant difference (HSD) test at 95% confidence level.

3.3. Results and discussion

3.3.1. pH

The pH of the samples from method 1 varies between 4.7 and 6.3 and in the case of method 2 from 4.3 to 6.1, with standard errors between 0.1 and 0.3 and 0.1 to 0.2, respectively (Table 3.3). Those values are in the range typically reported for soils of this area (Núñez & Recio 2007). Note that the sampling method had no noticeable impact on the size of the error. Using the Tukey test, the samples AZPB1 and AZQB1 were statistically different from AZPDB1. Also, unburnt AZQU2-1/2/3 can be statistically distinguished from the group, AZQB2-1/2/3 and that includes AZQDB2-1/2/3. However, comparing the two methods, no clear relationship between the number of fire events and pH can be deduced, although it is expected that fire leads to an increase of the soil pH (Gómez-Rey & González-Prieto 2014, Xu et al. 2012). It seems that 7 years after the last fire the liming effect was neutralized and local heterogeneity with respect to parent rock and vegetation cover had a greater impact on soil pH than the input of charcoal.

3.3.2. Electrical Conductivity (EC)

The electrical conductivity of a soil can be used as an indicator of the availability of plant micronutrients. After fires, this value commonly increases due to the accumulation of ash (Inbar et al. 2014). At our study sites, 7 years after the last fire, electrical conductivity (EC) values between 414 and 464 $\mu S\ cm^{-1}$ for the samples of method 1 and between 350 and 467 $\mu S\ cm^{-1}$ for those of method 2 with a standard error varying from 26 to 45 $\mu S\ cm^{-1}$ and 9 to 52 $\mu S\ cm^{-1}$ were determined (Table 3.3). Whereas the sites of method 1 cannot be distinguished with respect to EC, the unburnt sites of the sample set of method 2 shows lower values than all burnt soils.

Comparable to the measurements of the pH, we could not detect a major difference in the magnitude of the standard error resulting from the sampling methods.

3.3.3. C and N concentrations

With the exception of the material from site AZPB1, the mean organic C contents of the soils range between 57.2 and 58 mg C g $soil^{-1}$, indicating that the last fire event had no lasting impact on the SOM concentration of those soils. The soil of AZPB1 showed not only higher mean C contents but also higher standard errors then the others (Table 3.3). This is in line with a low homogeneity of the area after considerable post-fire restoration activities such as the removal of tree residues, terracing, and replanting of *Leguminosea*. Based on our ANOVA, the soils in AZPB1 had significantly higher C_{org} concentration than AZQB1 and AZPDB1 (p=0.045, Table 3.3). Preparing a box-plot diagram (Fig. 3.2a) or a media and standard error plot (Fig. 3.2b) revealed that two subsamples from AZPB1, with 35 and 180 mg C g $soil^{-1}$, are extreme values, most likely caused by the inhomogeneity of the relief. The sample with the lowest C content was collected at a slope where almost no vegetation grew, whereas the other one was obtained in an organic matter accumulation zone at the end of the slope. In the case of composite samples obtained with method 2, no differences were found. Differences with respect to C distributions have been reported for other hilly regions (Novara et al. 2011, Rumpel et al. 2006).

Table 3.3. pH (H_2O), electrical conductivity (EC), C_{org} and N contents and nitrate concentration in fire affected and unaffected Cambisols of the Sierra de Aznalcóllar and the respective standard errors

	Sampling site	**Nº fires**	**pH**	**EC (µS cm^{-1})**	**C_{org} (mg g $soil^{-1}$)**	**N (mg g $soil^{-1}$)**	**NO_3^- (mg kg $soil^{-1}$)**
Method 1	AZPB1	1	6.3 ± 0.3 a	442 ± 26 ns	130.4 ± 17.4 a	2.9 ± 0.7 ns	4.5 ± 0.5 ns
	AZQB1	1	5.8 ± 0.1 a	414 ± 34 ns	57.2 ± 11.7 b	3.6 ± 0.3 ns	5.7 ± 0.6 ns
	AZPDB1	2	4.7 ± 0.1 b	464 ± 45 ns	58.0 ± 4.6 b	2.8 ± 0.3 ns	6.2 ± 0.4 ns
Method 2	AZQU2-1/2/3	0	6.1 ± 0.2 a	350 ± 15 b	55.1 ± 5.0 ns	3.8 ± 0.1 a	2.2 ± 0.0 ns
	AZQB2-1/2/3	1	4.3 ± 0.2 b	467 ± 52 ab	57.0 ± 2.1 ns	2.4 ± 0.2 b	2.2 ± 0.2 ns
	AZQDB2-1/2/3	2	4.6 ± 0.1 b	463 ± 9 a	45.4 ± 8.1 ns	1.8 ± 0.3 b	2.9 ± 0.2 ns

Values followed with different letters within the same column are significantly different at $p < 0.05$ by Tukey´s Honestly Significant Difference (HSD) test. ns: no significant differences.

Considering the size of the error for AZPB1, it can be confirmed that topographical inhomogeneity greatly affects C_{org} contents, which are better averaged by the sampling method 2.

However, this method may be more appropriate if general patterns are needed and sampling method 1 for studies focusing on the variability of the soil in the same area. Total N values showed no statistical difference base on ANOVA, yielding a *p*-value of 0.555, for the samples obtained with method 1 (Table 3.3). For the set collected with method 2, we were able to identify two groups discerning the unburnt soils from the single and double burnt sites (Table 3.3).

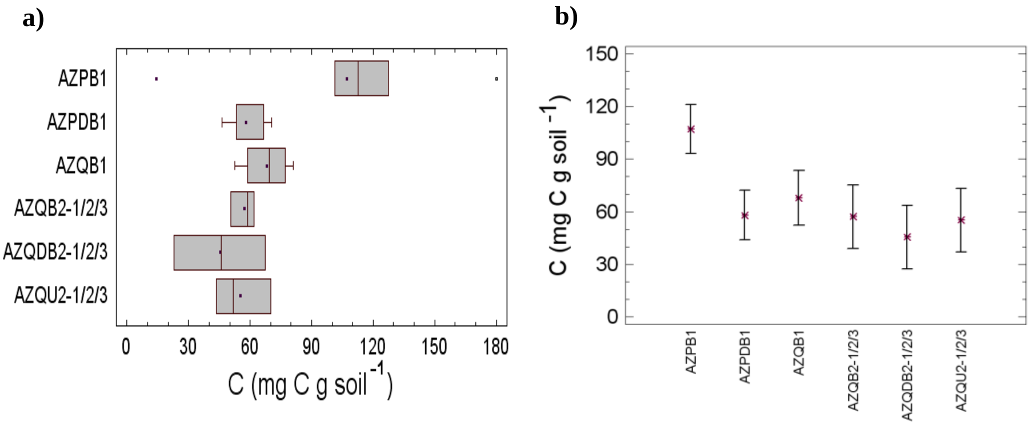

Fig.3.2. a) Box-plot of the values measured for the C contents in fire-affected and unaffected Cambisols from the Sierra de Aznalcóllar, by the method 1 and 2 (single and composite sample). The box represents the interquartile range (IQR= Q3-Q1), the horizontal line inside of the box is the median and the whiskers are extended until 1.5*IQR. The outlier was no included in the statistical analysis (in the diagram is represented with a filled square).
b) Medium and standard error of C content for fire-affected and unaffected area and the method used during sampling.

After and during low-intensity burning, biological and non-biological processes transform organic N into more available ammonium and nitrate N (Prieto-Fernández et al. 2004). Whereas ammonium is a direct product of combustion, nitrate forms after nitrification. Destruction of the vegetation and combustion of allelopathic compounds, such as terpenes and phenols, promotes the increase of nitrifiers and the transformation of available ammonium to nitrate (Andersson et al. 2004). However, in the soils from the Sierra de Aznalcóllar, higher nitrate levels were not maintained after the 7 years of post-fire recovery. This is in line with previous findings of Carter and Darwin Foster (2004) who highlighted that just after the fire the concentration of available N forms (NO_3^-, NH_4^+) increased, but that it decreased again with prolonged post-fire recovery time.

According to the statistical analysis, neither the samples obtained in June 2011 nor those collected in December 2011 showed differences which could be associated with the number of fire events. The standard errors for methods 1 and 2 range between 0.4 to 0.6 mg kg soil^{-1}and from 0.0 to 0.2 mg kg soil^{-1}respectively, showing that the second approach yields less variability.

3.3.4. Chemical characterization of soil organic matter

With the exception of those of the HF-treated soils from site AZPB1, all solid-state ^{13}C NMR spectra show their main intensity in the chemical shift region of *O/N*-alkyl C (110 to 45 ppm) (Fig. 3.3). The averaged contributions of each chemical shift region to the total spectrum are listed in Table 3.4. Among all soils, the SOM of unburnt soil AZQU2-1/2/3 contains the highest percentage of *O/N*-alkyl C (45% ± 2%) and the lowest content of aryl C (16 % ± 0%) which is in the typical range for forest soils unaffected by fire (Baldock et al. 1990, Knicker 2011b, Wilson 1981) and consistent with the reported absence of PyOM in soils (Ignacio Villar Movellan, CEDEFO, Sevilla, personal communication). The clear signal in the chemical shift region of *O*-aryl C (160 to 140 ppm) points to contributions of lignin residues, the methoxyl C of which contributes adds to the intensity between 60 and 45 ppm. Analyzing the set obtained with method 2 allowed the differentiation between the unburnt sites (AZQU2) and the group of burnt soils composed of AZQB21 and AZPDB2. No discrimination was achieved with respect to the number of fire events in the area. Within the samples obtained with method 1, the chemical composition of those from AZPB1 is significantly different from that of the other two sites. The high aryl C concentration of 46 % ± 2% of t AZPB1 together with minor intensity contributions to the *O*-aryl C region confirms a considerable abundance of charred residues. We can only speculate if this is due to a recent local fire or caused by the input of charcoal residues in the time frame of the recuperation measures. All other burnt sites reveal aryl C contents between 23 and 26 % of the total organic C. These amounts are clearly below those expected for fire-affected soils (Certini et al. 2011), but their aryl C contents are significantly higher than that of the unburnt site AZQU2-1/2/3. Thus, although the fire had no lasting effect on the quantity of the SOM, its quality still showed the typical pattern of elevated charcoal input. However, no major difference was revealed between the SOM composition of the single and double burnt sites for AZQB1 and AZPDB1 collected with method 1 and between AZQB2-1/2/3 and AZQDB-1/2/3 sampled with method 2. This suggests that the second fire did not lead to an extra gain of charred material into the SOM. The statistical analysis of the intensity in the chemical shift region attributed to *O/N*-alkyl C joins the site AZQU2-1/2/3 (unburnt) and AZQB1 into one group

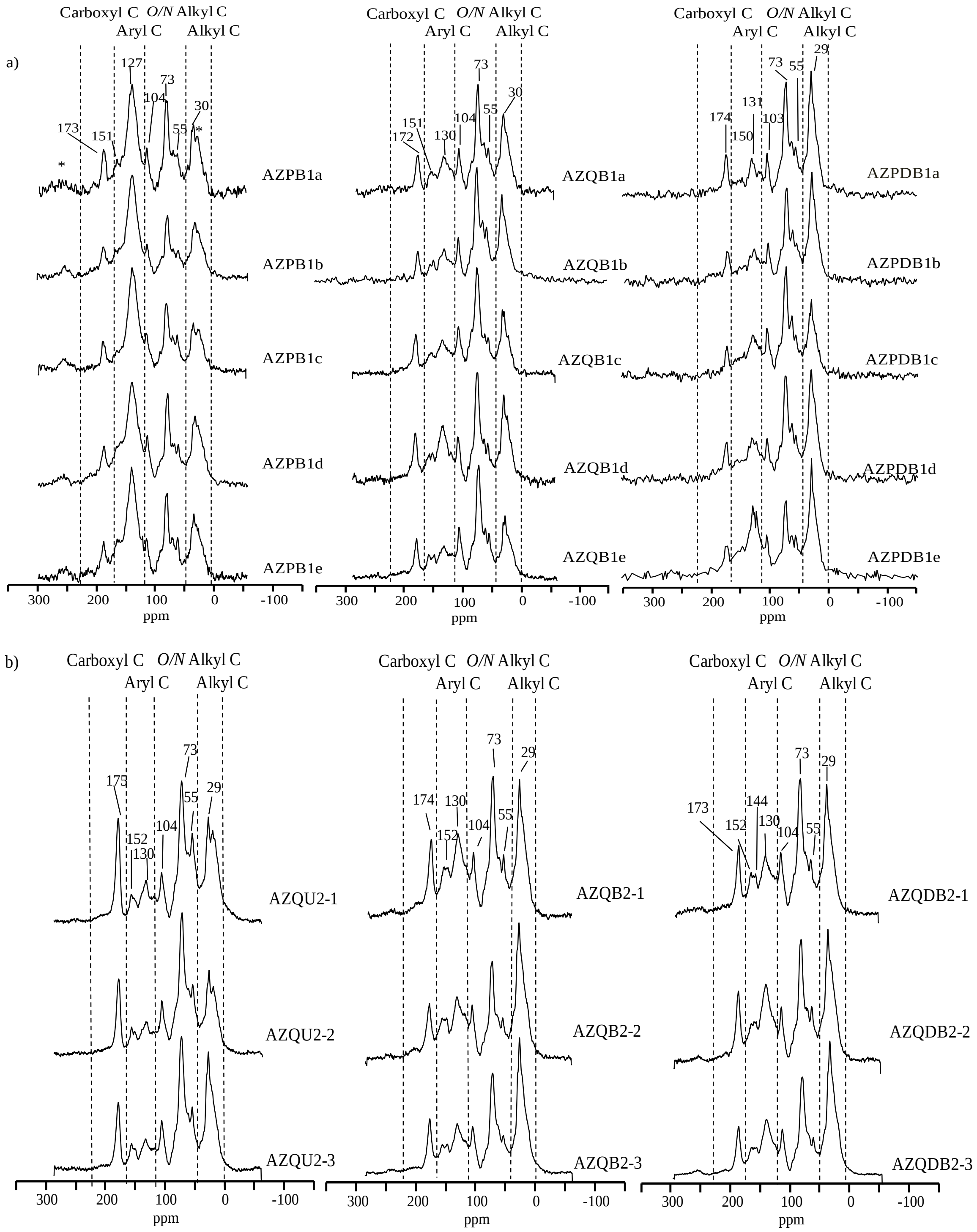

Fig. 3.3. Solid-state ^{13}C NMR spectra of HF-treated fire-affected and unaffected Cambisols obtained with sampling method 1 (a) and 2 (b) from the Sierra de Aznalcóllar.

Table 3.4. Relative intensity distribution of the solid-state ^{13}C NMR spectra of HF-treated fire-affected and unaffected Cambisols of the Sierra de Aznalcóllar, Southern Spain, the alkyl C/O/N-alkyl C ratios and the contribution of COREC$_{arom}$ to the initial C of the samples

	Sampling site	**Carboxyl C**	**Aryl C**	***O/N*-Alkyl C**	**Alkyl C**	**Alkyl C/ *O/N*-Alkyl C**	**COREC$_{arom}$ % of initial C**
Method 1	AZPB1	9.1 ± 0.4 ns	46.0 ± 1.5 a	28.8 ± 1.1 b	16.1 ±1.0 b	0.56 ± 0.04	36.4 ± 2.2, a
	AZQB1	10.0 ± 0.7 ns	22.6 ± 0.5 b	43.4 ± 1.6 a	24.1 ± 1.9 a	0.56 ± 0.07	5.2 ± 1.9, b
	AZPDB1	9.9 ± 0.4 ns	23.5 ± 2.5 b	37.8 ± 2.3 a	28.9± 1.7 a	0.78 ± 0.07	10.2 ± 3.1, b
Method 2	AZQU2- 1/2/3	12.0 ± 1.0 ns	15.7 ± 0.1 b	44.6 ± 2.0 a	27.8 ± 1.6,ns	0.63 ± 0.06	0.7 ± 0.4, b
	AZQB2- 1/2/3	11.5 ± 0.7 ns	25.8 ± 1.8 a	32.5 ± 1.5 b	30.2 ± 2.8,ns	0.94 ± 0.11	5.5 ± 1.1, a
	AZQDB2- 1/2/3	10.1 ± 0.6 ns	26.3 ± 0.9 a	34.4 ± 1.7 b	29.3 ± 2.1,ns	0.86 ± 0.10	6.2 ± 0.3, a

Letters within the same group are significantly different at $p < 0.05$ by HSD of Tukey test. ns: no significant differences.

Possibly, this is caused by the fact that in comparison to the other sites, a thick shrub layer was developed after the fire at AZQB1 leading to a higher input of partially decomposed litter material. Such material contains high amounts of carbohydrates, which decreases with ongoing microbial reworking of the litter concomitantly with a relative increase of alkyl C (Knicker & Lüdemann 1995b). This interpretation is supported by the alkyl C / *O/N*-alkyl C ratio, which increases with ongoing humification and was suggested by Baldock et al. (1997) as an index for the degradation degree of fire-unaffected SOM (Table 3.4). Comparably, no significant difference can be identified between single and double burnt sites.

Upon closer inspection of the solid-state ^{13}C NMR spectra shown in Fig. 4.3, it is evident that AZQB1d and AZPDB1e contain more aryl C then the other replicates. In contrast, sampling method 2 led to relatively consistent spectra within each treatment. Nevertheless, comparing the standard errors of the relative intensities of the different chemical shift regions (Table 3.4), these were no major differences between the two sampling methods.

3.3.5. Pyrogenic organic matter (PyOM) content

Figure 3.4b depicts the solid-state ^{13}C NMR spectra of the organic residues after their chemical oxidation. All spectra show a clear signal in the chemical shift region of alkyl C between 60 and 0 ppm. Considering the low contribution of carboxyl C to the overall intensity of the spectra (Table 3.4), this is best explained with paraffinic structures which survived the acid treatment due to their hydrophobicity. The spectra of COREC from the unburnt soils show almost no intensity in the aromatic C region. Less than 1% of the initial C_{org} of the original soils was recovered as $COREC_{arom}$ (Table 3.4; Fig. 3.4). In contrast, a clear and broad signal can be seen in this region in the spectra of COREC of the fire-affected soils. This aromatic C is best assigned to charcoal residues which, in contrast to lignin, survived the harsh chemical treatment. High $COREC_{arom}$ contents of 36% ± 2% were determined for the soils of AZPB1, whereas AZQB1 and AZPDB1 showed comparable but considerably lower values of < 10%, which did not allow to distinguish between single and double burning. The fact that the value for AZPB1 is in the range found for soils shortly after intense fire (13 to 41%) (Knicker et al. 2007) may give a further indication that at this site the charcoal input occurred only recently. Again, with respect to the sampling method, no major difference was observed in the standard errors.

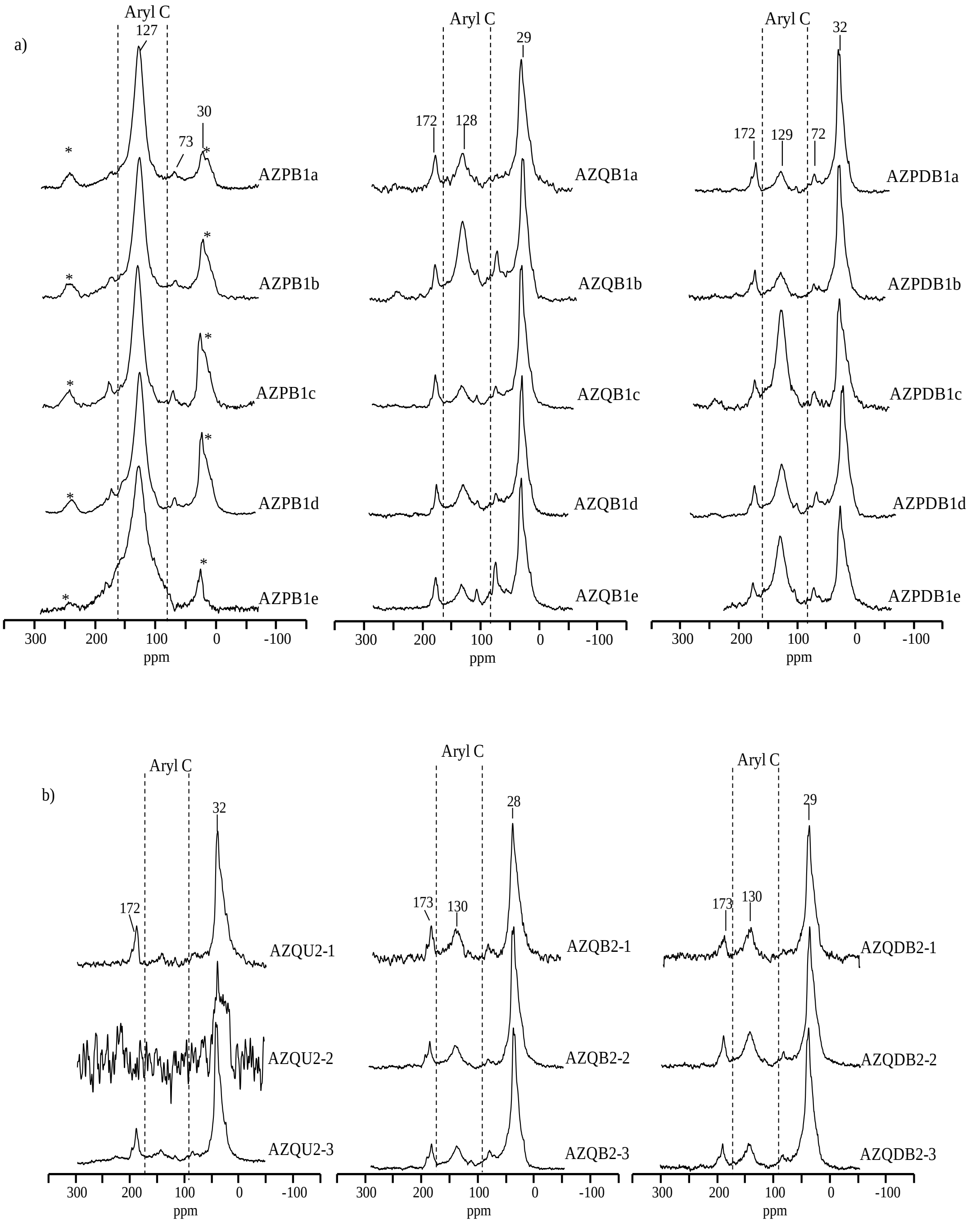

Fig. 3.4. Solid-state ^{13}C NMR spectra of fire-affected and unaffected Cambisols from Sierra de Aznalcóllar, obtained with method 1 (a) and 2 (b) after chemical oxidation with acid potassium dichromate.

3.4. Conclusions

The statistical evaluation of the results obtained for the elemental composition of soils from the mountainous region of the Sierra de Aznalcóllar revealed standard errors < 20 mg C and < 1 mg N g^{-1} soil irrespective of the used sampling methods. These low values indicate that in the studied area, relief and fuel distribution inhomogeneity had only minor impact on the variability of the C and N contents and that the more complex sampling approach 2 did not improve the reliability of the obtained data. However, on has to bear in mind that the material of sampling method 1 and 2 were collected at different sites at different times. Thus, method 1, the variation (expressed through standard error) is the result of small-scale spatial variability within a 15 x 15 m plot.

Applying method 2, we accessed the larger-scale spatial variability between plots. The fact that we did not observe large differences in the standard errors between the two sampling methods indicates that the small-scale variability ascertained in method 1 is similar in magnitude to the larger-scale variability observe in method 2.

Summarizing our data, we can not confirm a lasting impact of charcoal input on the size of the SOM pool, which is in contrast to the common belief that PyOM in soils represents an recalcitrant and efficient long-term C sink (González-Pérez et al. 2004).

With respect to the quality of SOM, our investigation showed that independently of the sampling method used, the standard error in each chemical shift region did not exceed 10% of its relative intensity. Thus, variability of the chemical composition due to relief and fuel distribution inhomogeneity could be satisfactorily averaged by using composite sampling. Again, sampling method 2 did not increase the reliability of the obtained data.

However, in contrast to the data of the elemental analysis, sampling method 1 was sufficient to allow differentiation between fire-affected and unburnt areas by means of solid-state NMR spectroscopy. Here, a small but clear enhancement of the aromaticity evidenced the fire history of the soil, although it was not discernible how often the burning occurred. However, comparing those aromatic C contents with those of soils sampled only 4 weeks after the fire in 2004 (Knicker et al. 2013) a considerable decrease of the charcoal content in the topsoils during 7 years of recovery occurred. Since comparable observations are reported by Guénon et al. (2011)Guénon et al. (2011) for a fire-affected region 17 years after the last wildfire, our results point to the conclusion that, at least in our study area, the typical pattern induced by former vegetation fires on soils will not persist beyond next decades.

3.5. References

Alexis MA, Rumpel C, Knicker H, Leifeld J, Rasse D, Péchot N, Bardoux G, Mariotti A (2010): Thermal alteration of organic matter during a shrubland fire: A field study. Organic Geochemistry 41, 690-697

Almendros G, Gonzalez-Vila FJ, Martin F (1990): Fire-induced transformation of soil organic matter from an oak forest: experimental approach to the effects of fire on humic substances. Soil Science 149, 158-168

Almendros G, Knicker H, González-Vila FJ (2003): Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state 13C- and 15N-NMR spectroscopy. Organic Geochemistry 34, 1559-1568

Almendros G, González-Vila FJ (2012): Wildfires, soil carbon balance and resilient organic matter in Mediterranean ecosystems. A review. Spanish Journal of Soil Science 2, 8-33

Andersson M, Michelsen A, Jensen M, Kjoller A (2004): Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. Soil Biology and Biochemestry 36, 849-858

Aponte C, Marañón T, García LV (2010): Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry 101, 77-92

Augustine DJ, Brewer P, Blumenthal DM, Derner JD, von Fischer JC (2014): Prescribed fire, soil inorganic nitrogen dynamics, and plant responses in a semiarid grassland. Journal of Arid Environments 104, 59-66

Bacon P (1995): Nitrogen Fertilization in the Environment. Taylor & Francis

Badía D, Martí C, Aguirre AJ, Aznar JM, González-Pérez JA, De la Rosa JM, León J, Ibarra P, Echeverría T (2014): Wildfire effects on nutrients and organic carbon of a Rendzic Phaeozem in NE Spain: Changes at cm-scale topsoil. CATENA 113, 267-275

Baldock JA, Oades JM, Vassallo AM, Wilson MA (1990): Solid state CP/MAS 13C N.M.R. analysis of particle size and density fractions of a soil incubated with uniformly labelled 13C-glucose. Australian Journal and Soil Respiration 28, 193-212

Baldock JA, Oades JM, Nelson PN, Skene TM, Golchin A, Clarke P (1997): Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Soil Research 35, 1061-1084

Baldock JA, Smernik RJ (2002): Chemical composition and bioavailability of thermally altered Pinus resinosa (Red pine) wood. Organic Geochemistry 33, 1093-1109

Bodí MB, Martin DA, Balfour VN, Santín C, Doerr SH, Pereira P, Cerdà A, Mataix-Solera J (2014): Wildland fire ash: Production, composition and eco-hydro-geomorphic effects. Earth-Science Reviews 130, 103-127

Braadbaart F, Poole I, van Brussel AA (2009): Preservation potential of charcoal in alkaline environments: an experimental approach and implications for the archaeological record. Journal of Archaeological Science 36, 1672-1679

Caon L, Vallejo VR, Coen RJ, Geissen V (2014a): Effects of wildfire on soil nutrients in Mediterranean ecosystems. Earth-Science Reviews 139, 47-58

Caon L, Vallejo VR, Ritsema CJ, Geissen V (2014b): Effects of wildfire on soil nutrients in Mediterranean ecosystems. Earth-Science Reviews 139, 47-58

Carter MC, Darwin Foster C (2004): Prescribed burning and productivity in southern pine forests: a review. Forest Ecology and Management 191, 93-109

Certini G (2005): Effects of fire on properties of forest soils: a review. Oecologia 143, 1-10

Certini G, Nocentini C, Knicker H, Arfaioli P, Rumpel C (2011): Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. Geoderma 167–168, 148-155

de la Rosa JM, Knicker H (2011): Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373

de la Rosa JM, Faria SR, Varela ME, Knicker H, González-Vila FJ, González-Pérez JA, Keizer J (2012): Characterization of wildfire effects on soil organic matter using analytical pyrolysis. Geoderma 191, 24-30

de la Rosa JM, González-Pérez JA, González-Vila FJ, Knicker H (2013): Medium term effects of fire induced soil organic matter alterations on Andosols under Canarian pine (Pinus canariensis). Journal of Analytical and Applied Pyrolysis 104, 269-279

Fakoussa RM, Hofrichter M (1999): Biotechnology and microbiology of coal degradation. Applied Microbiology and Biotechnology 52, 25-40

Fultz LM, Moore-Kucera J, Dathe J, Davinic M, Perry G, Wester D, Schwilk DW, Rideout-Hanzak S (2016): Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology 99, 118-128

Gärdenäs AI, Ågren GI, Bird JA, Clarholm M, Hallin S, Ineson P, Kätterer T, Knicker H, Nilsson SI, Näsholm T, Ogle S, Paustian K, Persson T, Stendahl J (2011): Knowledge gaps in soil carbon and nitrogen interactions – From molecular to global scale. Soil Biology and Biochemistry 43, 702-717

Gómez-Rey MX, González-Prieto SJ (2014): Short and medium-term effects of a wildfire and two emergency stabilization treatments on the availability of macronutrients and trace elements in topsoil. Science of The Total Environment 493, 251-261

Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003): The effect of 10% HF treatment on the resolution of CPMAS 13C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma 116, 373-392

González-Pérez JA, González-Vila FJ, Almendros G, Knicker H (2004): The effect of fire on soil organic matter—a review. Environment International 30, 855-870

Guénon R, Vennetier M, Dupuy N, Ziarelli F, Gros R (2011): Soil organic matter quality and microbial catabolic functions along a gradient of wildfire history in a Mediterranean ecosystem. Applied Soil Ecology 48, 81-93

Heydari M, Faramarzi M, Pothier D (2016): Post-fire recovery of herbaceous species composition and diversity, and soil quality indicators one year after wildfire in a semi-arid oak woodland. Ecological Engineering 94, 688-697

Hilscher A, Knicker H (2011): Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology and Biochemistry 43, 261-270

Hodge A, Robinson D, Fitter A (2000): Are microorganisms more effective than plants at competing for nitrogen?. Trends in Plant Science 5, 304-308

Inbar A, Lado M, Sternberg M, Tenau H, Ben-Hur M (2014): Forest fire effects on soil chemical and physicochemical properties, infiltration, runoff, and erosion in a semiarid Mediterranean region. Geoderma 221–222, 131-138

Johnson DW, Fenn ME, Miller WW, Hunsaker CF (2008): Chapter 18 Fire Effects on Carbon and Nitrogen Cycling in Forests of The Sierra Nevada. In: Andrzej Bytnerowicz MJAARR , Christian A (Editors), Developments in Environmental Science. Elsevier, pp. 405-423

Johnson DW, Turner J (2014): Nitrogen budgets of forest ecosystems: A review. Forest Ecology and Management 318, 370-379

Kara O, Bolat I (2009): Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecological Indicators 9, 1151-1155

Keeney DR, Nelson DW (1982): Nitrogen in organic forms. In: Page AL, Miller RH , Keeney DR (Editors), Methods of Soil Analysis, Part 2. Agronomy American Society of Agronomy, Madison, WI, pp. 643-698

Kloss S, Sass O, Geitner C, Prietzel J (2012): Soil properties and charcoal dynamics of burnt soils in the Tyrolean Limestone Alps. CATENA 99, 75-82

Knicker H, Lüdemann H-D (1995a): N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329-341

Knicker H, Lüdemann H-D (1995b): N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemestry 23, 329-341

Knicker H, Almendros G, González-Vila FJ, Martin F, Lüdemann HD (1996): ^{13}C- and ^{15}N-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology and Biochemistry 28, 1053-1060

Knicker H (2004): Stabilization of N-compounds in soil and organic-matter-rich sediments—what is the difference?. Marine Chemistry 92, 167-195

Knicker H, González-Vila FJ, Polvillo O, González JA, Almendros G (2005a): Fire-induced transformation of C- and N- forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (Pinus pinaster). Soil Biology and Biochemistry 37, 701-718

Knicker H, Totsche K-U, Almendros G, González-Vila FJ (2005b): Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS 13C NMR spectra obtained from pyrogenic humic material. Organic Geochemestry 36, 1359-1377

Knicker H, Almendros G, González-Vila FJ, González-Pérez JA, Polvillo O (2006): Characteristic alterations of quantity and quality of soil organic matter caused by forest fires in continental Mediterranean ecosystems: a solid-state ^{13}C NMR study. European Journal of Soil Science 57, 558-569

Knicker H (2007): How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85, 91-118

Knicker H, Müller P, Hilscher A (2007): How useful is chemical oxidation with dichromate for the determination of "Black Carbon" in fire-affected soils?. Geoderma 142, 178-196

Knicker H, Hilscher A, González-Vila FJ, Almendros G (2008a): A new conceptual model for the structural properties of char produced during vegetation fires. Organic Geochemistry 39, 935-939

Knicker H, Wiesmeier M, Dick DP (2008b): A simplified method for the quantification of pyrogenic organic matter in grassland soils via chemical oxidation. Geoderma 147, 69-74

Knicker H (2010): “Black nitrogen” – an important fraction in determining the recalcitrance of charcoal. Organic Geochemistry 41, 947-950

Knicker H (2011a): Solid state CPMAS ^{13}C and ^{15}N NMR spectroscopy in organic geochemistry and how spin dynamics can either aggravate or improve spectra interpretation. Organic Geochemestry 42, 867-890

Knicker H (2011b): Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. Quaternary International 243, 251-263

Knicker H (2011c): Soil organic N - An under-rated player for C sequestration in soils?. Soil Biology and Biochemistry 43, 1118-1129

Knicker H, González-Vila FJ, González-Vázquez R (2013): Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biology and Biochemistry 56, 31-39

Li Y (2010a): Can the spatial prediction of soil organic matter contents at various sampling scales be improved by using regression kriging with auxiliary information?. Geoderma 159, 63-75

Li Y (2010b): Can the spatial prediction of soil organic matter contents at various sampling scales be improved by using regression kriging with auxiliary information?, 159. Elsevier, Amsterdam, PAYS-BAS, 13 pp

Lombao A, Barreiro A, Carballas T, Fontúrbel MT, Martín A, Vega JA, 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

López-Martín M, Nowak KM, Milter A, Knicker H (2016): Incorporation of N from burnt and unburnt 15N grass residues into the peptidic fraction of fire affected and unaffected soils. Journal of Soils and Sediments, 1-11

López-Serrano FR, Rubio E, Dadi T, Moya D, Andrés-Abellán M, García-Morote FA, Miettinen H, Martínez-García E (2016): Influences of recovery from wildfire and thinning on soil respiration of a Mediterranean mixed forest. Science of The Total Environment 573, 1217-1231

Lloret F, Vilà M (1997): Clearing of vegetation in Mediterranean garrigue: response after a wildfire. Forest Ecology and Management 93, 227-234

Martín A, Díaz-Raviña M, Carballas T (2012): Short- and medium-term evolution of soil properties in Atlantic forest ecosystems affected by wildfires. Land Degradation & Development 23, 427-439

Mayor AG, Bautista S, Llovet J, Bellot J (2007): Post-fire hydrological and erosional responses of a Mediterranean landscpe: Seven years of catchment-scale dynamics. CATENA 71, 68-75

Mayor ÁG, Goirán SB, Vallejo VR, Bautista S (2016): Variation in soil enzyme activity as a function of vegetation amount, type, and spatial structure in fire-prone Mediterranean shrublands. Science of The Total Environment 573, 1209-1216

Nannipieri P, Eldor P (2009): The chemical and functional characterization of soil N and its biotic components. Soil Biology and Biochemistry 41, 2357-2369

Nocentini C, Certini G, Knicker H, Francioso O, Rumpel C (2010): Nature and reactivity of charcoal produced and added to soil during wildfire are particle-size dependent. Organic Geochemestry 41, 682-689

Novara A, Gristina L, Bodì MB, Cerdà A (2011): The impact of fire on redistribution of soil organic matter on a mediterranean hillslope under maquia vegetation type. Land Degradation & Development 22, 530-536

Núñez MA, Recio JM (2007): Kaolinitic paleosols in the south west of the Iberian Peninsula (Sierra Morena region, Spain). Paleoenvironmental implications. CATENA 70, 388-395

Prieto-Fernández Á, Carballas M, Carballas T (2004): Inorganic and organic N pools in soils burned or heated: immediate alterations and evolution after forest wildfires. Geoderma 121, 291-306

Rengel Z, Marschner P (2007): Nutrient cycling in terrestrial ecosystems. Springer

Rivas Y, Huygens D, Knicker H, Godoy R, Matus F, Boeckx P (2012): Soil nitrogen dynamics three years after a severe Araucaria–Nothofagus forest fire. Austral Ecology 37, 153-163

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006): Black carbon contribution to soil organic matter composition in tropical sloping land under slash and burn agriculture. Geoderma 130, 35-46

Tessler N, Wittenberg L, Greenbaum N (2016): Vegetation cover and species richness after recurrent forest fires in the Eastern Mediterranean ecosystem of Mount Carmel, Israel. Science of The Total Environment 572, 1395-1402

Velasco-Molina M, Berns AE, Macías F, Knicker H (2016): Biochemically altered charcoal residues as an important source of soil organic matter in subsoils of fire-affected subtropical regions. Geoderma 262, 62-70

Vergnoux A, Di Rocco R, Domeizel M, Guiliano M, Doumenq P, Théraulaz F (2011): Effects of forest fires on water extractable organic matter and humic substances from Mediterranean soils: UV–vis and fluorescence spectroscopy approaches. Geoderma 160, 434-443

Wang Q, Zhong M, Wang S (2012): A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. Forest Ecology and Management 271, 91-97

Wilson MA (1981): Applications of nuclear magnetic resonance spectroscopy to the study of soil organic matter. Journal Soil Science 32, 167-186

Xu Y, Sun J, Lin Q, Ma J, Shi Y, Lou K (2012): Effects of a surface wildfire on soil nutrient and microbial functional diversity in a shrubbery. Acta Ecologica Sinica 32, 258-264

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Capítulo 4.

