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Agricultural and Environmental Evaluation of Biochar Obtained by Pyrolysis from Different Organic Wastes.

Evaluación Agronómica y Ambiental del uso Agrícola del Biochar Obtenido por Pirólisis de Residuos Orgánicos

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La presentación de la Tesis Doctoral titulada "**Evaluación agronómica y ambiental del uso agrícola del biochar obtenido por pirólisis de residuos orgánicos**", realizada por Dª. Inés López Cano, bajo nuestra inmediata dirección y supervisión, y que presenta para la obtención del grado de Doctor por la Universidad de Murcia.

En Murcia, a 20 de junio de 2016

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[1] This poster received the "Best Poster" award at the III International Symposium on Organic Matter Management and Compost Use in Horticulture.

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ABBREVIATIONS

C	Carbon
C org	Organic carbon
CEC	Cation Exchange Capacity
CM	CellMatt
DAP	Diammonium phosphate
DTPA	Diethylenetriaminepentaacetic acid
EBC	European Biochar Certificate
EC	Electrical Conductivity
ECN	Stichting Energieonderzoek Centrum Nederland
F	Fertiliser
GH	Greenhouse waste
GW	Green waste
HTT	Highest Treatment Temperature
IBI	International Biochar Initiative
M	Manure
O	Holm Oak
OC	Holm Oak commercial
OS	TPOMW + Sheep manure
OS+B	TPOMW + Sheep manure+ Biochar
OWS	Organic Waste Systems
PC	Press cake
PM	Pig manure
S	Soil
TPOMW	Two-phase olive mill waste
XX_{FS}	XX Feedstock
XX400	Biochar at 400°C
XX450	Biochar at 450°C
XX600	Biochar at 600°C
XX650	Biochar at 650°C
WHC	Water Holding Capacity

RESUMEN

La incorporación de biochar a los suelos agrícolas está ganando importancia en los últimos años como resultado del estudio de la denominada “Terra Preta de Indio”, en la cuenca del Amazonas. Estos suelos, altamente fértiles, fueron formados por la actividad humana y contienen una alta concentración de carbón pirogénico similar al contenido en el biochar.

El término “biochar” fue introducido hace una década, para distinguirlo del carbón activo y del carbón vegetal. Actualmente, “biochar” es el término globalmente aceptado en el contexto de las enmiendas de suelo.

El biochar es un material sólido obtenido a través de la conversión termoquímica de la materia orgánica (tales como madera, estiércol, hojas, etc.) en un ambiente ausente de oxígeno a temperaturas relativamente bajas (<700°C). Durante el proceso de pirólisis, los polímeros naturales de la biomasa (ligninas, hemicelulosa, celulosa, grasas y carbohidratos) son térmicamente descompuestos en diferentes fracciones: bio-aceites (vapores condensados), biochar (fracción solida), hidrocarburos condensados, gases no condensables, energía o calor. Por lo tanto, el biochar

reduce aproximadamente un tercio de su masa respecto de la biomasa original y concentra su contenido de C, lo que provoca cambios en sus propiedades fisicoquímicas e incrementa su estabilidad.

Debido a que se pueden utilizar una amplia variedad de materiales para la producción de biochar, las propiedades fisicoquímicas del mismo vendrán determinadas por el origen y el tipo de biomasa utilizado para su producción. Además, factores como las condiciones de pirólisis, el tipo de suelo, el cultivo y las condiciones climáticas del lugar, entre otros, condicionarán las propiedades que aporta el propio biochar al suelo. Por lo tanto, según el uso agrícola al que vaya destinado el biochar, ya sea como enmienda de suelo, sólo o mezclado con fertilizantes, o como aditivo del proceso de compostaje, se diseñarán diferentes biochars con unas propiedades u otras.

La incorporación de biochar al suelo es considerada como una nueva estrategia para el secuestro de C. Debido a la baja tasa de descomposición del C recalcitrante presente en el biochar, el suelo puede actuar como un gran sumidero de C. Además, la pirólisis de residuos forestales y agrícolas supone una alternativa a las prácticas habituales de quema de residuos donde se liberan grandes cantidades de CO_2, CH_4 y/o NO_x a la atmosfera, siendo estos los principales gases de efecto invernadero.

Las propiedades físicas del biochar juegan un papel clave en su interacción con el suelo. La incorporación de biochar puede influir en la estructura del suelo, la textura, la porosidad, la distribución del tamaño de partículas y la densidad. Esto a su vez, puede alterar el contenido de oxígeno, la capacidad de almacenamiento de agua e incrementar la retención de nutrientes, mejorando la disponibilidad de N y P en el suelo, concretamente en la rizosfera.

El efecto del biochar sobre el rendimiento de los cultivos es variable dependiendo del tipo de biochar, la dosis de aplicación, las propiedades del suelo y las condiciones ambientales. Debido a tantos factores la respuesta de los cultivos no es uniforme. Los resultados encontrados en estudios previos fueron en unos casos positivos y en otros negativos. Dada la influencia compleja del biochar sobre la fertilidad del suelo, es necesario un mayor conocimiento de los procesos que tienen lugar y cómo afectan al crecimiento de las plantas.

Habitualmente el biochar es incorporado al suelo solo o en compañía de fertilizantes bien orgánicos o minerales. En unas ocasiones el biochar es mezclado con compost antes de incorporarlo al suelo y otras es incorporado al inicio del proceso de compostaje. La aplicación de biochar durante el compostaje de estiércol y otros materiales ricos en N ha revelado que tiene efectos positivos sobre el proceso compostaje, ya que reduce las pérdidas de N e incrementa la velocidad del proceso, entre otros beneficios. Sin embargo, el impacto del biochar no ha sido explorado en otras matrices orgánicas con bajo contenido de N en los que pueden dificultar el proceso.

Una de las limitaciones para la incorporación de biochar a las prácticas agrarias es el coste de su producción, encarecido mayormente por el uso de biomasa que ya posee un valor económico. El uso de biomasa ocasiona, además de costes sociales y ambientales, la rivalidad por la misma con otros usos (combustible, uso de la tierra para cultivos energéticos, etc.).

La utilización de residuos orgánicos como materias primas alternativas para producir biochar puede ser una solución para reducir costes. Su uso puede ayudar, a disminuir las emisiones de gases de efecto invernadero y el coste asociado de una mala gestión de los residuos orgánicos.

El objetivo global del presente trabajo de tesis consiste en estudiar la viabilidad de una serie de residuos orgánicos de origen agrícola y urbano para su transformación en biochar (mediante un proceso de pirólisis lenta) y evaluar su uso como (1) enmienda de suelos y como (2) aditivo del proceso de compostaje.

Para alcanzar dicho objetivo global, se han propuesto los siguientes objetivos parciales:

- Caracterización físico-química de un amplio rango de residuos orgánicos (madera de encina, residuos de invernadero, residuos de parques y jardines, la fracción celulósica resultante del tratamiento térmico de residuos sólidos urbanos (CellMatt), purínes de cerdo, y un digerido parcialmente deshidratado de residuos sólidos urbanos) y de los biochars obtenidos a partir de los mismos mediante pirólisis lenta a 400°C y 600°C.
- Evaluación agrícola y ambiental del uso de diversos biochars como enmienda de suelo, ya sean solos o en combinación con fertilizantes minerales y otras enmiendas orgánicas.
- Evaluación del biochar como aditivo durante el proceso de compostaje del alperujo y estiércol de oveja con el fin de valorar su impacto en el proceso de compostaje (degradación de la materia orgánica, emisión de gases, ciclo del N), y sobre la calidad del compost final en términos de su valor nutricional.

Así pues, el trabajo experimental de la tesis se estructuró del siguiente modo:

Capítulo 3: **Aptitud de diferentes residuos agrícolas y urbanos como materia prima para la producción de biochar**. Se realizan la caracterización química de seis residuos orgánicos de origen agrícola y urbano. También se caracterizan los biochar obtenidos mediante pirólisis lenta a 400°C y 600°C y dos biochars comerciales a 450°C y 650°C con el fin de explorar su idoneidad como enmiendas de suelo. La caracterización de los diversos biochars incluye el análisis de su composición elemental, la relación molar H/C org y O/C org, además del contenido de compuestos volátiles, cenizas y carbono fijado. En una segunda etapa, también se llevó a cabo una caracterización agronómica, donde se determinó la composición de macro y micronutrientes, el pH, la conductividad eléctrica y la capacidad de retención de agua de los diversos biochars. Por último, se analizan las limitaciones (potenciales) para su uso en agricultura debido a su contenido en metales pesados.

Capítulo 4: **Evaluación agronómica de los biochars obtenidos a partir de residuos agrícolas y urbanos.** Evaluación agronómica y medio ambiental sobre el uso de biochars pirolizados a 400°C y un biochar comercial a 650°C, solos o en combinación con dos fuentes de nitrógeno, bien un fertilizante mineral o una enmienda orgánica (estiércol). Con el fin de entender el impacto del origen del biochar (composición lignocelulósica y contenido en cenizas) sobre las dinámicas de nutrientes del suelo, el destino de los metales pesados y el crecimiento de las plantas. Así se efectúa el estudio de: la dinámica de C en el suelo y las emisiones de CO_2, la dinámica de N y P en el suelo y su tasa de mineralización, la biodisponibilidad de micronutrientes y el comportamiento de los metales pesados en el suelo. Además, se efectúa un ensayo de cultivo de lechuga en maceta para evaluar la respuesta de la planta tras la aplicación de biochar, el potencial impacto (negativo o positivo) sobre su crecimiento y la movilización de metales pesados.

Capítulo 5: **El biochar favorece las transformaciones de N y disminuye sus pérdidas durante el compostaje de alperujo y estiércol de oveja**. En este capítulo se ensaya el impacto de una pequeña cantidad de biochar (4%) sobre el proceso de compostaje de alperujo (el residuo sólido de la almazara de dos fases) mezclados con un 54% de estiércol de oveja a escala piloto durante 31 semanas. Se estudia la idoneidad del biochar como aditivo del compostaje y su impacto sobre todo el proceso, poniendo especial atención al ciclo del N, la emisión de gases de efecto invernadero (CO_2, CH_4, N_2O, CO y SH_2) y la calidad y estado nutricional del compost obtenido.

Recientemente se han publicado un gran número de estudios científicos evaluando el impacto de diversas materias primas lignocelulósicas y de otros materiales sobre las características de los biochars. Sin embargo, existe muy poca información sobre el uso de residuos lignocelulósicos alternativos de origen agrícola o urbano, que puedan reemplazar el uso de maderas para producir biochar.

A grandes rasgos la caracterización físico-química de los residuos orgánicos de origen agrícola y urbano (capítulo 3) muestra que la mayoría de los biochars presentan propiedades aceptables para su uso. Las características de los materiales de partida usados y la temperatura del proceso de pirólisis determinaron las propiedades físico-químicas. El incremento de la temperatura de pirólisis conllevó un aumento del contenido de cenizas, del C recalcitrante y mayores pérdidas de los grupos funcionales que contienen N y O. Desde el punto de vista agrícola, se espera que los biochars preparados a 400°C al ser más degradables en el suelo, desarrollen una mayor interacción con los ciclos de nutrientes del suelo. Todos los biochars fueron adecuados para su incorporación al suelo con excepción de los biochars procedentes de la fracción celulósica resultante del tratamiento térmico de residuos sólidos urbanos (CellMatt) debido a su alto contenido en metales pesados, que además se incrementó tras el proceso de pirólisis.

Antes de la incorporación del biochar a los suelos agrícolas es necesario entender mejor su comportamiento en las dinámicas de nutrientes del suelo, en su tasa de descomposición y en sus efectos potenciales, ya sean positivos o negativos, asociados a su origen a partir de residuos orgánicos.

En el cuarto capítulo se estudia la aplicación de biochar al suelo. A diferencia de los resultados encontrados en el tercer capítulo, la incorporación de biochar se vio poco influida por el tipo y el origen del biochar, mostrando apenas impacto sobre los principales ciclos de nutrientes (C, N y P). Las principales diferencias entre los biochars fueron originadas, por el contenido relativamente alto de cenizas, en los biochar preparados a partir de residuos orgánicos pre-tratados comparados con los obtenidos a partir de biomasa lignocelulósica. Finalmente, al incorporar los biochar al suelo durante los ensayos de incubación no se observarán impactos negativos sobre las dinámicas de nutrientes, ni efectos fitotóxicos que afectarán negativamente a las plantas ensayadas. Algunos biochar mostran necesidades menores de agua para mantener los niveles de humedad, pero es incierto si ésta estuvo disponible para la planta. El uso agrícola de los biochars preparados a partir de residuos municipales pre-tratados, en concreto CellMatt, se puede ver limitado por la presencia de metales pesados.

En el quinto capítulo se estudia cómo afecta la incorporación de una pequeña cantidad de biochar en el proceso de compostaje de alperujo. A pesar de la baja concentración de N en el alperujo, el biochar supuso un importante impacto sobre las transformaciones de N que tienen lugar durante el proceso de compostaje. Durante el ensayo, las concentraciones de NH_4^+ se mantienen bajas, sin embargo, en la pila con biochar se miden altas concentraciones de NO_3^-, mostrándose así una mayor nitrificación que no afectó a la cantidad total de emisiones de N_2O. Sin embargo, en la pila con biochar se reducen las pérdidas totales de N en un 15% respecto de la pila control, minimizándose estas pérdidas al 2%. Como consecuencia al final del proceso de compostaje, la aplicación de biochar aumento más del doble el contenido NO_3^-, además de la cantidad total de N, mientras que no se vio afectada la concentración de otros nutrientes ni de los metales pesados.

En resumen, la aplicación de un 4% de biochar al inicio del proceso de compostaje mejoró el valor agronómico del compost ya que se incrementó la disponibilidad de N en residuos lignocelulósicos pobres en dicho elemento.

Finalmente, las conclusiones generales que derivan de estos ensayos son:

1. Se han preparado una serie de biochars con propiedades adecuadas como enmiendas de suelo a partir de residuos orgánicos de origen agrícola y urbano, con la excepción de los biochar pirolizados a 600°C a partir de residuos urbanos y los biochars de CM.

2. Las propiedades fisicoquímicas de los biochars se determinan por los materiales de partida y la temperatura de pirolisis. Los materiales de partida lignocelulósicos dieron lugar a biochars con menor concentración de cenizas y mayor porcentaje de carbono fijado. Al incrementar la temperatura de pirolisis se incrementó el contenido de cenizas y las pérdidas de grupos funcionales que contienen nitrógeno y oxígeno.

3. El efecto de concentración de la materia orgánica y/o la contaminación del horno de pirólisis incrementa la concentración de metales pesados en el biochar respecto de las materias primas de origen. Por consiguiente, se debe poner especial atención a la selección de residuos orgánicos urbanos como materiales de partida, ya que la presencia de metales pesados puede representar una limitación para su uso en las prácticas agrícolas.

4. El origen diferente y las propiedades físico-químicas de los biochars (contenido de cenizas y carbono orgánico) tiene un impacto bajo sobre los principales ciclos de nutrientes (C, N y P), siendo la respuesta del suelo muy similar. No se han encontrado efectos fitotóxicos o impactos negativos sobre la dinámica de nutrientes del suelo.

5. La presencia de metales pesados en algunos biochars preparados a partir de residuos urbanos puede limitar su uso como enmienda del suelo (según los actuales criterios sobre biochar). Sin embargo, un experimento con lechuga mostró que la cantidad de metales pesados adsorbidos por la planta es menor que el máximo permitido en hortalizas de hoja.

6. El biochar es un aditivo efectivo para el compostaje de alperujo y favorece la activación inicial del proceso de compostaje, pero no tuvo un impacto claro sobre las emisiones de CO_2, CH_4, y N_2O.

7. La adición de un 4% de biochar disminuyó las pérdidas de N y duplicó el contenido mineral de N en el compost de alperujo, incrementando así su valor nutricional. Sin embargo, el biochar no afectó a la concentración de metales pesados en el compostaje.

Chapter 1: General introduction

1.1. General introduction

The application of biochar to agricultural land is receiving increasing attention in recent years as a result of the study of the so-called "*Terra Preta de Indio*" (Indian *black* earth) in the Amazon Basin. These soils, that are quite fertile, were formed by human activity and are known as containing a high concentration of pyrogenic carbon (similar to carbon in biochar), which gives them their characteristic dark colour (Shackley et al., 2014; Stahel, 2016).

At present, the scientific interest of biochar research has been trending upward for the last several years. The number of publications on biochar in the scientific references and their impact, in terms of the citations in the most-cited journals, exceeds that of the more established subject of compost science (Lehmann and Joseph, 2015).

The term “biochar” was introduced a decade ago, to distinguish activated carbon from charcoal, and by now this term is widely accepted globally in the context of soil amendment (Stahel, 2016). The aim of biochar addition is to improve soil functions such as: decreasing nutrient leaching, enhancing the soil fertility, increasing soil carbon (C) and maintain soil moisture. It has also been found to reduce greenhouse gas emissions (mainly N_2O and CH_4) and the odours of biomass decomposition that would otherwise degrade in soil (Sharpley and Moyer, 2000). The main benefits of biochar are summarised in Figure 1.1.

1.2 What is biochar?

Biochar *is a solid material obtained from thermochemical conversion of biomass in an oxygen limited environment* (IBI, 2014). Biochar is produced by thermal decomposition of organic material (*e.g.* wood, manure or leaves) under limited supply of oxygen (O_2), and at relatively low temperatures (<700°C) (Sharpley and Moyer, 2000). Thus this solid product can reduce about one third of its mass and being therefore highly is concentrated in C. The pyrolysis causes wide changes in its physicochemical properties and increases its stability.

A wide variety of feedstocks can be used to produce biochar. The origin and type of the biomass feedstock will define the properties of the obtained biochars, each with its own opportunities and constraints (Lehmann and Joseph, 2015). In addition, the benefits of a given biochar vary widely with the production conditions, soil type, crops, and climate factors (IBI, 2014). Therefore, different biochars characteristics can be required for different agricultural applications, either as soil amendment, admixture with fertilisers or as compost additive (Kleber et al., 2015).

In general, biochar may help in the prevention of climate warming and hence to increase soil fertility, reduce agricultural waste and produce renewable energy. Therefore the motivation of biochar soil

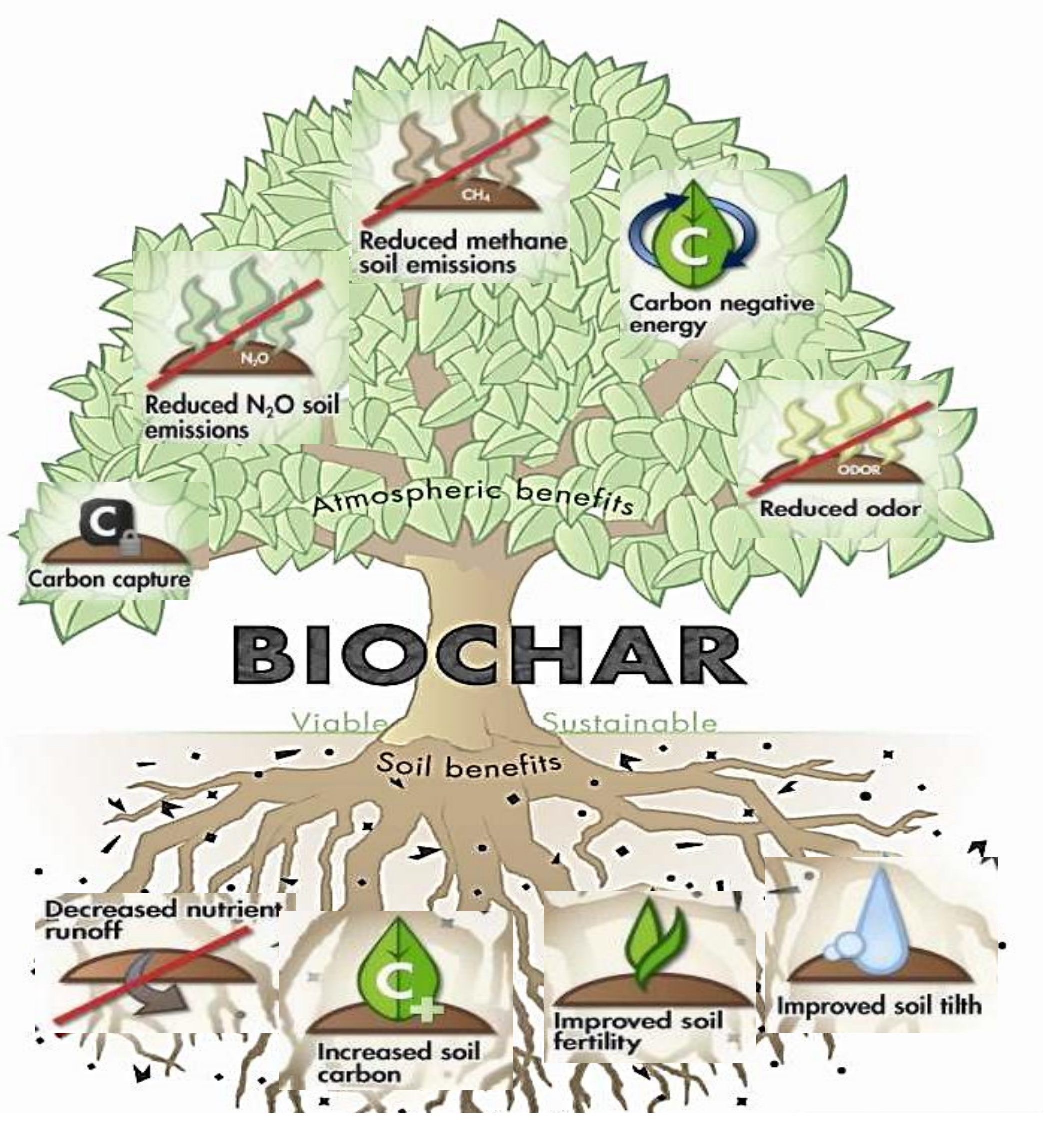

Figure 1.1: Main benefits of biochar to atmospheric and soil system (Sharpley and Moyer, 2000).

addition can be very different depending on the intended benefits (EuropeanCommission, 2015b; Lehmann and Joseph, 2015), see Figure 1.2. However, many of these issues are still debated as the foreseen effects and the mechanisms behind them are still uncertain (EuropeanCommission, 2015b).

1.3 Origin of biochar

The origin of biochar started with the agriculture during the Neolithic Age (EuropeanCommission, 2015a). In Europe, various indications were found suggesting that slash-and-burn cultivation

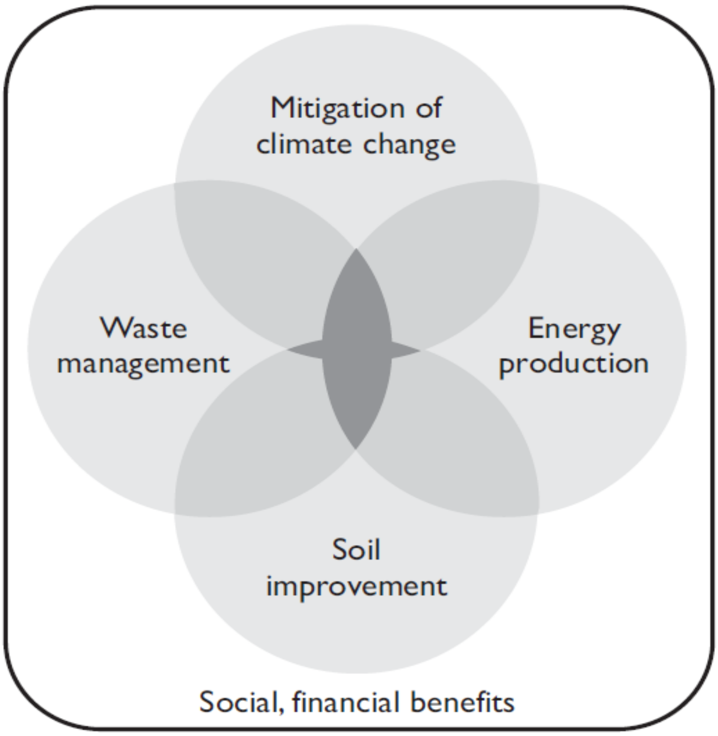

Figure 1.2: Motivation for applying biochar technology (Lehmann and Joseph, 2015).

might have played an important role to exploit suboptimal farming conditions (EuropeanCommission, 2015a). However, the use of biochar is inspired in the most famous ancient anthropogenic soils, *Terra Preta*, in the Amazonian from the pre-Columbian civilization times, where the biochar plays a prominent role, leaving behind sustainable fertile black earth-like soils (Glasser, 2001). These soils were created over several hundred to a few thousand years ago, maintaining their fertility largely due to the high proportion of biochar-type organic matter (Lehmann and Joseph, 2015). Although biochar is produced specifically for application to soil as part of agronomic or environmental management, in the case of *Terra Preta* it is not clear whether biochar application to soil was intentional or not (EuropeanCommission, 2015a).

Besides, there are other references about biochar application into agricultural lands around the world. For example, the production of rice husk biochar directly after harvesting is a common practice in many Asian countries and has been used since the beginning of rice cultivation (Ogawa and Okimori, 2010). Recently, Japan played a pioneering role in the agricultural use of biochar in combination with fermentation or composting techniques (Yoshizawa et al., 2007; Yoshizawa et al., 2005)

1.4 Production technology of biochar making

Pyrolysis is a thermal decomposition process occurring in the absence of oxygen. It is also the first step in combustion and gasification processes, which are followed by total or partial oxidation of the primary products (Bridgwater, 2003). During the pyrolysis process, the natural polymeric constituents of biomass (lignin, hemicellulose, cellulose, fats and carbohydrates) are thermally broken down into different fractions: bio-oil (condensed vapours), charcoal (solid fraction), condensable hydrocarbons, non-condensable gases, energy or heat (Bridgwater, 2003; Camps-Arbestain et al., 2015). Different distributions and yields of the aforementioned fractions depend mostly on the rate of heating and the final temperature during the process (Bridgwater, 2003).

The pyrolysis process includes a combination of time, heat and pressure exposure factors that can vary between processors, equipment and feedstocks. As a result, very different biochar systems emerge and, therefore, biochar can be produced from various types of processes including slow and fast pyrolysis, and gasification (Ronsse et al., 2013) (Table 1.1).

Table 1.1: Biomass thermochemical conversion technologies and product distribution adapted from Sohi et al. (2009).

Process	Temp. (°C)	Residence time	Liquid (bio-oil)	Solid (biochar)	Gas (syngas)
SLOW PYROLYSIS	Low-moderate 300-700 °C	Long (min-days)	30% (70% water)	35%	35%
INTERMEDIATE PYROLYSIS	Low-moderate 300-700 °C	Moderate (1-15 min)	50% (50% water)	25%	25%
FAST PYROLYSIS	Moderate 300-700 °C	Short (<2s)	75% (25% water)	12%	13%
GASIFICATION	High >800 °C	Long	5% (5% water)	10%	85%

That is, each pyrolysis process is distinguished by different ranges of temperature, heating rates, feedstock and vapour residence times. However, the original biomass structure, which can be very diverse (*e.g.* forestry products, agricultural residues, animal wastes, or municipal waste products), strongly influences the final biochar structure (Joseph et al., 2009). The challenge for biochar science is to predict and ensure the quality and potential agronomic and environmental benefits of biochars produced from any given feedstock by any given pyrolysis conditions (Steiner et al., 2008).

1.5 C sequestration, GHG emission reductions and renewable energy generation.

Biochar has been proposed as a new strategy for C sequestration in terrestrial ecosystems (Stahel, 2016). The very low decomposition of biomass-derived black C, *i.e.* biochar, in soil offers both a large and long-term C sink, which exceeds the potential C sequestration in plant biomass (Stahel, 2016). There is experimental evidence, by incubation soil experiments (Bruun et al., 2012; Novak et al., 2010) or in plant-soil system (Kammann et al., 2011), supporting that biochar is quite stable in soil and hence suitable for C sequestration. However, other studies reveal that in the short-term biochar increases soil CO_2 effluxes, that may result from SOC decomposition via “priming” of soil carbon (Whitman et al., 2015; Zimmerman et al., 2011).

Even though biochar is different from other soil organic carbon (C org) pools (Spokas, 2010), its physicochemical properties also contributes to the valuable functions of soil organic matter, such as soil stabilization by aggregation and retention of nutrients and water (DataMarket, 2012).

Nowadays large amounts of forestry and agricultural wastes and other biomass are currently burned or left to decompose thereby releasing CO_2 and/or CH_4, two main greenhouse gases (GHGs), into the atmosphere. These emissions could be avoided or at least reduced through the pyrolysis system, converting the biomass C into more aromatic and chemically stable structures in biochar and placed in soils (Woolf et al., 2010).

Biochar can also contribute to reduce the emission of N_2O, a potent GHG. Agriculture is the main source of the global anthropogenic N_2O emissions, contributing approximately to about 60% of the total emissions, due to the widespread use of synthetic N fertilizers (IPCC, 2014). A review based on recent studies have indicated that incorporating biochar into soil reduces N_2O emissions by 54% in laboratory and field studies (Cayuela et al., 2014). However, there are multiple factors influencing emissions of N_2O from soils, some of them derived from biochar type, such as feedstock or pyrolysis conditions, and others from agricultural practices, such as biochar and fertiliser application rate or soil texture (Cayuela et al., 2014).

In addition, during the pyrolysis process a significant fraction of the feedstock C is released, together with the hydrogen, in the form of combustible gases. These gases can be captured and utilised as a source of energy, displacing fossil energy and avoiding or reducing GHGs emissions to the atmosphere (Cowie et al., 2015).

In summary, there are several ways in which biomass pyrolysis systems, including the use of biochar as soil amendment, can contribute to reduce the impact of global warming, such as the potential for C sequestration, the reduction in GHG emission and the generation of renewable energy (Roberts et al., 2010).

1.6 Biochar impacts on agricultural practices

1.6.1 Biochar as soil amendment

Biochar can play a beneficial role in agriculture. As a soil amendment, biochar may act alone or in combination with mineral fertilisers and/or organic amendments, influencing on physical and biological components of the soil (Glaser et al., 2002), and consequently, having a positive effect on soil fertility (Spokas et al., 2012).

The physical properties of biochar play a key role in the different ways that biochar can interact with soil (Downie et al., 2009). Incorporation of biochar can influence soil structure, texture, porosity, particle size distribution and density, thereby potentially altering air oxygen content, water storage capacity (Ippolito et al., 2014, 2016), cation exchange capacity (CEC) (Liang et al., 2006) and an increase of nutrients retention capacity (Ippolito et al., 2014). There are some studies which relate the nutrient retention capacity with an enhancing N and P availability in soil (DeLuca et al., 2015; Lopez-Cano et al., 2013; Taghizadeh-Toosi et al., 2012), and specially within the plant rooting zone (Prendergast-Miller et al., 2014).

The effects of biochar on crop production are rather variable depending of biochar type, application rate, soil properties, and environmental conditions, resulting in different responses, mostly positive but also some negative (Alburquerque et al., 2014; Chan and Xu, 2009; Jeffery et al., 2011). In general, the results of a statistical meta-analysis showed a positive benefit of biochar application to soils on crop productivity, with a grand mean increase of 10% (Jeffery et al, 2011). In addition, these authors also showed that two of the main mechanisms for yield improvements may be a liming effect and the influence on the water holding capacity (Jeffery et al., 2011). Besides, recent studies have indicated a complex biochar and fertiliser interaction with respect to yield response (Spokas et al., 2012). Alburquerque et al. (2014) found that the combined addition of biochar and fertiliser led to about 20-30% increase in grain yield compared with the use of fertiliser alone, which is in agreement with other studies that showed similar results in wheat (Olmo et al., 2016) and oat (Schulz and Glaser, 2012). Therefore, the influence of biochar on soil fertility is affected by a complexity of conditions (Spokas et al., 2012). Thus, a considerable knowledge level is required to understand the processes involved in biochar's nutrients retention capacity and how they affect plant growth. This information is necessary to predict the biochar impact on nutrient use and crop production (Olmo et al., 2016), and also the long-term impact of biochar in soil, as a consequence of aging (DataMarket, 2012).

1.6.2 Biochar as an additive to composting process

The distinct properties of biochar such as cation exchange capacity, water holding capacity, porous structure, etc., have been demonstrated to influence soil properties. However, these interactions are also expected to occur within a composting matrix (Steiner et al., 2015). Although biochar can be mixed with matured compost, it yields more benefits when it is introduced into the composting pile at the beginning of the process (Meyer-Kohlstock et al., 2015).

There are several studies showing the benefits of the addition of biochar at the initial stage of the composting process (Table 1.2). The main effects of biochar include: impacts on the microbiology of composting process, leading to an accelerated composting process (Yoshizawa et al., 2007), increased CO_2 emissions (Ogawa and Okimori, 2010) and even reduced the volatilisation of NH_3 (Hua et al., 2009). According to these studies, it seems rational to suggest that biochar addition may influence important functions, such as organic matter degradation, mineralisation and immobilisation of nutrients and GHG emissions (Steiner et al., 2015). Considering the interaction of biochar with the organic matter during composting, it would be also expected that biochar can influence the performance of composting process and the quality of the end product (Wei et al., 2014).

Nevertheless, the impact of biochar in the microbial community structure and physicochemical properties during composting with organic wastes is not well defined yet (Wei et al., 2014) and it is necessary to further investigate the changes during composting and the effects of biochar on the quality of the compost.

Table 1.2: Main impacts of biochar as an additive to organic waste composting, adapted from Steiner et al. (2015).

Observed impacts	**Reference**
Impact on the microbiology of composting	
Enhanced microbial population (fungi)	(MacDonald et al., 2011)
Higher temperature and CO_2 emissions	(Ogawa and Okimori, 2010)
Accelerated composting process	(Yoshizawa et al., 2007)
Impact on biochar properties	
Changes in biochar surface chemistry	(Yoshizawa et al., 2005)
Impact on C and N dynamics	
Reduced N losses	(Hua et al., 2009)
Enhanced organic matter processing	(MacDonald et al., 2011)
Impact on GHG emissions	
Reduction of CH_4 emissions	(Sonoki et al., 2013)

1.7 Integration of biochar into waste management strategies

One of the limitations for the incorporation of biochar into current agricultural practices is the cost of biochar production, mostly associated to the use of clean wood as feedstock. Furthermore, there are other social and economic concerns associated to the use of wood for pyrolysis and its competition for other uses (fuel, use of land for dedicated energy crops, etc.).