Distribución y transformación del carbono y nitrógeno negro en las fracciones físicas de un suelo quemado después de siete años

Chapter 4.

Distribution and transformation of Black Carbon and Black Nitrogen in physical soil fractions from fire-affected soils after seven years of recuperation

Este trabajo ha sido enviado para su publicación a

Organic geochemistry

Distribution and transformation of Black Carbon and Black Nitrogen in physical soil fractions from fire-affected soils after seven years of recuperation

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Abstract

Incorporation of black nitrogen (BN), formed during charring of plant residues and litter into soil organic matter (SOM), shifts its soil organic nitrogen composition toward N-heterocyclic constituents. To investigate the medium term impact of BN on the quality of SOM, topsoils from Cambisols and Luvisols located in the Sierra de Aznalcóllar (Southern Spain) were sampled four weeks and seven years after a severe fire. The solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectra of the samples obtained four weeks after the fire prompted to a quick incorporation of charred residues into SOM. Correspondingly, pyrrole-type N dominated their soil organic N fraction. Seven years of recuperation led to a considerable decrease of aryl C, although its contribution to the total organic C was still higher at the burnt sites than in the unburnt reference soil. The fast loss of pyrogenic organic matter (PyOM) during soil recuperation calls the claimed longevity of charred residues in the soils into question. The BN showed even lower biochemical recalcitrance. Density and particle size fractionation of the organic matter (OM) of the retrieved soils indicated that the remaining char accumulated mainly in the particulate organic matter fractions (POM). Whereas the soils under *Quercus* showed comparable distribution of aromatic C in the free POM (fPOM) and occluded POM (oPOM) fractions, those under pine revealed a preferential aryl C accumulation in the fPOM fraction. However, to what extent, the lack of interaction between the mineral fraction and the BN and black carbon (BC) contributed to their low biochemical recalcitrance needs further research.

Keywords

Particulate organic matter, particle-size fractionation, solid-state ^{15}N NMR, biochemical recalcitrance of pyrogenic organic matter.

4.1. Introduction

During a wildfire event the vegetation and its litter suffer structural changes such as condensation and cyclization of organic structures (Almendros et al. 2003). The new mixture of heat-altered biomacromolecules belong to a continuum of pyrogenic organic matter (PyOM) (Masiello 2004), the structure and properties of which depends on the combustion conditions and its source (Knicker 2010). The PyOM is commonly seen as an important C sink due to its highly aromatic nature. This was supported by Vasilyeva et al. (2011) who observed that after 55 years in fallow Chernozems the quantity and quality of PyOM did not change. On the other hand, recent laboratory incubation studies reported mean resident times of PyOM in soils in the range of decades to centuries but not around millennia as previously claimed (Bird et al. 2015, Hilscher et al. 2009, Santos et al. 2012). Incubation experiments by Nocentini et al. (2010) showed that charcoal can be mineralized by microorganisms. Further indications for biochemical degradation of PyOM were also given by Hilscher and Knicker (2011) who performed laboratory incubation experiments with grass-derived charcoal and evidenced microbial attack of the aromatic network with a concomitant increase of carboxyl C. In fact, analyzing deep horizons of Ferralsols under Cerrado, revealed that those oxidized aromatic ring structures are transported into deeper soil horizons where they accumulate (Velasco-Molina et al. 2016). Abiven et al. (2011) reported that aged charcoal (10 years) released between 40-55 times more benzene polycarboxylic acids (BPCA) than fresh charcoal, possibly due to the higher oxidation state of aged sample. Moreover, the latter contributed with more BPCA where all six rings C were substituted by carboxylic C to the extract. Evidence for PyOM degradation was also obtained in natural ecosystems such as soils from a fire-prone region in the Sierra de Aznalcóllar in Southern Spain. Here, the turnover times of PyOM were only slightly higher than those obtained for humified SOM (Knicker et al. 2013). Analysis of the soils seven years after the last fire confirmed fast recuperation of most soil parameters to the pre-fire values. Comparably, the aromaticity was only slightly higher in the fire-affected soils than in the unburnt counterparts, indicating that only few pyrogenic organic C (PyOC) survived the post fire recovery time (López-Martín et al. 2016b).

Only recently, the PyOM research community recognized that nitrogen containing heterocyclic aromatic structures represent an important fraction of charred organic residues (Knicker 2010, Knicker et al. 2008). It is formed by cyclization of biogenic organic N sources such as peptides and amino sugars into mainly pyrrole-type N. Whereas early studies assumed a high recalcitrance of those structures (Knicker & Skjemstad 2000), newer incubation studies with ^{15}N-enriched source material evidenced their biochemical degradability and the subsequent use of the mobilized N for plant growth (de la Rosa & Knicker 2011). Experiments using ^{15}N-enriched PyOM as soil amendment in pot experiments further evidenced the incorporation of BN-derived nitrogen into the peptide pool of SOM (López-Martín et al. 2016a). Whereas our understanding of how and when BC is degraded in soils is still insufficient, even less is known about the fate and stability of BN during aging of PyOM.

Aside from chemical protection of organic residues due to their low biochemical accessibility, i.e, due to lack of adequate enzymes or steric hindrance (Knicker et al. 1996), survival of OM in soils and sediments was suggested to occur via physical encapsulation into hydrophobic organic networks (Knicker & Hatcher 1997). Alternatively, SOM can be part of the molecular structure and the chemical mechanisms, the SOM pools are protected from the decomposition via physical mechanism (Skjemstad et al. 1996, Sollins et al. 1996) either by adsorption, entrapment into micropores (Mayer 1994) or occlusion within aggregates. According to the latter concept, density and physical fractionation are powerful methods to distinguish different OM pools that differ in their degradability.

The particle size fractionation is based on the concept that SOM attached to particles of different size and mineralogy differs in structure and function (von Lützow et al. 2007). Using solid-state nuclear magnetic resonance spectroscopy, Baldock et al. (1992) observed an increasing degradation degree of SOM from coarse to fine particles.

The free particulate organic matter (fPOM) is part of the fraction which is floating on the aqueous phase of a soil suspended in a solution with a defined density between 1.6-1.8 g cm^{-3} (Cerli et al. 2012, Sohi et al. 2001, Sollins et al. 2006). This material is only scarcely degraded and does not interact with the mineral phase (unprotected C pool). Occluded particulate-organic matter (oPOM) shows a higher degradation degree and is protected within macroaggregates. It is liberated by disruption of the aggregates by ultrasonification with defined energy between 450 and 500 J ml^{-1} (Kölbl & Kögel-Knabner 2004). Cerli et al. (2012) reported that the intensity of dispersion vary with the aggregate stability.

Whereas the fPOM and oPOM are labile pools with turn-over times from days to years, turn-over times from years to centuries were observed for material associated with the fine fractions (clay) (Wagai et al. 2015). However, one has to keep in mind that fresh microbial material can also accumulate in the fine fractions (Clemente et al. 2011, Plaza et al. 2013).

Studies on Australian soils demonstrated that PyOM is preferentially associated with the fine fractions (Skjemstad et al. 1999). Singh et al. (2014), on the other hand, found the highest PyOM yields in the fPOM separate 10 months after PyOM addition to the soil. Comparably, during a short term incubation experiment Hilscher and Knicker (2011) using dual-isotopic (^{15}N, ^{13}C) labelled PyOM, recovered most of the label in the POM fraction, although in spite of the short time span of the experiment, some of the PyOM residues were already incorporated into the mineral-associated phase. Liang et al. (2008) evidenced physical protection by superficial interaction between PyOM and minerals, but suggested that chemical recalcitrance was more important. Heckman et al. (2014) found a preferential accumulation of PyOM into the oPOM fraction.

The intention of the present study was to bring some light on the stability of BN in fire-affected soils. Therefore, we characterized the chemical composition of SOM in soils sampled 4 weeks and 7 years after the last fire event, both by solid-state ^{13}C and ^{15}N NMR spectroscopy. In order to elucidate the impact of SOM-mineral phase interaction on PyOM stabilization, we assessed how the PyOM is distributed among the density and particle size fractions and if the interaction with the mineral phase can explain the survival of PyOM, in particular its BN constituents, 7 years after of a fire

4.2. Materials and Methods

4.2.1. Site description

The study site is located in the National Park of Sierra Aznalcóllar in Southern Spain, which was affected by wildfires in 1996 and 2004. The latter destroyed around 30000 ha of forest. A more detailed description of the sites and the fire history of this region was published elsewhere (López-Martín et al. 2016b).

The sampled soils are classified as Leptosols and Cambisols (IUSS Working Group WRB 2014). Before the last fire, the covering vegetation was pine (*Pinus pinaster*, and *Pinus pinea*) and *Quercus* (*suber and ilex*) forests, but the 2004 fire largely destroyed the vegetation. The first sample set, which was used to describe the short-term impact of fire on SOM decomposition of the bulk soils derived from a sampling campaign, conducted 4 weeks after the fire event in August 2004. The respective samples are marked with the code 04. The material was taken from the first 5 cm of a fire-unaffected (sample code: U) mineral soil under pine (sample code: P) (sample name: AZ04PU) and of the fire-affected (sample code: B) mineral soils under oak (sample code: Q) (AZ04QB), respectively, after removing the litter or ash layer (Knicker et al. 2013). A further composite sample derived from a double burnt (sample code: DB) Cambisol close to a pine which survived the fires in 1996 and in 2004 (AZ04PDB). In June 2011, those locations were visited again and material from the first 5 cm of the mineral soils were collected (López-Martín et al. 2016b). At the site under oak, the trees which survived the fire in 2004 recovered together with a dense shrub cover. Here, two samples were selected, one of which derived from a place under a shrub (AZQBd) and the second from place scarcely covered (AZQBa). At the double burnt site, from which samples of three soils were used for the present analysis, (AZPDBc,d,e) most of the burnt trees were removed and a maquia (*Cistus ladanifer, Retama sphaerocarpa*) had developed.

A further site formerly under pine was sampled (AZPB) remaining tree trunks and their roots were removed after the fire and the area was terraced for restoration. The four corners and the center of a randomly chosen square with a side length of 15 m were sampled. However, based on pre-experiments (López-Martín et al. 2016b), three samples (AZPBc,d,e) revealing detectable difference with respect to OM quality and quantity were chosen for further analysis in the present study. Note at this site, considerable amounts of charcoal particles were visually detected already during sampling. Most likely, they originate from an unregistered fire, which occurred between 2004 and 2011, or from charred wooden hunks that were disintegrated and incorporated into the soil during the restoration work.

In addition, composite samples from a third sampling event in December 2011 were used for the present study. Here, composite samples of sites under *Quercus*, which were burnt only once in 2004 (AZQB1) and twice in 1996 and 2004 (AZQDB1), were probed. An unburnt soil under *Quercus* (AZQU3) was used as a reference.

The soil textural classifications were done according the (FAO 2006) (Table 4.1). The soils of the first sampling in 2004 were air-dried whereas those taken in 2011 were dried at 40º C. Al samples were sieved through a mesh of ɵ < 2 mm to obtain the fine earth and subsequently stored in the dark until further analysis.

4.2.2. Soil fractionation

4.2.2.1. Density fractionation

In order to reveal the medium term fate of BC and BN, the topsoils taken in 2011 were subjected to a density fractionation procedure.in order to separate fPOM, oPOM and the mineral associated organic fractions (MAF) (Golchin et al. 1994b, Sohi et al. 2001). Briefly, 30 g of fine dry earth (ɵ < 2 mm) were weighted in a glass recipient and slowly covered with 200 ml of a sodium polytungstate (SPT; $3Na_2WO_4 \cdot 9WO_3 \cdot H_2O$) solution with a density of 1.8 g cm^{-3} using a

Table 4. 1. Texture, organic carbon (C_{org}) and total nitrogen (N_t) contents of the topsoil of Leptosols under an unburnt pine (AZ04PU) and a burnt (AZ04QB) *Quercus* forest, collected in 2004, four week after an intense fire, and topsoils derived from an unburnt (AZQU3), single (AZQB1, AZQBa,d) and double (AZQDB1) burnt Cambisol under *Quercus* and a single burnt Cambisol under pine (AZPBc,d,e). Those soils are from Sierra de Aznalcóllar, Southern Spain, and were obtained in 2011, seven years after a severe fire. nd: no data.

Sample	Texture	C_{org} (mg g^{-1})	N_t (mg g^{-1})
AZ04PU	Clay-Loam	22 ± nd	1.5 ± nd
AZ04QB	Loam	70 ± nd	3.6 ± nd
AZ04QDB	Clay-Loam	7.9 ± nd	0.4 ± nd
AZQU3	Sandy-Loam	69.9 ± 0.1	3.9 ± 0.0
AZQB1	Sandy-Loam	58.7 ± 0.0	2.6 ± 0.0
AZQDB1	Sandy-Loam	23.1 ± 0.0	0.8 ± 0.0
AZQBa	Sandy-Loam	14.4 ± 0.1	1.0 ± 0.0
AZQBd	Loam	65.4 ± 0.3	3.4 ± 0.0
AZPDBc	nd	53.1 ± 0.2	2.6 ± 0.0
AZPDBd	Loam	70.6 ± 0.0	3.8 ± 0.0
AZPDBe	Sandy-Loam	53.3 ± 0.4	2.3 ± 0.0
AZPBc	nd	12.8 ± 0.1	4.6 ± 0.0
AZPBd	Clay-Loam	10.2 ± 0.2	1.9 ± 0.1
AZPBe	Loam	3.5 ± 0.1	1.2 ± 0.0

Pasteur pipette. After an overnight rest, the floating fPOM was separated from the sediment, comprising MAF, by soaking and collected it in a side-arm flask using a vacuum system. Right after that, the fPOM was washed with deionizer water using ultrafiltration through a glass fiber filter (142 mm diameter, 0.22 µm, Millipore) until the solution reached an electrical conductivity of ≤ 5 µS cm^{-1}.

The sediment was resuspended in polyethylene bottle with SPT with a density of 1.8 g cm^{-3} and subjected to sonication with a probe submerging 15 mm of depth in order to disrupt the aggregates and separate oPOM from the mineral phase. The applied energy input was 400 J ml^{-1} by ultrasound (UP 400S ultrasonic processor, Hielscher Ultrasound Technology) and was adjusted according to calibration with a clay dispersion curve. The bottle was submerged in an ice bath for avoiding the thermal alteration of OM. The output of ultrasonic energy was carried out by calorimetric calibration and fixed to 26.28 J s^{-1}. After dispersing, the oPOM floating on

SPT was recovered after centrifugation for 10 min at 4000 rpm and subsequent decanting the liquid phase together with the floating OM. Cleaning of oPOM was achieved by vacuum filtration through a glass fiber filter and rinsing with deionized water until the solution reached 5 µS cm^{-1}. Lastly, the sediment remaining in the polyethylene bottle represents the MAF, which was repeatedly washed with the deionized water until the electrical conductivity of the solution reached 50 µS cm^{-1}. All fractions were freeze-dried and stored in the dark for further analysis.

4.2.2.2. Particle-size fractionation of MAF

The recovered MAF was further separated into sand-size (2000-63 µm), course silt-size (63-20 µm) and fine (< 20 µm) fractions. Therefore, 10 g of dry MAF were suspended during 1 h in 50 ml deionized water and then wet-sieved.

4.2.3. Carbon and nitrogen analysis

The bulk soils, the fPOM, oPOM, MAF and the particle size fractions were analyzed for their total carbon (C) and nitrogen (N) contents in triplicates using an elemental analyzer Carlo-Erba EA-1108-CHNS. All samples were free of carbonates hence the total C concentration was taken as the organic carbon (C_{org}).

4.2.4 Soil demineralization treatment

Prior to solid-state ^{13}C and ^{15}N solid-state NMR spectroscopy, the bulk soils and the particle size fractions were demineralized four times with 10% (*v/v*) of hydrofluoric acid (HF) according to (Gonçalves et al. 2003) in order to concentrate the SOM and to remove paramagnetic ions. Finally, the samples were freeze-dried.

4.2.5. Solid-state ^{13}C and ^{15}N NMR spectroscopy

The solid-state ^{13}C cross-polarization (CP) magic angle spinning (MAS) NMR spectra of the pretreated HF samples from the bulk soil and the physical particle-size fractions were acquired with a Varian 7.05 T Unity Inova 300 MHz and Bruker Avance III HD 400 MHz widebore spectrometer equipped with a broadband triple resonance probe for rotors with a diameter of 4 mm. A ramped ^{1}H-pulse was applied during the contact time of 1 ms, using a pulse delay time of

300 ms. The spinning speed of the rotor was 8 and 14 kHz. For quantification, the following five chemical-shift regions were integrated: alkyl C (45 to 0 ppm), *N*-alkyl C (60 to 45 ppm), *O*-alkyl C (110 to 60 ppm), aryl C (160 to 110 ppm), carbonyl C (220 to 160 ppm) (Knicker 2011b). The intensity of the spinning side bands of the aryl C region between 325 and 300 ppm and 0 and -50 ppm were added to that of the aryl C Knicker et al. (2005). The solid-state CPMAS ^{15}N NMR spectra were obtained using a widebore Bruker Avance III 600 MHz by spinning the 4 mm rotor at a speed of 15 kHz, a widebore Bruker Avance III HD 400 MHz equipped with a double resonance probehead for 7 mm rotors and a widebore Varian 7.05 T Unity Inova 300 MHz with a rotor of 6 mm and the spinning speed was 6 and 8 kHz. In the first two cases, a pulse delay of 200 ms and a contact time of 0.7 ms were applied. In the last equipment the pulse delay was time was 400 ms and the contact time 1 ms. The solid-state ^{15}N NMR spectra were not quantified due to the low signal-to-noise ratio.

4.3. Results and discussion

4.3.1. Chemical composition of the bulk soils

A detailed description and a statistical evaluation of the intensity distribution of the solid-state ^{13}C NMR spectra of the bulk soils were previously reported (Knicker et al. 2013, López-Martín et al. 2016b). In brief, the spectra of the unburnt soil under pine, sampled in 2004 (AZ04PU) and of the unburnt soil under *Quercus* taken in 2011 (AZQU3) are dominated by the signal intensity in the chemical shift region of *O*-alkyl C, which derives mainly from carbohydrates of biomass residues (Fig. 4.1). The high alkyl C content (45 to 0 ppm) of more than 20% (pine) and 30% (*Quercus*) of the total ^{13}C intensity indicates an advanced humification of the SOM. The proportions of aromatic C (160 and 110 ppm) of 21% (pine) and 30% (*Quercus*) are in the range expected for soils without or with low contributions of charred material (Knicker et al. 2007). The clear signals in the region from 160 to 140 ppm (*O*/*N*-aryl C) indicate the presence of lignin residues. Inspecting the respective ^{15}N NMR spectrum allows the assignment of almost the entire organic N to amide-N which confirms a mostly biogenic origin of the SOM (Fig. 4.1).

At the sampled site, the wildfire in 2004 resulted in a considerably increased of aromaticity both for organic C and N_t. The latter is revealed by the notable contribution of ^{15}N intensity in the chemical shift region assigned to pyrrolic N (-150 to - 250 ppm) in the solid-state ^{15}N NMR spectra of AZ04QB and AZ04PB (Fig. 4.1). The high aromaticity of the fire-affected soil

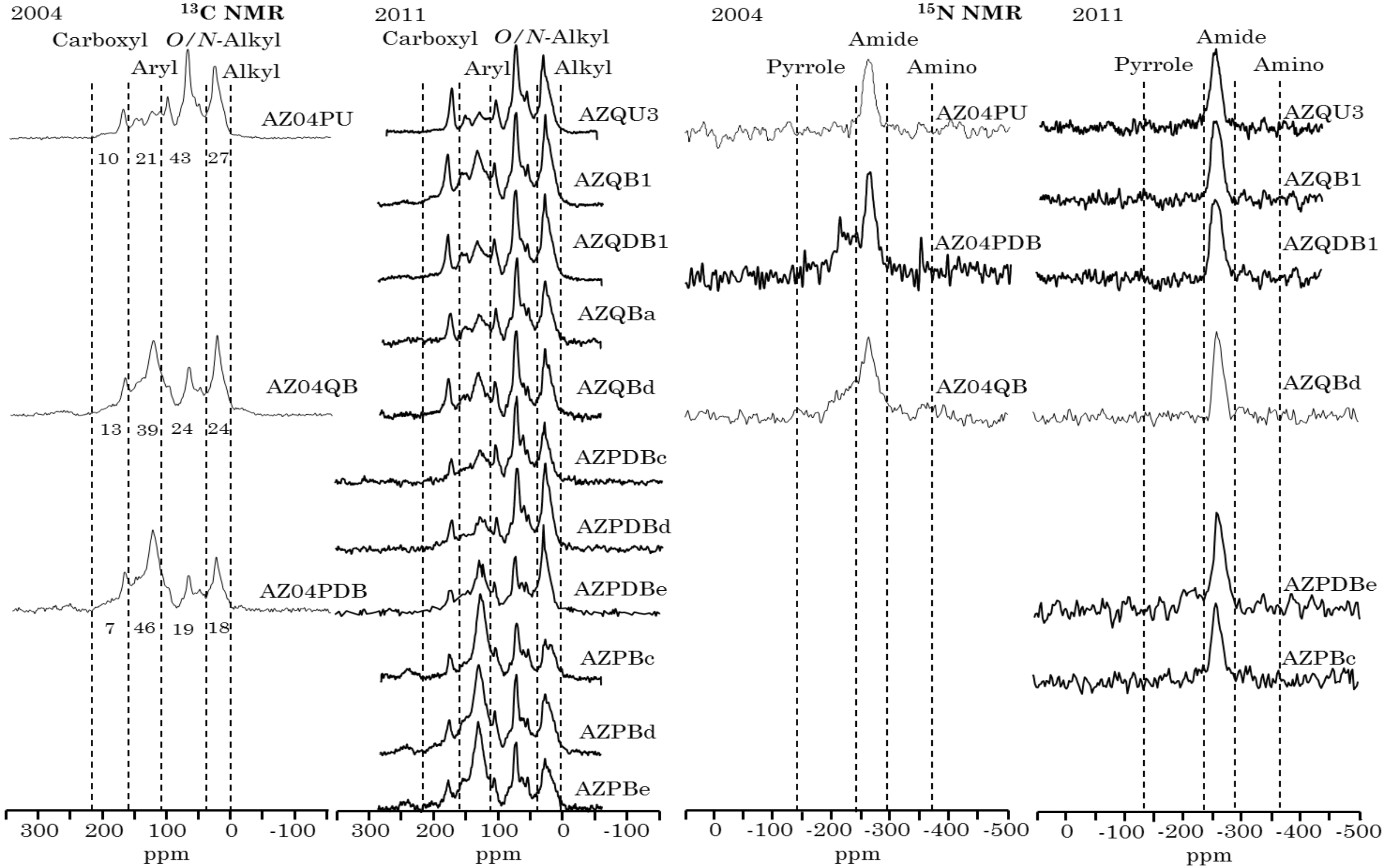

Fig. 4.1. Solid-state ^{13}C NMR and ^{15}N NMR spectra of HF-treated unburnt (U), burnt (B) and double burnt (DB) Leptosol and Cambisol sampled in 2004 (4 week after the fire) and in 2011 (7 years after the last fire) from the Sierra de Aznalcóllar (AZ), Southern Spain. The soils were formerly under pine forest (AZ04PU, AZ04PDB), AZPDBc,d,e and AZPBc,d,e and under *Quercus* stands (AZ04QB, AZQU3, AZQB1, AZQDB1 and AZQa,b).

sampled four weeks after the fire event is best explained by a high input of charred material, although the ash layer was removed prior to sampling. The presence of PyOM was confirmed by NMR spectra obtained after strong chemical oxidation with acid potassium dichromate (Knicker et al. 2007). Most tentatively, the fast incorporation of PyOM was facilitated by the high porosity of the soils caused by its high contents of gravel and stones. Seven years after the fire, the chemical composition of the bulk soils has changed considerably and was clearly expressed in a reduction of aromaticity and a concomitant increase of *O*/*N*-alkyl C. This indicates a substantial loss of PyOM, either by degradation or by erosion, during the seven years of recuperation after the fire event. A fast biochemical degradation of PyOM is in line with laboratory respiration studies indicating that under controlled conditions at 25°C the medium residence times of PyOM in the studied soil are around 40 years (Knicker et al. 2013).

Input of fresh OM from litter and microbial residues during recuperation is contributing to the masking of the remaining PyOM. However, compared to the unburnt soils, a small but significant elevation of aromaticity in the soils with fire history was confirmed by the application of a more detailed statistical analysis (López-Martín et al. 2016b). The soils (AZPBc,d,e) obtained from the site at which charcoal residues were found yielded in solid-state ^{13}C NMR spectra with comparable intensity distribution to those of the soils studied four weeks after the intense fire. Obviously, degradation of charcoal and masking by input of fresh litter had not occurred to a higher extend, yet. Thus, in contrast to the other samples, their partitioning pattern of PyOM in physical soil fractions is more likely to describe short-term effects. With respect to the organic N fraction, recovery of burnt soils leads to a decrease of the intensity of the typical resonance line for BN between -145 and -250 ppm (Knicker 2010) in favor of the amide N signal between -250 and -285 ppm. Only in the ^{15}N NMR spectrum of the sample AZPDBe a clear shoulder in the region of pyrrole N can still be discerned. This is in line with a fast degradation of N-heterocyclic aromatics of PyOM as it was recently observed in pot experiments (de la Rosa & Knicker 2011).

Note that in spite of the high aromaticity in the ^{13}C NMR spectra of the samples AZPBc,d,e, only low intensity in the pyrrole N region is identified in their ^{15}N NMR spectra. This may be an indication that the charred residues derive from N-poor sources such as wood char rather than from charred OM of the humus layer or leave residues.

4.3.2. Mass, C_{org} and N_t recoveries after density and particle size fractionation

After density separation, for all soils > 90% of their masses were recovered, most of which was assignable to the mineral fractions (Table 4.2). An exception is the sample AZPDBe of which only 81% of the total mass was yielded, probably due to losses of clay during the fractionation procedure.

After the particle-size fractionation the highest mass recovery was observed in the fraction of ɵ < 20 µm with values > 39% with the exception of AZPDBe and AZQDB1 of which 57 and 62% were found within the sand fraction size.

Both, N_t and C_{org} recovery after density fractionation was > 73% except for AZQBd and AZQU3 where the C_{org} recovery was 59 and 65%, respectively. The yields obtained after particle-size fractionation exceed of 71% of both, C_{org} and N_t with the exception of AZQDB1 which recovered of 64% of N_t.

Table 4.2. Mass recovery after density fractionation (sodium polytungstate at 1.8 g cm^{-3}) into free (fPOM) and occluded (oPOM) particulate organic matter and in mineral associated organic fraction (MAF) from unburnt (AZQU3), single (AZQB1, AZQBa,b) and double burnt (AZQDB1) soils under *Quercus* and under double (AZPDBc,d,e) and single (AZPBc,d,e) burnt Cambisol which were under pine before a severe fire event. The soils were sampled after 7 years of the last fire and derived from the Sierra de Aznalcóllar, Southern Spain. The recoveries of the particle-size fractions are given relative to the yields of MAF.

Sample	Recovery (%)					
	Density fractionation			Particle-size fraction (µm)		
	fPOM	oPOM	MAF	2000-63	63-20	< 20
AZQU3	8	5	82	38	10	52
AZQB1	7	7	83	38	6	55
AZQDB1	3	4	90	62	13	23
AZQBa	4	2	94	37	6	55
AZQBd	9	2	80	43	5	49
AZPDBc	16	2	80	33	4	62
AZPDBd	14	2	79	32	6	39
AZPDBe	15	7	60	57	9	34
AZPBc	31	10	55	20	7	73
AZPBd	16	5	71	28	6	65
AZPBe	6	2	89	27	6	65

4.3.3 Distribution of C_{org}, N_t among the fractions and its C/N ratio

For the soils derived from the sites formerly under pine, most of to the initial total organic carbon (C_i) and total nitrogen (N_i) is associated to the fPOM fraction (Table 4.3). Whereas the relative contribution of C_i and N_i between those sites is fairly comparable, lower amounts of C_i and N_i are recovered in the oPOM fraction of the double burnt site than in that of the single and recently burnt soil. The contrary is true for MAF. Note that the C/N ratios of the density fractions are clearly higher in those derived from the single burnt site than in those extracted from the double burnt soils. This may indicate that the SOM at the double burnt site was at a more developed degradation state. Alternatively, the single burnt site may have received a higher input of low-N sources. Separating the MAF into sand (2000-63 µm), coarse silt (63-20 µm) and the fine fraction (< 20 µm) showed that the latter represents the largest part with respect to contribution to the bulk soil and to total OM (Table 4.4), which is in line with (de Junet et al. 2013). The low C/N ratios (Table 4.4) of the mineral fractions are in the range expected for microbial biomass or well-humified SOM.

Compared to the soils under pine, in the soils under *Quercus* less C and N was recovered with fPOM, however a clear trend with respect to fire history was not revealed. Whereas the different fractions obtained from AZQBa,d show comparable C/N ratios to those of the unburnt composite soil AZQU3, those of AZQB1 and AZQDB1 were clearly higher. In line with the observation for the pine stand, after particle size fractionation, between 75 and 92% of the total C and N was recovered in the fine fraction. Thus, this fraction mainly determines the composition of MAF. The course silt fractions comprise less than 4% and 8% of the N_t and C_{org}, respectively of the bulk samples. Comparably, the sand fraction contains less than 17% of the C_{org} and between 8 and 16% of the N_t with respect to the bulk soil. A trend with fire history or stand was not observed.

4.3.4. Chemical characterization of SOM fractions

The solid state ^{13}C NMR spectra of the fPOM of almost all soils sampled from stands under *Quercus* are dominated by signals from *O*-alkyl C, alkyl C followed by aryl C as found by Plaza et al. (2012) and Golchin et al. (1994b) for soils with different properties and climate conditions (Fig. 4.2 and 4.3). Compared to the bulk soils, the fPOM fractions contain slightly more and the oPOM separate slightly less *O*-alkyl C (Fig. 4.2 and 4.3). This finding is in agreement with the fact that fPOM is composed mainly of plants debris, which are only partly degraded that fPOM

Table 4.3. Recovery of organic carbon (C_{org}) and total N (N_t) as carbon (C_f) and nitrogen (N_f) of the free (fPOM), occluded (oPOM) particle organic matter fractions and the mineral-associated organic fraction (MAF) from unburnt (AZQU3), single (AZQB1, AZQBa,b) and double burnt (AZQDB1) Cambisols under *Quercus* and from double (AZPDBc,d,e) and single (AZPBc,d,e) burnt Cambisols under pine sampled seven years after a fire in the Sierra de Aznalcóllar, Southern Spain.

Sample	**fPOM**			**oPOM**			**MAF**		
	C_f of C_{org} (%)	**N_f of N_t (%)**	**C/N**	**C_f of C_{org} (%)**	**N_f of N_t (%)**	**C/N**	**C_f of C_{org} (%)**	**N_f of N_t (%)**	**C/N**
AZQU3	46.0 ± 0.3	28.7 ± 4.1	24 ± 3	33.8 ± 1.0	25.3 ± 2.5	20 ± 1	21.2 ± 1.0	46.7 ± 5.9	7 ± 1
AZQB1	31.7 ± 1.6	17.9 ± 3.2	36 ± 6	48.3 ± 7.2	28.1 ± 5.5	35 ± 3	20.0 ± 0.2	54.0 ± 6.6	7 ± 1
AZQDB1	32.7 ± 1.4	16.1 ± 0.0	39 ± 5	43.9 ±0.6	26.3 ± 1.3	34 ± 4	24.1 ± 1.5	57.6 ± 6.8	11 ± 2
AZQBa	41.8 ± 4.0	17.1 ± 2.9	26 ± 3	26.9 ± 1.5	11.4 ± 1.7	26 ± 2	31.3 ± 0.0	71.6 ± 12.1	5 ± 1
AZQBd	58.5 ± 6.4	30.4 ± 5.0	26 ± 1	11.4 ± 0.4	6.8 ± 0.9	24 ± 2	30.1 ± 3.2	62.7 ± 6.5	7 ± 1
AZPDBc	67.7 ± 1.7	40.5 ± 5.7	29 ± 4	12.9 ± 0.3	8.5 ± 0.9	27 ± 3	19.4 ± 0.3	51.1 ± 3.6	7 ± 1
AZPDBd	63.1 ± 1.1	36.5 ± 7.4	31 ± 5	15.0 ± 0.4	9.9 ± 1.1	33 ± 3	21.9 ± 1.6	55.6 ±7.5	7 ± 0
AZPDBe	71.4 ± 2.1	47.3 ± 7.2	34 ± 5	16.7 ± 0.2	12.1 ± 2.3	32 ± 5	11.9 ± 2.0	40.7 ± 3.8	7 ± 1
AZPBc	65.6 ± 1.7	50.2 ± 7.0	40 ± 5	26.5 ± 2.4	21.7 ± 5.3	38 ± 5	7.9 ±0.2	28.1 ± 3.2	9 ± 1
AZPBd	62.0 ± 1.6	39.8 ± 5.1	47 ± 5	26.9 ± 0.5	17.8 ± 2.1	45 ± 4	11.1 ± 0.6	42.4 ± 3.9	8 ± 3
AZPBe	60.5 ± 2.3	26.6 ± 4.9	47 ± 6	25.3 ± 0.8	9.5 ± 1.1	55 ± 5	14.2 ±1.0	63.9 ± 12.1	5 ± 1

is composed mainly of plants debris, which are only partly degraded (Golchin et al. 1994a) whereas the material in oPOM underwent advanced microbial degradation (Christensen 2001, Dorodnikov et al. 2011). The alkyl C-to-*O*-alkyl C ratio (Table 4.5) suggested by (Baldock et al. 1997, Golchin et al. 1994a) as a degradation index is in line with this interpretation. Calculating the alkyl C-to-carboxyl C ratio points toward an increase of the average chain length from fPOM to oPOM, which is line with Plaza et al. (2012) who revealed an enrichment of unsubstituted-aliphatic material such as lipid structures (cutin and suberin) in oPOM. Note that those patterns are observed for all studied samples sets irrespective of the site and fire history. Comparably, the solid-state ^{15}N spectra of all studied POM fractions show no major difference (Fig.4.4). The only signal which unbiasedly can be discerned from the noise is attributed to amide N, indicating that Black Nitrogen did indeed not survive to a higher extent in these fractions. The soil organic carbon in MAF at particle sizes < 20 µm is composed mainly of *O*-alkyl C.