Besides the high cost of biochar, current studies also demonstrate that just one biochar addition does not turn each temperate fertile soil into a fertile miracle (Kammann, 2013). In addition, the positive impact on CO_2 emissions could be compromised since biochars are usually produced from woody materials, mainly forestry and clean agricultural waste.

The use of organic wastes, as alternative feedstock to produce biochar, could be a solution to avoid growing crops for their sole purpose of producing feedstock for pyrolysis.

There are currently vast amounts of organic wastes being generated annually in the EU (Panoutsou et al., 2009), which could serve as feedstock (Figure 1.3). One possibility is the use of bio-wastes, which include biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants. Meyer-Kohlstock et al. (2015) have recently estimated that only one third of the potential bio-waste, around 35 million tons, is currently used to produce compost.

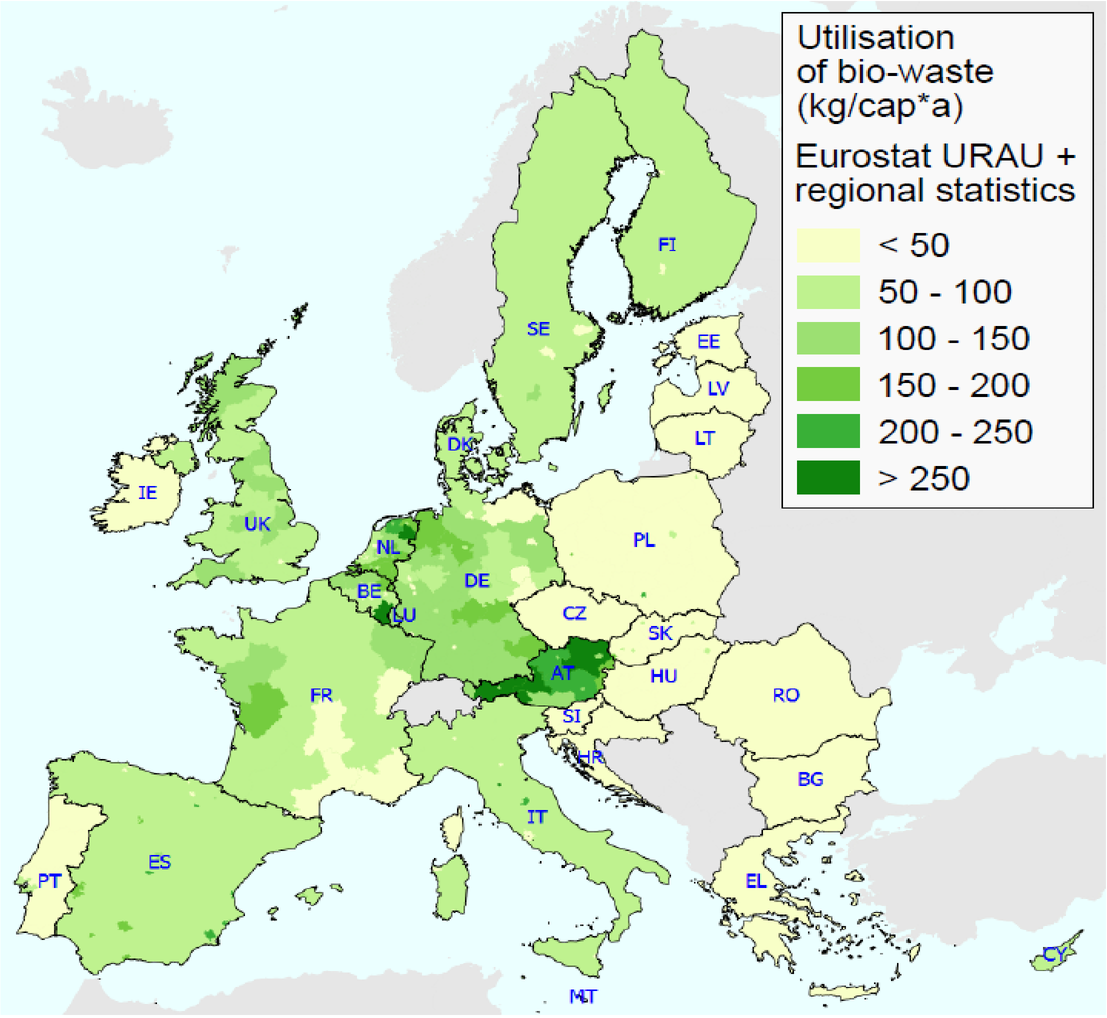

Figure 1.3: Bio-waste utilization in kg per capita and year in the EU-28, based on Eurostat statistics (Meyer-Kohlstock et al., 2015).

Another possibility is the use of agricultural wastes as feedstock. These wastes are generated in large amounts across the EU (Figure 1.4) and cover different vegetal and animal wastes, including manures, which could present favourable properties for the production of biochar (García et al., 2014; Pasangulapati et al., 2012). Thus, there remains a large potential for biochar production, for example the woody part of bio-waste (green waste) and other a wide range of lignocellulosic agricultural wastes.

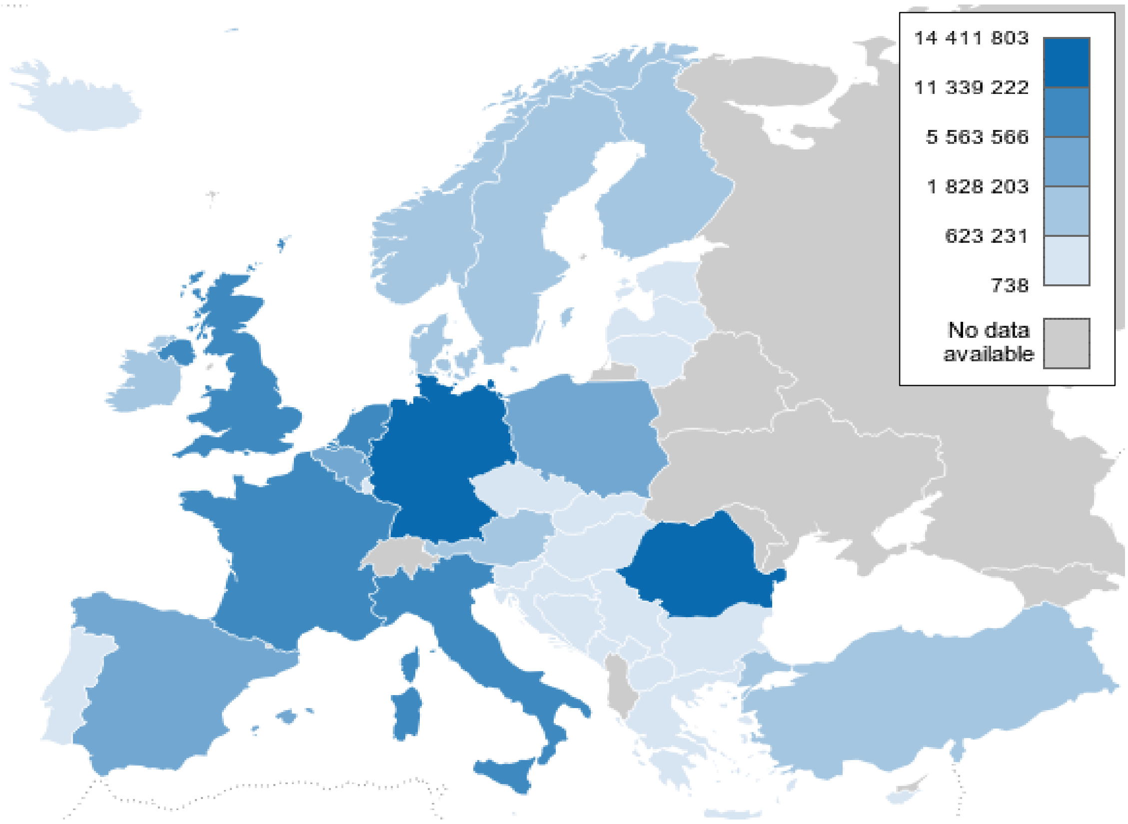

Figure 1.4: Generation of agricultural wastes across EU in Kilograms per capita/Tonne, based on Eurostat statistics (DataMarket, 2012).

Biochar production can become an attractive alternative to complement the classical way of waste management, especially to achieve closing the nutrients cycle through recycling C, N and P between urban and agricultural regions, where the long transportation distances must be avoided (Lehmann and Joseph, 2015).

Figure 1.5 shows how organic waste could be recycled into soil by replacing landfill and combustion with composting and pyrolysis

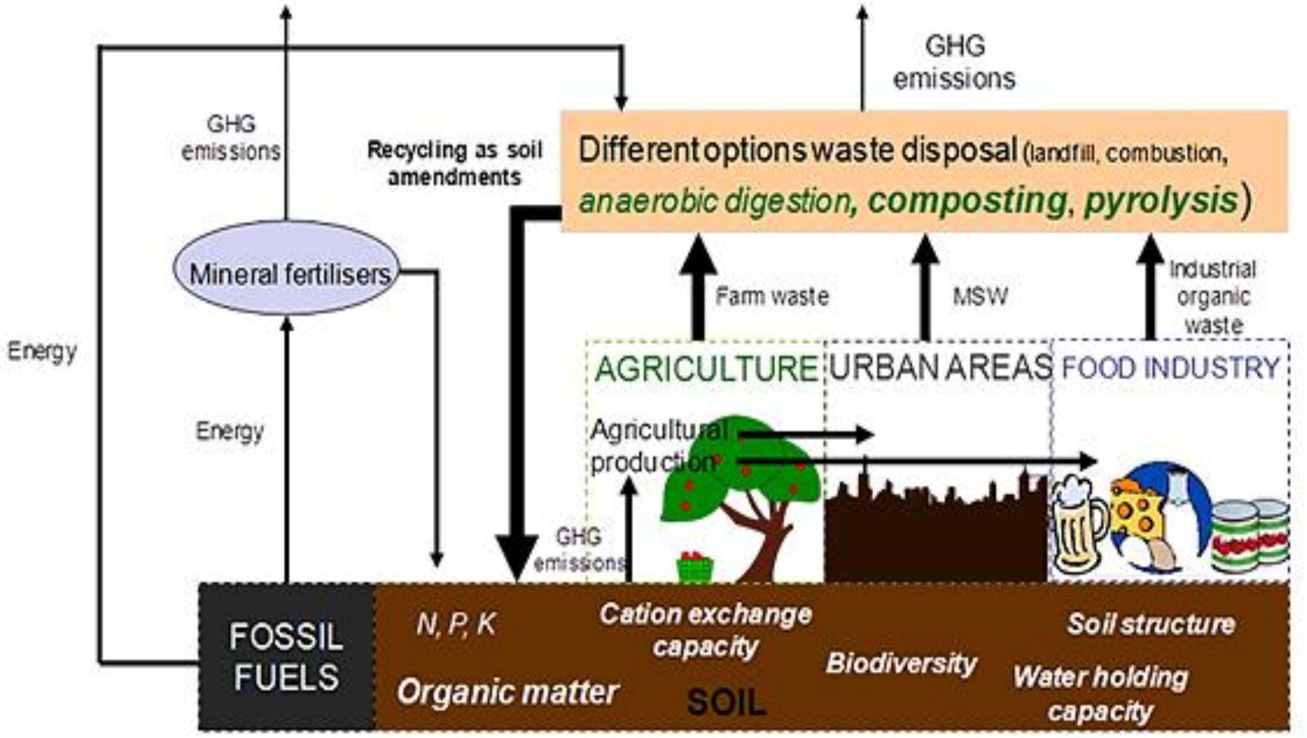

Figure 1.5: This diagram shows expected changes in nutrients, organic matter, greenhouse gases and energy flows in agricultural production systems due to the partial substitution of fertilising materials by compost or/and biochar (EuropeanCommission, 2015b).

and could affect the nutrients and organic matter flows decreasing the use of mineral fertilisers and therefore a reduction in fossil fuels and GHGs emissions (EuropeanCommission, 2015b).

In brief, incorporating the pyrolysis of organic waste as a management option can help to attenuate the effects of climate change, decreasing the emissions and cost associated from an inappropriate waste management. This strategy would have important implications at farm level, where agricultural wastes can be recycled on-site by pyrolysis.

Once biochar is obtained, it can be directly applied to the soil, to return the nutrients previously uptaken by plants, or it can be incorporated into traditional nutrient management practices such as manure composting or anaerobic digestion, improving the recycling process.

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Chapter 2: Objectives, hypothesis and structure of the thesis

2.1 Objectives, hypothesis and structure of the thesis

The recycling of organic wastes in agriculture is a valuable strategy to ensure the reuse of resources such as organic matter and nutrients and, at the same time, avoid the negative impacts associated to an inappropriate waste management (soil and water pollution and release of GHGs). Composting is nowadays the most widely accepted method for the stabilisation of organic wastes for their use in agriculture, but there are other waste treatment technologies gaining interest in recent years. Pyrolysis, the thermal conversion of biomass into bioenergy and biochar, has become the foremost studied process at present.

Biochar presents favourable physicochemical properties for its use in agriculture, either as organic amendment in agricultural soils or as an additive to the composting process. The properties of biochar are governed not only by the pyrolysis conditions abut also by the lignocellulosic composition of the woody biomass traditionally used as feedstock. The increasing interest in the use of pyrolysis for the recycling of organic wastes will generate different biochars with physicochemical properties which will be affected by the origin and chemical composition of the organic wastes used as feedstocks.

To date, most studies analysing biochar as soil amendment focused on biochars produced from woody materials, whereas other feedstocks have been studied to a lesser extent, or even not studied at all. This thesis has a strong focus on developing a better understanding of the influence of the original waste used for pyrolysis on the biochar characteristics and its behaviour in soil. Also, the use of biochar as a composting additive was tested. Our hypotheses can be summarized as follow:

The origin of the feedstocks will determine the biochar physicochemical properties, especially regarding the lignocellulosic composition, ash content and the presence of heavy metals, which will define the suitability of the biochars as soil amendments.

The lignocellulosic composition and ash content of feedstocks will regulate the behaviour of the biochars in soil and their interaction with soil nutrient dynamics.

The physicochemical properties of biochar will present favourable conditions for the composting process by accelerating the composting process, enhancing the efficient use of N and minimising its losses during composting.

The main objective of the present thesis was to explore the recycling in agriculture of a range of organic wastes of agricultural and urban origin through their transformation into biochar by pyrolysis and to evaluate its use as soil amendment and as additive to the composting process.

In this context, three general objectives were set:

- To evaluate the physicochemical characteristics of a series of biochars obtained by pyrolysis from a wide range of organic wastes (oak, greenhouse waste, green waste, cellulosic fraction of MSW, pig manure and food digestate) and at different pyrolysis conditions (slow pyrolysis at 400°C and 600°C).

- To perform an agricultural and environmental evaluation of the use of these biochars as soil amendment either alone or in combination with mineral fertilisers and other organic amendments.

- To evaluate the effect of biochar as additive during composting of olive mill wastes and sheep manure, in order to assess its impact on the composting performance (organic matter degradation, gas emission, N cycling), and on the quality of the final compost in terms of its nutritional value.

The aforementioned partial objectives correspond with each chapter:

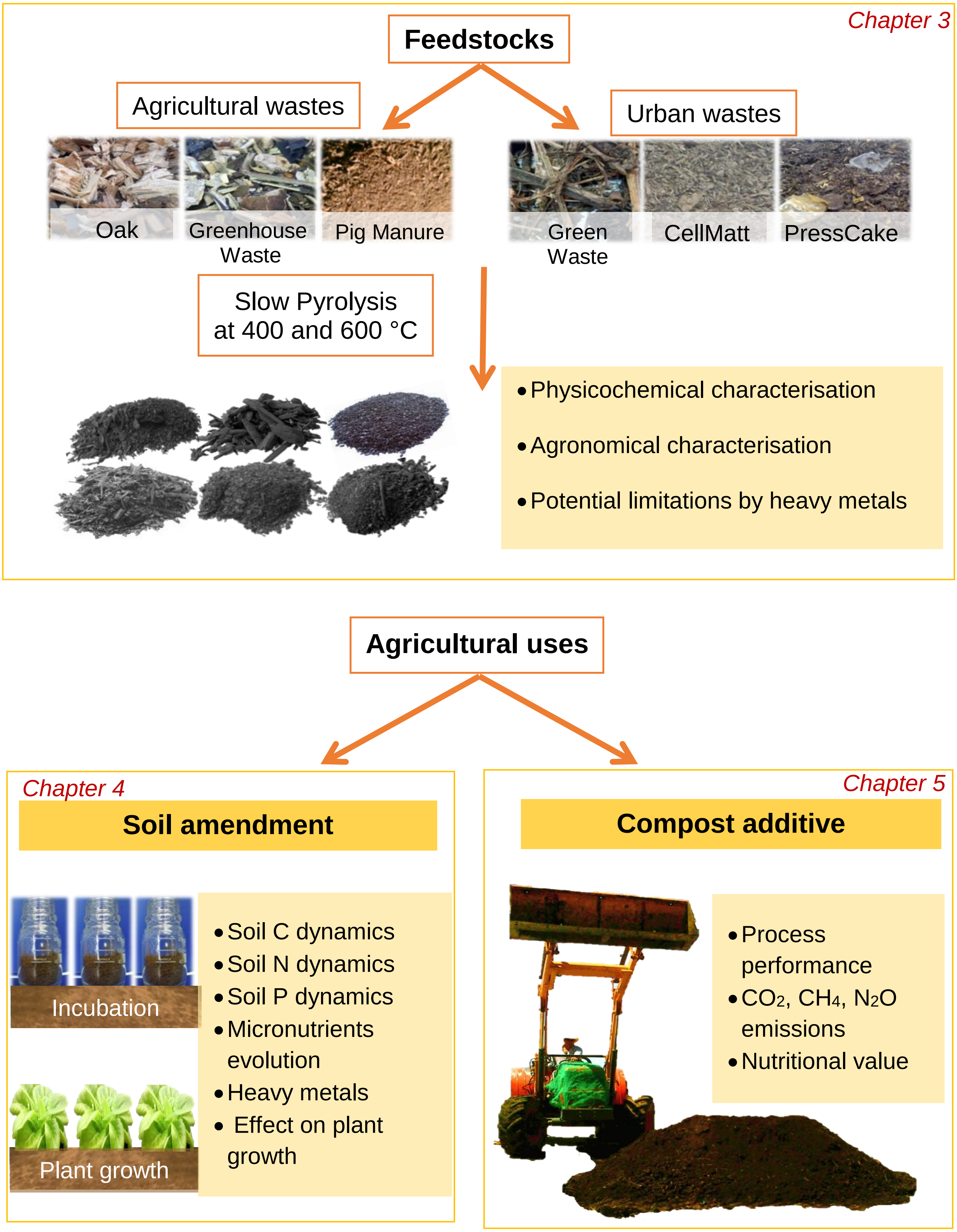
Chapter 3: **Suitability of different urban and agricultural organic wastes as feedstocks for the production of biochar**. In this chapter, a comprehensive chemical characterisation of six different organic wastes of agricultural and urban origin was performed. Similarly, their respective biochars, obtained by slow pyrolysis at 400 and 600°C, were characterised in order to explore their suitability as soil amendments.

Chapter 4: **Agronomical and environmental evaluation of biochars from urban and agroindustrial organic wastes.** Agronomical and environmental evaluation of the use of biochar as soil amendment in order to understand the impact of the origin of the biochar (lignocellulosic composition and ash content) on soil nutrient dynamics, the fate of heavy metals and plant growth.

Chapter 5: **Biochar improves N cycling during composting of olive mill wastes and sheep manure**. This chapter studied the suitability of biochar as composting additive, and its impact on the overall performance of the composting process, with special attention to the N cycle, the emission of greenhouse gases and the nutritional quality of the final compost.

The general outline of this thesis is schematically depicted in the diagram below:

Agricultural and environmental evaluation of biochar obtained by pyrolysis from different organic wastes.



Chapter 3

Feedstocks

Agricultural wastes

Oak

Greenhouse Waste

Pig Manure

Urban wastes

Green Waste

CellMatt

PressCake

Slow Pyrolysis at 400 and 600 °C

- Physicochemical characterisation
- Agronomical characterisation
- Potential limitations by heavy metals

Agricultural uses

Chapter 4

Soil amendment

Incubation

Plant growth

- Soil C dynamics
- Soil N dynamics
- Soil P dynamics
- Micronutrients evolution
- Heavy metals
- Effect on plant growth

Chapter 5

Compost additive

- Process performance
- CO_2, CH_4, N_2O emissions
- Nutritional value

Chapter 3: Suitability of different agricultural and urban organic wastes as feedstocks for the production of biochar

3.1. Introduction

The recent EU action plan for the circular economy sets clear targets for the reduction of waste and establishes an ambitious and reliable long-term strategy for waste management and recycling (EuropeanCommission, 2015a). The main environmental hazards associated to a poor organic waste management are soil and water pollution and also the release of greenhouse gases to the atmosphere, contributing to global warming. Besides, the disposal of organic wastes implies significant losses of natural resources such as organic matter, nutrients and energy (Stahel, 2016).

An appropriate management of organic waste can help to mitigate climate change by decreasing gas emission from landfills and also reducing indirectly other transport and energy efforts originated from a bad waste management (EuropeanCommission, 2010). The new circular economy business model adopted by the EU (EuropeanCommission, 2015a) enforces EU members to reduce the amount of biodegradable wastes disposed of in landfills, including agricultural residues, household, commercial and industrial wastes. The revised legislative proposal on waste aims at reducing the environmental impact from landfilling of wastes and to ensure their valorisation, reuse or recycling (EuropeanCommission, 2015b). Composting is the most common recycling option for organic waste around the EU, despite the fact that only one third of the potentially available feedstocks are used for compost production (Meyer-Kohlstock et al., 2015). Thus, composting and other biological treatments have an important role to play in helping the EU to move towards closing the loop of products lifecycle. More recently, the pyrolysis of organic waste, for biochar and bioenergy co-production, has gained considerable interest worldwide as an alternative recycling option.

According to the International Biochar Initiative (IBI), biochar is defined as *a solid material obtained from thermochemical conversion of biomass in an oxygen limited* environment (IBI, 2014). Thus, this solid product has about a third of the mass of the original biomass and contains half the original C, which is enriched in aromatic C and is highly resistant to decomposition. Biochar is intended for use as an agent for soil improvement and also for a broad range of environmental applications, including improved resource use efficiency, soil remediation and greenhouse gas mitigation (Kleber et al., 2015). The use of biochar as soil amendment is often considered as a mechanism to sequester C org, which remains in the soil for centuries or millennia depending on the degree of aromaticity and the chemical complexity (O/C org ratio) of the biochar (Spokas, 2010). The benefits of a given biochar material vary widely on the origin of the feedstocks as well as the pyrolysis conditions, which determine their physicochemical properties. The intended use as soil amendment also requires that biochars do not contain harmful levels of heavy metals or persistent organic contaminants (IBI, 2014). Different international initiatives such as the IBI certification (IBI, 2014) the Biochar Quality Mandate (Shackley et al., 2014) by the British Biochar Foundation and the European Biochar Certificate (EBC) (EBC, 2012), among others, are recently published guidelines to identify the main qualities and characteristics of biochar and setup quality standards for its use in agriculture.

Currently, biochars are mostly produced from forestry and clean agricultural waste, and especially from woody materials, which are considered as reference materials. The growth of lignocellulosic crops for biochar production is being questioned, since they can compete with food production. But there is a broad range of organic residues that could potentially be transformed to biochar, not representing a competition with food production systems. For example, the lignocellulosic residues generated after the harvesting of horticultural crops, and also clean wastes from household, commercial and industrial processes (Kleber et al., 2015; Sohi et al., 2015), could present favourable properties as potential feedstock for pyrolysis.

Recently, a large number of studies have been published in the scientific literature evaluating the impact of raw lignocellulosic and other feedstocks on the characteristics of biochars (Al-Wabel et al., 2013; Jindo et al., 2014; Rutherford et al., 2012; Yargicoglu et al., 2015). However, there is limited information on the use of alternative lignocellulosic residues of agricultural or urban origin, which could replace the traditional use of wood material to produce biochar. These alternative feedstocks may have undergone biological or physicochemical pre-treatments, which may alter their lignocellulosic composition and ash content, affecting the properties of the obtained biochars.

The aim of this work was to explore the suitability of alternative residues from agricultural and urban origin as feedstock for the production of biochar. To achieve this aim, a physicochemical and agronomical characterisation was performed on a series of biochars obtained by pyrolysis from a wide range of organic wastes of different origins (with different lignocellulosic composition and ash content) and at different pyrolysis conditions (slow pyrolysis at 400°C and 600°C).

3.2. Material and methods

3.2.1. Feedstock description

A range of organic wastes from agricultural and urban sources was chosen to provide a wide variety of feedstock for the pyrolysis process. The selection was based mainly on their origin but also on the different concentration of organic matter and its lignocellulosic composition (Table 3.1):

Holm Oak (O_{FS}) pruning is a forestry waste with a high concentration of lignin, cellulose and hemicellulose. This biomass is free of any potentially hazardous elements (like heavy metals or other toxic compounds) and it was supplied by Proininiso Inc. (Malaga, Spain).

Greenhouse waste (GH_{FS}) is an agricultural waste constituted by a mixture of horticultural crop residues (stalks and leaves), mainly from pepper (*Capsicum annum*) and aubergine (*Solanum* *melongena*), which were grown in a greenhouse in TECNOVA (Almería, Spain). This waste is a heterogeneous lignocellulosic biomass that also contains up to 2% plastic tags.

Green waste (GW_{FS})[2] is an organic waste from municipal origin comprised by a mixture of grass, leaves, branches, small stems, tree pruning, etc. separately supplied from gardens in urban areas, which usually is shredded and chipped. It is a highly heterogeneous residue, which can present a seasonal variable composition. It was collected by Stichting Energieonderzoek Centrum Nederland (ECN) (Petten, The Netherlands) from urban parks in the Netherlands.

CellMatt (CM_{FS}) is an organic waste from urban origin produced by Graphite Resources Limited (UK) from household, commercial and industrial wastes through steam autoclaving technology that sterilises wastes (>160°C and 6 bar) to achieve the complete elimination of pathogens. During the process, the biological fraction of the waste is broken down into a cellulose rich biomass.

Pig manure (PM_{FS}) is an agricultural waste generated in swine growing facilities, mainly containing the faeces and urine from the

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[2] The amount of feedstock available for analysis was limited and the chemical characterisation was completed with data from scientific literature (Donovan et al., 2010).

animals. It can include plant material, such as straw, typically used as bedding material. This waste was collected from farms in the Netherlands and used by ECN to produce biochar. The feedstock was not available for analysis. The physicochemical characterisation was obtained from the scientific literature (Huang et al., 2011; Santos et al., 2015; Xiu et al., 2010).

Press cake (PC_{FS}) is an organic waste of urban origin obtained from anaerobic digestion of source-separated municipal waste. Pressed cake contains largely fibrous and woody material. It was supplied by Organic Waste Systems (OWS) (Belgium).

3.2.2. Biochar production

Feedstocks were converted into biochar through slow pyrolysis in a Pyromaat Auger pyrolysis reactor, as described by Fryda and Visser (2015). Pyromaat is an indirectly heated screw conveyer reactor, in which the biomass is moved along the reactor length at a fixed speed using a screw thread feeder. At the end of the reactor, the biochar is collected, whereas the gas and the dust are directed to an afterburner cleaning system (Fryda and Visser, 2015). The slow pyrolysis biochars were produced at a highest treatment temperature (HTT) of 400°C and 600°C and at residence time of 60 min.

In addition, two commercial biochars from holm oak produced at HTT of 450°C and 650°C (OC450 and OC650) were obtained from Proininso Inc., where biochar is produced by continuous large-scale slow pyrolysers (at residence time of about 15 hours), using a mono retort reactor (Table 3.1).

Table 3.1: Feedstocks and pyrolysis conditions used to produce the biochars.

Feedstock	Biochar	Biochar producer	Process description	HTT
Holm oak	**OC450**	Proininso	Slow pyrolysis (> 15 h.)	**450°C**
(O_{FS})	**OC650**	Proininso	Slow pyrolysis (> 15 h.)	**650°C**
	O400	ECN	Slow pyrolysis (60 min.)	**400°C**
	O600	ECN	Slow pyrolysis (60 min.)	**600°C**
Greenhouse	**GH400**	ECN	Slow pyrolysis (60 min.)	**400°C**
waste (GH_{FS})	**GH600**	ECN	Slow pyrolysis (60 min.)	**600°C**
Green waste	**GW400**	ECN	Slow pyrolysis (60 min.)	**400°C**
(GW_{FS})	**GW600**	ECN	Slow pyrolysis (60 min.)	**600°C**
CellMatt	**CM400**	ECN	Slow pyrolysis (60 min.)	**400°C**
(CM_{FS})	**CM600**	ECN	Slow pyrolysis (60 min.)	**600°C**
Pig manure	**PM400**	ECN	Slow pyrolysis (60 min.)	**400°C**
(PM_{FS})	**PM600**	ECN	Slow pyrolysis (60 min.)	**600°C**
Press	**PC400**	ECN	Slow pyrolysis (60 min.)	**400°C**
cake (PC_{FS})	**PC600**	ECN	Slow pyrolysis (60 min.)	**600°C**

HTT: highest treatment temperature; ECN: Stichting Energieonderzoek Centrum Nederland.

3.2.3. Analytical methods

A series of analysis was conducted to evaluate the physicochemical properties of feedstocks and biochars. In the case of biochars, the analytical methodologies recommended by IBI were followed (IBI, 2014), whereas in the case of feedstock conventional analytical methodologies were used, as described below.

Both, feedstocks and biochars, were air-dried, ground and sieved (<1mm.) before analysis. Moisture content was obtained by drying at 105°C for 24h. The organic matter content in feedstocks was determined by loss on ignition at 550°C according to UNE-CEN/TS 14775:2010. In the case of biochars, the ash and volatile content were determined at 750°C and 950°C, respectively, according to ASTM D1762-84, and expressed as percentage of the total weight. Fixed carbon was calculated by subtraction, as follows:

$$\text{Fixed C (\%)} = 100 - \text{ash (\%)} - \text{volatile content (\%)}$$

To conduct ultimate analyses, the samples were ball milled to achieve a homogenised sample. Total nitrogen (N), Total carbon (C), C org, total hydrogen (H) and total sulphur (S) were analysed by automatic elemental analysis (LECO CHNS-932, USA) and expressed as a percentage of total dry weight. Total oxygen (O) was determined by difference, according to Enders et al. (2012), as follows:

$$O\ (\%) = 100 - ash\ (\%) - C\ (\%) - N\ (\%) - H\ (\%)$$

Feedstocks were subjected to biochemical analyses to assess the concentration of lignin, cellulose and hemicellulose. Lignin was determined by the Klason's method, described in ASTM D1106-96; cellulose was analysed by ASTM D1103-60 and hemicellulose concentration by subtracting the cellulose concentration from the delignified sample (hollocellulose) obtained by Browning's method (Browning, 1967).

Macro-, micro-nutrients and heavy metal concentrations of feedstocks and biochars were measured after microwave HNO_3/H_2O_2 digestion by Inductively Coupled Plasma spectroscopy (ICP-OES) (ICAP 6500 DUO THERMO, England).

Water holding capacity (WHC) of biochars was determined via mass difference after saturating the biochar with distilled water, which was kept at atmospheric pressure, allowing the water to completely drain (Kinney et al., 2012). The electrical conductivity (EC) and pH values of feedstock samples were determined in a 1:10 (w/v) aqueous extract, however in the case of biochars they were determined in a 1:20 (w/v) aqueous extract according to (IBI, 2014).

The thermal analysis of the feedstock was performed by using an SDT-2960 simultaneous DSC-TGA thermal analyser (TA instruments) under static-nitrogen atmosphere with the following temperature ramp: (1) temperature equilibration at 30°C, followed by linear heating (at a rate of 5 °C min^{-1}) from 30°C to 105°C; (2) isotherm for 10 min; (3) and ramping of 5 °C min^{-1} from 105°C to 1000°C.

3.2.4. Calculations and statistical analyses

The results of the chemical analyses are expressed on an oven-dry basis (105°C, 24h) and presented as the mean value and standard deviation of the replicates (at least duplicate analyses).

Statistical analyses of data were performed using the IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). The relationships between studied parameters were addressed through a correlation analysis (Pearson correlation coefficient) and significance defined as $p<0.05$.

3.3. Results and Discussion

3.3.1. Physicochemical characterisation of feedstocks

The suitability of the organic wastes as feedstocks for the production of biochar was evaluated considering their physicochemical and chemical properties (Tables 3.2; 3.3 and 3.4), their lignocellulosic composition (Figure 3.1) and thermal behaviour (Figure 3.2).

The selection included six organic wastes from agricultural and municipal sources. They were selected to provide a wide variety of feedstock for the pyrolysis process. They included residues that had been subjected to physicochemical or biological treatments (pre-treated) and other ones which did not undertake any treatment (raw). Pre-treatment had an impact on the lignocellulosic composition of residues and their ash concentration.

Holm oak prunings (O_{FS}) were mostly composed by lignin, cellulose and hemicellulose, which made up to 96% of its organic matter (Figure 3.1). This rich lignocellulosic composition reflects the origin of this waste, a clean hard wood material, and can be consider as “raw lignocellulosic” feedstock. This residue had the highest volatile content and C org concentration of all the wastes studied. It was also characterised by a high C/N ratio, low ash concentration and low electrical conductivity (EC) (Table 3.2). The pyrolytic behaviour of the waste is shown in Figure 3.2. The thermal analysis showed the classic thermal degradation of oak wood, characterised by a high pyrolytic decomposition assigned to celluloses and hemicelluloses below 400°C, the thermoxidation of lignin, which takes place in the temperature range between 400-500°C; and a final step with an almost complete degradation, remaining a residue of less than 0.07% of the initial weight (Emandi et al., 2011).