A comparable finding is reported by Sohi et al. (2001) and Boeni et al. (2014). Comparing the relative intensity distribution in the ^{13}C NMR spectra of the clay fractions with those of the oPOM, a higher contribution of *O*-alkyl C is observed for all sampling sites. A relationship with stand or fire history is not evident. The Alkyl C-to-*O*-alkyl C ratio increased in the following order fPOM, < 20 µm and oPOM (Table 4.5).

This is in contrast to the general assumption that the most degraded SOM is associated with the fine particle size fractions. On the other hand it is in line with Bimüller et al. (2014) who stated that in spite of the enrichment in polysaccharides, the SOM in the fine fractions are expected to be less bioavailable or/and accessible for microbial degradation due to the interaction or the OM with the mineral surfaces.

In the fine particle size fractions, the carboxyl C contributes with < 10% to the $C_{org,}$ which is in accordance with the assumption that their OM has not experienced extensive microbial degradation but has contribution of microbial residues (Clemente et al. 2012, Clemente et al. 2011, Six et al. 2002).

Table 4.4. Recovery of organic carbon (C_f) and nitrogen (N_f) from the organic carbon (C_{org}) and total nitrogen (N_t) of the bulk soils in the sand-size (2000-63 mm), course silt-size (63-20 mm) and fine-size (< 20 µm) fractions from unburnt (AZQU3), single (AZQB1, AZQBa,b) and double burnt (AZQDB1) soils under *Quercus* and from Cambisols under pine after two (AZPDBc,d,e) and one (AZPBc,d,e) forest fires and the C/N ratios of the different fractions. The samples derive from the Sierra de Aznalcóllar, Southern Spain, and were taken seven years after the last fire.

Sample	**2000-63 mm**			**63-20 mm**			**< 20 µm**		
	C_f of C_{org} (%)	**N_f of N_t (%)**	**C/N**	**C_f of C_{org} (%)**	**N_f of N_t (%)**	**C/N**	**C_f of C_{org} (%)**	**N_f of N_t (%)**	**C/N**
AZQU3	6.3 ± 0.8	8.4 ± 0.0	5 ± 0	2.9 ± 0.1	3.0 ± 0.4	7 ± 1	89.2 ± 6.6	91.6 ± 2.6	7 ± 0.3
AZQB1	13.2 ± 1.7	11.7 ± 2.9	10 ± 3	8.4 ± 1.2	4.4 ± 0.5	17 ± 0	78.6 ± 3.8	83.9 ± 2.0	8 ± 0.3
AZQDB1	16.8 ± 2.8	14.6 ± 7.5	13 ± 1	7.9 ± 1.4	4.0 ± 1.7	27 ± 8	74.9 ± 1.9	86.6 ± 0.0	11 ± 0.3
AZQBa	13.8 ± 1.5	15.5 ± 3.5	5 ± 1	5.8 ±0.2	3.9 ± 0.0	8 ± 0	80.1 ± 7.1	79.1 ± 14.7	6 ± 0.9
AZQBd	11.2 ± 1.3	16.0 ± 4.6	6 ± 2	2.5 ± 0.1	2.9 ± 0.0	7 ± 0	86.6 ± 1.2	80.9 ± 3.7	9 ± 0.3
AZPDBc	9.4 ± 0.2	10.6 ± 0.0	6 ± 0	4.0 ± 0.5	1.8 ± 0.2	15 ± 1	86.1 ± 5.3	87.8 ± 5.6	7 ± 0.0
AZPDBd	10.4 ± 2.5	14.6 ± 4.3	6 ± 2	4.0 ± 0.3	1.8 ± 0.7	11 ± 3	83.2 ± 3.9	82.6 ± 9.0	8 ± 0.6
AZPDBe	26.4 ± 3.0	30.4 ± 8.5	7 ± 1	8.0 ± 0.1	5.2 ± 0.0	12 ± 0	66.1 ± 0.6	64.5 ± 2.1	8 ± 0.2
AZPBc	5.3 ± 0.6	4.8 ± 0.6	8 ± 1	3.4 ± 0.3	3.2 ± 0.3	8 ± 0	91.7 ± 5.5	92.0 ± 5.9	8 ± 0.0
AZPBd	6.9 ± 0.3	8.2 ± 1.0	7 ± 1	3.2 ± 0.3	1.5 ± 2.2	8 ± 0	89.5 ± 7.7	90.3 ± 6.4	8 ± 0.1
AZPBe	12.7 ± 3.6	8.2 ± 1.1	7 ± 3	3.1 ± 0.1	3.4 ± 0.4	4 ± 0	84.5 ± 0.0	88.4 ± 0.0	4 ± 0.0

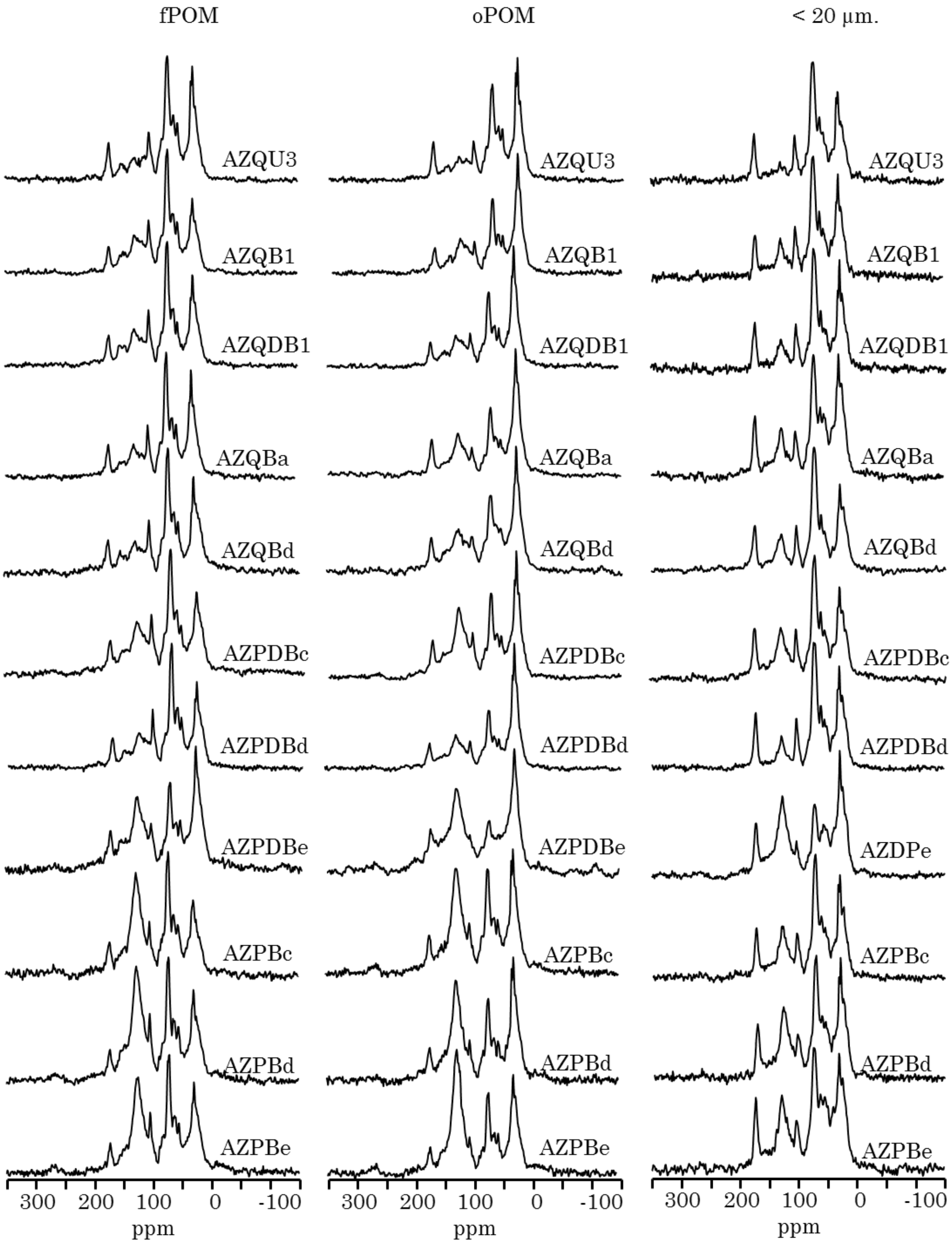

Fig. 4.2. Solid-state ^{13}C NMR spectra of the HF-treated fine particle-size fractions (< 20 µm) and the free and occluded particulate organic matter (fPOM, oPOM) after density fractionation (1.8 g cm-3) from Cambisols sampled seven years after a fire event in the Sierra de Aznalcóllar, Southern Spain. The latter were located under *Quercus* forests which were unburnt (AZQU3), burnt once (AZQB1, AZQa,d) and double burnt (AZQDB1). Further material was obtained from soils which were under pine before they were burnt once (AZPBc,d,e) or twice (AZPDBc,d,e).

The fact that the intensity in the methoxyl C/*N*-alkyl C (60 to 45 ppm) increased slightly, whereas the clear signals assignable to *O*-aryl of lignin between 160 and 140 ppm almost vanished underlines an accumulation of peptides in the mineral fraction. This is supported by their narrow C/N ratio and the dominance of the signal in the amide N region between -250 to -285 ppm of their solid-state ^{15}N NMR spectrum (Fig. 4.4). Accordingly, Zhou et al. (2014), suggested that the clay fraction adsorbs and protects N-rich OM such as peptides.

Table 4.5. *A*lkyl C/*O*-alkyl C and alkyl C/carboxyl C ratios of free (fPOM), occluded (oPOM) particle organic matter and fine fraction (< 20 µm) of the mineral associated organic matter derived from unburnt (AZQU3), single (AZQB1, AZQBa,b) and double burnt (AZQDB1) soils under *Quercus* and from double (AZPDBc,d,e) and single (AZPBc,d,e) burnt Cambisols under pine. All soils were sampled seven years after the last fire from the Sierra de Aznalcóllar, Southern Spain.

Samples	**Alkyl C/*O*-alkyl C**			**Alkyl C/carboxyl C**		
	fPOM	**oPOM**	**< 20 µm**	**fPOM**	**oPOM**	**< 20 µm**
AZQU3	0.8	1.0	0.7	4.2	3.7	4.0
AZQB1	0.6	1.3	0.8	3.7	4.7	4.4
AZQDB1	0.7	1.3	0.8	3.7	4.8	4.3
AZQBa	0.8	1.5	1.0	3.2	3.4	3.1
AZQBd	0.8	1.0	0.7	2.9	2.9	3.4
AZPDBc	0.7	1.1	0.8	3.6	3.4	2.5
AZPDBd	0.7	1.5	0.8	3.5	3.9	2.9
AZPDBe	1.3	1.4	1.4	2.8	1.8	2.6
AZPBc	0.6	1.1	0.8	2.0	2.4	2.6
AZPBd	0.7	1.3	0.9	2.7	3.6	3.4
AZPBe	0.7	1.1	0.9	3.3	3.8	2.7

With respect to the contribution of aryl C, a clear impact of the fire history is observed for the samples obtained from the stands under *Quercus* and pine (Fig. 4.3). Although for the bulk soils, the aromaticity decreased considerably during the seven years of recuperation (Fig. 4.1), the fPOM and oPOM fractions of the single and double burnt site seven years after the fire showed clearly higher aryl C contributions than the unburnt site AZQU3. With the exception of the samples from AZPBc,d,e, the aromaticity of oPOM from the soils of the burnt sites collected in 2011 was higher than that of fPOM, but no difference between double and single burning was revealed (Fig. 4.3). This is in contrast to studies of Singh et al. (2014) who found the highest

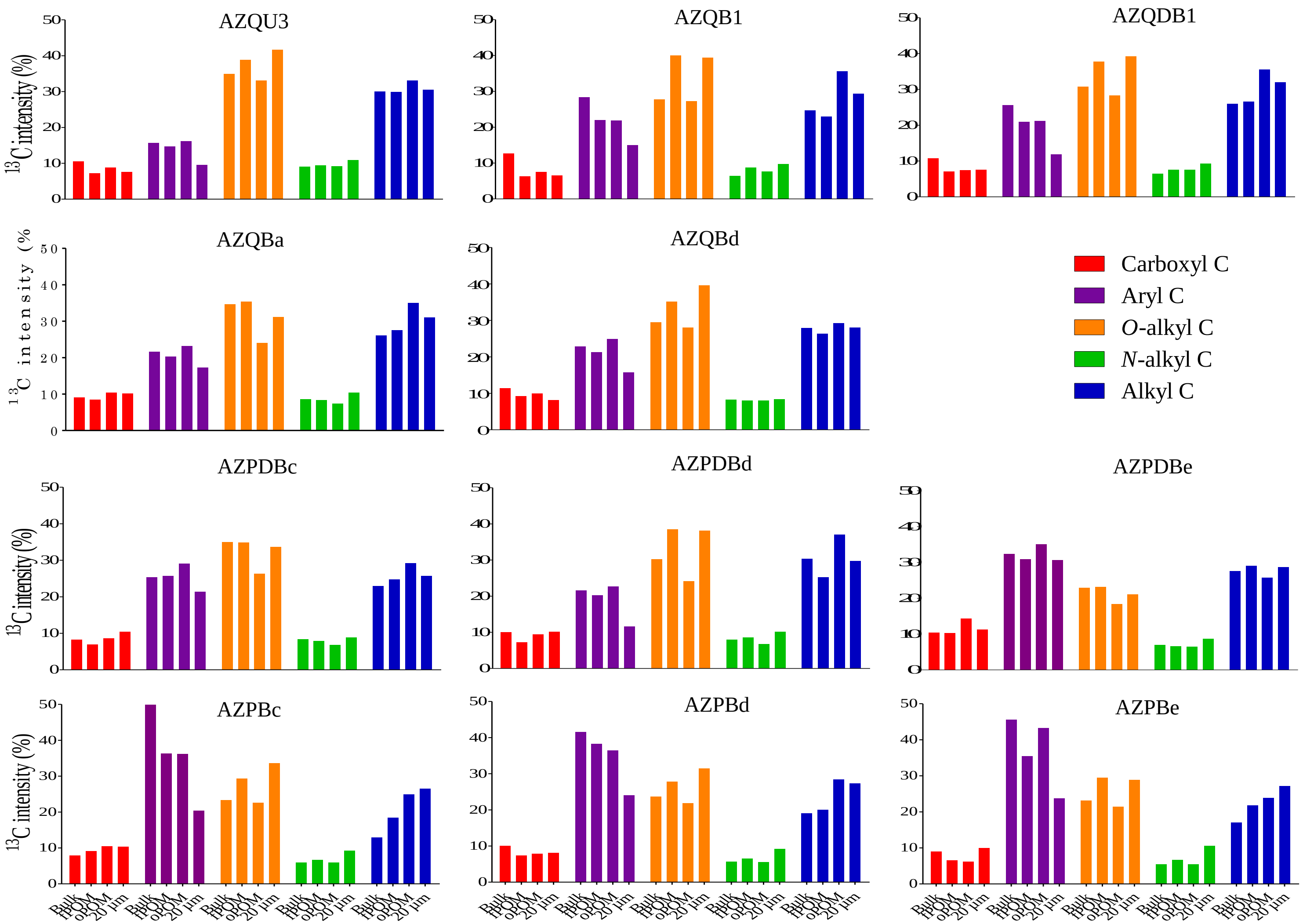

Fig. 4.3. Intensity distribution of the solid-state ^{13}C NMR spectra of the HF-treated bulk soils from Cambisols, their free and occluded particulate organic matter (fPOM, oPOM) obtained after density fractionation (1.8 g cm^{-3}) and their fine particle-size fraction (< 20 µm). The soils were sampled in the Sierra de Aznalcóllar seven years after a severe fire event and were located under *Quercus* stands which were unburnt (AZQU3), single burnt (AZQB1, AZQa,d) and double burnt (AZQDB1) and from soils which were under pine before they were burnt twice (AZPDBc,d,e) or once (AZPBc,d,e).

PyOM contribution in the fPOM fraction. Between 62 and 86% of the aromatic C of the bulk samples was recovered with both POM fractions (Table 4.6). A preference for oPOM or fPOM was not evidenced for the samples derived from the sites under *Quercus*, whereas those from the stands under pine indicated a preferential accumulation in the fPOM fraction.

A closer analysis of the aromatic region of the solid-state ^{13}C NMR spectrum of the < 20 µm fraction of the unburnt soils under *Quercus* (Fig. 4.2) discloses an almost compete disappearance of the signal in the chemical shift region between 160 and 140 ppm assignable to *O*-aryl C as it occurs in lignin. This is in accordance to other reports showing that lignin residues rarely enters this fractions (Dorodnikov et al. 2011, Heckman et al. 2014) and the aromatic contribution must originate from other sources. Alternative compounds are aromatic amino acids or olefinic structures in unsaturated lipids or charcoal contributions. Conclusively, in the case of the burnt soils, the higher aromatic C contribution is best explained with fine PyOM residues.

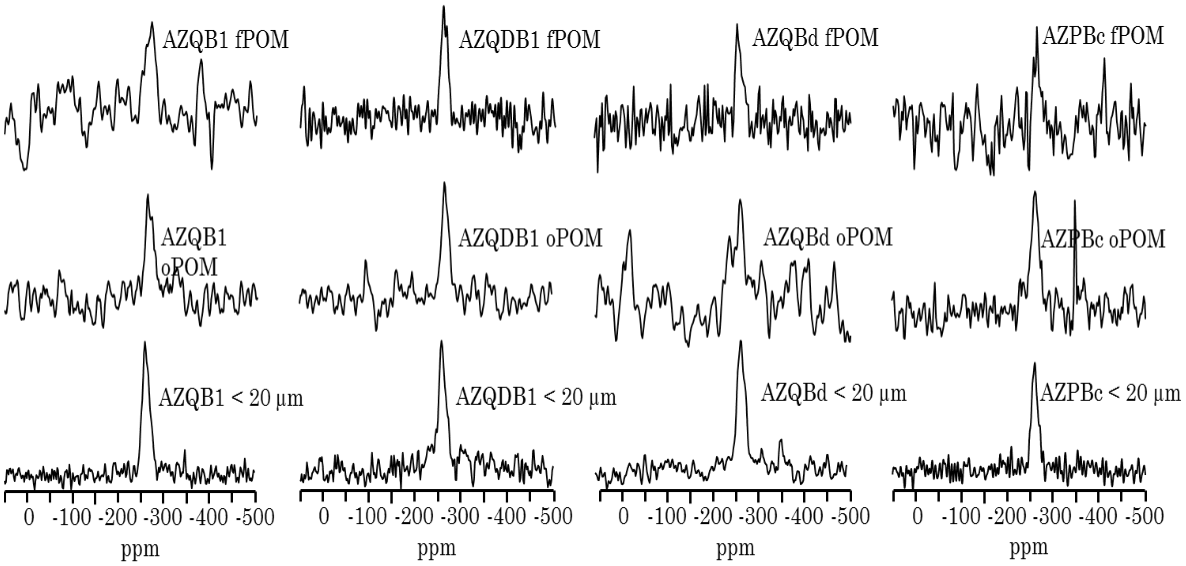

Fig. 4.4. Solid-state ^{15}N NMR spectra of free and occluded particulate organic matter fractions (fPOM, oPOM) and the HF-treated fine particle-size fraction (< 20 µm) of Cambisols from the Sierra de Aznalcóllar. They were sampled in 2011, 7 years after a wildfire of a single (AZQB1, AZQBd) and double (AZQDB1) burnt *Quercus* forest

However, their contribution is lower than 4 % of C_i in the bulk soils and less than 20% of its aromatic C moiety (Table 4.6). Less than 30% of the aromatic C has been calculated to be associated to the course mineral-associated fractions.

For the soils obtained from the pine stands, even lower amounts of C_i are recovered as aromatic C of the < 20 µm fractions. Note that here too, clear signal for lignin are not evidenced in the respective solid-state ^{13}C NMR spectra and the solid-state ^{15}N NMR spectra show most of their intensity in the amide N region (Fig. 4.4). Between 59 and 69% of the aromatic C of the bulk soils was recovered with the fPOM fraction, leaving 15 to 18% associated with oPOM.

Table 4.6. Recovery of organic C of the bulk soil as aromatic carbon (C_{arom}) in the free (fPOM), occluded (oPOM) particle organic matter, fine fraction (< 20 µm) and course fraction (2000 – 20 µm) of unburnt (AZQU3), single (AZQB1, AZQBa,b) and double burnt (AZQDB1) soils under *Quercus* and from double (AZPDBc,d,e) and single (AZPBc,d,e) burnt Cambisol under pine sampled seven years after a fire in the Sierra de Aznalcóllar. $C_{arombulk}$ corresponds to the contribution of aromatic C in each fraction to the total aromatic C content in the bulk soil.

Samples	**fPOM**		**oPOM**		**< 20 µm**		**2000-20 µm**
	C_{arom} (%)	**$C_{arombulk}$ (%)**	**C_{arom} (%)**	**$C_{arombulk}$ (%)**	**C_{arom} (%)**	**$C_{arombulk}$ (%)**	**$C_{arombulk}$ (%)**
AZQU3	6.8	43.0	5.4	34.6	1.8	11.5	11
AZQB1	7.0	24.5	10.6	37.3	2.4	8.4	30
AZQDB1	6.7	26.1	9.3	36.4	2.2	8.4	29
AZQBa	8.5	39.3	6.2	28.9	4.3	20.1	12
AZQBd	12.5	54.4	2.8	12.4	4.1	17.9	15
AZPBc	23.8	47.8	9.6	19.2	1.5	3.0	30
AZPBd	23.7	57.1	9.8	23.6	2.4	5.8	14
AZPBe	21.4	47.0	10.9	24.0	2.8	6.2	23
AZPDBc	17.4	68.8	3.8	14.8	3.6	14.2	2
AZPDBd	12.8	59.2	3.4	15.8	2.2	10.1	15
AZPDBe	22.1	68.2	5.9	18.1	2.4	7.4	6

In the samples with a recent charcoal input these numbers change to 47 to 57% for fPOM and 19 to 24% for oPOM. Less than 30% of the aromatic C can be attributed to the course mineral-associated fractions. However, note that at both pine sites, 60 to 70% of C_i is recovered with fPOM. Our results are in accordance with Soucémarianadin et al. (2014) evidencing that in fire affected soil the charcoal can be found in the fPOM. Heckman et al. (2014), on the other hand, identified the highest charcoal contributions in the oPOM fraction of soils derived from a temperate forest ecosystem under a conifer stand. They concluded that here fire was the main driver of the formation of the occluded fraction and that the selective preservation of PyOM is the main C stabilization mechanism in the oPOM fraction. Other studies report a preferential accumulation of BC in the light fraction ($\rho < 2$ mg m^{-3}) of agriculturally used topsoils (Brodowski et al. 2007, Vasilyeva et al. 2011). With increasing soil depth, the BC content in the light fraction decreased whereas that of the more dense fraction remained constant, possibly because the latter experienced a better protection due to interaction with the mineral phase. Protection by means of mineral-BC interaction was also suggested by Vasilyeva *et al.*, 2011 and Rovira et al. (2009). Liang et al. (2008) examining the stability of BC in the Anthrosol proposed further that chemical recalcitrance may have been more important than physical protection at their study site. Stabilization of PyOM via mineral-BC interactions in the fine size particles cannot be confirmed by our study (Table 4.6) since < 23% of the aromatic C of the bulk soils is recovered with the fraction < 20 µm (clay, fine and medium silt). It seems that the majority of char particles introduced into the soil after the fire were too big to enter the medium and fine silt fraction. Considering that for PyOM in these soils, relative short mean residence times in the range of centuries were determined (Knicker et al. 2013) ongoing recuperation of the ecosystem may lead to further degradation and thus disintegration of PyOM associated with POM. On the one hand, this would lead to a relative enrichment of mineral-associated PyOM, but also to the formation of smaller units, which can enter the fine particle size fractions and contain sufficient functional groups for providing adsorption sites for interaction with the mineral phase.

4.4. Conclusions

The present investigation confirmed low longevity of PyOM in fire-affected soils from the Sierra de Aznalcóllar. However, after seven years of recuperation from the last fire, the presence of PyOM was still revealed by higher aromatic C contents in the SOM of burnt than of unburnt soils. The observation that most aromatic structures were recovered in the POM may evidence high chemical recalcitrance of the remaining charred residues since POM is only weakly protected by mineral interactions and assumed to be quickly degraded. On the other hand, the fast loss of aromaticity during the seven years of recuperation argues against this interpretation of high chemical recalcitrance. Possibly, the lack of mineral-PyOM association may have allowed fast PyOM degradation. However, the functional groups of some partially oxidized PyOM may interact with the mineral surface before they were completely metabolized. Physically protected by this interaction the PyOM may be able to survive on a long-term scale. Following this argumentation, the BC detected in the fine fraction of Chernozems (Vasilyeva et al., 2011) and North Amercian (Glaser & Amelung 2003) and Australian grassland soils (Skjemstad et al. 1999) would be formed by such residual PyOM, and represent only a very small fraction of the original charcoal input.

A further interesting conclusion of our study is based on the observation that no indication for the survival of heterocyclic N was evidenced in soils seven years after the fire, with the exception of the bulk soils AZPDBe and AZPBc. Thus, although BC was still identified the contribution of BN has been diminished under the detection limited, which is in line with a lower biochemical stability of the latter. Possibly, charged N-groups in pyrrole-type structures are better accessible to enzymes than condensed polybenzene rings. However, the question to which extent the content of BN in PyOM determines its stability in soils has still to be answered in future research.

4.5. References

Abiven S, Hengartner P, Schneider MPW, Singh N, Schmidt MWI (2011): Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. Soil Biology and Biochemistry 43, 1615-1617

Almendros G, Knicker H, González Vila JF (2003): Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy. Organic Geochemestry. 34, 1559-1568

Baldock JA, Oades JM, Waters AG, Peng X, Vassallo AM, Wilson MA (1992): Aspects of the chemical structure of soil organic materials as revealed by solid-state^{13}C NMR spectroscopy. Biogeochemistry 16, 1-42

Baldock JA, Oades JM, Nelson PN, Skene TM, Golchin A, Clarke P (1997): Assessing the extent of decomposition of natural organic materials using solid-state ¹³C NMR spectroscopy. Soil Research 35, 1061-1084

Bimüller C, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, Haug S, Schroll R, Kögel-Knabner I (2014): Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biology and Biochemistry 68, 241-251

Bird MI, Wynn JG, Saiz G, Wurster CM, McBeath A (2015): The Pyrogenic Carbon Cycle. Annual Review of Earth and Planetary Sciences 43, 273-298

Boeni M, Bayer C, Dieckow J, Conceição PC, Dick DP, Knicker H, Salton JC, Macedo MCM (2014): Organic matter composition in density fractions of Cerrado Ferralsols as revealed by CPMAS ^{13}C NMR: Influence of pastureland, cropland and integrated crop-livestock. Agriculture, Ecosystems & Environment 190, 80-86

Brodowski S, Amelung W, Haumaier L, Zech W (2007): Black carbon contribution to stable humus in German arable soils. Geoderma 139, 220-228

Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012): Separation of light and heavy organic matter fractions in soil — Testing for proper density cut-off and dispersion level. Geoderma 170, 403-416

Clemente JS, Simpson AJ, Simpson MJ (2011): Association of specific organic matter compounds in size fractions of soils under different environmental controls. Organic Geochemestry 42, 1169-1180

Clemente JS, Gregorich EG, Simpson AJ, Kumar R, Courtier-Murias D, Simpson MJ (2012): Comparison of nuclear magnetic resonance methods for the analysis of organic matter composition from soil density and particle fractions. Environmental Chemistry 9, 97-107

Christensen BT (2001): Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52, 345-353

de Junet A, Basile-Doelsch I, Borschneck D, Masion A, Legros S, Marol C, Balesdent J, Templier J, Derenne S (2013): Characterisation of organic matter from organo-mineral complexes in an Andosol from Reunion Island. Journal of Analytical and Applied Pyrolysis 99, 92-100

de la Rosa JM, Knicker H (2011): Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373

Dorodnikov M, Kuzyakov Y, Fangmeier A, Wiesenberg GLB (2011): C and N in soil organic matter density fractions under elevated atmospheric CO2: Turnover vs. stabilization. Soil Biology and Biochemistry 43, 579-589

FAO (2006): Guidelines for Soil Description. Food and Agriculture Organization of the United Nations Rome

Glaser B, Amelung W (2003): Pyrogenic carbon in native grassland soils along a climosequence in North America. Global Biogeochemical Cylces 17, 1064

Golchin A, Oades J, Skjemstad J, Clarke P (1994a): Study of free and occluded particulate organic matter in soils by solid state ^{13}C Cp/MAS NMR spectroscopy and scanning electron microscopy. Soil Research 32, 285-309

Golchin A, Oades J, Skjemstad J, Clarke P (1994b): Soil structure and carbon cycling. Soil Research 32, 1043-1068

Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003): The effect of 10% HF treatment on the resolution of CPMAS ^{13}C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma 116, 373-392

Heckman K, Throckmorton H, Clingensmith C, González Vila FJ, Horwath WR, Knicker H, Rasmussen C (2014): Factors affecting the molecular structure and mean residence time of occluded organics in a lithosequence of soils under ponderosa pine. Soil Biology and Biochemistry 77, 1-11

Hilscher A, Heister K, Siewert C, Knicker H (2009): Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemestry 40, 332-342

Hilscher A, Knicker H (2011): Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology and Biochemistry 43, 261-270

IUSS Working Group WRB (2014) World Reference Base for Soils and Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Reference Base Nº. 106. FAO, Rome

Knicker H, Scaroni AW, Hatcher PG (1996): ^{13}C and ^{15}N NMR spectroscopic investigation on the formation of fossil algal residues. Organic Geochemestry. 24, 661-669

Knicker H, Hatcher PG (1997): Survival of protein in an organic-rich sediment - possible protection by encapsulation in organic matter. Naturwissenschaften 84, 231-234

Knicker H, Skjemstad JO (2000): Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state ^{13}C and ^{15}N NMR spectroscopy. Australian Journal Soil Respirometry, 113-127

Knicker H, Totsche KU, Almendros G, González-Vila FJ (2005): Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS 13C NMR spectra obtained from pyrogenic humic material. Organic Geochemestry. 36, 1359-1377

Knicker H, Müller P, Hilscher A (2007): How useful is chemical oxidation with dichromate for the determination of "Black Carbon" in fire-affected soils?. Geoderma 142, 178-196

Knicker H, Hilscher A, González-Vila FJ, Almendros G (2008): A new conceptual model for the structural properties of char produced during vegetation fires. Organic Geochemestry. 39, 935-939

Knicker H (2010): "Black nitrogen" - an important fraction in determining the recalcitrance of charcoal. Organic Geochemestry. 41, 947-950

Knicker H (2011): Solid state CPMAS ^{13}C and ^{15}N NMR spectroscopy in organic geochemistry and how spin dynamics can either aggravate or improve spectra interpretation. Organic Geochemestry. 42, 867-890

Knicker H, González-Vila FJ, González-Vázquez R (2013): Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biology and Biochemistry 56, 31-39

Kölbl A, Kögel-Knabner I (2004): Content and composition of free and occluded particulate organic matter in a differently textured arable Cambisol as revealed by solid-state 13C NMR spectroscopy. Journal of Plant Nutrition and Soil Science 167, 45-53

Liang B, Lehmann J, Solomon D, Sohi S, Thies JE, Skjemstad JO, Luizão FJ, Engelhard MH, Neves EG, Wirick S (2008): Stability of biomass-derived black carbon in soils. Geochimica et Cosmochimica Acta 72, 6069-6078

López-Martín M, Nowak KM, Milter A, Knicker H (2016a): Incorporation of N from burnt and unburnt 15N grass residues into the peptidic fraction of fire affected and unaffected soils. Journal of Soils and Sediments, 1-11

López-Martín M, Velasco-Molina M, Knicker H (2016b): Variability of the quality and quantity of organic matter in soil affected by multiple wildfires. Journal of Soils and Sediments 16, 360-370

Masiello CA (2004): New directions in black carbon organic geochemistry. Marine Chemistry 92, 201-213

Mayer LM (1994): Relationship between mineral surfaces and organic carbon concentrations in soils and sediments. Chemestry Geology 114, 347-363

Nocentini C, Guenet B, Di Mattia E, Certini G, Bardoux G, Rumpel C (2010): Charcoal mineralisation potential of microbial inocula from burned and unburned forest soil with and without substrate addition. Soil Biology and Biochemistry 42, 1472-1478

Plaza C, Fernández JM, Pereira EIP, Polo A (2012): A Comprehensive Method for Fractionating Soil Organic Matter Not Protected and Protected from Decomposition by Physical and Chemical Mechanisms. CLEAN – Soil, Air, Water 40, 134-139

Plaza C, Courtier-Murias D, Fernández JM, Polo A, Simpson AJ (2013): Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: A central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124-134

Rovira P, Duguy B, Ramón Vallejo V (2009): Black carbon in wildfire-affected shrubland Mediterranean soils. Journal of Plant Nutrition and Soil Science 172, 43-52

Santos F, Torn MS, Bird JA (2012): Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology and Biochemistry 51, 115-124

Singh N, Abiven S, Maestrini B, Bird JA, Torn MS, Schmidt MWI (2014): Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment. Global Change Biology 20, 1629-1642

Six J, Conant RT, Paul EA, Paustian K (2002): Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155-176

Skjemstad JO, Clarke P, Taylor JA, Oades JM, McClure SG (1996): The chemistry and nature of protected carbon in soil. Australian Journal Soil and Respirometry 34, 251-271

Skjemstad JO, Taylor JA, Smernik RJ (1999): Estimation of charcoal (char) in soils. Communication in Soil Scicience and Plant Analysis 30, 2283-2298

Sohi SP, Mahieu N, Arah JRM, Powlson DS, Madari B, Gaunt JL (2001): A procedure for isolating soil organic matter fractions suitable for modelling. Soil Science Society of America Journal 65, 1121

Sollins P, Homann P, Caldwell BA (1996): Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105

Sollins P, Swanston C, Kleber M, Filley T, Kramer M, Crow S, Caldwell BA, Lajtha K, Bowden R (2006): Organic C and N stabilization in a forest soil: Evidence from sequential density fractionation. Soil Biology and Biochemistry 38, 3313-3324

Soucémarianadin LN, Quideau SA, MacKenzie MD (2014): Pyrogenic carbon stocks and storage mechanisms in podzolic soils of fire-affected Quebec black spruce forests. Geoderma 217–218, 118-128

Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011): Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. Soil Biology and Biochemistry 43, 1985-1988

Velasco-Molina M, Berns AE, Macías F, Knicker H (2016): Biochemically altered charcoal residues as an important source of soil organic matter in subsoils of fire-affected subtropical regions. Geoderma 262, 62-70

von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007): SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183-2207

Wagai R, Kajiura M, Asano M, Hiradate S (2015): Nature of soil organo-mineral assemblage examined by sequential density fractionation with and without sonication: Is allophanic soil different?. Geoderma 241–242, 295-305

Zhou Z, Chen N, Cao X, Chua T, Mao J, Mandel RD, Bettis Iii EA, Thompson ML (2014): Composition of clay-fraction organic matter in Holocene paleosols revealed by advanced solid-state NMR spectroscopy. Geoderma 223–225, 54-61

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Capítulo 5.