Greenhouse waste (GH_{FS}) is a heterogeneous vegetable waste with a lignocellulosic composition over 85% of the organic matter, similar to oak. However, GH_{FS} has larger cellulose content (53%) and lower lignin (24%) and hemicellulose (7%) contents than O_{FS}, due to the lower amount of wood in these agricultural wastes, compared to O_{FS}. The rich lignocellulosic composition provides a high C org, and therefore a large volatile content, similarly to O_{FS}, and for this reason GH_{FS} could also be considered as a “raw lignocellulose” waste. However, due to its horticultural origin, it contains have a higher

Table 3.2.: Chemical properties of the organic wastes used as feedstock for the pyrolysis process. O_{FS}: oak holm pruning, GH_{FS}: greenhouse waste, GW_{FS}: green waste, CM_{FS}: CellMatt, PM_{FS}: pig manure and PC_{FS}: press cake.

Feedstock	**pH** [a]	**EC**[a]	**Volatile content**	**Ash**	**Fixed C**	**N**	**C**	**C org**	**H**	**S**	**O**	**H/C**	**C/N**	**O/C**
		(mS cm^{-1})	**(%)**			**(g 100g^{-1})**								
O_{FS}	5.7 *(0.1)*	0.7 *(0.0)*	79.6 *(0.2)*	2.9 *(0.3)*	17.5 *(0.0)*	0.53 *(0.01)*	48.7 *(0.2)*	46.4 *(0.7)*	8.1 *0.0*	0.00 *(0.00)*	39.8 *(0.2)*	2.07	87.6	0.75
GH_{FS}	5.4 *(0.0)*	9.8 *(1.1)*	72.1 *(0.1)*	14.2 *(0.5)*	11.1 *(0.0)*	1.44 *(0.01)*	41.1 *(0.0)*	41.0 *(0.2)*	7.5 *0.1*	0.87 *(0.07)*	34.9 *(0.0)*	2.17	28.4	0.75
GW_{FS}	n.a.	n.a.	n.a.	23.0 *(0.5)*	n.a.	1.41 *(0.13)*	38.7 *(0.2)*	36.2 *(0.9)*	4.8 *(0.1)*	0.06 *(0.04)*	37.3 *(0.0)*	1.57	25.6	0.77
CM_{FS}	7.1 *(0.0)*	4.3 *(0.2)*	63.8 *(0.8)*	25.1 *(0.6)*	13.7 *(0.0)*	1.43 *(0.01)*	40.9 *(0.0)*	39.0 *(1.7)*	7.5 *0.1*	0.87 *(0.07)*	24.2 *(0.0)*	2.30	27.2	0.56
PM_{FS}	7.3[b]	6.9[b]	60.2[c] *(0.9)*	18.3[c] *(2.0)*	21.5[c] *(1.4)*	2.81[d]	33.5[d]	n.a.	6.2 [d]	n.a.	57.5 [d]	2.19 [d]	11.9 [d]	1.29 [d]
PC_{FS}	8.0 *(0.2)*	1.8 *(0.3)*	29.1 *(0.7)*	63.8 *(0.4)*	14.1 *(0.0)*	1.20 *(0.00)*	20.8 *(0.0)*	20.4 *(0.6)*	2.2 *(0.1)*	0.26 *(0.02)*	11.7 *(0.0)*	1.31	17.0	0.50

[a] water extract 1:10 w:v 25°C; EC: Electrical Conductivity; [b]: these data were obtained from (Santos et al., 2015); [c]: data obtained from (Huang et al., 2011); [d]: data obtained from (Xiu et al., 2011); n.a.: not available; standard deviation in brackets (n=2).

concentration of total N and K, respect to O_{FS}, and soluble elements that give rise the highest EC of all the wastes studied (9.8 mS cm^{-1}). Thermal analysis revealed a similar degradation pattern to O_{FS} as a consequence of a similar lignocellulosic composition, with an additional degradation step after 400°C due to the plastic tags and a final residue that represent 14.73% of the original dry weight.

Green waste (GW_{FS}) is also a lignocellulosic feedstock, which can be consider a rich lignocellulosic material as O_{FS} and GH_{FS}. Its composition depends on the proportion of branches, grass and leaves

Table 3.3: Concentration of nutrients in feedstocks (dry-weight basis). O_{FS}: Holm oak waste, GH_{FS}: Greenhouse waste; CM_{FS}: CellMatt; PM_{FS}: Pig manure; PC_{FS}: Press cake.

Feedstock	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**	**Mo**	**B**
	(g $100g^{-1}$)					**(mg kg^{-1})**					
O_{FS}	0.06 *(0.00)*	0.20 *(0.00)*	0.91 *(0.05)*	0.06 *(0.00*	0.01 *(0.00*	136 *(5)*	7.3 *(0.9)*	220 *(5)*	19 *(1)*	<0.5 *(0.0)*	8.5 *(0.1)*
GH_{FS}	0.18 *(0.01)*	3.31 *(0.12)*	0.88 *(0.02)*	0.37 *(0.02*	0.03 *(0.00*	113 *(6)*	16.9 *(0.2)*	55 *(3)*	32 *(1)*	<0.5 *(0.0)*	16.6 *(0.8)*
GW_{FS}	0.10	0.74	0.72	0.13	0.03	2215	18.6	145	68	3.0	16.62
CM_{FS}	0.22 *(0.01)*	0.45 *(0.05)*	3.35 *(0.04)*	0.18 *(0.01*	0.64 *(0.03*	7415 *(1457)*	146.4 *(8.1)*	174 *(15)*	835 *(81)*	<0.5 *(0.0)*	38.6 *(15.3)*
PM_{FS}[a]	4.22	1.06	5.08	1.30	0.29	2055	156.2	351	623	3.7	747.0
PC_{FS}	0.36 *(0.02)*	0.74 *(0.02)*	2.60 *(0.30)*	0.29 *(0.02)*	0.18 *(0.01)*	8534 *(399)*	46.6 *(1.2)*	206 *(8)*	202 *(11)*	2.1 *(0.1)*	22.0 *(0.3)*

[a]: these data were obtained from Xiu et al. (2011); n.a.: not available; standard deviation in brackets (n=2).

Table 3.4: Heavy metal concentration in feedstocks. O_{FS}: Holm Oak waste, GH_{FS}: Greenhouse waste; CM_{FS}: CellMatt; PM_{FS}: Pig manure; PC_{FS}: Press cake.

Feedstock	**Cd**	**Cr**	**Ni**	**Pb**
	(mg kg^{-1})			
O_{FS}	<0.1 *(0.0)*	5.0 *(0.1)*	2.4 *(0.6)*	0.9 *(0.2)*
GH_{FS}	0.4 *(0.0)*	2.8 *(0.3)*	2.5 *(0.1)*	0.9 *(0.1)*
GW_{FS}	0.3 *(0.0)*	137.7 *(2.3)*	56.5 *(3.5)*	16.8 *(1.2)*
CM_{FS}	0.9 *(0.2)*	163.7 *(3.3)*	99.1 *(0.6)*	119.5 *(1.9)*
PM_{FS}[a]	n.a.	2.2	n.a.	18.8
PC_{FS}	1.1 *(0.0)*	67.1 *(8.0)*	30.5 *(3.8)*	98.3 *(16.6)*

[a]: these data were obtained from Xiu et al. (2011); n.a.: not available; standard deviation in brackets (n=2).

in the mixture, which define the relative proportions of lignin, cellulose and hemicellulose. The presence of branches and grass increases the proportion of lignin and cellulose, whereas the presence of leaves and grass will decrease the proportions of cellulose and hemicellulose (Donovan et al., 2010). In addition, this residue can have higher amounts of ash than O_{FS} and GH_{FS} feedstocks, which imply high concentrations of mineral constituents, including potentially toxic heavy metals such as Cr, Ni, Pb (Table 3.4) and Zn (Table 3.3) as a consequence of the urban origin of this waste and the presence of dirty wood. Despite the theoretical rich lignocellulosic composition of green waste, this waste presents differences form raw lignocellulosic feedstocks due to the presence of inert. The urban origin of green waste may be responsible of the high ash concentration of these waste, originated from their mixing with other residuals during collection.

CellMatt (CM_{FS}) is an organic waste that has undergone a pre-treatment, consisting in an autoclaving process that caused the partial degradation of the organic matter. CM_{FS} is characterised by a lignocellulosic composition over 74% of the organic matter (Figure 3.1), so it could be defined as a “pre-treated organic” feedstock. The main difference with the “raw lignocellulosic” residues is the absence of the hemicellulose fraction, which has been decomposed during the pre-treatment process. However, the C org concentration (40.9%) was not affected by autoclaving and was similar to GH_{FS} and GW_{FS} (Table 3.2). Another important characteristic of CM_{FS} is the high mineral composition reflected by the ash content (25%). The heavy metal concentration of CM_{FS} (Table 3.4) is the highest of all the waste studied and could represent a limitation for its use as soil amendment.

In Spain, the use of CM_{FS} waste as soil amendment would be limited to 5 ton ha^{-1} yr^{-1} (class C amendment), due to the high concentration of Cd, Cu, Ni, Pb, Zn and Cr (BOE, 2013). Hence, high heavy metal concentrations and ash content in the feedstock could limit its

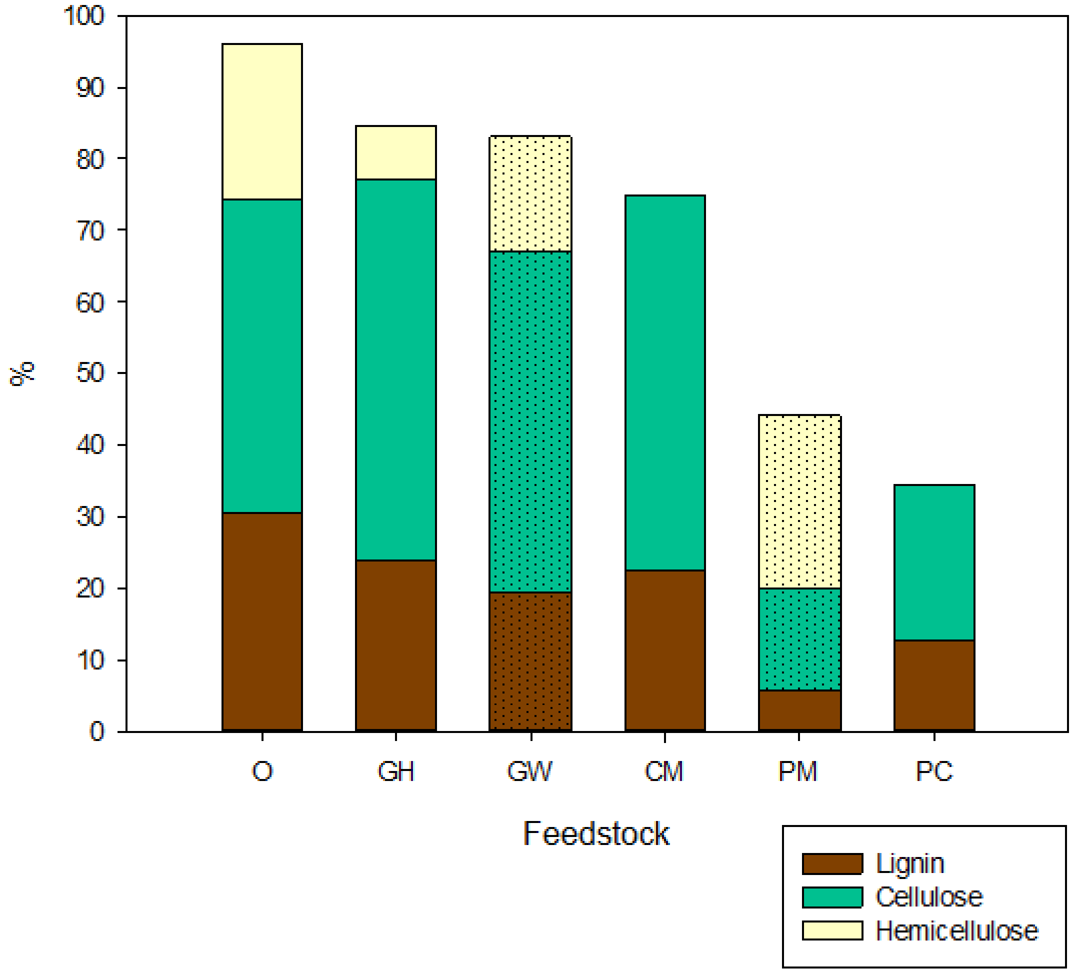

Figure 3.1: Lignocellulosic fractions of feedstocks: O_{FS}: Holm Oak waste, GH_{FS}: Greenhouse waste; CM_{FS}: CellMatt; PM_{FS}: Pig manure; PC_{FS}: Press cake. The dotted data of GW_{FS} and PM_{FS} were obtained as average of study of Donovan et al. (2010) and Xiu et al. (2010).

classification as biochar and land use as amendment (Enders et al., 2012; IBI, 2014). The thermal analysis showed the characteristic degradation pattern of lignocellulosic feedstock (Figure 3.2), and a high amount of final residue (25.08% of the total weight).

Pig manure (PM_{FS}) cannot be considered as a raw lignocellulosic residue, since the lignocellulosic fraction only constituted 44% of the

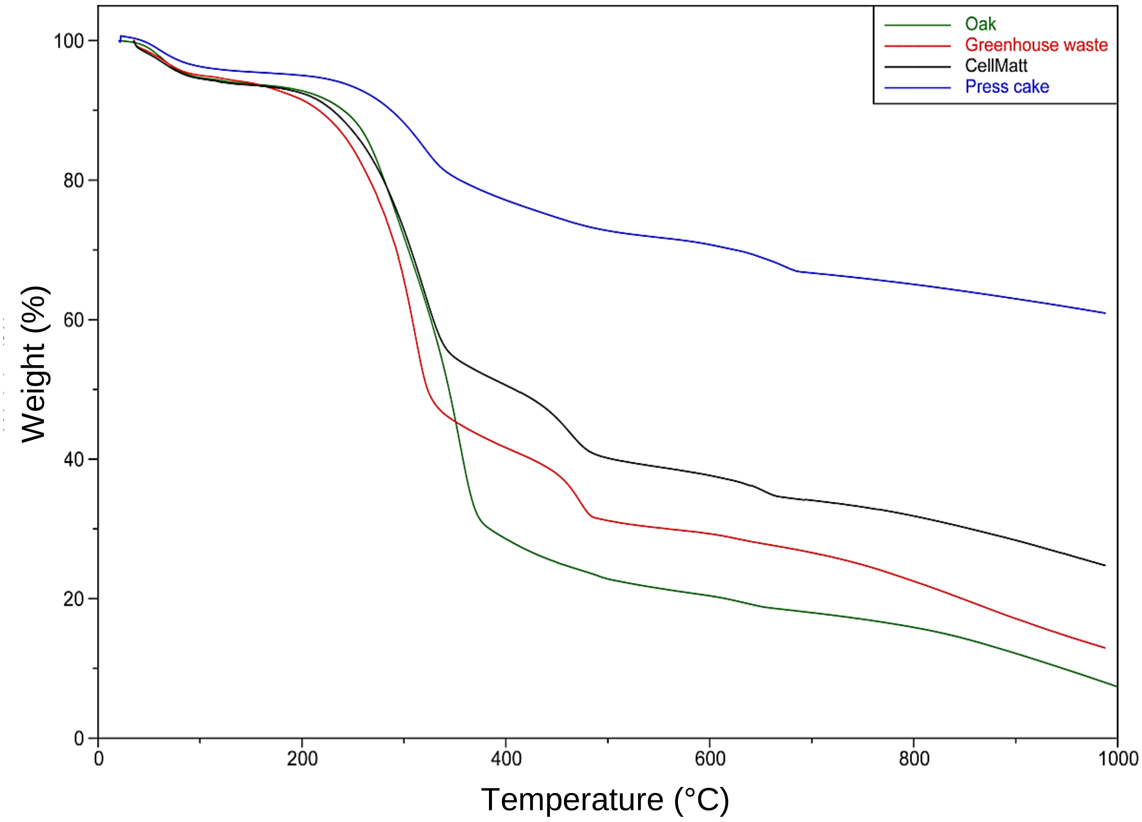

Figure 3.2: Weight loss profiles for four different biomass types: holm oak, greenhouse waste, CellMatt and press cake. The thermal analysis was performed with a DSC-TGA thermal analyser under static-nitrogen atmosphere.

organic matter (Huang et al., 2011). Thus, C org concentration is typically lower than in raw lignocellulosic residues (Table 3.2). The main characteristic of PM_{FS} is the high N concentration (2.8%) (Xiu et al., 2011) and consequently a low C/N ratio that probably will have an impact on the characteristics of biochars. In general, PM_{FS} is characterised by 18.3% of ash matter (Huang et al., 2011), with high EC and neutral pH values (Santos et al., 2015). Therefore, PM_{FS} has high nutrient concentrations, especially in P, Ca, Mg, Fe, Cu, Mn, Zn and B (Xiu et al., 2011). The high concentrations of Cu and Zn typically exceed the limits for agricultural use (BOE, 2013). However, depending on the separation technique used for the treatment of PM_{FS} (gravity settling, centrifugation or belt press) the amounts of different heavy metals could be decreased (Xiu et al., 2009). The thermal degradation profile displayed by Yanardag et al. (2014) showed that most of the organic matter degradation occurs before 500°C, and the final residue is lower than 20% of the total weight.