¿Es el Black Nitrogen una fuente de N para el crecimiento de las plantas en areas afectadas por el fuego y es usado eficientemete en presencia de N inorgánico?

Chapter 5.

Can Black Nitrogen serve as a N-source for plant growth in fire-affected areas and how is its use affected by the presence of inorganic N?

Este trabajo se está preparando para ser enviado para su publicación a

Soil Biology and Biochemistry

Can Black Nitrogen serve as a N-source for plant growth in fire-affected areas and how is its use affected by the presence of inorganic N?

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Abstract

In order to obtain a more profound understanding of the impact of pyrogenic organic matter (PyOM) on the N bioavailability in fire-affected soils, an incubation experiment was performed. Therefore, *Lolium perenne* was grown on soil material from unburnt and burnt forest areas in the Sierra de Aznalcóllar, Southern Spain after covering it with ^{15}N-enriched fresh grass litter (^{15}N-OM) or its PyOM (^{15}N-OM). In parallel, pots were additionally fertilized with non-enriched KNO_3 (N_i). Further pots contained unenriched PyOM or unburnt plant (OM) residues together with $K^{15}NO_3$ ($^{15}N_i$) and only $^{15}N_i$. The amount of added N was adjusted to avoid alteration of the C/N ratio of the soil as well as N-excess or limitation. After defined incubation times, the remaining litter layer was removed and soil, roots and leaves were separated. Determination of the recovered added ^{15}N ($^{15}N_{add}$) together with a statistical analysis of the results showed that fire history of the soil had no impact on the $^{15}N_{add}$ partitioning between plant leaves, roots and soils.

Whereas $^{15}N_{add}$-uptake into the roots showed no statistical difference between kind of amendment or with incubation time, in the plant leaves clearly higher $^{15}N_{add}$ recoveries were obtained for pots treated with $^{15}N_i$ and ^{15}N-OM than for those with ^{15}N-PyOM. Neither positive nor negative priming was detected, since addition of N_i did not alter the percentage of $^{15}N_{add}$ derived from ^{15}N-PyOM and ^{15}N-OM found in the leaves. Comparably, addition of PyOM and OM did not alter the efficiency of $^{15}N_i$ incorporation into plant residues. After 16 months of incubation, the soils with ^{15}N-PyOM amendment showed the highest $^{15}N_{add}$-sequestration. Addition of $^{15}N_i$ led to a considerable ^{15}N-loss until the end of the experiment. Solid-state ^{15}N nuclear magnetic resonance spectroscopy (NMR) suggests that some of the $^{15}N_{add}$ sequestered in the soils topped with ^{15}N-PyOM occurred as amide N, most likely of microbial biomass residues.

In summary, our results allow the conclusion that addition of N-rich PyOM and ash to soils after fires has a strong short-term impact on N-cycling in the affected soils. Accordingly, directly after the fire, N_i of the ash replaces the N which otherwise had been supplied by the decaying

plant litter. It provides a fast N-fertilization that is necessary for a quick recovery of the plant cover. Since Ni which is not directly used for biomass production can be quickly lost by volatilization or leaching, PyOM serves as an efficient N sink which only slowly releases N and thus provides additional fertilization on a medium and long-term scale. On a long-term scale, almost all of the N in PyOM will be transformed into soil organic N with comparable properties and functions as SON derived from unburnt litter.

5.1. Introduction

The biggest nitrogen (N) reservoir is the atmosphere where the most abundant N form dinitrogen (N_2) constitutes about 78 % of the air gas. As such, it is not directly available for most microorganisms or plants although in soils, nitrogen represents a limiting nutrient and is need for the built-up of their biomass.During the N cycle, N_2 is converted by microbial fixation or lightning into reactive forms (ammonia, NH_3) which can be used for biomass production. At the same time, bioavailable N forms are mobilized during the decomposition of organic matter. In soils, plants and microbes can assimilate the inorganic N forms such as nitrate (NO_3^-) and ammonium (NH_4^+), but also low molecular weight organic N, such as amino acids. In particular in nutrient-poor ecosystems, plants and microbes have established a competitive relationship, which is expected to be altered by ecosystem disturbance, as there are vegetation fires. Burning of plant debris or the humic layer is expected to alter the kind of litter refilling the SOM pool. Hence, PyOM produced during the incomplete combustion of vegetation and SOM is made up by aromatic structures and heterocycle-N rings formed by dehydration, decarboxylation, demethylation, condensation and cyclization (Almendros et al. 2003, Knicker 2007). The newly formed N-compounds known as Black Nitrogen (BN) (Knicker 2010) derive from N source of biogenic origin such as proteins and amino acids which are transformed into pyrroles, imidazoles and indoles (Almendros et al. 2003) as was demonstrated by solid-sate ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy. These structures were formerly considered to be hardly degradable by microorganisms and their formation from labile N-sources is expected to increase the N competition between plants, microbes and SOM. Although others detected mean residence times of BC of 300 years (Lehndorff et al. 2014). However, previous short term experiments using ^{15}N-enriched PyOM as a soil amendment showed that ^{15}N derived from BN (Hilscher & Knicker 2011) can be used for plant growth (de la Rosa & Knicker 2011). Subsequent analysis of the soil with solid-state ^{15}N NMR spectroscopy revealed that most of the remaining organic ^{15}N derived from the added BN was transformed into amide structures, which may accumulate either as primary degradation products of BN or as biogenic residues after the incorporation of ^{15}N mobilized during BN degradation into microbial biomass. However, it is commonly assumed that charring of N-rich biomass releases a considerable amount of ammonium, which is accumulating in the burnt soil (Mayor et al. 2016, Rodríguez et al. 2009). Since the fire has killed most of the vegetation, the competition between nitrifiers and plants for ammonium has shifted in favor to the nitrifying microbes leading to an accumulation of nitrate (Karhu et al. 2015). However, if not used by the plants, this nitrogen may be lost for the recovering ecosystem due to leaching during post-fire rains.

In order to obtain a more profound understanding of the bioavailability of N from PyOM by plants and to evaluate how the input of BN affects the N cycling in the plant-soil system an incubation experiment was performed. Therefore, *Lolium perenne* was grown on soil material from a fire-prone forest area in the Sierra de Aznalcóllar, Southern Spain after covering it with ^{15}N-enriched PyOM from grass residues, to simulate the accumulation of charred material. In parallel, pots were prepared with ^{15}N-enriched but unburnt grass residues and with inorganic ^{15}N. In addition, by amending pots with mixtures of ^{15}N-enriched and non-enriched N sources (fresh plant material; OM, PyOM and KNO_3), we intended to obtain some insights into the importance of N from PyOM as a potential N source in fire-affected soil. After defined incubation times, the remaining litter layer was removed and soil, roots and leaves were separated. Analyzing their ^{15}N content allowed the monitoring of the incorporation of BN-derived ^{15}N between the different compartments. Solid-state ^{15}N NMR spectroscopy was used to identify the organic N forms sequestered in the soil. In order to reveal the impact of previous fires on the N-cycling, we used soil material from both an unburnt and a burnt forest region.

5.2. Material and methods

5.2.1. Soils

The matrix for the incubation experiments derived from Cambisols (IUSS Working Group WRB, 2014) located in the Sierra de Aznalcóllar, close to Sevilla in the Southwest of Spain. In December of 2011, two different soils were sampled, both covered with *Quercus* forest. The first derived from an unburnt (37° 32´N and 6° 15´W) and the second from a burnt area (37° 30´N and 6° 19´W) which suffered an intense fire in 2004, seven year prior of our sampling. The soil was sieved (2 mm) and oven-dried (40 ºC). A more detailed description of the soil characteristic and the soil organic matter (SOM) composition is given in (López-Martín et al. 2016b).

5.2.2. Production of ^{15}N-enriched organic matter (^{15}N-OM) and pyrogenic organic matter (^{15}N-PyOM)

A detailed description of the production of the plant and char residues is given in (López-Martín et al. 2016a). In brief, *Lolium perenne* was grown, under stable greenhouse conditions at 24 ± 2/17 ± 2 ºC (16 h day/ 8 h night) in two different trays using material from the unburnt soil as substrate. One tray was irrigated with deionized water in order to produce unlabeled organic matter (OM) and the second tray was watered every week with a $K^{15}NO_3$ solution (99 atom %, 0.5 g L^{-1}) to obtain ^{15}N-enriched organic matter (^{15}N-OM). The aboveground mass of both experiments was cut every two weeks and dried at 40 ºC. The harvests of every cut were homogenously mixed. Pieces of ^{15}N-OM and OM were placed in separate preheated ceramic trays and charred in a Muffle furnace at 350 ºC for 8 minutes in the presence of oxygen in order to obtain ^{15}N-PyOM and PyOM. The temperature was selected to produce char comparable to that remaining on soil surface after natural wildfires. After the heat treatment all ^{15}N-PyOM were combined and homogenized. The PyOM yields were treated comparably. Both samples were ball-milled and had the following characteristics OM (6.2 ± 0.1% N_t, 0.373±0.002 atom % ^{15}N), PyOM (2.8 ± 0.2 N_t, 0.401±0.000 atom % ^{15}N), ^{15}N-OM (3.0± 0.2 N_t, 54.495±0.332 atom % ^{15}N) and ^{15}N-PyOM (4.3± 0.4 N_t, 20.641±0.261 atom % ^{15}N).

5.2.3. Incubation experiment

For the pot experiment, 0.25 g of *L. perenne* seeds were added on 100 g soil material which either derived from the burnt or the unburnt area. In total sixteen plastic pots were prepared. After seeding, both the unburnt and burnt soils were amended with either (1) 30 mg of $K^{15}NO_3$ ($^{15}N_i$), (2) 600 mg of ^{15}N-OM, (3) 300 mg of non-enriched plant residues and 30 mg of $K^{15}NO_3$ (OM + $^{15}N_i$), (4) 300 mg of ^{15}N-OM plus 30 mg of KNO_3 (^{15}N-OM + $^{14}N_i$), (5) 600 mg of ^{15}N-PyOM, (6), 300 mg of PyOM plus 30 mg $K^{15}NO_3$ (PyOM+$^{15}N_i$), or (7) 300 mg of ^{15}N-PyOM plus KNO_3 (^{15}N-PyOM+$^{14}N_i$). As controls, soils without amendment (8) were prepared. For each variation, two series of six samples were prepared. The amount of added amendment was adjusted in such a manner that the C/N ratio of the soil + amendment remained constant. For further details of the amendments see (López-Martín et al. 2016a). The pots were watered every 3rd day with 30 mL with deionized water without extra nitrogen inputs during the incubation under controlled greenhouse conditions at 24 ± 2/17 ± 2 ºC (16 h day/ 8 h night). The pots were perforated and the leachate was recovered once per months after watering in excess. However, the yields were too low for further analysis. During the first experimental months, the plant shoots were harvested monthly due to the fast aboveground-plant growth but thereafter the leaves were cut every four months. After 0.5, 1, 5, 8, 12 and 16 months, two pots of each variation were taken, and the remaining litter or ash layer carefully removed. From the pots, entire plant, root and soil were separated. Roots were gently washed in order to completely remove the soil. All materials were dried in an oven at 40 °C. Afterwards they were ground and stored for further analysis.

5.2.4. Elemental composition and determination of ^{15}N content

The C_{org} and N_t contents of the soil matrix were determined with an elemental analyzer (EA 1108; Carlo Erba, CHNS). The C_{org}, N_t and δ^{15}N of the incubated soil, the harvested plants and the roots were measured with a Flash 2000 HT combustion elemental microanalyzer and a Flash HT Plus elemental analyzer coupled to a Delta-V advantage isotopic ratio mass spectrometer (IRMS) via ConFlo IV interfase (Thermo Scientific, Bremen, Germany), with an analytical measurement error of ± 0.2‰. The % atom ^{15}N excess of the each measured material was expressed in % of labeled amendment added ($^{15}N_{add}$) recovery in leaves, roots and soil.

5.2.5. Solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy

In order to concentrate OM and remove paramagnetic ions prior to the NMR analysis, samples were treated with 10 % (*v*/*v*) hydrofluoric acid (HF) (Gonçalves et al. 2003). Briefly, 10 g of dried soil sample were weighed into a polyethylene bottle and 40 ml of HF were added. After 2 hours shaking, the samples were centrifuged and the supernatant discarded. The same procedure was repeated four times. Subsequently the concentrated OM was flushed with deionized water and freeze-dried.

The solid-state cross-polarization (CP) magic angle spinning (MAS) ^{13}C and ^{15}N NMR spectra of the HF-treated soils and the ash/litter layer were acquired with a Bruker Avance III HD 400 MHz using a triple resonance probe for rotors with a diameter of 4mm. The rotor was rotated with 14 kHz. A ramped ^{1}H- pulse was applied during the contact time of 1 ms, using a pulse delay time of 300 ms for ^{13}C and 400 ms in the case of ^{15}N.

The quantification of the ^{13}C NMR spectra was performed by the integration of the following five chemical-shift regions: alkyl C (45 to 0 ppm), *N*-alkyl C (60 to 45 ppm), *O*-alkyl C (110 to 60 ppm), H/C aryl C (110 to 140 ppm), O/C aryl C (140-160) carbonyl C (220 to 160 ppm) (Knicker et al. 2005). The spinning side bands of the aryl C region were considered by adding the intensity between 325 to 300 ppm and 0 to -50 ppm to that of the aryl C region. Quantification of the solid-state ^{15}N NMR spectra was only performed when considerable signal intensity was obtained in the chemical shift region of pyrrolic N (-145 ppm to -245 ppm). In this case, the detectable intensity between -145 and -245 ppm was set to 100% and the contribution of pyrrolic N and amide N (-245 ppm to -285 ppm) were determined.

5.2.6. Statistical analysis

The statistical analysis was conducted with the software SPSS Statistic 17.0. The % of $^{15}N_{add}$ in soil, leaves and roots was transformed by square root in order to improve homogeneity of variance and normality. The comparisons between impact of soil type, and the different treatments for each incubation months were performed using 2-way-ANOVA approaches followed by Tukey's b Post Hoc. We used a $p \leq 0.05$ for significance difference between means.

5.3. Results

5.3.1. $^{15}N_{add}$ recovery in leaves, roots and soil

After 2 weeks and 1 months of incubation, the aboveground and belowground biomass yields were still too low for an unbiased analysis of $^{15}N_{add}$ with the IRMS equipment. (Fig. 5.1a/b).

During the incubation experiment, no significant impact of the kind of treatment or soil on biomass production was detected. After five months, the N-contents of the harvested grass residues were between 1.0 and 3.2% of the total dry weight. After eight months the values increased to 2.1 and 3.6% s and accounted for 1.7 to 2.9 % of the dry weight at the end of the experiment after sixteen months (Table A of the appendix). After 5, 8, 12 and 16 months, the total amount of ^{15}N, which was incorporated into all aboveground plant residues or roots of each amendment, was determined. In order to identify the contribution of $^{15}N_{add}$ to the total ^{15}N of the harvested biomass, the amount of ^{15}N, which had been accumulated in the plants of the control was subtracted and the difference is given as the percentage of ^{15}Nadd in Table 5.1a. Here, the different small letters at each single incubation month (per column) indicate significant differences among $^{15}N_{add}$ recovery from the applied amendments.

Even after five months, only small amounts of $^{15}N_{add}$ were recovered within the roots. Statistical analysis revealed that neither fire history of the used soil matrix, nor sampling date had an impact on the accumulation of $^{15}N_{add}$ in the root system. Statistical differences are only

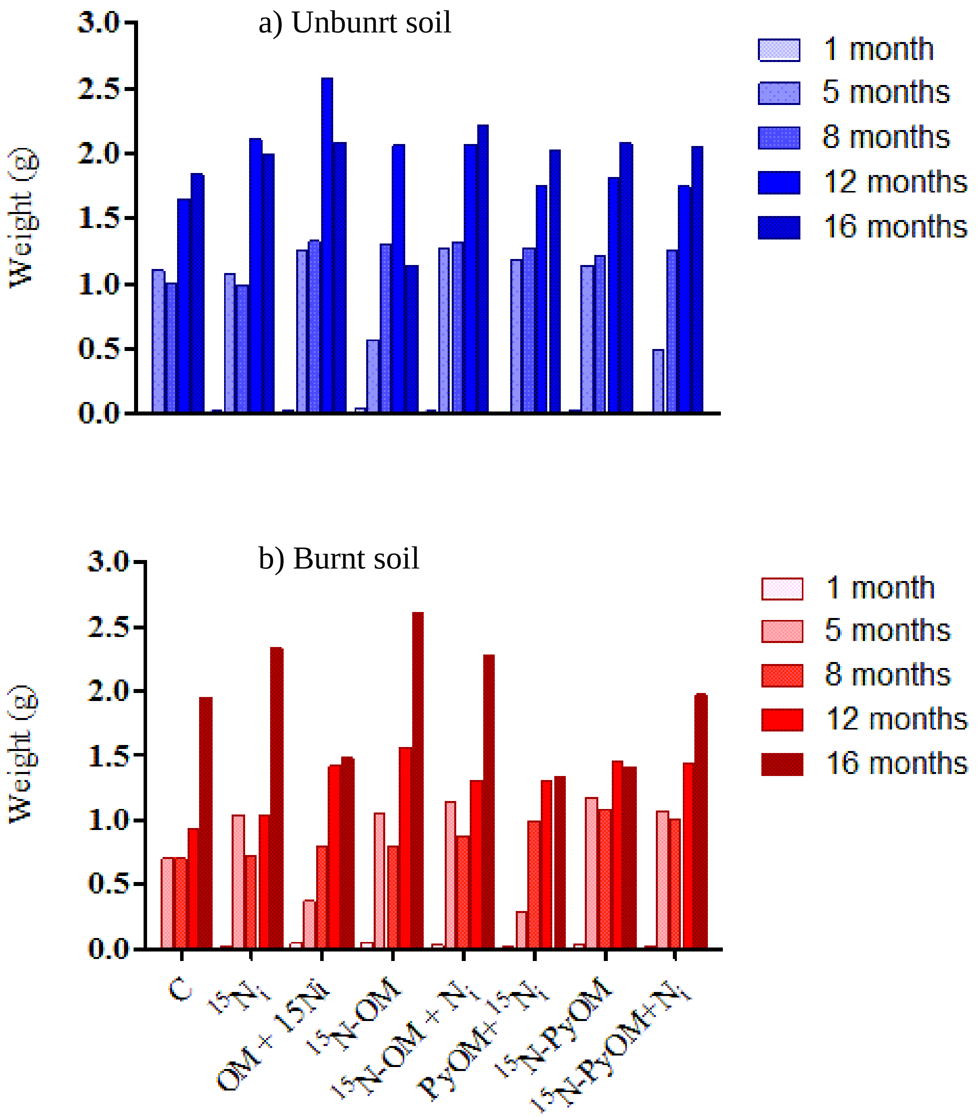


Fig. 5.1.a: Mass recovery leaves in a) unburnt b) burnt soil at the end of each incubation period

observed with respect to the used amendments (p=0.000) (Fig. 5.2a). Accordingly, the roots in the pots with ^{15}N-PyOM+N$_i$ and ^{15}N-PyOM toppings showed the lowest ^{15}N uptake accounting in both amendments with 0.3 % of $^{15}N_{add}$ whereas the ^{15}N-OM+N$_i$ and ^{15}N amendments led to higher values of 1.1 and 1.3 % of $^{15}N_{add}$.

Comparable to the statistical analysis of $^{15}N_{add}$ uptake by the roots, that of the $^{15}N_{add}$ recovery in the soil unveiled no impact of the fire history of the soils but differences are observed with respect to incubation time (p=0.000) (Fig. 5.2b/c).

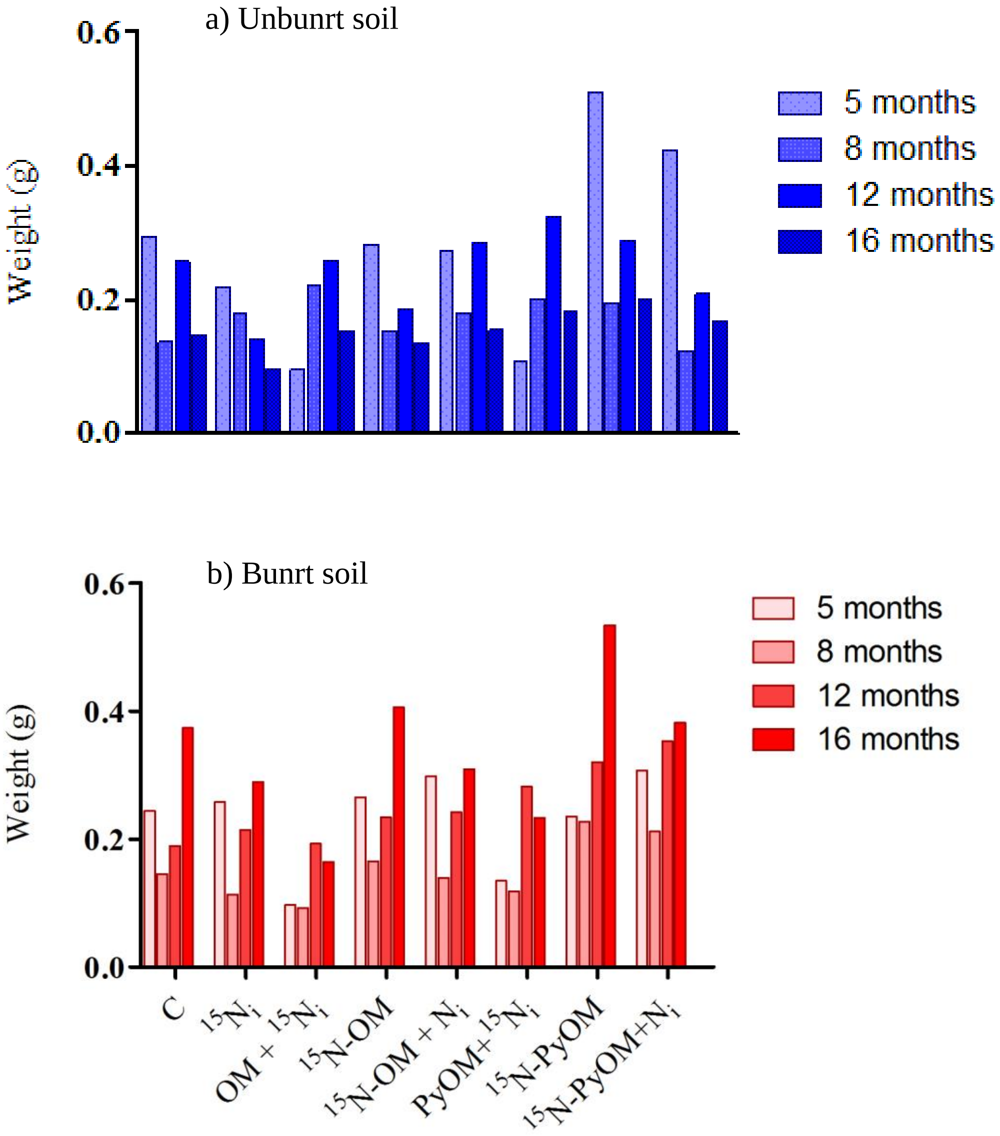


Fig. 5.1.b: Mass recovery roots in a) unburnt b) burnt soil at the end of each incubation period

With the exception of the pots with ^{15}N-OM+N$_i$ and ^{15}N-OM amendments, the recovery of ^{15}N$_{add}$ with the soil matrix diminished with incubation time. This decrease is most clearly expressed in the experiment with ^{15}N$_i$ additions. This decrease of the latter is partially caused by the quick use of easily available ^{15}N for plant growth but leaching may also contribute to the ^{15}N$_{add}$ loss. The highest ^{15}N$_{add}$ incorporations into the soil were reached with the ^{15}N-PyOM+N$_i$ and ^{15}N-PyOM toppings whereas ^{15}N-OM+N$_i$ and ^{15}N-OM amendments accounted for the smallest ^{15}N$_{add}$ recovery until the first month. However, here, one has to bear in mind that only ^{15}N$_{add}$, which had already entered the soil matrix was considered and some of the ^{15}N$_{add}$ of the burnt and unburnt OM still remained in the litter layer.

Table 5.1.a. Recovery (%) of ^{15}N from^{15}N$_{add}$ applied as labeled and unlabeled fresh (OM) and pyrogenic organic (PyOM) with and without labeled (^{15}N$_{i}$) and unlabeled (N$_{i}$) N and its control in roots, leaves and soil at the end of each incubation time (5, 8, 12 and 16 months).

Amendments	**Roots Months**			
	5	**8**	**12**	**16**
C	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
^{15}N$_{i}$	2.7 ± 2.4	0.9 ± 0.1	0.9 ± 0.4	1.3 ± 1.6
OM+^{15}N$_{i}$	0.4 ± 0.2	0.5 ± 0.4	1.3 ± 0.5	0.1 ± 0.3
^{15}N-OM	1.3 ± 0.2	1.0 ± 0.1	0.7 ± 0.2	1.0 ± 1.0
^{15}N-OM+N$_{i}$	1.7 ± 0.4	1.0 ± 0.1	1.3 ± 0.6	0.8 ± 0.7
PyOM+^{15}N$_{i}$	0.6 ± 0.1	0.5 ± 0.1	1.3 ± 0.2	0.4 ± 0.0
^{15}N-PyOM	0.5 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
^{15}N-PyOM+N$_{i}$	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.3	0.3 ± 0.3

Amendments	**Leaves Months**			
	5	**8**	**12**	**16**
C	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
^{15}N$_{i}$	23.3 ± 13.6	26.2 ± 4.3	14.4 ± 2.2	39.6 ± 23.4
OM+^{15}N$_{i}$	15.6 ± 9.4	32.7 ± 11.2	17.4 ± 1.7	6.1 ± 5.0
^{15}N-OM	10.2 ± 0.4	11.0 ± 3.7	7.4 ± 0.7	17.5 ± 4.3
^{15}N-OM+N$_{i}$	12.8 ± 0.3	14.7 ± 3.7	9.9 ± 1.5	15.6 ± 7.3
PyOM+^{15}N$_{i}$	12.5 ± 6.5	23.1 ± 3.8	22.8 ± 1.3	11.5 ± 7.8
^{15}N-PyOM	2.7 ± 1.1	2.2 ± 0.2	1.0 ± 1.1	2.0 ± 0.6
^{15}N-PyOM+N$_{i}$	2.2 ± 0.9	2.0 ± 0.0	1.5 ± 1.0	3.2 ± 1.2

Table 5.1.b. Recovery (%) of ^{15}N from ^{15}N$_{add}$ applied as labeled and unlabeled fresh (OM) and pyrogenic organic (PyOM) with and without labeled (^{15}N$_{i}$) and unlabeled (N$_{i}$) N and its control in soil at the end of each incubation time (0.5 and 1 months).

Amendments	**Soil Months**					
	0.5	**1**	**5**	**8**	**12**	**16**
C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
^{15}N$_{i}$	40.7±16.5	55.3±1.6	19.5±3.0	17.5±1.9	13.0±1.1	21.7±9.3
OM+^{15}N$_{i}$	63.2±3.6	39.6±15.3	9.9±4.5	16.1±7.4	14.4±1.6	7.0±5.0
^{15}N-OM	10.5±0.4	18.8±8.1	24.7±9.5	26.7±1.2	24.4±0.9	19.0±11.6
^{15}N-OM+N$_{i}$	15.9±5.7	17.4±6.1	35.2±2.3	38.3±7.2	25.6±1.2	25.4±23.0
PyOM+^{15}N$_{i}$	45.7±19.2	53.1±5.1	9.2±5.9	10.4±10.7	11.7±1.5	3.1±0.4
^{15}N-PyOM	23.3±7.3	100.0±0.0	87.5±47.1	35.9±7.0	31.7±2.3	61.7±8.5
^{15}N-PyOM+N$_{i}$	97.6±3.4	100.0±0.0	36.8±5.9	45.2±5.4	58.1±18.7	65.1±9.0

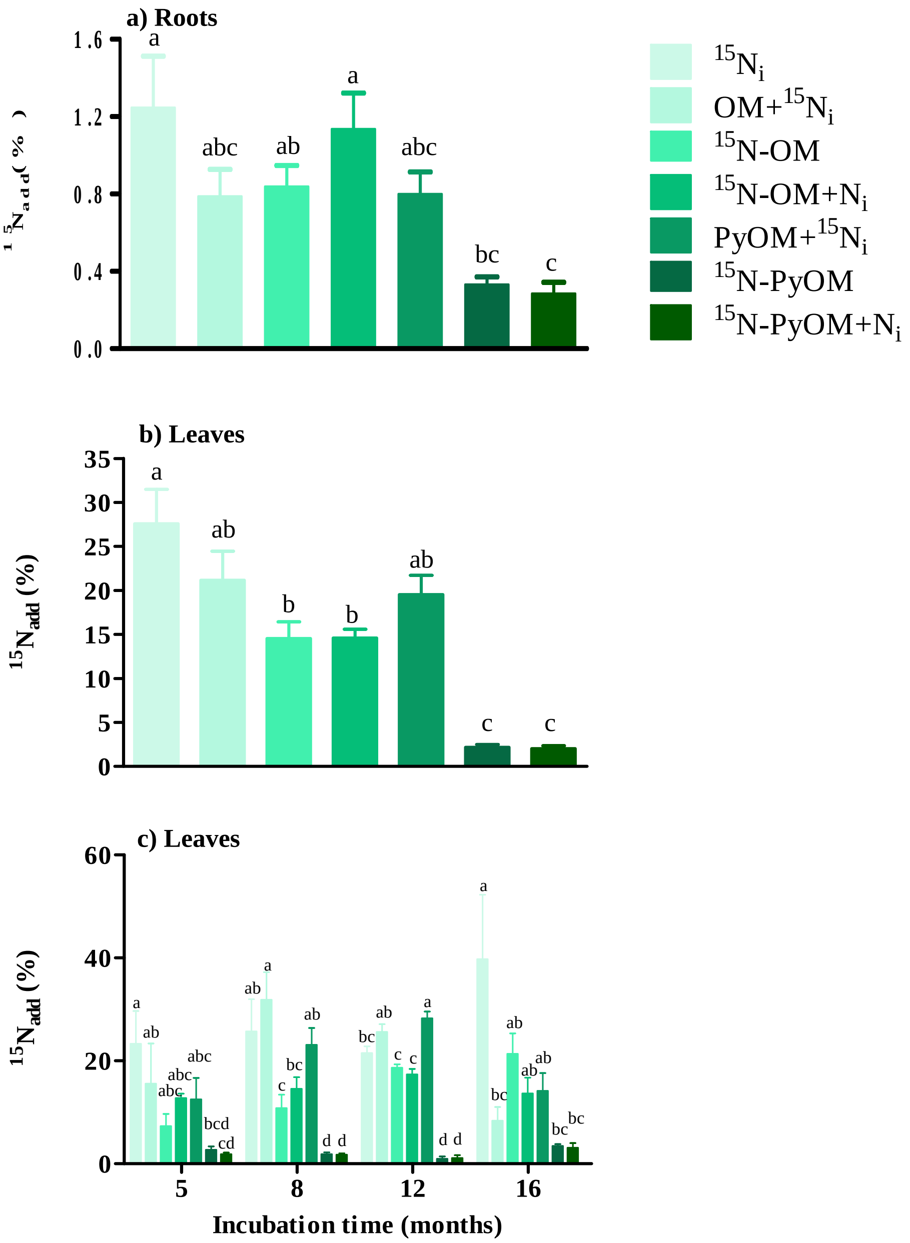
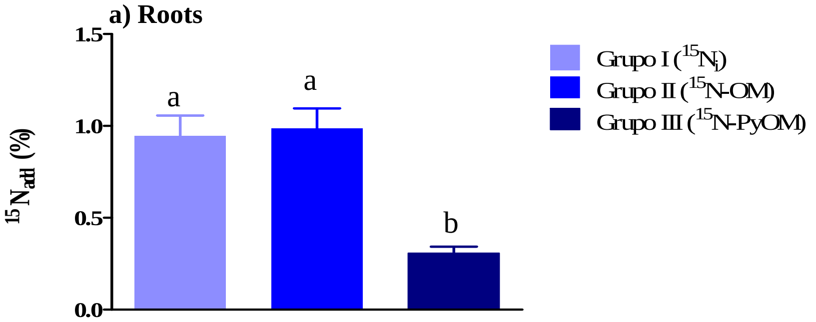


Fig. 5.2. Average recovery of ^{15}N from ^{15}N-enriched amendments ($^{15}N_{add}$) in a) roots and b) leaves according to the amendments used in the pot experiment as ^{15}N-enrich fresh (^{15}N-OM) and pyrogenic (^{15}N-PyOM) organic matter with and without inorganic N (N_i) and unlabeled fresh (OM) and pyrogenic (PyOM) organic matter with enriched inorganic N and $^{15}N_i$. The average was calculated by the sum of all ^{15}N recoveries determined of each treatment without distinguishing between burnt and unburnt soil matrix. c) Average of $^{15}N_{add}$ recovery in leaves according the incubation times. Each column is mean ± standard error. Significant differences are indicated by letters (ANOVA, $p \leq 0.005$, n= 16).

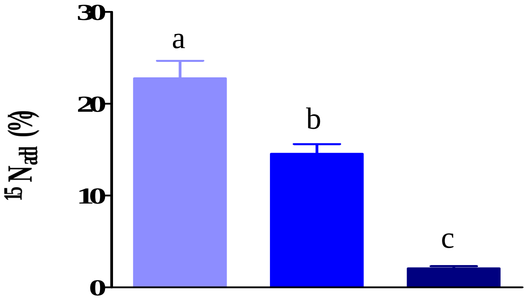
5.3.2. Impact of the N sources on the $^{15}N_{add}$ content in leaves, roots and soil

With the aim of studying how the chemical quality of $^{15}N_{add}$ affects the efficiency of its incorporation into leaves, roots and soil, the amendments were statistically combined as function of their ^{15}N source in the following four groups: (I: $^{15}N_i$) inorganic source ($^{15}N_i$, OM+$^{15}N_i$, PyOM+$^{15}N_i$), (II: ^{15}N-OM) organic litter (^{15}N-OM+N_i, ^{15}N-OM) and (III; ^{15}N-PyOM) charred organic matter (^{15}N-PyOM+N_i, ^{15}N-PyOM) (Fig. 5.3).

a) Roots



b) Leaves



c) Leaves

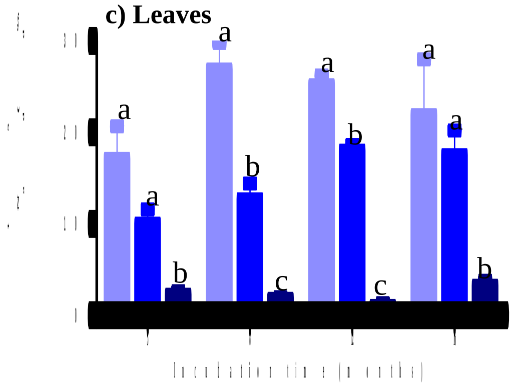


Fig. 5.3. Recovery of source (^{15}N) from the different ($^{15}N_{add}$) applied as labeled fresh (^{15}N-OM) and pyrogenic (^{15}N-PyOM) organic matter plus inorganic N (N_i) and $^{15}N_i$, up-take by a) roots and b) leaves. The average was calculated by the sum of all ^{15}N recoveries determined of each treatment without distinguishing between burnt and unburnt soil matrix. c) Average of $^{15}N_{add}$ recovery in leaves according the incubation times. Each column is mean ± standard error. Significant differences are indicated by letters (ANOVA, $p \leq 0.005$, n= 16).

This analysis points out that for the roots the incorporation efficiency of ^{15}N from inorganic sources and unburnt plant remains are comparable, whereas a clearly lower ^{15}N availability was observed for PyOM ($p = 0.000$).

On the other hand, clearly more $^{15}N_{add}$ (20.81 ± 1.9% of $^{15}N_{add}$) from the inorganic source than from unburnt OM accumulated in the leaves (12.4 ± 0.9% of $^{15}N_{add}$) (Fig. 4.2b). Although, $^{15}N_{add}$ from PyOM was mobilized for the build-up of leaf biomass, the incorporation efficiency was low (2.2 ± 1.9% of $^{15}N_{add}$), most likely due to the higher biochemical stability of burnt versus unburnt N-sources.

The statistical analysis of the $^{15}N_{add}$ content in the soil confirmed differences between each ^{15}N source and at each sampling months ($p = 0.000$). The highest $^{15}N_{add}$ sequestration is observed for the samples with ^{15}N-PyOM additions (89.4 ± 13.2 to 44.9 ± 7.5 % of $^{15}N_{add}$), but no clear alteration is detected with respect to the duration of the incubation (Fig. 5.4).

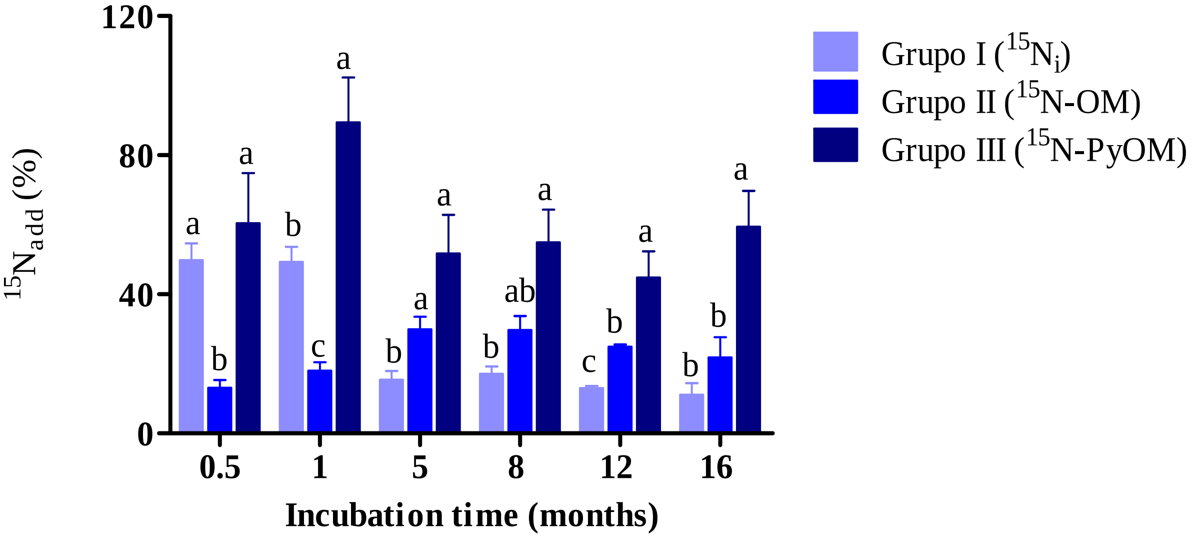


Fig. 5.4. Contribution of the enriched N (^{15}N) from three different ^{15}N-enriched sources ($^{15}N_{add}$) grouped as ^{15}N-enriched fresh (^{15}N-OM), pyrogenic (^{15}N-PyOM) organic matter and inorganic $^{15}N_i$ after different incubation times of soil material from burnt and unburnt Cambisol (Sierra Aznalcóllar, south Spain). The burnt and unburnt soil were statistically equal, therefore the $^{15}N_{add}$ were combined. Each column is mean ± standard error. Significant differences are indicated by letters (ANOVA, $p \leq 0.005$, n= 4).

It seems, once the PyOM has entered the soil, only a small portion of its N is mobilized and quickly used for plant growth. Thus, N-mobilization was not fast enough to allow for N-losses by leaching or volatilization. The second highest $^{15}N_{add}$ sequestration was achieved with application of organic N with unburnt plant residues. An increasing incorporation of $^{15}N_{add}$ is observed until the 5th month. Possibly, this is due to ongoing degradation of the litter layer which allows continous incorporation of organic residues into the soil matrix. However, the fact that between 10 and 20% of the $^{15}N_{add}$ were used for leaf formation underlines a considerably faster turnover of the organic N in OM than PyOM. After the 8th months the $^{15}N_{add}$ recovery in the soil decreased again until the end of the experiment. Considering that the total $^{15}N_{add}$ recovery (plant, soil, roots) did not change notable (Table 5.1a/b), losses by leaching must have been low. Clear losses of $^{15}N_i$ from the soil system are observed between the 1st and 5th month. This is best explained with its use for plant growth.

5.3.3. Preferential use of either N_i or N_{org} N_i and N_{org}

So far, the statistical analysis of the $^{15}N_{add}$ recovery from OM, PyOM and N_i confirmed that inorganic ^{15}N had the highest incorporation efficiency, whereas ^{15}N from PyOM showed the lowest losses from the soil and the highest sequestration potential. However, in order to reveal if the presence of inorganic N affects the turnover of organic N from OM and PyOM, the $^{15}N_{add}$ recoveries in the plant residues and the soil of the pots fertilized with only $^{15}N_i$ and with $^{15}N_i$ and PyOM and OM were statistically compared (Figure 5.5.a/b) and no difference was evidenced. Comparably, the addition of N_i did not affect the uptake of ^{15}N from unburnt OM nor that derived from PyOM. However, each amendment showed differences with respect to the control roots. From those results, it may be concluded that in our experiments, we were able to avoid N-limitation, since the lack of quickly available N_i did not increase the uptake rate of ^{15}N from burnt and unburnt organic sources. Thus, the availability of N_i had no major impact on the mobilization of N from organic sources. The efficiency of ^{15}N use for plant growth seems to be mainly determined by the degradation rate of the N-containing source and the latter was seemed to be unaffected by the fertilization with N_i.

The leaves group I and III did not show $^{15}N_{add}$ recovery differences according to fire history and incubation time therefore the data were combined, in order to evaluated the impact of the their application in leaves.

The study of the sequestration of $^{15}N_i$ with and without additional application of OM or PyOM into the organic matter within the soil found differences between the control and the other amendments until the 12th month of incubation but none between burnt and unburnt soil. The addition of organic residues had no impact on ^{15}Ni immobilization until the 12th month. At months 16, however, it was possible to distinguish between the ^{15}N recovery ($p = 0.000$), for OM and PyOM plus $^{15}N_i$ with 3.52 ± 2.1% and 3.4 ± 1.0% and $^{15}N_i$ with 21.0 ± 5.1% of $^{15}N_{add}$ (Fig. 5.4c). However, only the latter was statistically different from the control.

Adding N_i to amendments of ^{15}N-OM and ^{15}N-PyOM did not result in statistically relevant difference to the ^{15}N sequestration in soils without N_i fertilization, nor did we detect a dependency of $^{15}N_{add}$-sequestration from the organic additives with incubation time.

5.3.4. ^{15}N balance at the end of the experiment

In order to assay the total ^{15}N recovery which has been incorporated into the soil, the roots and the leaves, the $^{15}N_{add}$ values from burnt and unburnt soil were combined and statistically analysis, since no significant difference was attributable to the fire history of the soil. Both, kind of amendments and incubation time, affected the total $^{15}N_{add}$ recovery ($p= 0.003$). However, due to yield of plant material after 2 weeks and 1 month incubation time, the statistical analysis included only the samples obtained after 5 months of experiment time. After 16 months of incubation, the highest total $^{15}N_{add}$ recovery was obtained for the systems amended with ^{15}N-PyOM both with and without additional inorganic N, followed by the pots treated with ^{15}N-OM. The fact that most of the recovered $^{15}N_{add}$ accumulated in the soil underlines the importance of N-immobilization into organic residues if uncontrolled loss of N with leaching or volatilization is intended. The lowest total recovery of $^{15}N_{add}$ was yielded for the pots treated with $^{15}N_i$ and burnt or unburnt OM, confirming the high risk of N-loss if present as nitrate.

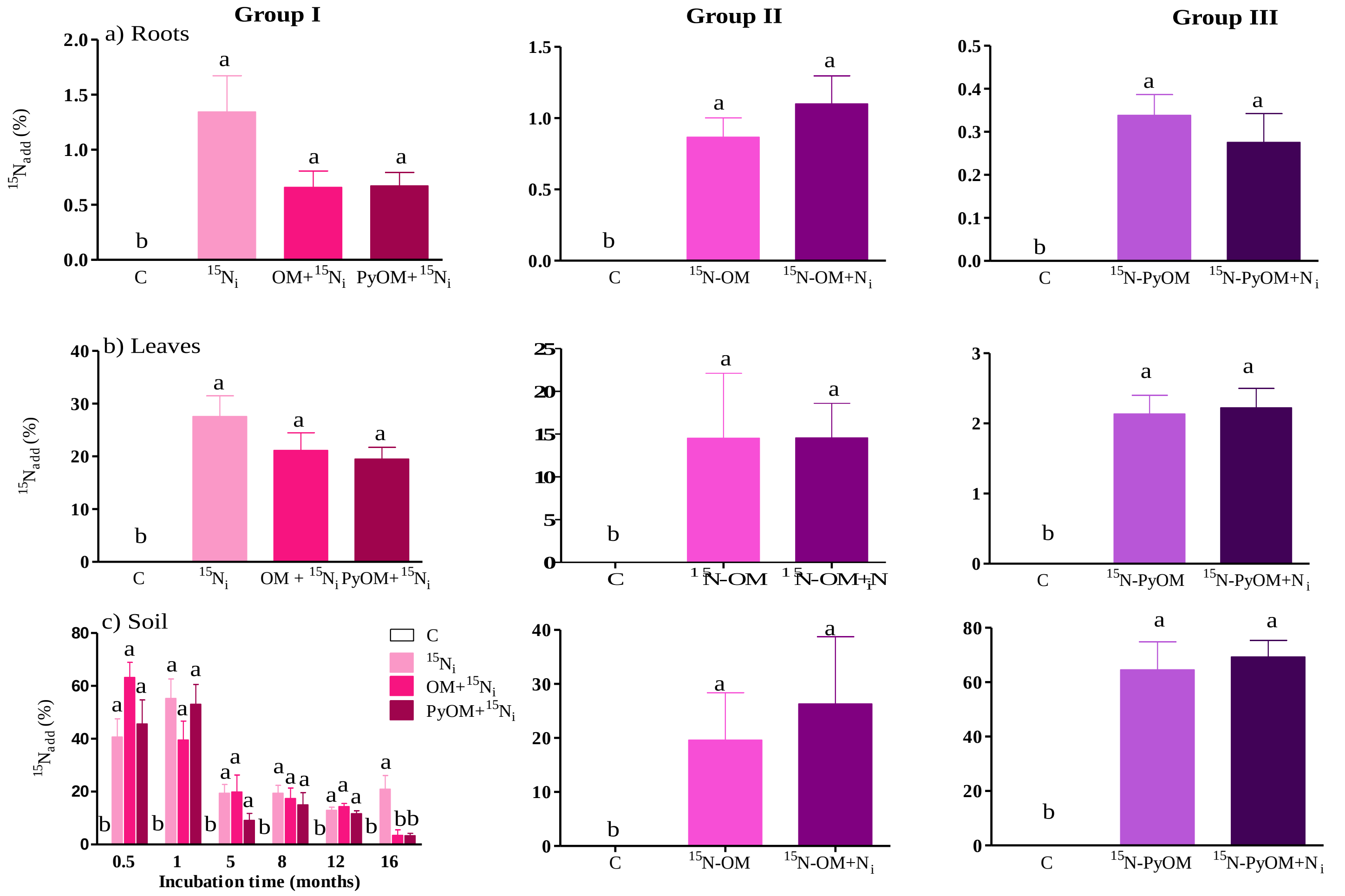


Fig. 5.5. Recovery of labeled nitrogen (^{15}N) in a) roots b) leaves and c) soil from three ^{15}N-enriched groups ($^{15}N_{add}$): I) inorganic $^{15}N_i$, fresh (OM) and pyrogenic (PyOM) organic matter plus $^{15}N_i$, II) ^{15}N-OM with and without N_i and III) ^{15}N-PyOM with and without N_i, applied in burnt and unburnt Cambisol (Sierra Aznalcóllar, south Spain) after different incubation times. Each column is mean ± standard error. Significant differences are indicated by letters (ANOVA, $p \leq 0.005$). The data were combined according to statistical results.

5.3.5. Solid state of ^{15}N and ^{13}C NMR

The solid-state ^{13}C NMR spectrum of unburnt soil from the control pot after 2 weeks of incubation shows its main intensities in the chemical shift region of alkyl C (45 to 0 ppm) and *O*-alkyl C (110 to 60 ppm) (Fig. 5.6, Table 5.2). The alkyl C-to-*O*-alkyl C ratio of 1.2 indicates an advanced humification stage of its OM. Comparable ^{13}C intensity distributions were determined for the unburnt soils amended with organic and inorganic N forms after 1 month of incubation. Note that the addition of PyOM or OM was not high enough to affect the bulk chemical composition of the SOM. After eight months, a slight decrease of the relative intensity in the *O*-alkyl C region from an average value of 28% to 25% and slight increases in that of carboxyl C and alkyl C are evidenced.

Table 5.2. Intensity distribution (%) of different C groups by ^{13}C NMR measurement in soil at the end of the experiment for each amendment at month 1th and 8th

Time (month)		carboxyl C	*O*-aryl C	aryl C	*O*-alkyl C	*N*-alkyl C	alkyl C
0.5 s	Control	6	5	18	29	8	34
1	$^{15}N_i$	11	6	18	29	8	28
	OM+$^{15}N_i$	9	5	16	29	8	34
	^{15}N-OM	9	5	17	27	9	32
	^{15}N-OM+N_i	9	5	18	27	9	33
	^{15}N-PyOM	9	5	17	27	7	34
	^{15}N-PyOM+N_i	8	6	14	30	8	35
	PyOM+$^{15}N_i$	12	6	16	26	7	33
8	$^{15}N_i$	11	6	18	27	8	31
	OM+$^{15}N_i$	7	5	19	25	9	36
	^{15}N-OM	8	5	14	25	10	38
	^{15}N-OM+N_i	10	6	17	26	8	33
	^{15}N-PyOM	10	6	18	25	7	34
	^{15}N-PyOM+N_i	11	5	16	25	9	34
	PyOM+$^{15}N_i$	11	6	18	25	8	33

Figure 5.7 shows the respective solid-state ^{15}N NMR spectra of the HF-treated bulk soils after 1 month and 8 months of incubation. All are dominated by the signal in the amide-N region

(-245 to -285).

Signals from nitrate N (-3.5 ppm) are even not observed in the spectra fertilized with $^{15}N_i$, indicating an efficient transformation of Ni into organic N forms, mostly peptides during the experiment. However, here one has to bear in mind that the used NMR acquisition parameters were optimized for organic N and inorganic N-salts may be underestimated. The ^{15}N NMR spectra of the soils of pots to which ^{15}N-PyOM was applied show a pronounced shoulder in the chemical shift region between -145 and - 245 ppm. The latter is assigned to pyrrole-type N and is typical for BN. Its appearance clearly demonstrates the incorporation of BN into the soil.

However, its relative contribution to the detectable ^{15}N-intensity between -145 and -285 ppm decreases between the 1st and the 8th months of incubation, from 44% and 38% to 24% and 31% in the spectra of soils amended with ^{15}N-PyOM and with ^{15}N-PyOM + N_i, respectively. It seems that some of the incorporated BN has been degraded and the mobilized N was used for the built-up of peptides most tentatively of microbial biomass. The latter was recently indicated by studying the ^{15}N-recovery in the amino acid/peptide fraction of those soils (López-Martín et al. 2016a).

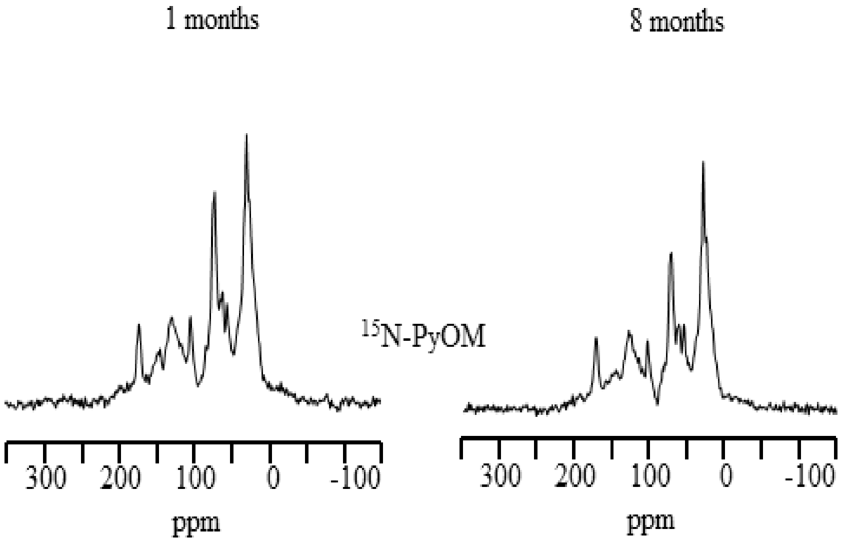


Fig.5.6. Solid-state ^{13}C NMR spectra of soils obtained from pots treated with ^{15}N-PyOM after 1 and 8 months of incubation.

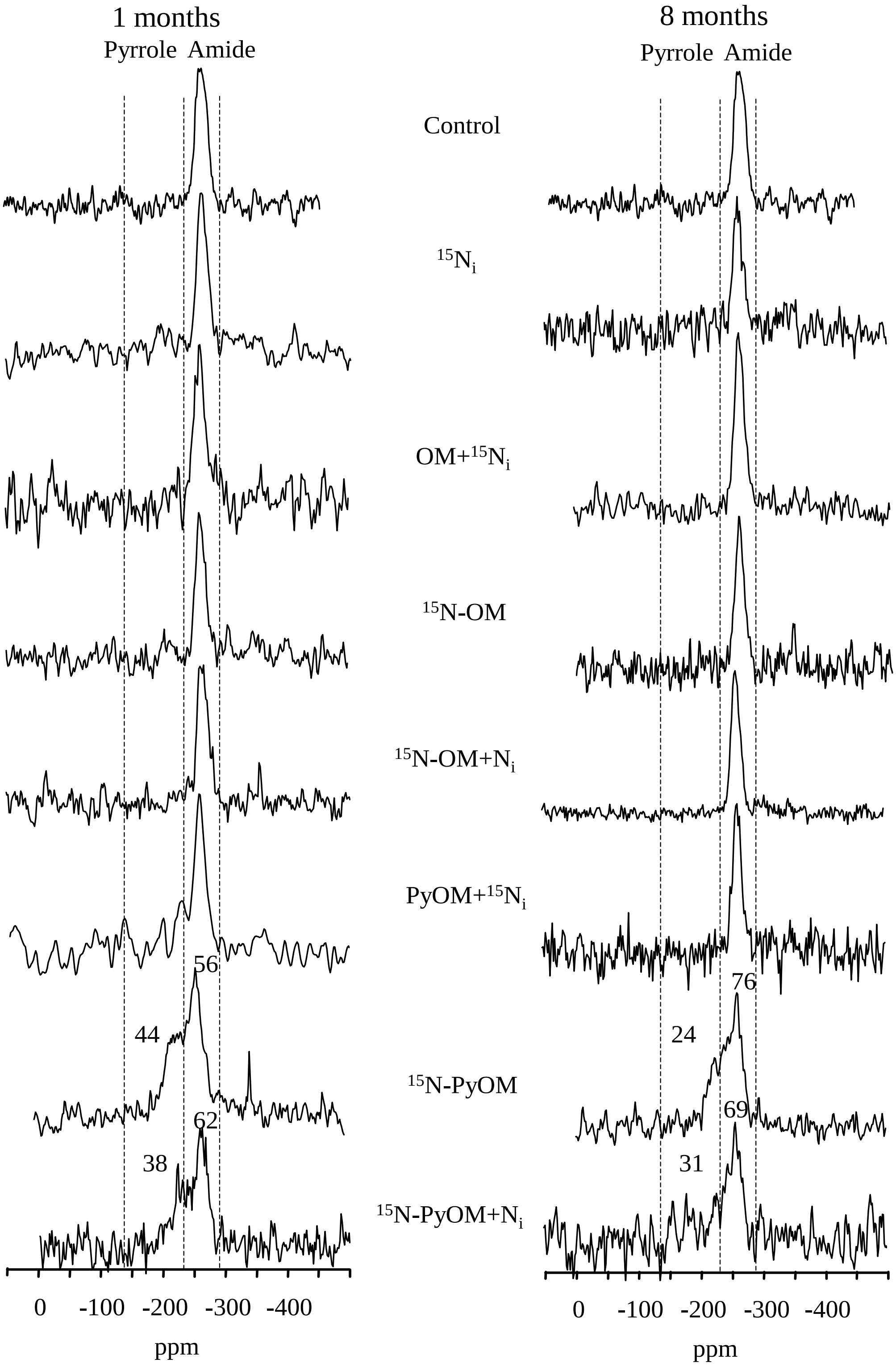


Fig. 5.7. Solid-state ^{15}N NMR spectra of the HF-treated bulk soils after 1 month and 8 months of incubation. The numbers refer to the relative contribution of pyrrole-N and amide N to the total ^{15}N intensity.

5.3.6. Biochemical transformation of PyOM in the litter layer

For a more detailed study of biochemical reworking of the PyOM residues, the litter layers of the pots amended with ^{15}N-PyOM with and without additional N_i fertilization were subjected to solid-state ^{13}C and ^{15}N NMR spectroscopy. Whereas the ^{13}C NMR spectrum of the fresh PyOM (Fig. 5.8, Table 5.3) revealed the typical pattern of burnt N-rich grass residues with high intensity in the aromatic C (160 to 110 ppm) and alkyl C (45 to 0 ppm), the spectra of the litter after 8 months incubation depicted a considerable increase of the O-alkyl C region (110 to 60 ppm) and a doubling of the intensity in the N-alky C region (60 to 45 ppm).

The first region is assigned to carbohydrates and the latter either to N-alkyl in amino acids and peptides or amino sugars. Considering that input of plant litter was neglectable, the appearance of those signals is best explained with the development of microbial biomass, soil algae or moss. Their presence was indicated by thin green spots covering the litter layer. The contribution of the latter even increases until the end of the experiment. The respective ^{15}N NMR spectra supports the formation of new biomass by the decrease of the relative intensity assigned to pyrrole and the enhancement of the contribution of the amide N intensity with increasing incubation time. In contrast to the carbon of the new biomass, which may have entered the litter layer by photosynthesis, the ^{15}N contributing to the additional ^{15}N intensity in the chemical shift of amide N must derive from BN (Fig.5. 9).

Table 5.3. Intensity distribution (%) in the ^{13}C NMR spectra of the litter layer obtained from the pots treated with ^{15}N-PyOM and ^{15}N-PyOM+N_i after 8 and 16 months of incubation

	carboxyl C	***O*-aryl C**	**aryl C**	***O*-alkyl C**	***N*-alkyl C**	**alkyl C**
^{15}N-PyOM (Control)	10	12	48	6	3	22
^{15}N-PyOM+N_i (8 months)	13	10	31	19	6	21
^{15}N-PyOM+N_i (16 months)	11	8	23	28	7	23
^{15}N-PyOM (8 months)	12	10	33	18	5	23
^{15}N-PyOM (16 months)	10	9	30	20	6	26

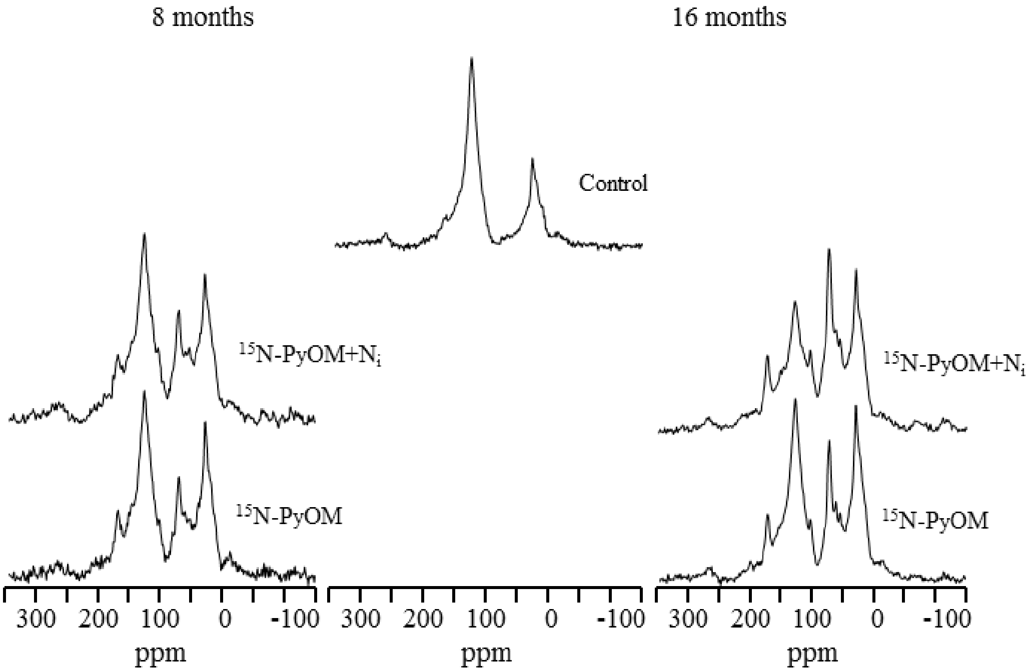


Fig. 5.8. Solid-state ^{13}C NMR spectrum of the fresh PyOM and the litter after 8 and 16 months of incubation

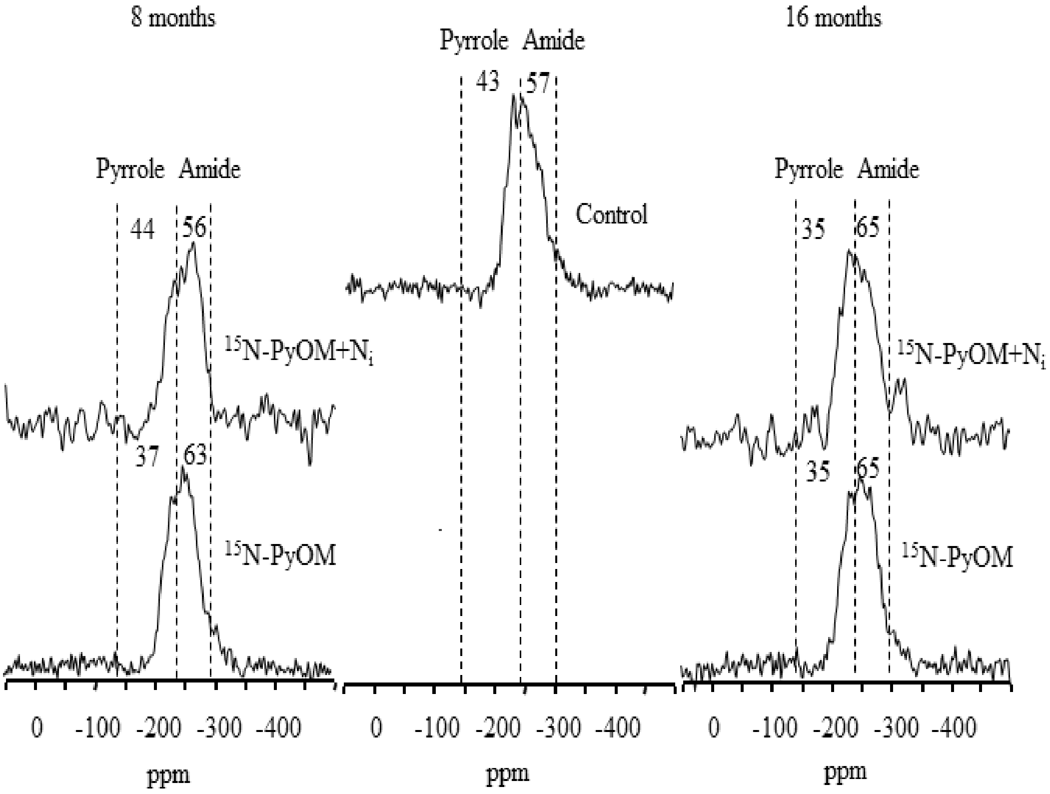


Fig. 5.9. Solid-state ^{15}N NMR spectra of the fresh PyOM and the litter after 8 and 16 months of incubation. The numbers refer to the relative contribution of pyrrole N and amide N to the total ^{15}N intensity.

5.4. Conclusion

Our studies on the short-term effect of PyOM addition after vegetation fires on the distribution of N between plants and soil did not reveal notable alterations due to fire history of the soil, although an impact of the latter on N-sequestration into soil peptides were recently observed (López-Martín et al. 2016a). The fact that regardless of the kind of N-amendment the amount of produced biomass and its N content were comparable to that of the control pots confirms that our treatments did not alter the overall N cycling and that the system was not under N-limitation. Under those conditions, the availability of additional N_i did not decrease the efficiency of ^{15}N uptake from unburnt or burnt OM. Thus, negative priming of N_i is not evidenced and it can be concluded that the kind and amount of ^{15}N which is taken up by the plants depends mainly on the bioavailability of the ^{15}N, which again is determined by the overall degradability of the ^{15}N-containing organic source. Although slower than N in unburnt litter, we were able to evidence degradation of BN and thus the mobilization of its BN.

In summary, our results point to the conclusion that incorporation of charred organic residues and ash into the soil after a forest fire can indeed alter N-cycling in soil, although this alteration will be mainly effective on a short and medium term. With the ash, inorganic N enters the system which refills the N-stocks which without the fire had been supplemented by mobilized N from decaying litter. This fertilization provides faster bioavailable N than only litter degradation alone and allows a quick recovery of the ground vegetation and microbial activity. Unfortunately, N_i, which has not been recycled into plant and microbial biomass, will be lost by volatilization or by leaching during upcoming raining events. On the other hand, the charred residues provide with BN an additional N source, which will be available only after efficient degradation of PyOM.

Thus, this PyOM can be seen as a slow-N release fertilizers which prevents N-losses from the ecosystem and provides additional N for the next vegetation cycles. Since with each N-cycle, a certain part of the BN is converted into biomass N, on a long-term scale, almost all of the BN will be transformed into SON with comparable properties and functions within the N-cycle in soils as SON derived from unburnt litter.

However, considering the importance of a fast development of a new plant cover after fire events to avoid soil erosion and desertification, our study clearly underlined the important ecological role of BN in fire-prone ecosystems, a fact that certainly asked for an improved consideration of BN in environmental research.

5.5. References

Almendros G, Knicker H, González-Vila FJ (2003): Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy. Organic Geochemistry 34, 1559-1568

de la Rosa JM, Knicker H (2011): Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373

Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003): The effect of 10% HF treatment on the resolution of CPMAS 13C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma 116, 373-392

Hilscher A, Knicker H (2011): Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. Organic Geochemistry 42, 42-54

Karhu K, Dannenmann M, Kitzler B, Díaz-Pinés E, Tejedor J, Ramírez DA, Parra A, Resco de Dios V, Moreno JM, Rubio A, Guimaraes-Povoas L, Zechmeister-Boltenstern S, Butterbach-Bahl K, Ambus P (2015): Fire increases the risk of higher soil N2O emissions from Mediterranean Macchia ecosystems. Soil Biology and Biochemistry 82, 44-51

Knicker H, Totsche KU, Almendros G, González-Vila FJ (2005): Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS 13C NMR spectra obtained from pyrogenic humic material. Organic Geochemestry. 36, 1359-1377

Knicker H (2007): How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85, 91-118

Knicker H (2010): “Black nitrogen” – an important fraction in determining the recalcitrance of charcoal. Organic Geochemistry 41, 947-950

Lehndorff E, Roth PJ, Cao ZH, Amelung W (2014): Black carbon accrual during 2000 years of paddy-rice and non-paddy cropping in the Yangtze River Delta, China. Global Change Biology 20, 1968-1978

López-Martín M, Nowak KM, Milter A, Knicker H (2016a): Incorporation of N from burnt and unburnt 15N grass residues into the peptidic fraction of fire affected and unaffected soils. Journal of Soils and Sediments, 1-11

López-Martín M, Velasco-Molina M, Knicker H (2016b): Variability of the quality and quantity of organic matter in soil affected by multiple wildfires. Journal of Soils and Sediments 16, 360-370

Mayor AG, Valdecantos A, Vallejo VR, Keizer JJ, Bloem J, Baeza J, González-Pelayo O, Machado AI, de Ruiter PC (2016): Fire-induced pine woodland to shrubland transitions in Southern Europe may promote shifts in soil fertility. Science of The Total Environment 573, 1232-1241

Rodríguez A, Durán J, Fernández-Palacios JM, Gallardo A (2009): Short-term wildfire effects on the spatial pattern and scale of labile organic-N and inorganic-N and P pools. Forest Ecology and Management 257, 739-746

Capítulo 6.

Incorporación del N procedente de residuos quemados y sin quemar de césped marcado con ^{15}N en la fracción péptidica de suelos afectados y sin afectar por incendios

Chapter 6.

Incorporation of N from burnt and unburnt ^{15}N- grass residues into the peptidic fraction of fire affected and unaffected soils

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Incorporation of N from burnt and unburnt ^{15}N- grass residues into the peptidic fraction of fire affected and unaffected soils

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Abstract

Purpose. To reveal if the input of pyrogenic organic matter (PyOM) affects the nitrogen (N) cycling in soil and its N can be used for the synthesis of microbial biomass, we investigated the incorporation of ^{15}N from ^{15}N-enriched grass residues (OM) or their PyOM into extractable amino acids (AAs) of soil organic matter (SOM) from an unburnt and a burnt soil amended with those residues.