Press cake (PC_{FS}) is another organic waste that has undergone a pre-treatment process (anaerobic digestion), so it could be defined as a “pre-treated organic” feedstock. The lignocellulosic composition of PC_{FS} constituted 33% of the organic matter, with only 22% of cellulose and 13% of lignin. This material contained very low levels of hemicellulose, due to the partial degradation of the organic matter during the anaerobic digestion. The pre-treatment also causes the low C org concentration and a high ash content (63.8%), which could limit its qualification as biochar (Enders et al., 2012; IBI, 2014).Therefore PC_{FS} has a high EC and high concentration of inorganic elements (Ca, Fe, Cu, Mn, Zn, B, Cd, Cr, Ni and Pb), that in the case of Cd, Ni and Zn, could limit its agricultural use in Spain (BOE, 2013). The thermal degradation profile reflected the presence of a large proportion of mineral elements with a massive final residue (61.09% of the initial weight).

3.3.2. Properties of biochars

The preparation of biochars from organic wastes was aimed at the recycling of these wastes as soil amendments in agriculture. Consequently, the biochars characterisation included a general physicochemical characterisation, the evaluation of their agronomical properties and an assessment of potential limitations associated to the presence of persistent pollutants.

3.3.2.1. Physicochemical characterisation.

a. Elemental composition

The elemental composition of biochars, including C org, H, N, S and O is shown in Table 3.5. The C org content is one of the most important properties of biochars. The amount of C org stored in aromatic structures is directly related to C sequestration potential, a key feature of biochars when used as soil amendment. In this study C org has been used for comparison, rather than total C that would also include carbonates from the ash fraction.

The amount of C org is one of the most relevant parameters considered by the IBI quality standards for the classification of biochars (IBI, 2014). According to these standards, biochars can be classified into three categories: Class 1 for biochars containing ≥60% C, class 2 ≥30% and <60% C, and class 3 ≥10% and <30% C. Thus, the biochars obtained in this study can be classified as follows: O and GH in class 1; OC, GW, CM and PM in class 2; and PC in class 3. This classification reflects the importance of the feedstock, rather than HTT, on the concentration of C org in the biochar. Only raw lignocellulosic feedstocks gave rise to class 1 biochars, whereas those biochars obtained from pre-treated organic wastes or green wastes from urban origin could only fall into the classes 2 or 3.

The pyrolysis conditions (time of residence and HTT) also affected the characteristics of biochars. The use of a long residence time in the reactor of Proininso decreased the amount of C org of the holm oak biochar compared to the short residence time (1 hour) in the Pyromat reactor (Table 3.5). Also, C org increased with temperature in raw lignocellulosic biochars and PM biochars whereas H, S and O concentrations decreased. However, CM and PC biochars (characterised by a high ash content, Figure 3.4) exhibited a different trend, characterised by a loss of C org and a rise in S concentration with increasing temperature, which is accumulated in the ash fraction as a concentration effect from the organic matter degradation.

The increase of C org with temperature in lignocellulosic biochars is due to the aromatization of C, which mostly begins at 220°C (Zhao et al., 2013) when the decomposition of hemicelluloses (220-315°C) and celluloses (315-400°C) take place. The decomposition of lignins (150-900°C) mainly occurs above 400°C, due to its aromatic structure (Novak, 2009; Yang et al., 2007). Thus, in biochars prepared at 400°C, mostly the hemicellulose and cellulose fractions have been degraded, whereas the pyrolysis at 600°C implies also a higher decomposition of lignin (Keiluweit et al., 2010; Kleber et al., 2015; Rutherford et al., 2012). However, the C losses during the pyrolysis of lignin are lower than for cellulose (Knicker, 2010). The presence of lignin in the feedstock increases C content in the biochar through dehydration of hydroxyl groups and thermal degradation of lignocellulose structures (Novak, 2009).

The effect of the temperature of pyrolysis was not so evident in CM and PC biochars. The C org concentrations in these biochars were similar to those found in the original feedstocks. Furthermore, the increase in the temperature of pyrolysis from 400°C to 600°C caused a decrease in the concentration of C org (Table 3.5). This phenomenon could be due to the pre-treatment of both feedstocks, where the most labile fraction of the C org had been partially degraded.

Table 3.5: Physicochemical properties of the biochars obtained at low and high temperature. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**pH**[a]	**EC**[a]	**WHC**	**N**	**C org**	**H**	**S**	**O**	**H/C**	**O/C**
		(mS cm^{-1})	**%**			**(g 100g^{-1})**				
OC450	9.5 *(0.1)*	0.6 *(0.0)*	94.2 *(3.8)*	0.73 *(0.08)*	56.6 *(0.1)*	3.05 *(0.08)*	0.0 *(0.0)*	16.2 *(0.2)*	0.64	0,21
OC650	9.4 *(0.0)*	0.4 *(0.0)*	84.4 *(1.8)*	1.08 *(0.08)*	59.7 *(1.6)*	1.39 *(0.03)*	0.0 *(0.0)*	15.7 *(0.6)*	0.28	0,20
O400	9.6 *(0.1)*	0.6 *(0.1)*	90.6 *(10.1)*	0.33 *(0.00)*	70.5 *(0.6)*	4.73 *(0.19)*	0.0 *(0.0)*	15.0 *(0.2)*	0.80	0.16
O600	8.8 *(0.0)*	0.7 *(0.0)*	96.7 *(3.7)*	0.34 *(0.00)*	75.8 *(0.3)*	2.45 *(0.01)*	0.0 *(0.0)*	6.1 *(0.1)*	0.39	0.06
GH400	9.9 *(0.0)*	5.6 *(0.0)*	198.3 *(8.3)*	1.14 *(0.05)*	61.5 *(0.6)*	3.27 *(0.14)*	0.0 *(0.0)*	14.9 *(1.0)*	0.63	0.18
GH600	9.7 *(0.0)*	6.3 *(0.0)*	152.7 *(5.6)*	0.88 *(0.02)*	71.0 *(0.6)*	1.54 *(0.02)*	0.1 *(0.0)*	7.5 *(0.1)*	0.26	0.08
GW400	9.5 *(0.0)*	1.3 *(0.1)*	n.a.	1.39 *(0.00)*	36.0 *(0.4)*	2.75 *(0.02)*	0.0 *(0.0)*	0.0 *(0.0)*	0.91	n.a.
GW600	10.4 *(0.0)*	0.7 *(0.0)*	55.4 *(3.5)*	0.90 *(0.01)*	42.7 *(1.4)*	1.25 *(0.01)*	0.0 *(0.0)*	0.0 *(0.0)*	0.35	n.a.
CM400	9.4 *(0.1)*	2.2 *(0.1)*	82.5 *(4.7)*	1.25 *(0.00)*	36.5 *(0.5)*	4.01 *(0.17)*	0.4 *(0.3)*	6.3 *(0.3)*	1.31	0.13
CM600	10.4 *(0.0)*	3.5 *(0.1)*	68.5 *(3.0)*	1.21 *(0.01)*	34.6 *(0.3)*	1.26 *(0.05)*	0.7 *(0.1)*	0.8 *(0.2)*	0.43	0.02
PM400	9.8 *(0.0)*	6.6 *(0.2)*	n.a.	2.28 *(0.06)*	52.2 *(0.4)*	3.26 *(0.05)*	0.1 *(0.0)*	13.0 *(0.7)*	0.74	0.19
PM600	10.7 *(0.0)*	6.8 *(0.1)*	n.a.	1.45 *(0.12)*	54.9 *(0.3)*	1.18 *(0.05)*	0.1 *(0.1)*	4.9 *(0.3)*	0.26	0.07
PC400	9.9 *(0.1)*	1.1 *(0.1)*	47.3 *(0.8)*	0.90 *(0.01)*	16.2 *(1.4)*	0.69 *(0.01)*	0.3 *(0.0)*	3.9 *(0.1)*	0.51	0.18
PC600	10.4 *(0.0)*	0.7 *(0.0)*	49.6 *(2.7)*	0.64 *(0.01)*	14.8 *(0.0)*	0.31 *(0.01)*	0.4 *(0.1)*	1.6 *(0.4)*	0.25	0.08

[a] water extract 1:20 w:v 25°C; EC: Electrical Conductivity; WHC: Water Holding Capacity; n.a.: not available; standard deviation in brackets (n=2).

The concentration of the rest of the elements (N, H, O and S) was also affected by feedstock composition and temperature of pyrolysis. In the case of N, PM biochars recorded the highest concentrations of N (2.28% and 1.21% for PM400 and PM600, respectively), compared to the rest of biochars (Table 3.5). The large amount of N in PM biochars can be explained by the high protein content in the feedstock (Tsai et al., 2012). Oak biochars registered the lowest N concentration due to the low concentration of this element in the original feedstock (0.53%N). Knicker (2010) showed that N compounds affected the thermal and chemical recalcitrance of biochar. Thus, depending on the feedstock composition, biochars will have different amounts and thermally altered N components incorporated into the biochar structure (Knicker, 2010). For instance, it has been revealed that N-rich biochars from GW, with a relatively high N concentration mainly due to the grass and leaves composition, is more stable to oxidation respect to N-poor wood biochar like oak biochar (Knicker, 2010). These differences in the N composition would have consequences for the N availability in soil (Knicker, 2007).

In general, all biochars showed a reduction in the concentration of N with increasing temperature, which agrees with observations in previous studies (Enders et al., 2012; Jindo et al., 2014). Oak biochars were the exception, which might be explained by the inclusion of N within the aromatic C structures. Preliminary studies suggested that high temperatures of pyrolysis could preserve N in wood biochars by the formation of heterocyclic N (Al-Wabel et al., 2013; Gaskin et al., 2008).

The response of H and O to changes in the HTT of pyrolysis was similar for all biochars, and tended to decline with increasing temperature. This has been reported in previous studies and it is due to the scission of weaker bonds (C-O and C-H compared to C-C) in the carbonaceous material, so that only a recalcitrant oxygen fraction remains fixed in the biochar (Al-Wabel et al., 2013; Rutherford et al., 2012). This effect decreases the presence of O-containing functional groups decreasing the surface acidity and polarity of biochars (Ahmad et al., 2012).

The decreased in S effect during the pyrolysis could be a volatilization effect even at low pyrolysis temperature (Al-Wabel et al., 2013; Enders et al., 2012).

b. Molar H/C org and O/C org ratios

The comparison between the relative contributions of two different elements may provide useful information on the chemical characteristics of biochars. Thus, the molar H/C org ratio has been proposed as an index of aromaticity of biochars, and also to classify the stability of biochars, in combination with the O/C org ratio (Enders et al., 2012; IBI, 2014). Molar ratios H/C org and O/C org are shown in Table 3.5 and plotted on a Van Krevelen diagram (Krevelen, 1961) in Figure 3.3. The molar H/C org ratio shows the degree of thermochemical alteration that produces fused aromatic ring structures in the material (IBI, 2014). Thus, a H/C org ratio below 0.7 indicates that the biochars have been thermochemically altered or “thermochemically converted” (IBI, 2014) (Figure 3.3).

The study of the Van Krevelen diagram allows differentiating the properties of feedstocks and biochars prepared under different conditions. Results showed great differences between feedstocks, located in the top-right corner of the diagram, and the biochars, divided in two different groups (prepared at 400°C and 600ºC), located in the bottom-left corner. When the temperature of pyrolysis increased, H/C org and O/C org ratios tended to decrease and homogenise. A negative correlation between production temperature and H/C org ratio has been found (p<0.01). Feedstocks showed the largest differences for H/C org ratios; being CM_{FS} the highest value

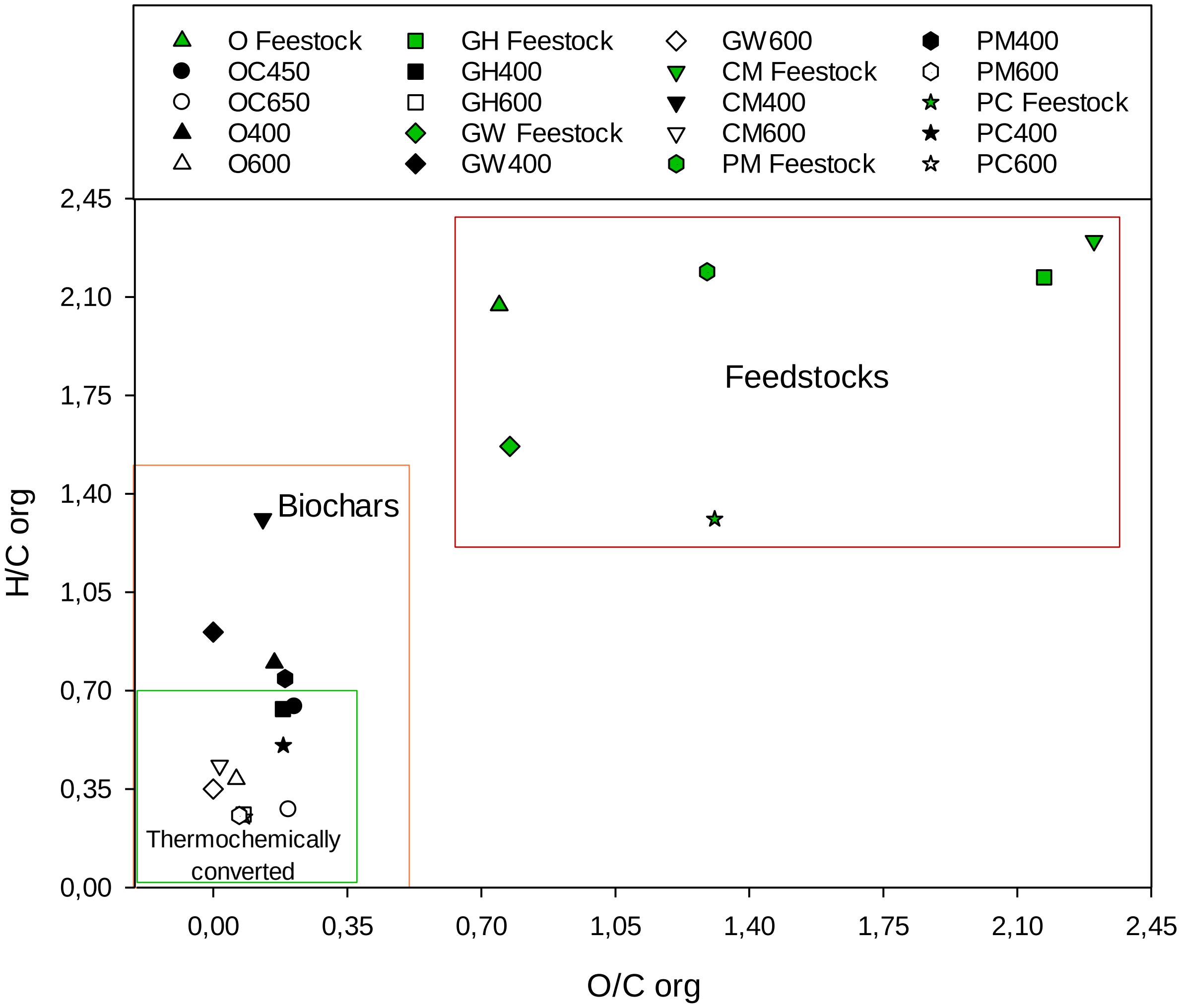

Figure 3.3: H/C org and O/C org ratios for feedstocks (red square) and pyrolysed residues in Van Krevelen diagram. The orange square contains all biochars, and the green one includes only the thermochemically converted materials (H/C org < 0.7), according to IBI (2014). OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

(2.30) and PC_{FS} the lowest (1.31) (Table 3.2 and Figure 3.3). The differences decreased in biochars at 400°C, ranging from 0.51 to 1.31, which showed that some of them were not completely “converted” (O400, GW400, CM400 and PM400). However, at 600ºC all biochars registered similar H/C org ratios, in the range 0.26-0.43. Thus, the highest H/C org of CM400 can be explained by the large proportion of the cellulosic fraction in the feedstock, characterised by a high amount of H and O-containing functional groups (Spokas, 2010).

According to Spokas (2010) and Enders et al. (2012) a H/C org below 0.4 or O/C org below 0.2 indicate a high C sequestration potential, whereas biochar above these limits are considered to have a moderate sequestration ability. According to this, all biochars prepared at 600ºC (except CM600) can be classified as "high sequestration potential" materials, whereas the rest of biochars are classified with "moderate sequestration ability", except GW biochars that have "no C sequestration ability" due to their high ash content. Also, according to Spokas (2010) the O/C org ratio can also help to predict the life time of biochar. Biochars with an O/C org ratio under 0.2 will possess a half-life of >1000 years. All biochars prepared at both temperatures are in this class, except OC400, which possess a half-life between 100-1000 years. This effect has implications on biotic and abiotic interactions in amended soils, being biochars with low O/C org ratio values less easily biodegradable (Spokas, 2010). However, the relationship between material properties and decomposers and environmental conditions could determines the greater persistence of biochar on soil, rather than only its recalcitrance (Lehmann et al., 2015).

c. Volatile matter, ash and fixed carbon content.