Materials and methods. Pots seeded with *Lolium perenne* and filled with soil from a burnt and an unburnt Cambisol from Southern Spain were topped either with ^{15}N-enriched grass residues (^{15}N-OM), its ^{15}N-PyOM, mixtures of KNO_3 (N_i) and ^{15}N-OM or ^{15}N-PyOM as well as $K^{15}NO_3$ mixed with non-enriched OM or PyOM. After incubation of the pots for up to 16 months under controlled conditions, the AAs, extracted from the litter-free soil were quantified by gas chromatography mass spectrometry (GC/MS). The fate of the added ^{15}N ($^{15}N_{add}$) was followed by isotopic ratio mass spectrometry (IRMS) and analyzed by statistical means.

Results and discussion. The contribution of extractable AAs to SOM of the non-amended burnt soil was twice of that for the unburnt soil. After amendment, their yields and the percentage of $^{15}N_{add}$ recovered in AAs was always higher for the burnt soil. Stabilization of proteinaceous residues during the incubation was indicated. Already after 2 weeks, $^{15}N_{add}$ from ^{15}N-PyOM was recovered within the AAs.

Conclusions. Our experiment confirmed that N from PyOM is incorporated into the peptidic fraction of SOM of post-fire soils. The efficiency of this incorporation is not altered by the

presence of N_i and vice versa. We demonstrated further a short and medium term impact of fire on N-cycling in soils, expressed by alteration of the contribution of acid-extractable AAs to the soil organic N (SON) pool.

Keywords

Amino acids; isotope ratio mass spectrometry; incubation experiment; black nitrogen; pyrogenic organic matter; N turnover in post-fire soils.

6.1. Introduction

It is widely known that N is a limiting nutrient for plants and microorganisms. As such, it represents an important entity not only in the overall N cycle but also for C sequestration in soils. Aside from inorganic N sources provided by biotic and abiotic N-fixation, N-precipitation and N mineral fertilizers, the N supply in soils is refilled by organic N of decaying biomass. Here, it is mainly bound in peptides and AAs (Friedel and Scheller, 2002). Only small amounts of N can be assigned to amino sugars, nucleic acids, alkaloids or tetrapyrroles. Most of these compounds enter the soil during litter fall. Applying common extraction approaches using acid hydrolysis between 7 and 50% of the total SON of fire-unaffected soils can be assigned to AAs in peptides and proteins (Stevenson 1982). According to the non-destructive method of solid-state ^{15}N NMR spectroscopy, more than 80% of SON in fire-unaffected soils are attributable to peptide-like compounds (Knicker et al. 1993). They may be part of decaying microbial residues, plant debris or exo-enzymes but are also constituents of non-hydrolyzable residues and the slow turning SON pool (Hilscher & Knicker 2011). The mechanisms responsible for the high chemical and biochemical recalcitrance of the latter, however, are still to be revealed.

It is widely accepted that a change of N availability for soil organisms, i.e. by alteration of input amounts or the kind of source quality can have a major impact on the biological productivity of a soil (Gärdenäs et al. 2011). In particular, in fire-prone regions, such alterations are commonly induced by vegetation fire. High intensity fires can increase the inorganic N content of soils, but reduce considerably the input of organic N due to the complete destruction of the organic layer (Rovira et al. 2012, Varela et al. 2015) and underwood vegetation. Moderate and prescribed wildfires, on the other hand, can increase the organic carbon (C_{org}) and total nitrogen (N_t) contents (Scharenbroch et al. 2012) by the incorporation of partly charred residues and production of fresh litter from regrown plants after release of nutrients from the ash. As demonstrated by solid-sate ^{13}C and ^{15}N NMR spectroscopy, partial combustion of leaves, litter and humified material in the O horizons leads to a transformation of the proteinaceous material into so called black nitrogen (BN) (Knicker 2007). This fraction is composed of heterocyclic aromatic structures such as pyrroles, imidazoles and indoles (Almendros et al. 2003, Knicker 2010) which represent an integrated part of the polyaromatic network of PyOM (Knicker et al. 2008). As such, they were considered to contribute to the stable SOM pool, once they have been incorporated into the soil (Knicker & Skjemstad 2000). Indeed, earlier studies reported that the mean residence time of PyOM in soil is in the range of millennia or even more (DeLuca & Aplet 2008) although others detected mean residence time of 300 years (Lehndorff et al. 2014). Thus, the replacement of biogenic organic N sources by BN shortly after the fire may markedly decrease the fraction of bioavailable SON. However, previous experiments have demonstrated that the presence of BN can reduce the chemical recalcitrance of charred residues (Knicker 2010). It was shown that it is attacked by microorganisms, already after a few weeks after its addition to soils (Hilscher & Knicker 2011). It was further reported that the N mobilized during the degradation of BN can be taken up by plants (de la Rosa & Knicker 2011). These findings cast doubts on the role of BN as efficient N sink and raise the questions on how it is involved in the N cycling in soils. In order to fill this knowledge gap, we were interested if and to which extend N from BN is recycled by soil microorganisms for the built-up of their biomass. Therefore, we performed pot experiments in which the incorporation of ^{15}N from ^{15}N-enriched charred grass residues amended to a soil matrix into soil AAs was monitored for a period of 16 months. This design simulated the fate of BN during the time directly after the fire. Amino acids were chosen as markers for biological turnover of BN, since they are almost absent in BN but represent as a building block of peptides and proteins a major fraction of the soil biomass and its organic residues. In order to take the competition between plants and microorganisms for bioavailable N into account, *Lolium perenne* was grown on the amended soils. However, possible incorporation of ^{15}N into the plants were determined but not considered in the presence study since its focus was the inclusion of BN derived nitrogen into the biogenic proteinaceous fraction of SON. By including experiments with amendments composed of either only ^{15}N-enriched plant residues or ^{15}N-enriched plant residues mixed with unenriched PyOM or N_i and vice versa we hoped to identify potential preferential use of one or the other N-source. Whereas the amendment of the fresh PyOM provided information on the short-term impacts of BN addition on the N cycling, the medium-term effects were approached by comparing the ^{15}N incorporation into AAs of soil materials derived from the same region but either from an unburnt site or from a location having experienced an intense fire seven years before sampling.

6.2. Materials and methods

6.2.1. Sample material

The soil matrix for the incubation experiments derived from the first 5 cm of a burnt (N 37º 30´ W 6º 19´ AZQB2-1) and an unburnt Cambisol (IUSS Working Group WRB 2014) (N 37º 32´ W 6º 15´, AZQU2-3) from the Sierra de Aznalcóllar Southern Spain. The soils were sampled 7 years after an intense fire which occurred in this region in 2004. A more detailed description of the fire history of this region and the medium-term impact of the fire on soil parameters and organic matter composition can be obtained in López-Martín et al. (2016).

After removing visible root and plant residues, the soil was sieved through a 2 mm sieve, oven-dried at 40 °C and stored at a dark and dry place before use. The respective soil parameters (pH, electrical conductivity, C_{org}, N_t and nitrate) were previously determined (López-Martín et al. 2016). Briefly, the pH-values of the sandy-loamy soils are 5.7 and 4.2 for the unburnt and burnt soil. The C_{org} and N_t content of the unburnt soil are 69.2 ± 1.4 mg C_{org} g soil $^{-1}$ and 4.1 ± 0.3 mg N g soil $^{-1}$, whereas concentrations of 57.6 ± 1.9 mg C_{org} g soil $^{-1}$ and 2.6 ± 0.0 mg N g soil $^{-1}$ were determined for the material from the burnt area.

The plant residues (OM) were produced by growing rye grass (*Lolium perenne*) on material from the unburnt soil under greenhouse conditions at 24 ± 2 ºC/17 ± 2 ºC (16 h day/ 8 h night) and a relative humidity of 60 ± 10%. The only N-source of the non-enriched plants were the N available in the soil, whereas the ^{15}N-enrichment of ^{15}N-OM was achieved by a weekly watering with a $K^{15}NO_3^-$ solution (99 atom %, 0.5 g l^{-1}) to avoid access of N fertilization. In both experiments the grass was harvested every second week by cutting the aboveground biomass with scissors. After drying it in paper bags, in an oven at 40 ºC, the material was stored on a dark and dry place for further use. Since the N content of plants depends not only on the N-availability during growth but also on the age of the plants, the yields after each cut were combined and homogenized before storing. The non-enriched (PyOM) and the ^{15}N-enriched residues (^{15}N-PyOM) were obtained after cutting the grass into pieces of approximately 10 cm and charring them in preheated ceramic trays (Ø = 15.5 cm) in a Muffler furnace at 350 ºC, during 8 min and under oxic conditions. Whereas enough raw material for the production of non-enriched PyOM was yielded from the first growing experiment, a second sowing but with the same soil material was performed to obtain sufficient ^{15}N-OM for the production of ^{15}N-PyOM. The harvested grass of the respective cutting events was combined and homogenized before charring to overcome fluctuations in the efficiency of ^{15}N incorporation during plant growth and to yield in homogeneously enriched PyOM. In order to ensure homogenous charring conditions, the grass layer in the trays did not exceed 0.5 cm. At the end of the heating period, the charred organic matter was cooled down staying in the switched-off furnace of which the door was kept open. For the ^{15}N-PyOM, the charred material of both sowings was mixed thoroughly.

6.2.2. Incubation experiment

Sixteen plastic pots were filled with 100 g of sieved burnt or unburnt soil. Subsequently, 0.25 g of *Lolium perenne* seeds were planted and covered with the following amendments: (1) 30 mg of $K^{15}NO_3$ ($^{15}N_i$), (2) 600 mg of ^{15}N-OM, (3) 300 mg of non-enriched plant residues and 30 mg of $K^{15}NO_3$ (OM + $^{15}N_i$), (4) 300 mg of ^{15}N-OM plus 30 mg of KNO_3 (^{15}N-OM + $^{14}N_i$), (5) 600 mg of ^{15}N-PyOM, (6)), 300 mg of PyOM plus 30 mg $K^{15}NO_3$ (PyOM+$^{15}N_i$), (7) 300 mg of ^{15}N-PyOM plus KNO_3 (^{15}N-PyOM+$^{14}N_i$). The amounts of total N (N_{add}), ^{15}N ($^{15}N_{add}$) and organic C ($C_{org/add}$) to each pot are summarized in Table 6.1. Note that the amount of amendments was adjusted to ensure comparable C/N ratios of material (soil + amendment) in the pots and to avoid excess of N, both of which could have resulted in alterations of the N cycling. Additionally, control pots filled with soil matrix and planted with seeds but without any amendment were prepared. All pots were incubated in a greenhouse operating at the same conditions as used for the production of the grass residues. The pots were watered with 30 ml of deionized water every three days. The pots were perforated at the bottom in order to remove excess of water. Every month we simulated a bigger rain event by adding 100 ml of water. After 0.5, 1, 8 and 16 months two pots per amendment were sampled and treated as duplicates. Except for the 2-weeks experiment, the above-ground biomass was removed by cutting monthly until the 4th month of incubation. Thereafter, the cutting was every 4 months, due to slower plant growth. At the end of the experiment, after removing the grass, the remaining litter layer, which was mainly composed of residual amendment, and the roots were separated from the mineral soil. All materials were dried at 40°C in an oven and stored for further analysis.

6.2.3. Elemental composition and determination of ^{15}N content

The C_t and N_t contents and the ^{15}N isotopic signatures of the soils and the soil amendments were determined using a Flash 2000 HT combustion elemental micro-analyzer and Flash HT Plus combustion elemental analyzer via a ConFlo IV unit to a continuous flow Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany).

Since the pH of both soils were below 7 (6 for the unburnt, 5 for the burnt), the determined C_t corresponds to C_{org}.

Table 6.1. Amount of ^{15}N ($^{15}N_{add}$), total N (N_{add}) and $C_{org/add}$, amended per pot to the unburnt (U) and burnt (B) soil. Note that N_{add} includes both the ^{14}N and ^{15}N fraction. The C_p/N_p (w/w) ratios correspond to the total organic C and N of the soils in the pots after amendment (burnt and unburnt litter or inorganic N) but before their incubation. Here, the C_p content of the pot was calculated by summing up C_{org} of the original soil and the organic C added with the amendment ($C_{org/add}$), and N_p corresponds to the sum of the N_t of the original soil plus the N added with the amendment (N_{add})

Amendment	**$K^{15}NO_3$ ($^{15}N_i$)**	**^{15}N-OM**	**OM + $^{15}N_i$**	**^{15}N-OM +N_i (KNO_3)**	**^{15}N-PyOM**	**PyOM + $^{15}N_i$**	**^{15}N-PyOM + N_i**
$^{15}N_{add}$ (mg)	4.36	10.20	4.43	5.11	5.51	4.39	2.77
N_{add} (mg)	4.41	17.46	22.80	12.88	24.90	12.33	10.38
$C_{org/add}$ (mg)	0.0	334.8	115.5	117.4	332.2	111.6	117.4
U C/N (w/w)	17	17	16	17	17	17	17
B C/N (w/w)	22	22	21	22	21	22	22

6.2.4. Extraction and analysis of amino acids yielded after hydrolysis

Approximately 1 g of incubated soil or 0.2 g of fresh and charred organic amendments were weighted into a glass bottle (15 ml) and mixed with 5 ml of 6 M HCl and 0.05 mg of L-norleucine as an internal standard. L-norleucine was used for quantification of the AAs loss during the purification steps. The soils and the organic amendments were hydrolyzed at 110 °C, for 22 hours and under N_2 atmosphere. After the hydrolysis, the samples were filtered through glass-fiber membrane filters (0.07 µm, Wicom Perfect Flow, Germany) and the hydrolysate was dried under a flow of N_2 to remove the HCl. The dried hydrolysates were re-dissolved in 4 ml of 0.1 M HCl and 0.05 mg of trans-4-(aminomethyl) cyclohexane carboxylic acid was added as a second internal standard Nowak et al. (2011). The solution containing the hydrolyzed and free AAs was purified by passage over H^+ exchanged DOWEX 50 W X8 resin. Prior to elution of the AAs with 2.5 M ammonium hydroxide, the impurities were washed out with 25 ml 0.1 M oxalic acid and then with 5 ml 0.01 M HCl and 5 ml of distilled water which were used to eliminate residues of oxalic acid. The carboxylic groups of AAs were esterified with isopropanol / acetylchloride (1:4 v/v; 1 h, 110 ºC) and the amino groups were trifluoroacetylated with 1 ml of trifluoroacetic anhydride/dichlormethane (1:1 v/v; 1 h, 60 ºC) (Miltner et al. 2009). After derivatization the impurities were extracted into the aquatic phase of a mixture of chloroform/ phosphate buffers, and the chloroform phase was dried under N_2 (Ueda et al. 1989) and stored at -4 °C for subsequent analysis.

Samples were reconstituted in 100 µl dichloromethane, and the derivatized AAs were identified and quantified in duplicates by means of gas chromatography-mass spectrometry (GC–MS). A BPX5 column (30 m × 250 µm × 0.25µm; SGE, TOWN, COUNTRY) and 7890A GC System (Agilent Technologies, Waldbronn, Germany) with a detector 5975C inert XL MSD (Agilent Technologies) were used. The initial temperature of 50 ºC was kept for 5 min. Then 100 ºC was reached with 30 ºC min^{-1} and held for 5 min. After that the temperature increased at 10 ºC min^{-1} to 175 ºC were it remained constant for 5 min, then further heated to 250 ºC at 10 ºC min^{-1}, held for 5 min, and to 325 ºC at 30 ºC min^{-1}, hold for 5 min. The injection was performed at a split ratio of 1:20 and at an injector temperature of 280 ºC. For identification and quantification of the individual AAs an external standard (200 µl) containing alanine, glycine, threonine, serine valine, leucine, isoleucine, cysteine, proline, aspartic acid, methionine, glutamic acid, phenylalanine, tyrosine, lysine, histidine and cysteine at a concentration of 2.5 µmol ml^{-1} for all AAs except for cysteine which had a concentration of 1.25 µmol ml^{-1} was used. For the calculation of the contribution of AAs, the recovery of individual AAs summed up after they have been identified by comparing the retention time and the mass spectra with the external standard, using the MSD ChemStation software (Agilent Technologies). In order to determine the content of ^{15}N of the total AAs ($^{15}N_{AAs}$), the ^{15}N of the individual derivatized AAs were measured by GC–combustion–isotope ratio–MS (GC–C–irMS) and summed up. Therefore, the AAs were separated with a 7890 A GC System (Agilent Technologies) equipped with a BPX5 column (50 m × 0.32 mm × 0.5 µm) using the following temperature program: Increase of the temperature with 10 ºC min^{-1} from 50 ºC to 80 ºC, which was kept for 7 min before 120 ºC were reached with 3 ºC min^{-1} and hold for 5 min; heating to 210 ºC at 3 ºC min^{-1} After 5 min, the temperature was increased at 20 ºC min^{-1} to 300 ºC for 5 min. The samples were injected in the splitless mode at an injector temperature of 250 ºC. The eluting compounds were combusted and the resulting N_2 was analyzed for its isotopic composition by means of a Finnigan MAT 253 IRMS (Thermo Finnigan, Bremen, Germany). In order to determine the amount of ^{15}N recovered from $^{15}N_{add}$ of each treatment in the respective AAs fractions, the $^{15}N_{AAs}$ content of the control soil with natural ^{15}N abundance was subtracted from the $^{15}N_{AAs}$ contents of the amended soils.

6.2.5. Solid-state ^{15}N NMR spectroscopy

Prior to NMR analysis the soil samples were demineralized with 10 % (v/v) hydrofluoric acid (HF) (Gonçalves et al. 2003) in order to remove paramagnetic ions and to concentrate the OM. Briefly, 10 g of dried soil sample were weighed into a polyethylene bottle and 40 ml of HF were added. The closed bottles were shaken for 2 hours. After centrifugation the supernatant was removed and discarded. The same procedure was repeated four times. The concentrated OM was washed with deionized water and freeze-dried.

The solid-state cross-polarization (CP) magic angle spinning (MAS) ^{15}N-NMR spectra of the HF-treated soils were acquired with a Varian 7.05T Unity Inova (^{15}N resonance frequency: 60.8 MHz) and the fresh and charred OM were obtained with a Bruker DMX 400 (^{15}N resonance frequency: 40.6 MHz) Bruker Avance III 600 (^{15}N resonance frequency: 60.8 MHz) using a spinning speed of 8, 4 and 15 kHz, respectively. A ramped ^{1}H- pulse was applied during the contact time of 0.7 ms. Using a pulse delay time of 200 ms, 7500 scans were accumulated for the spectra of the labeled fresh and charred OM. For the ^{15}N NMR spectra of the soil samples, 1,000,000 scans were acquired with a contact time of 1 ms and a delay time 0.4 s.

6.3. Statistical analysis

The statistical analyses were accomplished using the software SPSS Statistic 17.0. Differences between the results obtained from different sampling events were evaluated using Mann-Whitney test. The impact of soil type and the effect of the substrate amendment on the change of the amount of newly synthesized AAs were analyzed using the Wilcoxon test. In order to reveal the impact of the fire history on N_{AAs} extractability and ^{15}N incorporation into N_{AAs}, we statistically combined the results of all variations (both with amendment and with incubation time) of each soil. For more detailed information about the impact of the source material on the incorporation of $^{15}N_{add}$ in AAs of soils, the treatments were grouped into the following four sets, I) control (C), II) inorganic source ($^{15}N_i$, OM+$^{15}N_i$, PyOM+$^{15}N_i$), III) organic litter (^{15}N-OM+$^{14}N_i$, ^{15}N-OM and III) charred organic matter (^{15}N-PyOM+$^{14}N_i$, ^{15}N-PyOM). Finally, with the aim to study the effect the presence of inorganic nitrogen on the use and degradation of organic N we compared the relative contribution of $^{15}N_{AAs}$ to N_{AAs} in the experiments with addition of $^{15}N_i$, ^{15}N-OM or ^{15}N-PyOM from burnt and unburnt soil. A p-value ≤ 0.05 was considered as statistically significant.

6.4. Results and discussion

6.4.1. Elemental composition of organic C, N_t and ^{15}N enrichment in the soils, OM and the PyOM

Table 6.2 lists the organic C, N_t and ^{15}N contents of PyOM, OM and the burnt and unburnt soils. Recent statistical evaluation of SOM alterations due to fire in the probed area indicated that 7 years after the event, the C_{org} and N_t values are only slightly higher in the unburnt area. A comparable observation is reported by Alcañiz et al. (2016) for soils recovered for 9 years after a prescribe fire in a Mediterranean area.

The non-enriched and ^{15}N-enriched *Lolium perenne* showed C/N-values between 6 and 13, which is in the range found in other studies (de la Rosa & Knicker 2011, Hilscher & Knicker 2011, Knicker & Lüdemann 1995). Note, the respective values for the PyOM and ^{15}N-PyOM are within this range, which indicates that in spite of N-losses during heating a considerable fraction of the organic N was incorporated as BN into the charred material. For both, unburnt and charred plant residues, the ^{15}N content is 0.373 and 0.401 atom %, which agrees with the natural ^{15}N abundance. The slightly higher value found for PyOM may be caused by the loss of N-volatile compounds during combustion leading to a depletion of the lighter isotope (Fraser et al. 2013). The atom % ^{15}N for unburnt and burnt soil range between 0.355 to 0.377 which also corresponds with the natural ^{15}N abundance in organic material (Robinson 2001). The ^{15}N abundance in ^{15}N-OM is with 54.495 ± 0.332 atom % considerably higher than in ^{15}N-PyOM (20.641 ± 0.261 atom %), which is best explained with the fact that the source material of those residues derived from two different growing experiments.

6.4.2. Distribution of N-forms in the starting materials

The ^{15}N NMR spectra (Fig. 6.1) of the burnt and unburnt soils are dominated by the signal between -240 and -285 ppm which is assigned to amide N (Knicker & Lüdemann 1995). Note, indole-type N and proline N may also contribute to the region between -240 and -250 ppm. A small signal appears at -346 ppm which is most likely caused by N bound to Cε in lysine and by N in other free amino groups of amino acids and amino sugars (Knicker 2011c, Witanowski et al. 1993). The solid-state ^{15}N NMR spectrum of the burnt soil shows no major intensity in the BN-typical region of pyrrole-type and indole-type N between -145 to -250 ppm ppm (Knicker 2010). Since in other studies of the same sampling area, signals of BN were clearly dominating the solid-state ^{15}N-spectrum of a burnt soil collected 4 weeks after an intense fire (Knicker

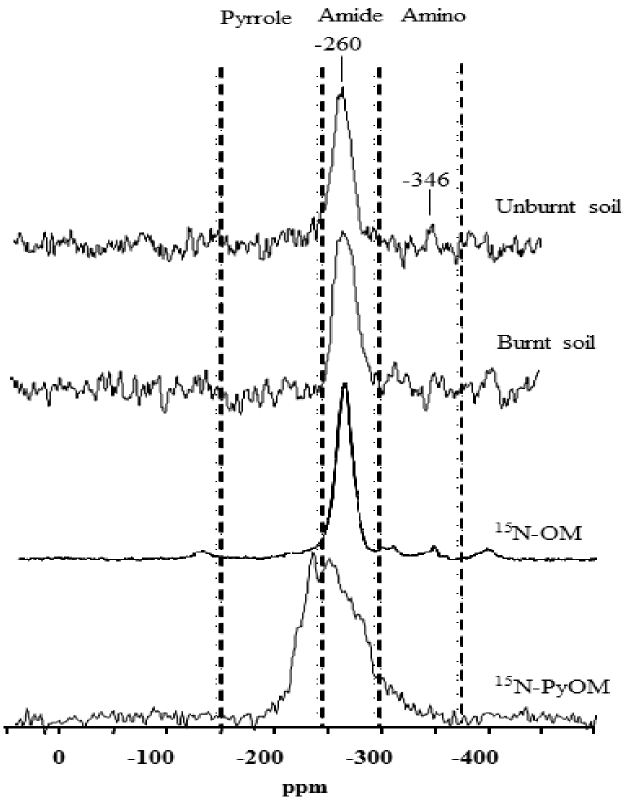

Fig. 6.1. Solid state ^{15}N NMR spectra of an unburnt and a burnt Cambisol from the Sierra Aznalcóllar (Spain), ^{15}N-labeled organic matter (^{15}N-OM) and pyrogenic organic matter (^{15}N-PyOM) produced from *Lolium perenne* at 350°C for 8 min.

2011b), the low intensity in the chemical shift region of heterocyclic N may indicates that BN has been partially remove either by degradation, erosion or leaching. The dominance of amide N in this spectrum, most tentatively of biogenic origin, is in line with recent results obtained by ^{13}C NMR spectroscopy, revealing a fast recovery of the SOM to its pre-fire composition (López-Martín et al. 2016).

In contrast to the solid-state ^{15}N NMR spectrum of the fresh grass material, which is dominated by signals of peptides (de la Rosa & Knicker 2011, Knicker & Lüdemann 1995), the ^{15}N NMR spectrum of PyOM confirms the presence of BN by clear signals peaking at -235 and -245 ppm which are typical for pyrrole N and indole N. However, some intensity is still recovered between -250 and -285 ppm which may indicate that not all peptides were transformed into heterocyclic N.

6.4.3. Total amino acids and total N of the extracted AAs in the starting materials

The contents of extracted AAs in the control soil of the unburnt area and in that of the nearby burnt region were 2.9 ± 0.2 mg AAs g soil $^{-1}$ and 4.0 ± 0.6 mg AAs g soil $^{-1}$, respectively (Table 6.2). These values are in the range of those found for soils studied by (Amelung and Zhang (2001) where the total AAs varied between 0.5 and 16.0 mg AAs g soil $^{-1}$. In general, only a

Table 6.2. C_{org}, N_t, atom % ^{15}N, extractable AAs contents and contribution of the extractable AAs and its nitrogen (N_{AAs}) to total N (N_t) in ^{15}N-enriched and non-enriched organic matter and charred material and in an unburnt and a burnt Cambisol from Sierra de Aznalcóllar (Spain)

	C_{org} (mg g dry matter $^{-1}$)	N_t (mg g dry matter $^{-1}$)	atom % ^{15}N	Total AAs (mg g dry matter^{-1})	AAs (mg g N_t $^{-1}$)	N_{AAs} (% of the N_t)
Unburnt soil	69.2 ± 1.4 (a)	4.1 ± 0.3 (a)	0.369 ± 0.002	2.9 ± 0.2	701.48 ± 39.55 (b)	8.79 ± 0.73 (b)
Burnt soil	57.6 ± 1.9 (b)	2.6 ± 0.0 (b)	0.370 ± 0.001	4.0 ± 0.6	1480.75 ± 228.29 (a)	18.90 ± 3.08 (a)
OM	385.4 ± 4.4	61.7 ± 1.3	0.373 ± 0.002	142.2 ± 45.6	2305.11 ± 522.99 (b)	30.45 ± 7.85 (b)
^{15}N-OM	391.2 ± 2.5	29.6 ± 2.2	54.495 ± 0.332	112.4 ± 3.4	3990.31 ± 276.18 (a)	53.01 ± 3.53 (a)
PyOM	371.2 ± 16.5	27.8 ± 1.9	0.401 ± 0.000	4.9 ± 1.1	175.06 ± 40.76 (ns)	2.51 ± 0.62 (ns)
^{15}N-PyOM	386.9 ± 19.4	43.0 ± 3.6	20.641 ± 0.261	5.8 ± 0.0	134.25 ± 0.90 (ns)	1.93 ± 0.02 (ns)

Values followed with different letters within the same column are significantly different at $p < 0.05$. ns: no significant differences.

Table 6.3. Average contents of the extractable AAs in the burnt (B) and unburnt (U) soils (mg AAs g soil^{-1}), which were incubated with different amendments as a function of incubation time

	0.5 month		1 month		8 months		16 months	
	B	U	B	U	B	U	B	U
C	5.7 ± 0.2	6.4 ± 0.0	4.9 ± 0.2	4.8 ± 1.0	3.0 ± 0.5	4.8 ± 0.2	2.4 ± 0.0	3.9 ± 0.0
^{15}N-PyOM+N_i	2.6 ± 0.8	5.7 ± 0.4	2.9 ± 0.7	4.7 ± 0.1	3.7 ± 0.2	4.3 ± 0.5	2.8 ± 0.0	4.6 ± 0.1
^{15}N-OM+N_i	2.7 ± 0.1	5.8 ± 0.8	2.5 ± 0.1	4.7 ± 0.5	3.7 ± 0.3	4.6 ± 0.6	2.8 ± 0.0	4.9 ± 0.1
^{15}N-PyOM	3.5 ± 0.5	13.2 ± 2.4	3.5 ± 1.2	9.5 ± 6.6	3.8 ± 0.1	4.1 ± 0.2	2.7 ± 0.0	4.4 ± 0.1
^{15}N-OM	6.5 ± 0.8	11.2 ± 0.8	6.8 ± 3.8	5.1 ± 1.2	3.6 ± 0.3	5.1 ± 0.2	3.5 ± 0.0	5.5 ± 0.2
PyOM+$^{15}N_i$	4.3 ± 0.5	4.5 ± 1.6	2.2 ± 0.4	4.1 ± 0.0	3.0 ± 0.2	3.9 ± 0.3	2.5 ± 0.0	4.6 ± 0.0
OM+$^{15}N_i$	4.7 ± 0.6	7.1 ± 0.8	2.5 ± 0.9	5.9 ± 1.3	3.5 ± 0.1	4.8 ± 0.2	2.4 ± 0.0	4.7 ± 0.1
$^{15}N_i$	4.5 ± 1.2	7.8 ± 0.5	4.1 ± 2.3	6.0 ± 0.5	3.5 ± 0.0	4.6 ± 0.0	3.0 ± 0.1	5.0 ± 0.0

small proportion of N_t of soils is hydrolyzed and recovered as N_{AAs} by the used method. (Friedel and Scheller (2002) or (Amelung et al. (2006) recovered 28 to 50 % and 22 to 46 % of N_t as N_{AAs}. In our approach, the recovery of N_t as N_{AAs} was between 9 and 19 % for the soils (Table 6.2). Higher AAs contents and N_{AAs} recoveries were obtained for OM and ^{15}N-OM. Here the contribution of N_{AAs} to N_t ranged from 31 to 53%. It seems that due to the higher humification degree of SOM, their peptides are better protected from hydrolysis than those in fresh litter. Considerably low amounts of AAs were obtained for PyOM (4.9 ± 1.1 mg g dry material^{-1}). In PyOM and ^{15}N-PyOM 2.51 ± 0.62 and 1.93 ± 0.02 % of N_t accounted for $N_{AAs.}$ Comparably, only 2% of the total $^{15}N_{add}$ in ^{15}N-PyOM was amended as $^{15}N_{AAs}$ to the soil before starting the experiment.

Statistical analysis of the extractable N_{AAs} contents normalized to N_t (Table 6.2) for both soils confirmed that in the fire-affected area the percentage of N_t attributable to AAs is twice the amount determined for the unburnt soil ($p = 0.01$). Thus, although the N content and the dominance of peptide-N in the soils of the burnt region recovered to the status of the unburnt soil, (López-Martín et al. 2016), the quality of the present peptides seems to be still affected by the former fire. Possibly, the fire history resulted in the production of fresh peptides which are more accessible to hydrolysis than those commonly accumulated in soils during humification.

6.4.4. Extractability of total AAs and N_{AAs} as a function of incubation time

During the incubation, the mean concentration of extracted AAs in the soils treated with the different amendments varied between 2.4 to 13.2 mg g soil $^{-1}$ (Table 6.3). In order to assess the relationship of the AAs extractability with either incubation time, we statistically compared the combined treatments of the unburnt with those of the burnt applying the Wilconxon test. As it can be revealed from Table 6.4, this analysis confirms decreasing recovery of AAs with incubation time. Comparing the results between burnt and unburnt soils showed further that the yields were always higher for the latter. A comparable approach was used for the statistical analysis of the relationship between N_{AAs} extractability with either incubation time or soil type (Fig. 6.2). Here, it should be noted that with ongoing incubation, the N_t content of the soils did not change significantly, neither between unburnt and burnt material nor between the different amendments, indicating that no major N loss by volatilization occurred. NMR spectroscopic data (data not shown) confirmed that no heterocyclic N was formed during the incubation, although for both soils the amount of extractable N_{AAs} decreased from the beginning until the end of the experiment (Fig. 6.2). Thus, the decline of extractable AAs with incubation time points toward ongoing AAs sequestration and transformation as it was suggested by (Miltner et al. 2009) and also agrees with findings by Creamer et al. (2012) and Nowak et al. (2013). Comparing the experiments with burnt and unburnt soils, we observed a general lower extractability of the N_{AAs} in the burnt soils. However, this difference is only statistically confirmed for the samples analyzed after 8 ($p = 0.000$) and 16 ($p = 0.013$) months of incubation. These observations may evidence that in our soils the fire history can indeed affect the N-cycling, although the impact was only clearly discernable at the end of the experiment.

6.4.5. Incorporation of $^{15}N_{add}$ into N_{AAs}

Figure 6.3 shows that already two weeks after addition of the amendment, $^{15}N_{add}$ is incorporated into the N_{AAs}. Its amount varied between 2 and 4 % of $^{15}N_{add}$. The low values may be explained by the facts that (1) part of $^{15}N_{add}$ was also incorporated into the growing plant residues and that (2) not all AAs of the soil were in an extractable form. For the experiment with ^{15}N-PyOM, the recovery of $^{15}N_{AAs}$ derived from $^{15}N_{add}$ varied between 0.67 and 12.62 % of $^{15}N_{add}$ which in average is slightly higher than the amount of $^{15}N_{AAs}$ added at the beginning of the experiment. This may allow the conclusion that that BN underwent a microbial transformation into non-heterocyclic residues. This is supported by the observation that already after 4 months approximately 2% of $^{15}N_{add}$ were recovered in the leaves of the freshly grown grass (data not shown). The $^{15}N_{add}$ incorporated into N_{AAs} decrease with time. The assimilation of $^{15}N_{add}$ into N_{AAs} was a bit higher in burnt than in unburnt soil with statistical differences at month 8 ($p = 0.039$) suggesting that the fire history after 7 years no affected the incorporation of organic N derived from fresh litter into peptideous material of SOM and microbial biomass.

6.4.6. Impact of the N sources on the ^{15}N content of N_{AAs}

In order to obtain more detailed information about the impact of the source material on the incorporation of $^{15}N_{add}$ in AAs of soils, the contribution of ^{15}N to the total N of the amino acids (N_{AAs}) was determined and the results were statistically analyzed after grouping the treatments according to their $^{15}N_{add}$ source into the following four sets: I) control, II) inorganic source ($^{15}N_i$, OM+$^{15}N_i$, PyOM+$^{15}N_i$), III) organic litter (^{15}N-OM+$^{14}N_i$, ^{15}N-OM and III) charred organic matter (^{15}N-PyOM+$^{14}N_i$, ^{15}N-PyOM). Note the value of 0.366% corresponds to the natural ^{15}N abundance and was found for the control set I (Fig. 6.4). The fact that relative to the control set I,

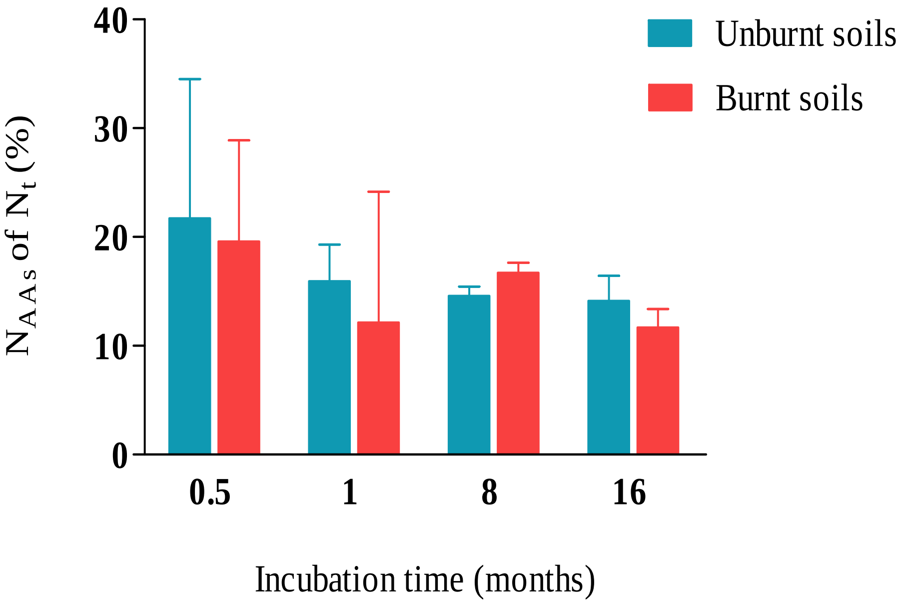

Fig. 6.2. Effect of time on the percentage of N_t which is extractable with the AAs (N_{AAs}) from burnt and unburnt soils amended with N-rich burnt and unburnt organic matter or inorganic N. For statistical reason, the impact of the kind of the amendment was not considered. Median values ± interquartile range (Mann-Whitney test, p < 0.005, n = 16).