The percentages of ash, fixed C and volatile matter of biochars at 400°C and 600°C are given in Figure 3.4. These three parameters define the main physicochemical properties of biochars, especially a high proportion of fixed C (>50%), low ash (<20%) and volatile content (<30%) observed in raw lignocellulosic biochars (O and GH), that are typical characteristics of biochars prepared from woody materials (Enders et al., 2012; Zhao et al., 2013). However, biochars prepared from other sources (PC, CM and GW biochars) showed higher ash (>50%), similar volatile content (<30%), and lower concentration of fixed C (<40%), whereas PM biochars had intermediate values (Figure 3.4).

A negative correlation between volatile content and production temperature was found for the studied biochars (*p*<0.01), which reflects the dependence of fixed C and volatile content on both the

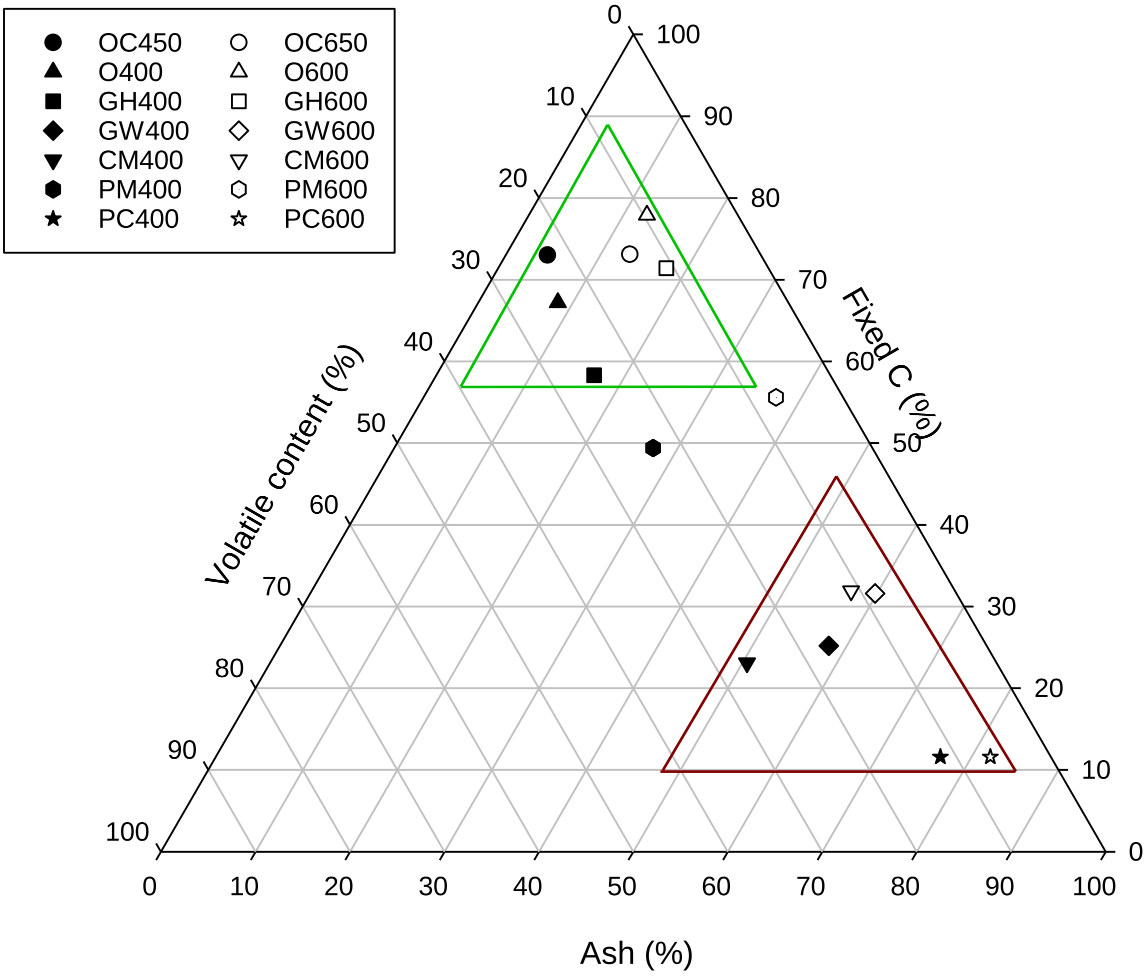

Figure 3.4: Triangle plot of ash, fixed C, and volatile content percentages of tested biochars and effect of pyrolysis temperature. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake. The black symbols represent the biochars at low temperature and the white symbols at high temperature (400°C and 600°C respectively). The green triangle contains the raw lignocellulosic biochars, and the red one includes the pre-treated lignocellulosic biochars.

chemical characteristics of feedstocks and the pyrolysis conditions. This relationship is in agreement with results found by other authors (Chia et al., 2015; Enders et al., 2012). Cellulose, hemicellulose and lignin degrade within 150-900°C and are affected by the mineral compounds in feedstocks (Chia et al., 2015). Thus, increasing the pyrolysis temperature increases the carbonaceous skeleton of biochars, which is a pore structure (Keiluweit et al., 2010) that mimics the cellular structure of wood in the original feedstock (Chia et al., 2015; Wildman and Derbyshire, 1991).

The largest proportions of ashes were found in PC, GW and CM and then in PM biochars, whereas, the lowest proportions were found in raw lignocellulosic biochars (O, GH). This effect is due to the original mineral composition of feedstocks. It should be noted that higher pyrolysis temperature increases ash contents and decreases volatile contents due the losses of the organic matter during the thermal decomposition, and the concentration of mineral constituents from feedstocks (Zhao et al., 2013). In addition, fixed C tends to increase with higher pyrolysis temperature due to the increase of aromatic C inside of C atoms rings and their condensation and growth into larger sheets and stacks (Kleber et al., 2015). However, PC biochars did not show an increase in fixed C at 600°C, the reason can be that high ash contents hinder the formation of aromatic C forms; this effect was observed by Enders et al. (2012). Xu and Chen (2013) also found that a large mineral content can prevent the diffusion of heat and therefore the release of volatile matter during pyrolysis.

According to the volatiles content, ash and fixed C results, it is expected that biochars prepared at 400°C would be more reactive in soil than biochars prepared at higher temperatures. In general, biochars at 400°C showed a higher H/C org and O/C org ratio that imply more diversified organic molecules, including aliphatic and cellulose type structures and containing more O-containing functional groups, being more easily biodegradable and also exhibiting a higher ion exchange capacity (Kleber et al., 2015; Novak, 2009; Spokas, 2010).

3.3.2.2. Agronomical characterisation

a. Macro and micro nutrients composition

The mineral composition of biochars (Table 3.5) is an indication of their potential as a nutrient source. The presence of nutrients in biochars, which are directly related to the ash content, are governed by the composition of the original feedstocks and the pyrolysis temperature. Wood and raw lignocellulosic biochars are characterised by a low amount of nutrients. On the other hand, CM, PC and also GH and PM biochars are rich in cation exchange capacity. In addition, CM, PC and PM biochars have a high content in Fe, Cu, Mn and Zn, as expected from the concentrations in the original feedstocks. In general, the concentration of macro and micro nutrients was enriched with the increasing pyrolysis temperature, especially in the case of raw lignocellulose biochars. However, biochars from PC_{FS} reversed this trend, and biochars from CM_{FS} did not show a clear tendency. While CM biochars showed an opposite behaviour for different nutrients (reductions in P and K and increments in the rest) PC biochars showed a reduction in the concentration of all nutrients.

As already mentioned in the case of N, the level of this element was low in all biochars, except in the case of PM (Table 3.5). Furthermore, the incorporation of N into the biochar structure reduces its availability for plants. P tended to increase during the pyrolysis process originating an enrichment of this element (Table 3.5). It is known that P only starts to volatilise when the pyrolysis temperature is greater than 800°C (Knicker, 2007).

The sum of the cations K, Na, Ca, and Mg reflected an increase respect to the feedstocks and with the temperature in both pyrolysis processes for all biochars with high lignocellulosic compounds and manure except in the case of PC biochars. The enrichment in nutrients could be due to a concentration effect, especially in raw lignocellulosic biochars as observed by Enders et al. (2012).

Table 3.6: Total macro and micro nutrient concentrations in the studied biochars. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**	**Mo**	**B**
	(g/100g)					**(mg kg^{-1})**					
OC450	0.04 *(0.00)*	0.99 *(0.04)*	1.39 *(0.11)*	0.13 *(0.01)*	0.02 *(0.00)*	160 *(14)*	4.5 *(0.4)*	152.1 *(6.3)*	8.4 *(1.2)*	<0.1 *(0.00)*	6.8 *(0.0)*
OC650	0.20 *(0.01)*	0.67 *(0.03)*	3.82 *(0.22)*	0.25 *(0.01)*	0.02 *(0.00)*	542 *(40)*	9.9 *(0.6)*	463.3 *(25.6)*	30.1 *(2.0)*	<0.5 *(0.00)*	16.1 *(0.8)*
O400	0.07 *(0.01)*	0.90 *(0.01)*	2.14 *(0.13)*	0.12 *(0.02)*	0.09 *(0.01)*	1351 *(152)*	18.3 *(2.8)*	44.5 *(4.7)*	74.7 *(13.5)*	2.46 *(1.10)*	13.4 *(0.2)*
O600	0.08 *(0.01)*	1.07 *(0.08)*	2.64 *(0.24)*	0.15 *(0.01)*	0.15 *(0.02)*	1667 *(372)*	22.0 *(2.4)*	61.8 *(4.8)*	85.8 *(13.9)*	1.30 *(0.62)*	16.1 *(0.7)*
GH400	0.24 *(0.00)*	3.39 *(0.00)*	2.18 *(0.00)*	0.76 *(0.00)*	0.28 *(0.00)*	1059 *(97)*	17.9 *(0.4)*	68.2 *(0.3)*	38.2 *(1.2)*	2.01 *(0.23)*	24.0 *(0.3)*
GH600	0.25 *(0.01)*	4.13 *(0.10)*	2.59 *(0.06)*	0.91 *(0.03)*	0.30 *(0.01)*	544 *(21)*	16.5 *(0.4)*	60.7 *(1.6)*	26.1 *(1.2)*	0.82 *(0.01)*	27.3 *(0.5)*
GW400	0.26 *(0.01)*	0.82 *(0.02)*	2.60 *(0.16)*	0.43 *(0.02)*	0.13 *(0.00)*	9124 *(124)*	41.4 *(2.9)*	1803.9 *(124.9)*	200.0 *(6.8)*	3.33 *(0.13)*	38.8 *(0.1)*
GW600	0.32 *(0.03)*	0.99 *(0.05)*	3.23 *(0.07)*	0.48 *(0.02)*	0.15 *(0.01)*	9550 *(264)*	50.1 *(0.8)*	321.1 *(18.2)*	263.5 *(21.3)*	3.99 *(0.28)*	41.0 *(2.2)*
CM400	0.42 *(0.00)*	0.84 *(0.00)*	4.26 *(0.07)*	0.34 *(0.00)*	0.70 *(0.01)*	10053 *(1612)*	96.2 *(4.0)*	225.5 *(15.0)*	357.5 *(13.3)*	5.63 *(0.37)*	29.6 *(0.2)*
CM600	0.37 *(0.03)*	0.70 *(0.07)*	5.17 *(0.54)*	0.36 *(0.06)*	0.99 *(0.14)*	10182 *(287)*	128.2 *(5.3)*	275.6 *(28.1)*	490.3 *(102.8)*	3.20 *(0.50)*	32.3 *(2.1)*
PM400	1.96 *(0.07)*	2.49 *(0.07)*	0.92 *(0.06)*	0.33 *(0.01)*	2.44 *(0.07)*	3213 *(116)*	111.7 *(3.9)*	365.4 *(16.2)*	452.6 *(11.5)*	3.58 *(0.09)*	63.9 *(1.1)*
PM600	2.65 *(0.04)*	2.92 *(0.09)*	1.14 *(0.01)*	0.45 *(0.01)*	2.97 *(0.04)*	2890 *(483)*	138.6 *(4.9)*	483.9 *(6.8)*	653.9 *(82.0)*	4.52 *(0.12)*	76.4 *(1.9)*
PC400	0.55 *(0.01)*	1.07 *(0.01)*	3.31 *(0.66)*	0.45 *(0.01)*	0.28 *(0.00)*	12217 *(560)*	97.7 *(16.7)*	282.5 *(17.6)*	324.2 *(85.1)*	4.90 *(0.80)*	29.2 *(0.1)*
PC600	0.51 *(0.01)*	0.99 *(0.01)*	2.99 *(0.07)*	0.40 *(0.04)*	0.26 *(0.01)*	11399 *(496)*	76.0 *(9.8)*	259.7 *(2.2)*	265.4 *(8.3)*	3.87 *(0.24)*	28.8 *(1.9)*

Standard deviation in brackets (n=2).

b. pH and EC values

All biochars showed alkaline pH, which ranged from 8.8 to 10.7 (Table 3.5). Two groups of biochars could be distinguished: one group including OC, O and GH biochars with values lower than 9.7, and a second group consisting of GW, CM, PM and PC biochars with values above 10.4. Increasing pyrolysis temperature increased pH values in CM, PC, GW, and PM biochars with high-ash content, a trend that was also observed by Enders et al. (2012). The decomposition during pyrolysis removes acidic functional groups and increases the ash content (Ahmad et al., 2012; Novak, 2009), causing the hydrolysis of Ca, K and Mg salts in presence of water (Gaskin et al., 2008) and leading to more alkaline biochars. In contrast, this effect was not so important in raw lignocellulose biochars such as O, OC and GH.

EC is a measurement that correlates with salinity and therefore is an important parameter to determine in any organic amendment before land use. The EC values in the studied biochars ranged from 0.4 to 6.8 mS cm^{-1} (Table 3.5). The EC results were correlated with the original EC values of the feedstocks. Thus, O and OC biochars showed the lowest EC values and PM and GH the highest. Increasing pyrolysis temperature also tended to increase the EC values. This effect was clearly observed in other studies at higher temperatures (600°C and 800°C) (Al-Wabel et al., 2013).

c. Water holding capacity

The hydraulic properties of biochars were studied by the WHC, which ranged from 47.3 to 198.3%, expressed on dry weight basis (Table 3.5). Raw lignocellulosic biochars, with low-ash content, showed the highest WHC (from 84.4 to 198.3%), whereas high-ash biochars showed lower values (from 47.3 to 82.5%). In general, high temperature biochars presented greater water uptake than low temperature biochars, which is in agreement with Gray et al. (2014). In addition, Spokas et al. (2015) related the WHC of biochars with the pore size of materials, which increases with the temperature production (see Section: 3.3.2.1.c; Volatile matter, ash and fixed carbon content).

Water holding capacity is a key feature of biochars to improve the agronomic quality of soils, but the hydrological properties of biochars cannot be fully assessed until they are added to the soils, where it will be possible to assess the amount of water that is accessible to plants (Masiello et al., 2015; Novak, 2009).

3.3.2.3. Potential limitations for the agricultural use of biochars: heavy metal contents

The presence of heavy metals in biochar may represent a limitation for its agricultural use, especially in the case of biochars prepared from feedstocks of urban or agroindustrial origins, which can contain high levels of heavy metals. The heavy metal concentration of the different biochars is shown in Table 3.7.

Due to the absence of European legislation about biochar, the suitability of biochars was assessed according to the European Biochar Certificate (EBC), a guideline on the production of biochar developed by the European biochar Foundation, and the Spanish legislation for organic amendments (BOE (2013)) (Table 3.8).

In general, the heavy metal concentration of the biochars increased respect to the original feedstock, and with the pyrolysis temperature, because of a concentration effect.

Table 3.7: Heavy metal concentration in the studied biochars. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**Cd**	**Cr**	**Ni**	**Pb**
	(mg kg^{-1})			
OC450	<0.1	5 *(1)*	3.3 *(0.9)*	1.1 *(0.2)*
OC650	<0.1	3 *(0)*	9.2 *(0.9)*	1.2 *(0.1)*
O400	<0.1	32 *(7)*	26.0 *(7.6)*	17.9 *(5.0)*
O600	<0.1	58 *(3)*	39.1 *(8.4)*	27.1 *(3.3)*
GH400	<0.1	29 *(0)*	15.8 *(1.3)*	5.8 *(0.5)*
GH600	<0.1	21 *(1)*	10.2 *(0.4)*	3.5 *(0.0)*
GW400	0.8 *(0.1)*	93 *(7)*	35.4 *(2.2)*	66.2 *(2.4)*
GW600	0.1 *(0.0)*	125 *(6)*	44.1 *(1.9)*	86.3 *(19.0)*
CM400	1.4 *(0.0)*	126 *(4)*	72.5 *(2.0)*	212.4 *(35.1)*
CM600	1.3 *(0.3)*	129 *(26)*	66.1 *(2.8)*	209.5 *(72.7)*
PM400	<0.1	8 *(1)*	7.3 *(0.2)*	4.8 *(0.1)*
PM600	<0.1	10 *(1)*	9.5 *(0.2)*	6.1 *(0.0)*
PC400	1.4 *(0.0)*	92 *(1)*	46.4 *(1.8)*	100.0 *(0.5)*
PC600	1.0 *(0.0)*	102 *(35)*	48.1 *(16.1)*	111.5 *(6.2)*

Standard deviation in brackets (n=2).