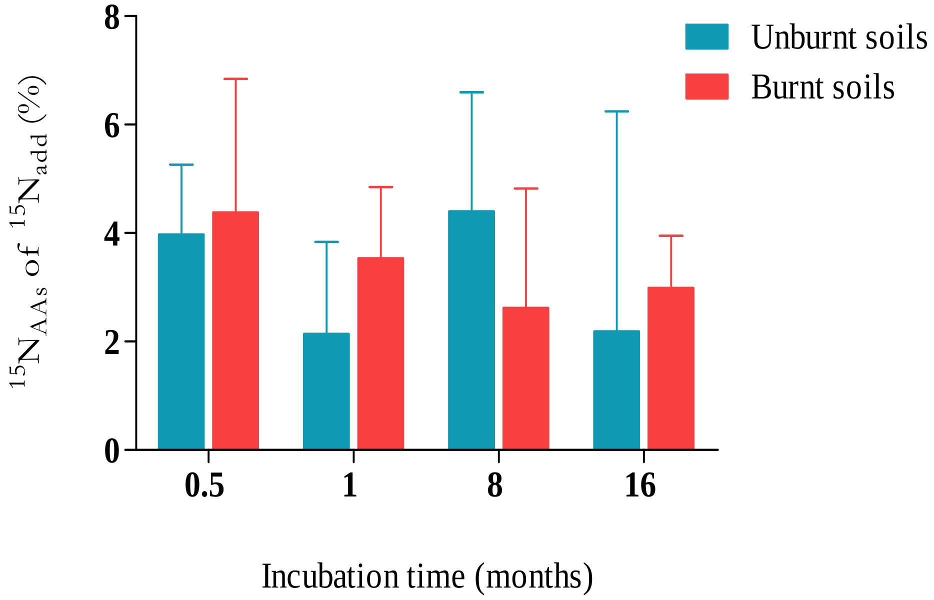

Fig. 6.3. Contribution of ^{15}N from ^{15}N-amendments ($^{15}N_{add}$) recovered as ^{15}N of AAs ($^{15}N_{AAs}$) after incubation of soil material from an unburnt and burnt Cambisol amended with ^{15}N-enriched burnt and unburnt organic matter and inorganic ^{15}N. For statistical reason, the impact of the kind of amendment was not considered. Median values ± interquartile range (Mann-Whitney test, p < 0.005, n = 16).

all other sets had higher $^{15}N_{AAs}$ recoveries indicates that $^{15}N_{add}$ has been incorporated into the extractable AAs fraction of the soil. However, since in average the amount of $^{15}N_{add}$ in N_{AAs} in the soil is only slightly higher than the percentage of N_{AAs} in PyOM we cannot unbiasedly differentiate if the recovered $^{15}N_{AAs}$ originate from the accumulation of PyOM-derived AAs or from AAs which were newly synthesized by microorganisms from BN.

In general, the contribution of $^{15}N_{AAs}$ to the total N_{AAs} increased continuously until the 8th month (Fig. 4) although the recovery of the latter decreased with incubation time (Fig. 6.2). At the end of the experiment time, the contribution of $^{15}N_{AAs}$ to total N_{AAs} is approximately 46% higher than that obtained after two weeks. From this it can be concluded that peptidic pool suffered a fast turnover in which the original AAs were mobilized and replaced by new peptides with AAs containing $^{15}N_{add}$. Whereas the first was most likely used for the synthesis of new biomass, the latter may have been released from biomass which already had been incorporated ^{15}N from the amendment. Possible sources of the released material are decaying residue, exudates or as exo-enzymes. Statistical analysis confirmed that differences also can be discerned between samples analyzed after the same incubation time but with different amendments ($p < 0.05$) (Table 6.4). Compared with the experiments amended with ^{15}N-PyOM those with the addition of ^{15}N-OM always showed higher $^{15}N_{add}$ contribution to N_{AAs} (Fig. 6.4). However, here

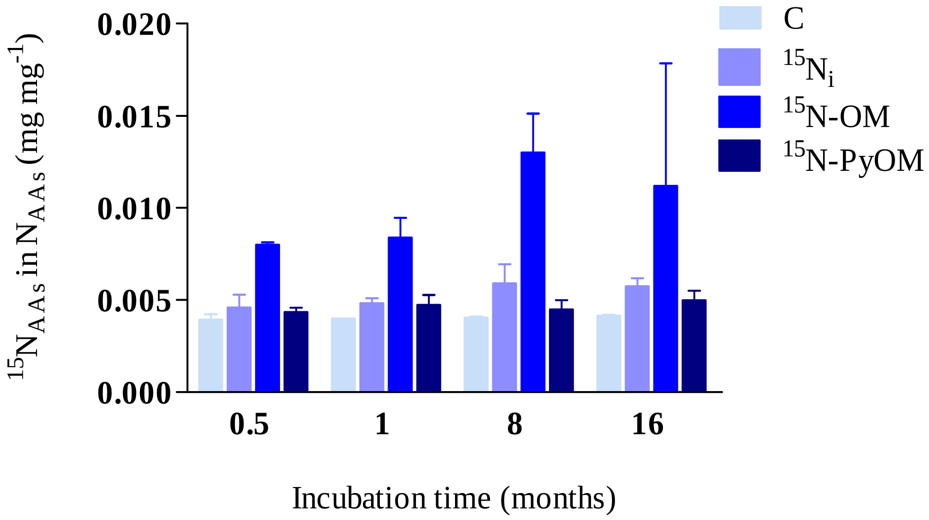

Fig. 6.4. Contribution of ^{15}N ($^{15}N_{AAs}$) to N of AAs (N_{AAs}) after incubation of soil material from an unburnt and burnt Cambisol amended with ^{15}N-enriched burnt and unburnt organic matter and inorganic ^{15}N. In order to evaluate the impact of the source material, the impact of the fire history of the soil was not considered and the results were grouped depending on the N source: control (C), inorganic N ($^{15}N_i$), fresh organic N (^{15}N-OM) and charred organic N (^{15}N-PyOM) Median values ± interquartile range (Wilcoxon test, $p < 0.005$, $n = 8$).

one has to bear in mind that we cannot discriminate if the extracted AAs derived from the ^{15}N-enriched plant residues which were incorporated into SOM or from newly synthesized biomass.

The $^{15}N_{add}$ contribution to N_{AAs} increases for the N_i and OM treatments only until the 8th month. After that we could not reveal any significant statistical difference. However, the $^{15}N_{AAs}$ recovery from the PyOM source augments slower but continuously until the end of the experiment ($p = 0.002$) 0.0050 ± 0.0004 mg $^{15}N_{AAs}$ mg N_{AAs} $^{-1}$. This value is slightly but significantly higher than the natural abundance of ^{15}N. Note that the incorporation of $^{15}N_{add}$ from N_i was only little bit higher than from PyOM which is interesting considering that inorganic N forms are commonly highly bio-available (Hu et al. 2016), whereas BN is commonly assumed to be biochemically more recalcitrant.

Table 6.4. Comparison of the p value obtained with the Wilcoxon test (n = 8) of ^{15}N incorporated into AAs extracted from an unburnt and a burnt Cambisol which was incubated after amendment with different N sources. For the statistical analysis, the experiments were grouped according to ^{15}N source (Fig. 6.4) as a function of incubation time. For comparison the values of the control (C) are also given

^{15}N source	**Time (months)**		
	0.5-1	**1-8**	**8-16**
C	0.109	0.109	0.068
$^{15}N_i$	0.236	**0.008**	0.398
^{15}N-OM	0.138	**0.028**	0.123
^{15}N-PyOM	**0.043**	0.066	**0.011**

The p value printed in bold represent statistical differences

6.4.7. Competition between N_i and N_{org}

In order to investigate how the presence of inorganic nitrogen affects the turnover and degradation of organic N, we statistically compared the relative contribution of $^{15}N_{AAs}$ to N_{AA} in the experiments with addition of $^{15}N_i$, ^{15}N-OM or ^{15}N-PyOM, respectively (Fig. 6.5). Note, if two different N sources are used and one is ^{15}N-enriched and the other not, the increase of the relative contribution of $^{15}N_{AAs}$ to N_{AAs} indicates the us e of the ^{15}N source and vice versa. The immobilization of $^{15}N_i$ increases until the 8th month which is confirmed by the statistical difference between control and the combination of N_i with fresh or charred material at each month ($p < 0.05$) (Fig. 6.5a). However, statistically, additional amendment of unenriched OM and PyOM did not affect the contribution of AAs formed after immobilization of $^{15}N_i$. Concomitantly, no significant impact of alternative organic N sources on the percentage of added $^{15}N_i$ which is incorporated into AAs (Fig. 6.6a) was revealed.

With respect to the incorporation of ^{15}N from ^{15}N-OM (Fig. 6.5b) there is a tendency that more ^{15}N is incorporated into N_{AAs} if no N_i is present. This trend would suggest that if both inorganic and organic N are present both sources are used at the same time. However, according to Fig. 6.6b, the efficiency of the use of ^{15}N derived from ^{15}N-OM (expressed as the percentage of ^{15}N derived from $^{15}N_{add}$ which was recovered in AAs) was statistically not affected by an additional N_i source which is in line with Zhang et al. (2015) reporting that there they observed no preferential incorporation of N_i over plant residue-N into microbial amino sugars.

With respect to the control, addition of ^{15}N-PyOM with and without N_i does not significantly alter the abundance of ^{15}N in N_{AAs} until two weeks after starting the experiment (Fig. 6.5c), but incorporation of ^{15}N from ^{15}N-PyOM starts to be statistically relevant after 1 month and

Table 6.5. Comparison of the p value of the amount of ^{15}N incorporated into AAs extracted from a burnt and an unburnt Cambisol after amendment with different N-sources and using the Mann-Whitney test (n = 4) between treatments as a function of incubation time.

Compared treatments	**Time (months)**			
	0.5	**1**	**8**	**16**
C / $^{15}N_i$	0.600	**0.013**	**0.019**	**0.019**
C / OM+$^{15}N_i$	**0.020**	**0.019**	**0.018**	**0.019**
C / PyOM+$^{15}N_i$	**0.020**	**0.028**	**0.019**	**0.019**
^{15}N / OM+$^{15}N_i$	0.240	1.000	0.243	0.149
^{15}N / PyOM+$^{15}N_i$	0.240	0.623	1.000	0.465
OM+^{15}N / PyOM+$^{15}N_i$	0.885	1.000	0.372	0.561
C / ^{15}N-OM	0.060	**0.019**	**0.019**	**0.019**
C / ^{15}N-OM+$^{14}N_i$	**0.020**	**0.019**	**0.019**	**0.019**
^{15}N-OM / ^{15}N-OM+$^{14}N_i$	0.643	0.127	0.083	0.248
C / ^{15}N-PyOM	0.384	**0.019**	**0.019**	**0.019**
C / ^{15}N-PyOM+$^{14}N_i$	0.108	**0.019**	**0.019**	**0.019**
^{15}N-PyOM / ^{15}N-PyOM+$^{14}N_i$	0.885	**0.005**	**0.038**	**0.020**

The p value printed in bold represent statistical differences continues until the end of the incubation ($p < 0.05$) (Table 6.5). The contribution of ^{15}N from ^{15}N-PyOM in N_{AAs} is significantly higher in the experiments without additional N_i amendment, confirming that in the presence of both, both N-sources are used simultaneously. Comparable to the unburnt N source, we have no statistical proof for a decrease of the efficiency of incorporating $^{15}N_{add}$ from PyOM if additional N_i is present (Fig. 6.6c).

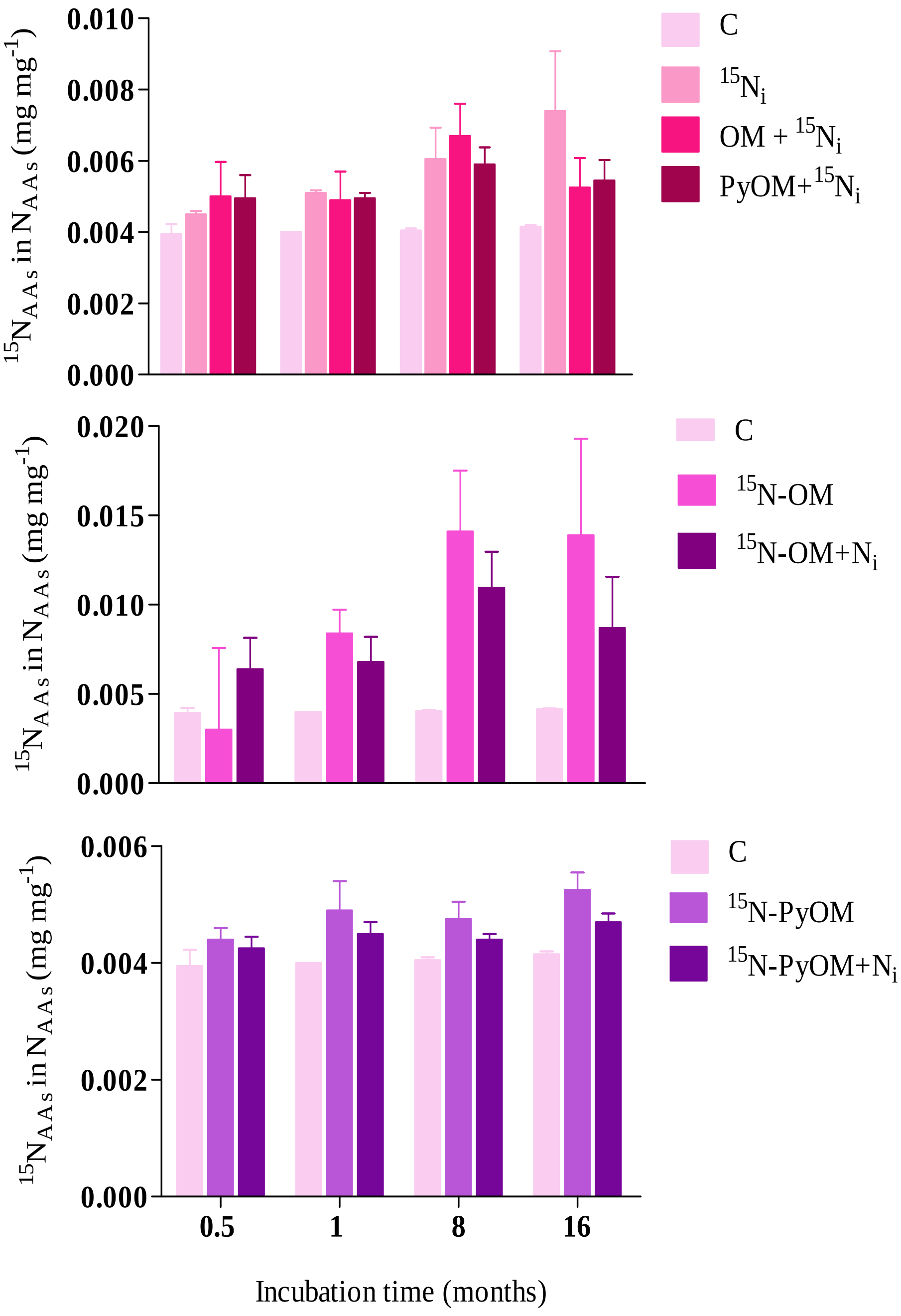

Fig. 6.5. Contribution of ^{15}N to the N of AAs (N_{AAs}) after incubation of soil material (both from an unburnt and burnt Cambisol) amended with a) inorganic ^{15}N ($^{15}N_i$) and with $^{15}N_i$ together with unburnt (OM) or burnt organic matter (PyOM) b) ^{15}N-OM and ^{15}N-OM together with N_i c) ^{15}N-PyOM and ^{15}N-PyOM together with N_i. Median values ± interquartile range (Wilcoxon test, $p < 0.005$, $n = 4$).

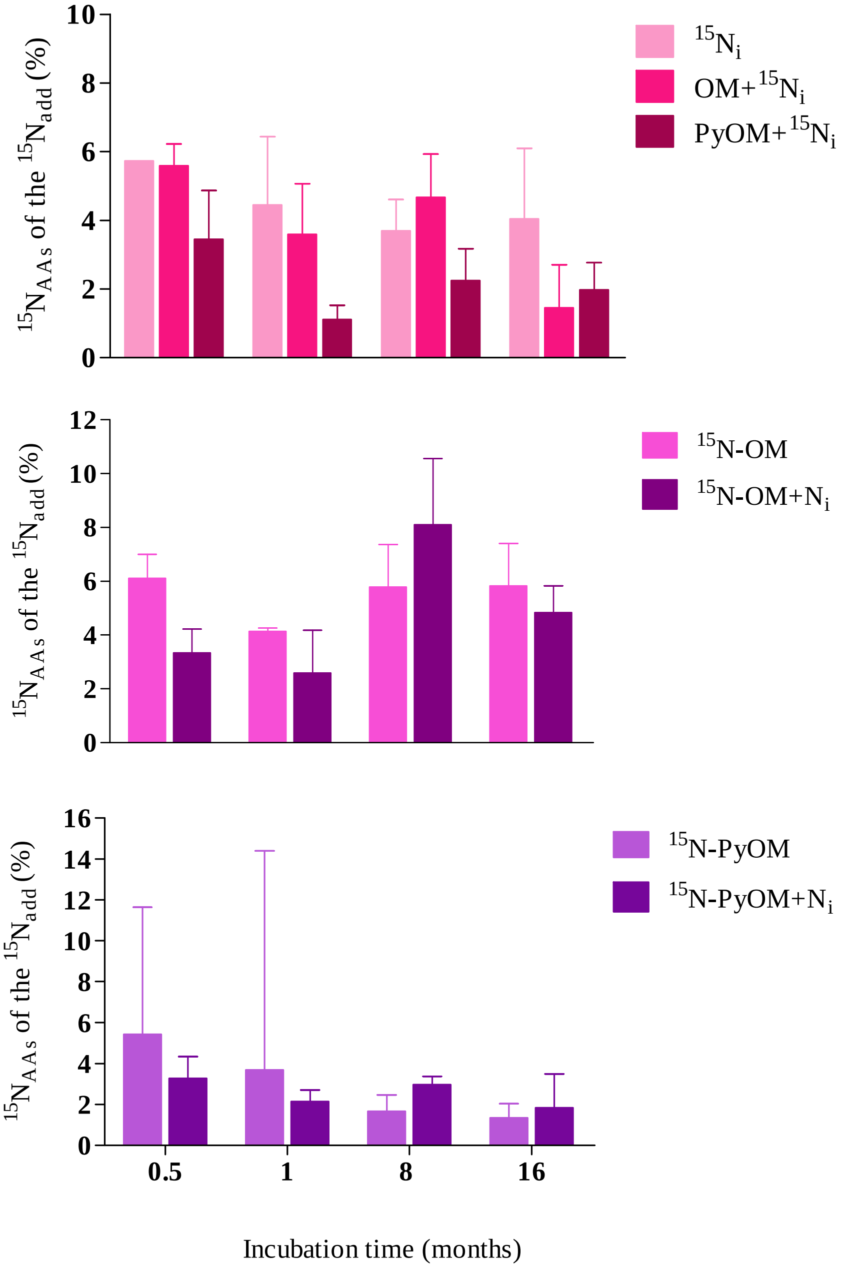

Fig. 6.6. Recovery of ^{15}N from ^{15}N-enriched amendments ($^{15}N_{add}$) in AAs ($^{15}N_{AAs}$) after incubation of soil material from an unburnt and burnt Cambisol amended with a) $^{15}N_i$ and with $^{15}N_i$ together with unburnt (OM) or burnt organic matter (PyOM) b) ^{15}N-OM and ^{15}N-OM together with N_i c) ^{15}N-PyOM and ^{15}N-PyOM together with N_i. Median values ± interquartile range (Mann-Whitney test, $p < 0.005$, $n = 4$).

6.5. Summary and conclusions

The performed pot experiment carried out for 16 months, using burnt and unburnt soil clearly demonstrated that former fire events can have an impact on N-cycling in soils. Although the extraction of AAs from both soils resulted in comparable yields, the relative contribution of extractable N_{AAs} to N_t is significantly higher in the fire affected soils. Considering that the NMR spectra attributed almost all of the organic N to peptide, these results allow the conclusion that after fire events, the newly synthesized peptides have a lower resistance against acid hydrolysis than peptides immobilized in soil organic matter formed without fire impact. Addition of fresh litter seems to shift the higher extractability of AAs towards the unburnt soil, possibly because the first provides additional labile AAs and allows for a higher microbial activity leading to new biomolecules with low resistance against acid hydrolysis. The fact that in several pots more ^{15}N from ^{15}N-PyOM was recovered in AAs than added as $^{15}N_{AAs}$ suggests that aside from incorporation of PyOM-derived AAs into the soil AAs pool, at least some ^{15}N of BN was recycled for the built-up of peptides in newly synthesized microbial biomass. With this step, the BN-derived nitrogen has transformed into a biogenic N source and is expected to behave as such during N cycling within the SON pool. Statistical analysis of our data did not reveal a significant impact of the presence of organic N on the percentage of added inorganic N which was incorporated into the peptidic N pool and vice versa. Although we could not prove unbiasedly a preferential immobilization of the N of any of the tested sources into AAs, our studies indicate that both inorganic N and organic N are simultaneously used as N supply. Thus, the presence of easily bioavailable N is not hindering the synthesis of new soil peptides from N in bound charred organic residues.

6.6. References

Alcañiz M, Outeiro L, Francos M, Farguell J, Úbeda X Long-term dynamics of soil chemical properties after a prescribed fire in a Mediterranean forest (Montgrí Massif, Catalonia, Spain). Science of The Total Environment

Almendros G, Knicker H, González-Vila FJ (2003): Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and 15N-NMR spectroscopy. Organic Geochemistry 34, 1559-1568

Amelung W, Zhng X, (2001) Determination of amino acid enantiomers in soils. Soil Biology and Biochemestry 33:553-562

Amelung W, Zhang X, Flach KW (2006) Amino acid in grassland soils: climatic effects on concentrations and chiraliy. Geoderma 130:207-217

Creamer CA, Filley TR, Olk DC, Plante A, Peltre C, Top SM, Boutton TW (2012): Degree of woody encroachment into grasslands controls soil carbohydrate and amino compound changes during long term laboratory incubation. Organic Geochemistry 52, 23-31

de la Rosa JM, Knicker H (2011): Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373

DeLuca TH, Aplet GH (2008): Charcoal and carbon storage in forest soils of the Rocky Mountain West. Frontiers in Ecology and the Environment 6, 18-24

Fraser RA, Bogaard A, Charles M, Styring AK, Wallace M, Jones G, Ditchfield P, Heaton THE (2013): Assessing natural variation and the effects of charring, burial and pre-treatment on the stable carbon and nitrogen isotope values of archaeobotanical cereals and pulses. Journal of Archaeological Science 40, 4754-4766

Friedel JK, Scheller E (2002): Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biology and Biochemistry 34, 315-325

Gärdenäs AI, Ågren GI, Bird JA, Clarholm M, Hallin S, Ineson P, Kätterer T, Knicker H, Nilsson SI, Näsholm T, Ogle S, Paustian K, Persson T, Stendahl J (2011): Knowledge gaps in soil carbon and nitrogen interactions - From molecular to global scale. Soil Biology and Biochemestry 43, 702-717

Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003): The effect of 10% HF treatment on the resolution of CPMAS 13C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma 116, 373-392

Hilscher A, Knicker H (2011): Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. Organic Geochemistry 42, 42-54

Hu G, He H, Zhang W, Zhao J, Cui J, Li B, Zhang X (2016): The transformation and renewal of soil amino acids induced by the availability of extraneous C and N. Soil Biology and Biochemistry 96, 86-96

IUSS Worling Group WRB (2014) World Reference Base for Soils and Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Reference Base Nº. 106. FAO, Rome

Knicker H, Fründ R, Lüdemann H-D (1993): The chemical nature of nitrogen in soil organic matter. Naturwissenschaften 80, 219-221

Knicker H, Lüdemann HD (1995): N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Organic Geochemistry 23, 329-341

Knicker H, Skjemstad JO (2000): Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state 13C and 15N NMR spectroscopy. Australian Journal Soil Respirometry 113-127

Knicker H (2007): Vegetation fires and burnings, how does char input affect the nature and stability of soil organic nitrogen and carbon? - a review. Biogeochemistry 85, 91-118

Knicker H, Hilscher A, González-Vila FJ, Almendros G (2008): A new conceptual model for the structural properties of char produced during vegetation fires. Organic Geochemistry 39, 935-939

Knicker H (2010): “Black nitrogen” – an important fraction in determining the recalcitrance of charcoal. Organic Geochemistry 41, 947-950

Knicker H (2011a): Soil ornagnic N- an under-rated player for C sequestration in soils?. Soil Biology and biochemestry 43:1118-1129

Knicker H (2011b): Solid state CPMAS ^{13}C and ^{15}N NMR spectroscopy in organic geochemistry and how spin dynamics can either aggravate or improve spectra interpretation. Organic Geochemestry 42, 867-890

Knicker H (2011c): Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. Quaternary International 243, 251-263

Lehndorff E, Roth PJ, Cao ZH, Amelung W (2014): Black carbon accrual during 2000 years of paddy-rice and non-paddy cropping in the Yangtze River Delta, China. Global Change Biology 20, 1968-1978

López-Martín M, Nowak KM, Milter A, Knicker H (2016): Incorporation of N from burnt and unburnt 15N grass residues into the peptidic fraction of fire affected and unaffected soils. Journal of Soils and Sediments, 1-11

Miltner A, Kindler R, Knicker H, Richnow H-H, Kästner M (2009): Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. Organic Geochemistry 40, 978-985

Nowak KM, Miltner A, Gehre M, Schäffer A, Kästner M (2011): Formation and Fate of Bound Residues from Microbial Biomass during 2,4-D Degradation in Soil. Environmental Science & Technology 45, 999-1006

Nowak KM, Girardi C, Miltner A, Gehre M, Schäffer A, Kästner M (2013): Contribution of microorganisms to non-extractable residue formation during biodegradation of ibuprofen in soil. Science of The Total Environment 445–446, 377-384

Robinson D (2001): δ15N as an integrator of the nitrogen cycle. Trends in Ecology & Evolution 16, 153-162

Rovira P, Romanyà J, Duguy B (2012): Long-term effects of wildifires on the biochemical quality of soil organic matter: a study on Mediterranean shrublands. Geoderma 179-180:9-19

Scharenbroch BV, Nix B, Jacobs KA, Bowles ML (2012): Two decades of low-severity prescribed fire increases soil nutrient availability in a Midwestern, USA oak (Quercus) forest. Geoderma 179-184:80-91

Stevenson FJ (1982): Organic forms of soil nitrogen. In: Stevenson FJ (editors) Nitrogen in Agricultural Soils. ASA-CSSA-SSSA, Madison, pp. 67-122

Ueda K, Morgan SL, Fox A, Gilbart J, Sonesson A, Larsson L, Odham G (1989): D-Alanine as a chemical marker for the determination of streptococcal cell wall levels in mammalian tissues by gas chromatography/negative ion chemical ionization mass spectrometry. Analytical Chemistry 61, 265-270

Varela ME, Benito E, Keizer JJ (2015): Influence of wildfire severity on soil physical degradation in two pine forest stands of NW Spain. CATENA 133, 342-348

Witanowski M, Stefaniak L, Webb. GA (Editors), 1993: Nitrogen NMR Spectroscopy. Annual Reports on NMR Spectroscopy, 25. Academic Press, London, 480 pp

Zhang H, Ding W, Luo J, Bolan N, Yu H (2015) The diynamics of glucose-derived ^{13}C incroporation into aggregates of a sandy loam soil following two-decade compost or inorganic fertilizer amendments. Soil and Tillage Research 148:14-19

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Capítulo 7.

Resultados generales

Chapter 7.

General results

<u>RESULTADOS GENERALES</u>

Objetivo 1: Estudio de las propiedades y variabilidad de la MOS de la Sierra de Aznalcóllar (Capítulo 3)

Se estudió el impacto del fuego sobre las propiedades de los suelos, y la variabilidad de la materia orgánica del suelo después de incendios, comparando dos tipos de muestreo (método 1: recogiendo muestras individuales y método 2: utilizando muestras compuestas).

Los resultados indicaron que ambos métodos dan lugar a valores similares en las propiedades físicas y químicas (pH y conductividad eléctrica (CE)), encontrándose dichos valores dentro del rango de los valores observados en los suelos de la Sierra de Aznalcóllar (Tabla 3.3). Sin embargo, dentro de cada tipo de método empleado, sí existieron diferencias entre los suelos. Así, las muestras del método 1 recogidas en un área afectada por el fuego bajo bosque de pinos (AZPB1) o *Quercus* (AZQB1) presentaron valores de pH más altos que las muestras procedentes del área quemada dos veces bajo un bosque de pinos (AZPDB1). En el caso del método 2, el mayor valor de pH se observó en el área control (AZQU2-1/2/3), que era estadísticamente diferente de las muestras bajo *Quercus* una (AZQB2-1/2/3) y dos veces quemadas (AZQDB2-1/2/3). En el caso de la CE no hubo diferencias dentro del tipo de muestreo.

En cuanto al contenido de carbono (C), la muestra AZPB1 del método 1, presentó el mayor valor y diferente estadísticamente al de las muestras AZQB1 y AZPDB1. Respecto de las cantidades de nitrógeno (N) y nitrato no se encontraron diferencias. En el método 2, sólo hubo diferencias en el contenido de N siendo mayor en las parcelas control AZQU2-1/2/3 (Tabla 3.3).

En cuanto a la calidad de la materia orgánica del suelo (MOS) estudiada mediante resonancia magnética nuclear de ^{13}C en estado sólido, se observó que todos los espectros a excepción de la muestra AZPB1, estaban dominados por la región *O/N*-alquílica (Fig. 3.3). Las muestras del método 2 no presentaron diferencias en cuanto a la calidad de la MOS teniendo en cuenta el número de fuegos, pero sí en comparación con la zona control (AZU2-1/2/3), la cual presentó la mayor intensidad en la región del *O/N*-alquílica con un 45% y la menor en la región arílica con 16%. En el método 1, la muestra AZPB1 presentó la mayor contribución en la región arílica con 46% y la menor en la región *O*-arílica.

Después de la oxidación química de todas las muestras, independientemente del método empleado, los espectros estaban dominados por la region alquílica (Fig. 3.4, Tabla 3.4), a excepción de la muestra AZPB1 la cual presentó la mayor intensidad en región arílica con un 36% (Fig. 3.4a) y la muestra procedente de la zona no quemada (AZU2-1/2/3) que contaba con menos del 1% de C arílico (Fig. 3.4).

Objetivo 2: Evaluación de la asociación órgano-mineral en la supervivencia del PyOM en un Cambisol y un Leptosol (Capitulo 4)

Los resultados obtenidos sobre la calidad de la MOS mediante resonancia magnética nuclear (RMN) de estado sólido de ^{13}C y ^{15}N, tras evaluar el impacto de la incorporación del *Black Nitrogen* (*BN*) al suelo después de cuatro semanas (2004) y siete años (2011) desde el último incendio, mostraron que los suelos control AZ04PU y AZQU3 bajo un bosque de pino y *Quercus* respectivamente estaban dominados por la región de *O*-alquílica y el N pirrol (Fig.4.1). Sin embargo los suelos quemados en 2004 (AZ04QB y AZ04PB) presentaron un alto contenido aromático tanto en C como en N (Fig. 4.1), mientras que los suelos muestreados siete años después del fuego, redujeron el N pirrolítico y la aromaticidad a expensas de un aumentando en el N amida y la región *O/N*-alquílica.

Las muestras recogidas en 2011, AZPDBe,a y AZPBc,d,e mostraron una baja intensidad en la región del N pirrol al igual que AZPBc,d,e, aunque éstas últimas contenían un elevado contenido aromático.

Después del fraccionamiento por densidad, la fracción recuperada fue mayor del 90% para todas las muestras (Tabla 4.2), asignada principalmente a la fracción mineral, con excepción de AZPDBe que recupero un 81%.

La masa recuperada tras el fraccionamiento por tamaño de partícula se encontró principalmente en la fracción fina (20 µm) con un valor mayor del 39% con excepción de AZPDBe y AZQDB1 donde la mayor parte de la masa fue recuperada en la fracción de tamaño arena con 57 y 62%.

El contenido de C y N recuperado tras el fraccionamiento por densidad fue superior al 73% menos en el caso del C recuperado para las muestras AZQBd y AZQU3 que fue de un 59 y 65%. El 71% de C y N fue medido para las muestras después del fraccionamiento de tamaño de partícula, con excepción de AZQDB1 que recuperó un 64% de N.

La cantidad de C (C_i) y N (N_i) inicial medido en los suelos desarrollados bajo pino se asociaron a la fracción de materia orgánica libre (*fPOM*, del acrónimo del inglés: *free particulate organic matter*) (Tabla 4.3). En la fracción de materia orgánica particulada ocluida (*oPOM*, del acrónimo del inglés, *occluded particulate organic matter*) las muestras quemadas dos veces fueron inferiores que en las muestras quemadas una vez y quemadas recientemente. El ratio C/N de las fracciones obtenidas por densidad procedentes de los suelos quemados una vez fueron mayores que las procedentes de suelos afectados por dos incendios. En el caso de las fracciones separadas por tamaño de partícula todas presentaron un ratio C/N bajo y la fracción fina fue la que más contribuyó a la MOS (Tabla 4.4).

La fracción *fPOM* de los suelos desarrollados bajo *Quercus* presentaron menor contenido de C_i y N_i que los desarrollados bajo pino. Las fracciones de las muestras AZQBa,d tuvieron ratios de C/N comparables a la muestra control AZQU3 mientras las muestras AZQB1 y AZQDB1 presentaron valores mayores.

Tanto para el suelo bajo *Quercus* como bajo pino el contenido de C y N fue recogido en la fracción fina osciló entre un 75 y 92%. Por lo tanto, no se observó un efecto del número de fuegos en el contenido de C y N en el suelo.

La caracterización química de las fracciones de MOS mediante RMN de ^{13}C mostró que la fracción *fPOM* está dominada por las regiones *O*-alquílica, alquílica y por último arílica. Comparando la fracción *fPOM* y la *oPOM* con el suelo total, se obtuvo que la primera presentaba mayor intensidad en la región *O*-alquílica mientas que la segunda menos, comportamiento que se observó para todos los suelos muestreados independientemente del número de fuegos (Fig. 4.2 y 4.3).

El RMN de ^{15}N de todas las fracciones se caracterizó por la presencia de N amida (Fig.4.4).

Las fracciones finas estuvieron dominadas por la región *O*-alquílica y no se observaron diferencias entre las muestras ni por los efectos del fuego ni del tipo de bosque.

Aunque después de 7 años la aromaticidad disminuyó en las muestras de suelo, aún se pudo observar que bajo *Quercus* y pino presentaban impacto del fuego en dicha región (Fig. 4.1). De manera que las fracciones *fPOM* y *oPOM* de las muestras procedentes de suelos quemados una y dos veces mostraron una mayor contribución en la región arilíca que la muestra control, AZQU3. Sin embargo, AZPBc,d,e presentaron más intensidad en la región arílica para la fracción *oPOM* que para la *fPOM*.

Con respecto a la acumulación de *PyOM*, las muestras bajo *Quescus* no presentaron diferencia teniendo en cuenta el número de fuegos, sim embargo bajo el bosque de pinos hubo una acumulación preferencial en la fracción fPOM.

El espectro de RMN de ^{13}C de la fracción fina de la muestra control AZQU3 reveló la desaparición de la señal de la región *O*-arílica, asignada a la lignina. La contribución de C_i recuperado como aromático en la fracción < 20 µm fue menor en las muestras procedentes del suelo bajo pino. Sin embargo, el RMN de ^{15}N puso de manifiesto que dicha región estaba dominada por N amida.

Objetivo 3: Obtener información del impacto del PyOM en la biodisponibilidad del nitrógeno en suelos afectados por incendios (Capítulo 5)

Los resultados obtenidos tras el experimento de incubación en invernadero indicaron que la producción de biomasa de plantas y raíces no estuvo influenciada ni por el tipo de tratamiento ni por el suelo (quemado o no quemado) (Fig. 5.1), y el contenido de N_t fue parecido a lo largo de los meses de incubación.

Con respecto al contenido de ^{15}N recuperado del añadido con cada una de las enmiendas ($^{15}N_{add}$) en suelo fue pequeño. Estadísticamente no tuvo impacto en el $^{15}N_{add}$ recuperado ni el tipo de suelo ni mes de incubación, sólo en el tipo de enmienda utilizado (Fig. 5.2a). Las enmiendas *^{15}N-PyOM+N_i* y *^{15}N-PyOM* fueron las que menos $^{15}N_{add}$ acumularon en las raíces mientras que las enmiendas que recuperaron más fueron ^{15}N-MO+N_i y $^{15}N_i$. Comparable al análisis de raíces, el del suelo reveló que sólo hubo diferencia en el contenido de $^{15}N_{add}$ para los meses de incubación. En general, el $^{15}N_{add}$ recuperado disminuyó con el tiempo de incubación con excepción de las enmiendas ^{15}N-MO+N_i y ^{15}N-MO. Las enmiendas que más $^{15}N_{add}$ incorporaron fueron *^{15}N-PyOM+N_i* y *^{15}N-PyOM* mientras que las que menos ^{15}N-MO+N_i y ^{15}N-MO.

A la hora de estudiar como afecta la calidad química de las enmiendas a la hora de incorporar $^{15}N_{add}$ en las hojas, raíces y suelo se establecieron tres grupos en función del origen del de ^{15}N: (I: $^{15}N_i$) inorgánico ($^{15}N_i$, MO+$^{15}N_i$, *^{15}N-PyOM*), (II: ^{15}N-MO) orgánico (^{15}N-MO+ N_i, ^{15}N-MO) y (III: *^{15}N-PyOM*) material quemado (*^{15}N-PyOM+N_i*, *^{15}N-PyOM*).

En el caso de las raíces, tanto la enmienda con origen inorgánico como orgánico tuvieron una recuperación de ^{15}N comparable, mientras que la que menos fue *^{15}N-PyOM*. Sin embargo, cuando el origen de la fuente de ^{15}N fue inorgánico se produjo la mayor recuperación de $^{15}N_{add}$ en hojas y la menor recuperación cuando el N enriquecido se aplicó como *^{15}N-PyOM* (Fig. 5.3b), tendencia que se repite para cada mes de incubación entre las tres fuentes de ^{15}N (Fig. 5.3c).

En el suelo, el mayor secuestro de $^{15}N_{add}$ se observó en las muestras donde se adicionó *^{15}N-PyOM* (Fig. 5. 4).

Del estudio de si la presencia de N_i afectaba a la descomposición del N_{org} de las enmiendas de residuo vegetal fresco (MO) como quemado (*PyOM*) en raíces y hojas se puso de manifiesto que no existió diferencias entre la adicción o no (Fig. 5.5a/b), pero en ambos casos siempre el $^{15}N_{add}$ $_{fue}$ mayor que el suelo control.

Para el suelo, se observó que el $^{15}N_{add}$ siempre fue mayor que el control hasta el mes 12 cuando se estudió el secuestro de ^{15}N en la combinación $^{15}N_i$ con MO o *PyOM* (Fig. 5.5c). Por otro lado, la adicción de N_i a las enmiendas *^{15}N-PyOM* y ^{15}N-MO, no produjo diferencias en el secuestro de ^{15}N.

El balance total de $^{15}N_{add}$ recuperado al final del experimento combinando los valores del suelo quemado y no quemado indicó que el tipo de enmienda empleada y el mes de incubación afectaba dicha recuperación y que *^{15}N-PyOM* con y sin N_i fueron las enmiendas que mostraron mayores valores de recuperación de N enriquecido (Tabla 5.1).

Con respecto a la calidad de la MOS medida mediante RMN de ^{13}C del suelo control después de dos semanas de incubación mostró que las regiones con mayor intensidad fueron la alquílica y la *O*-alquílica (Fig. 5.6, Tabla 5.2) y que su ratio alquílico/*O*-alquílico era de 1.2, indicando un avanzado estado de humificación. Los espectros de las distintas enmiendas empleadas al mes 1 presentaron espectro semejantes Después de 8 meses se apreció una disminución de la intensidad en la región *O*-alquílica y un pequeño aumento en las regiones carboxílica y alquílica. Los espectros de ^{15}N (Fig. 5.7) están dominados por la región de N amida incluso para la enmienda de $^{15}N_i$, indicando que el N_i se ha trasformado en formas de N orgánico. En los espectros de ^{15}N-*PyOM* con y sin N_i se apreció una pequeña señal en la región de N pirrolítico, típico del *BN*, intensidad que disminuyó desde el mes 1 al 8.

Finalmente, se estudió la transformación bioquímica de las enmiendas aplicadas a los maceteros en forma *PyOM* con y sin N_i mediante RMN de ^{13}C y ^{15}N. El espectro del ^{13}C del *PyOM* control se caracterizó por una elevada intensidad en la región aromática y alquílica (Fig. 5.8, Tabla 5.3). Después de 8 meses de incubación las regiones que experimentaron un incremento fueron la *O*-alquílica y la *N*-alquílica, intensidades que aumentaron hasta el mes 16. El correspondiente espectro de ^{15}N de dichas enmiendas se caracterizó por la disminución de señal en la región de N pirrolítico y un aumento en la región de N amida al final del experimento.

Objetivo 4: Revelar el papel del BN en el ciclo del N y si este N puede ser usado para la producción de biomasa microbiana (Capítulo 6)

El contenido de C orgánico (C_{org}) y N total (N_t) del suelo no quemado después de 7 años desde el incendio dio valores ligeramente más altos que es suelo quemado. El ratio C/N para los residuos de *Lolium perenne* tanto enriquecidos (^{15}N-MO) como no (MO) así como sus respectivos residuos quemados: *PyOM* y ^{15}*N-PyOM* mostraron valores entre 6 y 13. El contenido de ^{15}N en MO y *PyOM* se encontró en los valores de abundancia natural con 0.373 y 0.401 atom% al igual que el suelo control y quemado con 0.355 y 0.377 atom%. Sin embargo, el enriquecimiento en ^{15}N-MO y el ^{15}N-*PyOM* fue de 54.495 y 20.641 atom% (Tabla 6.2).

El espectro de RMN de ^{15}N puso de manifiesto que el suelo quemado y el no quemado estaban dominados por N amida, al igual que la MO, mientras que en el *PyOM* confirmó la presencia de *BN* y señal peptídica.

El contenido de N recuperado en forma de amino ácidos (N_{AAs}) extraíbles fue bajo para los materiales de partida (Tabla 6.2), en el caso del suelo quemado se extrajo el doble que en el suelo control y mientras que para el *PyOM* y el ^{15}*N-PyOM* se recuperó sólo el 2 %.

El estudio de la cantidad de amino ácidos extraíbles (AA_s) así como de N_{AAs} con el tiempo de incubación fue evaluado y se obtuvo que la cantidad de AA_s extraídos para cada tratamiento disminuyó con el tiempo (Tabla 6.3) y fue mayor siempre en el suelo no quemado al igual que el N_{AAs} (Fig. 6.2).

Con respecto a la cantidad de ^{15}N incorporado en el N_{AAs} ($^{15}N_{AAs}$) se puso de manifiesto que a las dos semanas de la adicción de las enmiendas enriquecidas en ^{15}N se había incorporado en el N_{AAs} (Fig. 6.3), que el contenido disminuía con el tiempo de incubación y que fue un poco mayor en el suelo quemado. Para la enmienda *^{15}N-PyOM* la recuperación del $^{15}N_{AAs}$ osciló entre 0.67 y 12.62 %.

Teniendo en cuenta la procedencia del ^{15}N medido en N_{AAs} se establecieron 4 grupos: (I) control (C), (II) inorgánico (N_i), (III) orgánico (MO) y (IV) quemado (*PyOM*). Los grupos II, III y IV recuperaron más $^{15}N_{AAs}$ que el grupo I (Fig. 6.4) y dicha recuperación aumentó hasta el mes 8 para luego disminuir, menos en el caso del grupo IV el cual aumento hasta el mes 16. Al final del experimento la contribución del $^{15}N_{AAS}$ al total de N_{AAs} fue aproximadamente un 46% más que a las dos semanas. La recuperación de ^{15}N procedente del N_i ($^{15}N_{add}$), fue sólo un poco mayor que la recuperación de $^{15}N_{add}$ en el grupo del *PyOM*.

Del estudio de como la presencia del N_i afecta a la degradación y tasa de descomposición del N_{org}, se obtuvo que el ^{15}N fue inmovilizado hasta el mes 8 y hubo diferencia estadísticas entre el control y la combinación de N_i y MO o *PyOM* (Fig. 6.5a). Sin embargo, estadísticamente, la adicción de MO o *PyOM* no enriquecidos no afectó a la contribución de los AA_s formados después de la inmovilización del $^{15}N_{i.}$ Paralelamente, no hubo un impacto en el porcentaje $^{15}N_{add}$ cuando se usó el N de fuentes orgánicos (Fig. 6.6a). Con respecto a la incorporación de ^{15}N procedente del ^{15}N-MO (Fig.6.5b) se observó que más ^{15}N fue incorporado cuando el N_i no estaba presente. La adicción de *^{15}N-PyOM* con y sin N_i no afectó a la incorporación de ^{15}N en el N_{AAs} hasta después de dos semanas (Fig. 6.5c, Tabla 6.5), la cual incrementó hasta finales del experimento y fue estadísticamente diferente. La contribución del ^{15}N del *^{15}N-PyOM* es mayor en ausencia de $N_{i,}$ lo que indica que en presencia de las dos fuentes de N, ambas son usadas simultáneamente.

Capítulo 8.

Discusión general

Chapter 8.

General discussion

DISCUSIÓN GENERAL

8.1. Discusión general

El relieve y la distribución no homogénea de material combustible no tuvieron impacto notable en la variabilidad del contenido de carbono (C) y nitrógeno (N) en suelos quemados y no quemados del área de estudio en la Sierra de Aznalcóllar, Andalucía, España.

Considerando los datos obtenidos, no podemos confirmar un impacto duradero de la entrada de restos carbonizados en el tamaño del compartimento (o *pool*) de materia orgánica del suelo (MOS), lo que contrasta con trabajos previos que presentan la materia orgánica pirolizada (*PyOM*) como un material recalcitrante y un eficiente sumidero de C a largo plazo (González-Pérez et al. 2004).

La calidad de la MOS, estudiada mediante espectroscopía de resonancia magnética nuclear (RMN), se ha mostrado independiente del método de muestreo empleado (error standard inferior al 10%). Así, la variabilidad de la composición química debida al relieve y a la distribución del material combustible se podría alcanzar de manera satisfactoria mediante el uso de muestras compuestas.

En contraste con los datos obtenidos mediante análisis elemental, los datos de espectroscopía de RMN permitieron una diferenciación entre áreas quemadas y no quemadas debido al incremento de la aromaticidad en las primeras. Resultados comparables han sido mostradas por Guénon et al. (2011) en una región quemada hacía 17 años. Así, podemos concluir que los efectos típicos de los incendios sobre el suelo no persistirán en las próximas décadas en nuestra zona de estudio.

En la búsqueda del mecanismo de estabilización del *Black Carbon* (*BC*) y del *Black Nitrogen* (*BN)* en suelos desarrollados bajo clima mediterráneo, suelos seleccionados de nuestra área de estudio fueron sometidos a fraccionamiento por densidad y tamaño de partícula. Con este enfoque se pretendía dilucidar el papel de las interacciones fracción mineral-*PyOM* en la conservación de la *PyOM* en el suelo.

La mayor parte de las estructuras aromáticas fueron recuperadas en la fracción de materia orgánica particulada (*POM*, del acrónimo del inglés*; particulate organic matter*), lo que puede evidenciar una elevada recalcitrantica química de los residuos carbonizados que no interaccionaron con la fracción mineral. Esto apoyaría la alta recalcitrancia química de la *PyOM* considerando que la *POM* de suelos no quemados está sólo débilmente protegida y puede ser rápidamente degradada. Por otra parte, la rápida pérdida de aromaticidad durante los siete años de recuperación tras el fuego se opone a esta interpretación de la alta recalcitrancia química. Posiblemente, la ausencia de asociación mineral-*PyOM* ha podido posibilitar la rápida degradación de la *PyOM*. Sin embargo, los grupos funcionales de la *PyOM* parcialmente oxidada podrían interactuar con la superficie mineral antes de ser completamente metabolizados. La protección física de esta interacción podría hacer que la *PyOM* sea capaz de perdurar a largo plazo. En línea con esta idea, el *BC* detectado en la fracción fina de Chernozems (Vasilyeva et al., 2011) y en suelos de praderas de norte América (Glaser & Amelung 2003) y de Australia (Skjemstad et al. 1999) podría formarse por este tipo de *PyOM* residual y representaría sólo una pequeña fracción del *input* inicial de carbón.

Otra conclusión de nuestro trabajo se basa en la baja resistencia de N heterocíclico durante el tiempo de recuperación tras un incendio, indicando una menor estabilidad bioquímica del *BN* que el *BC*. Esto podría deberse a la carga de los grupos nitrogenados en estructuras del tipo del pirrolíticas las cuales son más accesibles a las enzimas que los anillos condensados de polibenceno. No obstante, la pregunta de cuál es el papel del *BN* contenido en la *PyOM* en su estabilidad en los suelos deberá ser respondida en futuras investigaciones.

La baja longevidad del *BN* cuestionó si este N es disponible para las plantas y cómo su presencia afecta al ciclo del N en el suelo. Nuestros estudios sobre el efecto a corto plazo de la adición de *PyOM* tras incendios en la distribución suelo-planta del N no revelaron alteraciones considerables derivadas del historial de fuegos del suelo. Aunque, sí se observó cierto impacto de éste en el secuestro de N en los péptidos del suelo. Una alteración generalizada del ciclo del N no fue evidenciada, posiblemente porque las condiciones experimentales no estaban sometidas a limitación de N. Bajo estas consideraciones, la disponibilidad de Ni adicional no provocó un decrecimiento de la eficiencia de absorción de ^{15}N procedente de materia orgánica quemada o sin quemar. Por tanto, un *priming effect* negativo del N_i no fue observado. Por ello, podemos asumir que el tipo y la cantidad de ^{15}N que puede ser absorbido por las plantas depende principalmente de la biodisponibilidad de dicho ^{15}N. Al mismo tiempo, ésta es determinada por la degradabilidad de la fuente orgánica de ^{15}N. Aunque más lentamente que el N de hojarasca no quemada, fue posible evidenciar la degradación de *BN* y, por tanto, la movilización del *BN*.

En resumen, nuestros resultados nos permiten concluir que la incorporación en el suelo de residuos orgánicos carbonizados y de cenizas tras un incendio forestal puede ciertamente alterar el ciclo del N en el suelo, aunque dicha alteración se dará a corto y medio plazo. La fertilización con N_i procedente de cenizas directamente tras un incendio aporta nutrientes para una rápida recuperación de la vegetación y de la actividad microbiana, mientras que el secuestro de N en la *PyOM* permite una liberación lenta del N para el crecimiento de la biomasa a medio plazo. Esto previene la pérdida de N del ecosistema y proporciona N adicional para los siguientes ciclos de crecimiento de las plantas. Considerando la importancia de un rápido desarrollo de la cobertura vegetal tras un incendio a fin de evitar la erosión del suelo y procesos de desertificación, nuestro estudio claramente enfatiza la importancia ecológica del papel del *BN* en ecosistemas con riesgos de incendios, hecho éste que pone de manifiesto la necesidad de reconsiderar el papel del *BN* en las investigaciones ambientales.

El estudio detallado del N, procedente de *BN*, recuperado en los aminoácidos (AA_s) del suelo, reveló que la entrada de carbón tuvo un cierto impacto en el ciclaje del N. Tras los incendios, parece que los péptidos nuevamente sintetizados tienen menor resistencia frente a la hidrólisis ácida que aquellos péptidos inmovilizados en la MOS formada sin influencia del fuego. La adición de material vegetal fresco parece cambiar la alta extractabilidad de AA_s en suelos no quemados, posiblemente debido a que el primero proporciona AAs lábiles adicionales y permite una mayor actividad microbiana conduciendo a la formación de nuevas biomoléculas con baja resistencia frente a la hidrólisis ácida.

De acuerdo con nuestros resultados, al menos parte del N procedente del *BN* fue utilizado para la formación de péptidos de la biomasa microbiana nuevamente sintetizada. En esta etapa, el N derivado del *BN* se ha transformado en una fuente de N y es esperable que se comporte como tal durante el ciclaje del N dentro del *pool* de nitrógeno orgánico del suelo.

Nuestro estudio muestra además que el N inorgánico, el N orgánico de *PyOM* y la MO son utilizados simultáneamente como fuente de N. Así, la presencia de N fácilmente biodisponible no está entorpeciendo la síntesis de nuevos péptidos procedentes del N relacionado con residuos orgánicos carbonizados.

GENERAL DISCUSSION

8.2. General discussion

The statistical evaluation of the extent to which relief and pre-fire fuel distribution affects soil properties and composition of soil organic matter (SOM) in fire-affected and unburnt soils of the hilly area of the Sierra de Aznalcóllar, Andalusia, Spain indicated that in the studied area, relief and fuel distribution inhomogeneity had only minor impact on the variability of the carbon (C) and nitrogen (N) contents.

Summarizing our data, we cannot confirm a lasting impact of charcoal input on the size of the SOM pool, which is in contrast to the common belief that pyrogenic organic matter (PyOM) in soils represents an recalcitrant and efficient long-term C sink (González-Pérez et al. 2004).

With respect to the quality of SOM, our investigation showed that independently of the sampling method used, the standard error in each chemical shift region did not exceed 10% of its relative intensity. Thus, variability of the chemical composition due to relief and fuel distribution inhomogeneity could be satisfactorily averaged by using composite sampling.

However, in contrast to the data of the elemental analysis, nuclear magnetic resonance (NMR) spectroscopic analysis allowed a differentiation between fire-affected and unburnt areas by an increase of the aromaticity in the first. Since comparable observations are reported by Guénon et al. (2011) for a fire-affected region 17 years after the last wildfire, our results point to the conclusion that, at least in our study area the typical pattern induced by former vegetation fires on soils will not persist beyond the next decades.

In search of the mechanism involved in the stabilization of Black Carbon (BC) and Black Nitrogen (BN) in the Mediterranean soils of our research areas, selected soils were subjected to density and physical fractionation. With this approach it was intended to bring some light on the role of PyOM-mineral interactions for the survival of PyOM in soils. It was observed that most aromatic structures were recovered in the POM, which may evidence high chemical recalcitrance of the remaining charred residues without interaction of the mineral fraction. This would support high chemical recalcitrance of PyOM since unburnt particulate organic matter (POM) is only weakly protected and thus quickly degraded. On the other hand, the fast loss of aromaticity during the seven years of recuperation argues against this interpretation of high chemical recalcitrance. Possibly, the lack of mineral-PyOM association may have allowed fast PyOM degradation. However, the functional groups of some partially oxidized PyOM may interact with the mineral surface before they were completely metabolized. Physically protected by this interaction the PyOM may be able to survive on a long-term scale. Following this argumentation, the BC detected in the fine fraction of Chernozems (Vasilyeva et al., 2011) and North American (Glaser & Amelung 2003) and Australian grassland soils (Skjemstad et al. 1999) would be formed by such residual PyOM, and represent only a very small fraction of the original charcoal input.

A further interesting conclusion of our study is based on the low survival of heterocyclic N during the post-fire recuperation time, indicating a lower biochemical stability of BN than BC. This may be due to charged N-groups in pyrrole-type structures which are better accessible to enzymes than condensed polybenzene rings. However, the question to which extent the content of BN in PyOM determines its stability in soils has still to be answered in future research.

The low longevity of BN puts into question of its N is available for plant growth and how its input affects the soil N cycle. Our studies on the short-term effect of PyOM addition after vegetation fires on the distribution of N between plants and soil did not reveal notable alterations due to fire history of the soil, although an impact of the latter on N-sequestration into soil peptides were recently observed. An alteration of the overall N cycling was not evidenced, possibly because the experimental conditions avoided N-limitation. Under those conditions, the availability of additional N_i did not decrease the efficiency of ^{15}N uptake from unburnt or burnt OM. Thus, negative priming of Ni was not observed. Based on this we can assume that kind and amount of ^{15}N which is taken up by the plants depends mainly on the bioavailability of the ^{15}N, which again is determined by the overall degradability of the ^{15}N-containing organic source. Although slower than N in unburnt litter, we were able to evidence degradation of BN and thus the mobilization of its BN. In summary, our results point to the conclusion that incorporation of charred organic residues and ash into the soil after a forest fire can indeed alter N-cycling in soil, although this alteration will be mainly effective on a short and medium term. Fertilization with N_i from the ash directly after the fire provides nutrients for a quick recovery of the ground vegetation and microbial activity, whereas sequestration of N in PyOM allows a slow release of N for biomass growth on a medium term scale. This prevents N-losses from the ecosystem and provides additional N for the next vegetation cycles. Considering the importance of a fast development of a new plant cover after fire events to avoid soil erosion and desertification, our study clearly underlined the important ecological role of BN in fire-prone ecosystems, a fact that certainly asked for an improved consideration of BN in environmental research.

However, going into more detail with respect to the fate of BN in soils by analyzing the recovery of its N in soil amino acids (AA_s), some impact of charcoal input on N-cycling was observed. It seems that after the fire events, the newly synthesized peptides have a lower resistance against acid hydrolysis than peptides immobilized in soil organic matter formed without fire impact. Addition of fresh litter seems to shift the higher extractability of AAs towards the unburnt soil, possibly because the first provides additional labile AAs and allows for a higher microbial activity leading to new biomolecules with low resistance against acid hydrolysis. According to our results, at least some N of BN was recycled for the built-up of peptides in newly synthesized microbial biomass. With this step, the BN-derived nitrogen has transformed into a biogenic N source and is expected to behave as such during N cycling within the soil organic nitrogen (SON) pool. Our studies indicated further that inorganic N, organic N of PyOM and OM are simultaneously used as N supply. Thus, the presence of easily bioavailable N is not hindering the synthesis of new soil peptides from N in bound charred organic residues.

8.3. References

Glaser B, Amelung W (2003): Pyrogenic carbon in native grassland soils along a climosequence in North America. Global Biogeochemical Cycles 17, n/a-n/a

González-Pérez JA, González-Vila FJ, Almendros G, Knicker H (2004): The effect of fire on soil organic matter—a review. Environment International 30, 855-870

Guénon R, Vennetier M, Dupuy N, Ziarelli F, Gros R (2011): Soil organic matter quality and microbial catabolic functions along a gradient of wildfire history in a Mediterranean ecosystem. Applied Soil Ecology 48, 81-93

Skjernstad J O, Taylor J A, Smernik R J (1999): Estimation of charcoal (char) in soils. Communications in Soil Science and Plant Analysis 30, 2283-229

Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011): Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. Soil Biology and Biochemistry 43, 1985-1988

Capítulo 9.

Conclusiones

Chapter 9.

Conclusions

CONCLUSIONS

The examination of the soil organic matter (SOM) in fire-affected and unburnt soils in the Sierra de Aznalcóllar clearly confirmed that the longevity of pyrogenic organic matter (PyOM) in their soils is by far shorter than commonly assumed for Black Carbon (BC). Although being longer than fresh litter, mean residence times in the range of humified SOM were recently determined for PyOM (Knicker et al. 2013). Possibly, the mild climatic conditions of the Mediterranean areas during winter seasons together with soil conditions, which are favorable for microbial activity, support a fast turnover rate not only of naturally formed SOM but also of PyOM. However, the relatively fast loss of PyOM by biodegradation during post-fire recovery time can solve the contradiction observed by (Masiello & Druffel 2003). They stated that “If Black Carbon has been produced since the last glacial maximum via biomass burning at the same rate as it is now produced, BC should account for 25 – 125% of the total soil organic carbon pool”.

The fact that in topsoil, the majority of PyOM does not survive for millennia has been also observed in other studies (De la Rosa et al. 2013, Velasco-Molina et al. 2016), and asks for a more detailed analysis of the role of BC as an important long-term carbon (C) sink within the global C cycling. The fact that at archeological sites and sediments considerably old PyOM has been discovered suggests that longevity of PyOM is closely related to the conditions under which it is produced and accumulated. However, the involved mechanisms still need to be examined in more detail.

Comparable to other studies, most of the surviving PyOM after seven years of the fire was recovered with the particulate organic fractions (POM). This seems in contrast to a suggested protection of partially degraded PyOM from microbial degradation by interaction with the mineral phase as it was proposed for Australian soils (Qi et al. 2017). However, it may be speculated that the unprotected PyOM in the POM fraction may be finally degraded whereas the protected PyOM in the mineral fractions will accumulate. According to this scenario, only the latter may be still present on a long-term time scale.

The organic nitrogen (N) in PyOM was commonly neglected and only recently the scientific community started to become interested in this issue. In contrast to views, in which heterocyclic aromatic N represents biochemically recalcitrance forms, the present study confirmed that Black Nitrogen (BN) can be degraded and its N can be used both by microorganism and by plants for the built-up of new biomass. Here, one has to consider that the incorporation rate of N from BN was considerably lower than those from inorganic N or unburnt organic N, however, in fire-affected areas it plays certainly an important role for N-storage. Whereas, after the fire Ni released during combustion of OM represents an N source which is directly available for the recovery of the vegetation, BN can act as a slow-release fertilizer preventing considerable N losses due to leaching of nutrients after the first post-fire rains. From this point of view, BN accumulating after fires on the topsoil may play a more important role for ecosystem recovery than formerly thought which certainly needs more attention not only for a better understanding of fire-prone ecosystems but also for the development of improved post-fire management strategies.

References

De la Rosa JM, González-Pérez JA, González-Vila FJ, Knicker H (2013): Medium term effects of fire induced soil organic matter alterations on Andosols under Canarian pine (Pinus canariensis). Journal of Analytical and Applied Pyrolysis 104, 269-279

Knicker H, González-Vila FJ, González-Vázquez R (2013): Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. Soil Biology and Biochemistry 56, 31-39

Masiello CA, Druffel ERM (2003): Organic and black carbon ^{13}C and ^{14}C through the Santa Monica Basin oxic-anoxic transition. Geophysical Research Letters 30, 34/1-34/4

Qi F, Naidu R, Bolan NS, Dong Z, Yan Y, Lamb D, Bucheli TD, Choppala G, Duan L, Semple KT (2017): Pyrogenic carbon in Australian soils. Science of The Total Environment 586, 849-857

Velasco-Molina M, Berns AE, Macías F, Knicker H (2016): Biochemically altered charcoal residues as an important source of soil organic matter in subsoils of fire-affected subtropical regions. Geoderma 262, 62-70

Apéndice

Appendix

Table A.1. Total nitrogen content (%) in soil, leaves and roots at the end of each sampling date.

Months	Soil type	Treatments	Soil	Leaves	Roots
0.5	U	C	0.39	n.m.	n.m.
0.5	U	^{15}N-PyOM+N$_i$	0.40	n.m.	n.m.
0.5	U	^{15}N-OM+N$_i$	0.37	n.m.	n.m.
0.5	U	^{15}N-PyOM	0.37	n.m.	n.m.
0.5	U	^{15}N-OM	0.40	n.m.	n.m.
0.5	U	PyOM+^{15}N$_i$	0.40	n.m.	n.m.
0.5	U	OM+^{15}N$_i$	0.43	n.m.	n.m.
0.5	U	^{15}N$_i$	0.35	n.m.	n.m.
0.5	B	C	0.31	n.m.	n.m.
0.5	B	^{15}N-PyOM+N$_i$	0.31	n.m.	n.m.
0.5	B	^{15}N-OM+N$_i$	0.29	n.m.	n.m.
0.5	B	^{15}N-PyOM	0.29	n.m.	n.m.
0.5	B	^{15}N-OM	0.23	n.m.	n.m.
0.5	B	PyOM+^{15}N$_i$	0.35	n.m.	n.m.
0.5	B	OM+^{15}N$_i$	0.33	n.m.	n.m.
0.5	B	^{15}N$_i$	0.31	n.m.	n.m.
1	U	C	0.33	n.m.	n.m.
1	U	^{15}N-PyOM+N$_i$	0.39	n.m.	n.m.
1	U	^{15}N-OM+N$_i$	0.35	n.m.	n.m.
1	U	^{15}N-PyOM	0.38	n.m.	n.m.
1	U	^{15}N-OM	0.33	n.m.	n.m.
1	U	PyOM+^{15}N$_i$	0.43	n.m.	n.m.
1	U	OM+^{15}N$_i$	0.36	n.m.	n.m.
1	U	^{15}N$_i$	0.36	n.m.	n.m.
1	B	C	0.30	n.m.	n.m.
1	B	^{15}N-PyOM+N$_i$	0.32	n.m.	n.m.
1	B	^{15}N-OM+N$_i$	0.25	n.m.	n.m.
1	B	^{15}N-PyOM	0.33	n.m.	n.m.
1	B	^{15}N-OM	0.29	n.m.	n.m.
1	B	PyOM+^{15}N$_i$	0.29	n.m.	n.m.
1	B	OM+^{15}N$_i$	0.29	n.m.	n.m.
1	B	^{15}N$_i$	0.29	n.m.	n.m.

n.m. = not measured because of lack of material.

Table A.1. Total nitrogen content (%) in soil, leaves and roots at the end of each sampling date (*continuation*).

Months	Soil type	Treatments	Soil	Leaves	Roots
5	U	C	0.34	1.12	0.94
5	U	^{15}N-PyOM+N$_i$	0.37	1.16	0.95
5	U	^{15}N-OM+N$_i$	0.39	1.09	0.92
5	U	^{15}N-PyOM	0.39	1.13	1.05
5	U	^{15}N-OM	0.42	0.99	0.94
5	U	PyOM+^{15}N$_i$	0.38	3.23	1.21
5	U	OM+^{15}N$_i$	0.42	2.89	1.12
5	U	^{15}N$_i$	0.39	1.23	1.00
5	B	C	0.35	1.30	0.79
5	B	^{15}N-PyOM+N$_i$	0.33	1.10	0.79
5	B	^{15}N-OM+N$_i$	0.32	1.03	2.13
5	B	^{15}N-PyOM	0.38	1.01	0.81
5	B	^{15}N-OM	0.31	1.16	0.75
5	B	PyOM+^{15}N$_i$	0.29	2.60	0.82
5	B	OM+^{15}N$_i$	0.00	2.66	0.74
5	B	^{15}N$_i$	0.34	0.89	0.88
8	U	C	0.32	3.38	0.98
8	U	^{15}N-PyOM+N$_i$	0.33	2.81	1.13
8	U	^{15}N-OM+N$_i$	0.38	2.83	1.17
8	U	^{15}N-PyOM	0.34	2.99	0.97
8	U	^{15}N-OM	0.36	2.83	1.04
8	U	PyOM+^{15}N$_i$	0.41	3.08	0.98
8	U	OM+^{15}N$_i$	0.41	3.19	1.02
8	U	^{15}N$_i$	0.40	3.59	0.89
8	B	C	0.31	3.05	0.88
8	B	^{15}N-PyOM+N$_i$	0.32	2.07	0.83
8	B	^{15}N-OM+N$_i$	0.31	3.06	0.81
8	B	^{15}N-PyOM	0.25	2.14	0.76
8	B	^{15}N-OM	0.28	2.82	0.85
8	B	PyOM+^{15}N$_i$	0.27	2.64	0.91
8	B	OM+^{15}N$_i$	0.29	3.20	0.99
8	B	^{15}N$_i$	0.28	3.05	0.88

Table A.1. Total nitrogen content (%) in soil, leaves and roots at the end of each sampling date (*continuation*).

Months	Soil type	Treatments	Soil	Leaves	Roots
12	U	C	0.33	2.87	1.24
12	U	^{15}N-PyOM+N$_i$	0.33	2.03	1.12
12	U	^{15}N-OM+N$_i$	0.35	2.62	1.22
12	U	^{15}N-PyOM	0.32	1.83	1.06
12	U	^{15}N-OM	0.36	2.24	1.28
12	U	PyOM+^{15}N$_i$	0.35	2.03	1.05
12	U	OM+^{15}N$_i$	0.35	2.26	1.30
12	U	^{15}N$_i$	0.36	2.45	1.31
12	B	C	0.27	2.72	0.91
12	B	^{15}N-PyOM+N$_i$	0.27	1.82	0.89
12	B	^{15}N-OM+N$_i$	0.25	2.47	0.92
12	B	^{15}N-PyOM	0.25	1.67	0.90
12	B	^{15}N-OM	0.26	2.29	0.93
12	B	PyOM+^{15}N$_i$	0.27	2.02	0.92
12	B	OM+^{15}N$_i$	0.26	2.24	0.87
12	B	^{15}N$_i$	0.27	2.26	0.90
16	U	C	0.25	2.76	1.18
16	U	^{15}N-PyOM+N$_i$	0.19	2.47	1.35
16	U	^{15}N-OM+N$_i$	0.23	2.40	1.29
16	U	^{15}N-PyOM	0.24	2.43	1.26
16	U	^{15}N-OM	0.25	2.35	1.26
16	U	PyOM+^{15}N$_i$	0.23	2.71	1.29
16	U	OM+^{15}N$_i$	0.18	2.25	1.29
16	U	^{15}N$_i$	0.31	2.80	1.42
16	B	C	0.34	1.95	0.89
16	B	^{15}N-PyOM+N$_i$	0.34	1.88	0.87
16	B	^{15}N-OM+N$_i$	0.36	1.90	1.00
16	B	^{15}N-PyOM	0.33	1.66	0.86
16	B	^{15}N-OM	0.30	2.08	0.97
16	B	PyOM+^{15}N$_i$	0.28	2.86	0.89
16	B	OM+^{15}N$_i$	0.31	2.77	0.90
16	B	^{15}N$_i$	0.36	1.98	0.93