According to EBC (2012), biochars can be classified in three different quality grades: “premium”, “basic” and “not recommended”, depending on their heavy metals concentrations (Table 3.8). O and GH biochars fall into the category of "premium" quality. However, the use of biochars from feedstocks of urban origins might be restricted. CM biochars would not be recommended as soil amendment in agriculture due to the high concentration of Cr, Pb and Ni. The rest of biochars (GW and PC) fall within the category of "basic", with the only concern of biochars prepared at high temperature (600°C), where the concentration of Cr may exceed the legal limits. Special attention should be paid to the use of Ni-Cr steel in construction of pyrolysis reactors to avoid metal contamination from abrasion of the equipment (Camps-Arbestain et al., 2015).

According to the Spanish legislation BOE (2013) organic amendments can be classified in three different categories (A, B and C). Only the use of class C amendments is limited to 5 tons ha^{-1} yr^{-1}. In the case of the studied biochars, all of them could be used as soil amendment with the exception of CM, which is not allowed by the high concentration of Pb. Similarly, according to the EBC guidelines, the rich lignocellulosic biochars fall into the top quality category, class A, except in the case of O biochars, which fall within class B due to the high concentration of Ni (probably originated during the pyrolysis process). The rest of biochars of urban origin are classified as class B amendments.

Table 3.8: Quality grades of biochars according to EBC (2012) and classification as organic amendment according to the Spanish legislation on fertilizers BOE (2013).

	Quality grade	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
European guidelines	**Basic**	<1.5	<90	<100	<50	<150	<400
EBC (2012)	**Premium**	<1	<80	<100	<30	<120	<400
Spanish	**A**	0.7	70	70	25	45	200
legislation	**B**	2	250	300	90	150	500
BOE (2013)	**C**	3	300	400	100	200	1000

3.4. Conclusions

A series of biochars was produced from organic wastes of agricultural and urban origins. Most of them presented suitable properties for its use. The physicochemical properties of biochars were driven by the characteristics of feedstocks and the pyrolysis temperature. The use of pre-treated lignocellulosic residues led to biochars with a high concentration of ash, macro and micronutrients, whereas raw lignocellulosic residues produced biochars with similar characteristics to traditional wood biochars. The pyrolysis temperature established differences between biochars obtained at 400°C and 600°C. The use of high temperatures increased the ash content and recalcitrant C and increased the losses of N and O containing functional groups. Consequently, those biochars prepared at 400°C presented the highest mineral and volatile organic matter content, which are favourable properties for their agricultural use and the interaction with soil nutrient cycles.

According to the national legislation for organic amendments and the international standards for the use of biochars as soil amendments, all the biochars were suitable for its agricultural use, with the exception of CM, which presented high levels of Cr and Pb.

The presence of heavy metals in the feedstock and the contamination during the pyrolysis can limit their use in agriculture.

Before the use of these biochars as soil amendments, an agronomical evaluation should be performed to assess their impact on soil biogeochemical cycles and plant growth.

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Chapter 4: Agronomical and environmental evaluation of biochars from agricultural and urban organic wastes

4.1. Introduction

The development of new strategies for sustainable waste management is essential in order to divert organic wastes from landfills (EuropeanCommission, 2015). The challenge is to transform these organic wastes in organic amendments so that can be incorporated into agricultural practices. Therefore, the pyrolysis of organic wastes, for the production of bioenergy and biochar, has recently gained considerable interest as a recycling option, becoming an alternative strategy to composting and other biological waste treatments.

Biochar is attracting great attention as a C sequestration potential agent because C is transferred from the fast biological atmospheric C cycle into the much slower geological cycle due to its recalcitrant nature (Hamdan et al., 2010; Spokas, 2010). Concomitant with the enhanced C sequestration, there is a wide range of potential benefits related to the addition of biochar to soil, which are associated to the impact on soil physicochemical and biological equilibria (Glaser et al., 2002).

Some of the agronomical benefits of biochar include: an improvement in soil physical properties, and particularly in soil water-holding capacity, which would be beneficial in areas with limited rainfall (Ippolito et al., 2014, 2016); a change in soil nutrient dynamics, especially when used in combination with mineral fertilization or other organic amendments, affecting their bioavailability such as the case of N and P (DeLuca et al., 2015; Lopez-Cano et al., 2013; Taghizadeh-Toosi et al., 2012); a limitation of the bioavailability of heavy metals (Park et al., 2011). All these benefits might lead to an enhanced crop productivity, which is highly dependent on types of feedstocks and agroclimatic conditions (Jeffery et al., 2011).

The environmental and agronomical benefits of biochar are dependent on the type of biochar. Consequently, considerable variation in plant and soil responses are expected with different biochars (Biederman and Harpole, 2013; Hamdan et al., 2010). For instance, the macro and micro nutrient content of the biochar itself plays an important role in plant mineral nutrition, and plant growth. Although raw lignocellulosic biochars usually contain rather low nutrient concentrations, there are other biochars with high mineral content, especially those obtained from manures and other ash-rich feedstocks (Enders et al., 2012). In addition, low pyrolysis temperatures can also improve nutrient solubility of biochar, improving plant growth (Gunes et al., 2015).

At present, large scale implementation of biochar as soil amendment in agriculture is mainly limited by the cost of biochar production and application, that is higher than the cost of other organic materials typically used as soil amendment (*i.e.* compost, manures, etc. (Fornes et al., 2015)). The cost of the feedstock is a crucial input for economy analysis, especially when biochar is prepared from clean wood biomass. To reduce production cost, other alternative feedstocks are being evaluated, such as farm and urban wastes (Meyer-Kohlstock et al., 2015). In developed countries, the use of organic wastes as cheap feedstock has several advantages, since these wastes are available for free or even generate revenue and/or save disposal cost (Biederman and Harpole, 2013).

The evaluation of the agricultural use of biochar as soil amendment has been almost exclusively performed for biochars produced from woody feedstocks or from manures (Gunes et al., 2015; Jeffery et al., 2011; Mandal et al., 2016; Olmo et al., 2016). It is needed a better understanding of the soil nutrient dynamics, biochar decomposition and potential positive and negative effects associated to soil application of biochar prepared from organic wastes.

The aim of this work was to perform an environmental and agronomical evaluation of a selection of biochars prepared from different urban and agroindustrial organic wastes. The evaluation of biochars as soil amendments was performed when biochars were used alone or in combination with two different N sources: mineral fertilization and an organic amendment (manure). The assessment was based on the study of i) soil C dynamics and CO_2 evolution; ii) soil N and P dynamics and their mineralisation rate; iii) bioavailability of micronutrients and iv) the fate of heavy metals in soil. With this aim a pot trial was performed to evaluate also the response of lettuce to biochar addition and evaluate the potential negative impact on plant growth and heavy metal mobilisation.

4.2. Material and methods

4.2.1. Soil description

An agricultural soil, classified as Haplic Calcisol (WRB, 2014) was sampled from an organic olive orchard in Murcia Region (SE Spain) (coordinates 38°23’ N 1°22’ W). The soil was characterised by a sandy loam texture (57% sand, 27% silt and 16% clay), total C org 1.7 g 100g^{-1} and alkaline pH 8.1, with 30% $CaCO_3$. The characterisation of the soil is presented in Table 4.1. Before its use, the soil was sampled from the topsoil (0-20 cm), was air-dried and sieved (<2 mm).

Table 4.1: Main physicochemical properties of soil and the N sources; sheep manure as organic amendment and diammonium phosphate (DAP) as mineral fertiliser.

	pH [a]	**EC** [a]	**OM**	**C org**	**C/N Ratio**	**C ext**	**N**	**P**	**K**
		(mS cm^{-1})	**(g 100g^{-1})**			**(mg g^{-1})**	**(g 100g^{-1})**		
Soil	8.1	0.51	2.9	1.7	5.7	10.9	0.3	-	-
Manure	9.1	3.9	45.6	18.1	9.1	40.4	2.0	1.6	2.4
Fertiliser (DAP)	-	-	-	-	-	-	18.0	26.0	0.0

[a] water extract 1:10 w:v 25°C; EC: Electrical Conductivity; C ext: alkaline extractable C (0.1 NaOH; 1:10 w:v); DAP: DiAmmonium Phosphate

4.2.2. Biochars description

A wide range of biochars prepared by ECN at 400ºC were used: holm oak (O400), greenhouse waste (GH400), green waste (GW400), dried CellMatt (CM400), pig manure (PM400) and press cake

(PC400). In addition, two commercial biochars, from holm oak feedstock by Proininso Inc. were used: OC450 and OC650, produced at 450ºC and 650ºC, respectively. The main characteristics of the biochars are presented in Tables 3.1, 3.4, 3.5 and 3.6 (in Chapter 3).

4.2.3. Soil incubation experiments

The soil incubation experiments were performed under aerobic conditions (at 40% of its water holding capacity (WHC)) at 25 °C and during 30 days. The soil was pre-incubated at the same conditions for 7 days prior to use. Glass flasks (100 ml) were used for the incubation, containing 40 g (oven dry basis) of each treatment in triplicate and covered with a polyethylene sheet that allows gas exchange but minimizes evaporation. Moisture was gravimetrically adjusted by weight loss every two days with the addition of deionised water to each flask.

Also to biochar, two N sources (manure (M) and mineral fertilisation (F)) were selected for their addition to the soil incubation experiments. Sheep manure was selected as a widely used organic amendment in agriculture containing a relatively high proportion of N (1.98%), in organic form. M was added to the soil as a finely grounded (<1mm) dry material. Diammonium phosphate (F) was selected as one of the most important mineral fertilisers used worldwide as N and P source. The mineral fertiliser was applied to the soil in solution at the required concentration to equal the amount of N added in the manure treatments (198 mg N kg^{-1} soil). Their properties are reported in Table 4.1.

The incubation experiments consisted in seven treatments for each biochar: S, S+B (1%), S+B (2%), S+M (1%), S+B+M (1%), S+F (1%), and S+B+F (1%), expressed as dry weight, with the exception of the mineral fertiliser treatments. Each treatment included three parallel incubations for destructive soil sampling after 3, 7 and 30 days for chemical analysis (NO_3^--N, NH_4^+-N, P_{olsen}, and DTPA extract).

Table 4.2: Description of the soil incubation treatments with biochar and organic and inorganic amendments.

Treatments	Biochar	Manure (M)	Fertiliser (DAP)
S (Control)	-	-	-
S+B (1%)	1% w/w	-	-
S+B (2%)	2% w/w	-	-
S+M (1%)	-	1% w/w	-
S+B+M (1%)	1% w/w	1% w/w	-
S+F (1%)	-	-	**198 mg N kg^{-1} soil ***
S+B+F (1%)	1% w/w	-	**198 mg N kg^{-1} soil ***

*Same amount of N provided by 1% of manure.

4.2.4. Pot trials

Plant growth experiments were carried out in a growth chamber (19-24°C temperatures night/day) at CEBAS-CSIC, Murcia, Spain. The pot trials were used to compare the plant growth in soil amended with six different biochars at 2% dry weight basis: OC450, O400, GH400, GW400, CM400 and PC400, and a soil without biochar that was used as control. All pots received the mineral fertilisation recommended for lettuce crops (*Lactuca sativa* L., cultivar: Baby) (100 N kg ha^{-1} and 111 P kg ha^{-1}) (López et al., 2010).

Black plastic pots (900 ml), with the bottom sealed with cotton, were used for the experiment. Six pots were filled with 1000 g of soil (dry weight basis) for each treatment and the control and them were laid out on six trays. Each tray comprised seven pots, one pot from each treatment and one control pot, in a randomised design. In order to avoid the influence of the microclimate variability the location of the trays inside the growth chamber was changed every two days. Soil moisture was checked three times a week by measuring weight loss for each individual pot and adjusting with deionised water when necessary.

Three seeds were sown in each pot and placed in the growth chamber. After 12 days, the germination was evaluated, and plants were thinned to one lettuce per pot.

The leaf chlorophyll concentrations were measured with a Chlorophyll Meter (SPAD-502Plus, Konica Minolta Inc.) on the same pair of leaves at day 56. Two SPAD readings were taken at the same time of the day to minimize the potential effects of light intensity on chloroplast. Chlorophyll fluorescence was measured through Fv/Fm (ratio of variable to maximum fluorescence) that reflects the quantum efficiency of open photosystem II centres (Maxwell and Johnson, 2000). Chlorophyll fluorescence was measured on the same pair of leaves at day 56 using a fluorometer (OS30p+, Opti-Sciences, Inc., USA). Plants were adapted for 30 min in total darkness prior to measurements, and dark-adapted Fv/Fm was estimated using the saturation pulse method (Maxwell and Johnson, 2000).

After 72 days, plants were cut close to the surface above ground and biomass was harvested, cleaned with deionised water and dried at 60°C for 72 hours. After dried, the shoot dry weight was determined and milled to perform nutritional analyses. Soil was homogenised before sampling to determine NH_4^+-N NO_3^--N and PO_4^{3-}-P concentration and after that was air-dried to obtain the lettuce's roots remnants which were cleaned to determine the root dry weight.

4.2.5. Soil CO_2 evolution analyses

CO_2 evolution was determined during 15 days in the soil incubation experiments with two biochars (OC650 and CM400). The impact of the simultaneous addition of different N sources: mineral fertilisation (F) and sheep manure (M) was also studied. The CO_2 evolved from the treatments was measured by means of an automated system for continuous gas sampling and analysis (Mondini et al., 2010). The system operates as an "open chamber" system in which the glass containers of incubation experiments are continuously aerated. At regular time, a single container is made a "close chamber" for a selected period of 30 min by means of two valves and the gas accumulated is recirculated to homogenise it. The gas concentration in the chamber is automatically measured at the beginning and the end of this accumulation period by gas chromatography (Varian CP-4900 Micro-GC, Palo Alto, CA, USA). Gas fluxes are calculated using the rate of change in gas concentration during the measurement period, the molecular weight of the gas, the dynamic chamber volume and the weight of the soil sample by the follow equation:

$$F\ (\mu g\ g^{-1} soil\ min^{-1}) = (\Delta C \times M \times P \times V) / (R \times T \times W)$$

Where ΔC is the change in gas concentration (ppmv) in time interval Δt (min), M is the molecular weight of the gas, P is the atmospheric pressure (atm), V is the volume of the enclosed chamber, R is the ideal gas constant (0.082054 atm L mol^{-1} $°K^{-1}$), T is the temperature (°K) and W is the weight of the soil sample (g).

The volume of the chamber (including all the interconnecting tubing and valves dead end) without soil sample was 113.4 mL. The actual volume is calculated by subtracting from the volume of the chamber the volume occupied by the soil samples with glass flaks. The latter is calculated taking into account soil weight, density and water content and the containers.

Cumulative gas production or cumulative CO_2 net respiration (µg CO_2-C g^{-1} soil) was calculated from single measurements by integrating the area under the curve of gas efflux versus time as it follows:

$$\%C\ mineralized = (cumulative\ CO_2\text{-}C\ emissions\ of\ biochars\ treatment - cumulative\ CO_2\text{-}C\ emissions\ of\ control)/(C\ supplied)*100$$

4.2.6. Chemical analyses

NO_3^--N and PO_4^{3-}-P concentration in soil was determined by ionic chromatography (HPLC) (DIONEX ICS-2100, Sunnyvale, CA, USA) in the 1:10 (w:v) aqueous extract and NH_4^+-N by a colorimetric method based on the Berthelot´s reaction in the 1:10 (w:v) 2M KCl extract (Sommer et al., 1992).

Plant-available P in soil was determined by Olsen's method, measured in 0.5M $NaHCO_3$, adjusted to pH 8.5, extract by molybdenum blue colorimetry (Kuo, 1996).

Extractable micronutrients (Fe, Cu, Mn, Zn, Mo, and B) and heavy metals (Ni, Cd, Cr and Pb) concentrations in soil incubation and in biochars were measured after their extraction with DTPA/$CaCl_2$/TEA pH 7.3, the methodology was adapted to alkaline soils (Lindsay and Norvell, 1978), by Inductively Coupled Plasma spectroscopy (ICP-OES) (ICAP 6500 DUO THERMO, England).

Total concentrations of heavy metals (Cd, Cr, Ni and Pb) in lettuces were measured after HNO_3/H_2O_2 digestion by using inductively coupled plasma (ICAP 6500 DUO THERMO, England).

Chemical analyses are expressed on an oven-dry basis (105ºC, 24h) and represent the mean of at least triplicate analyses.

Phytotoxicity in biochars was performed by the *Lepidium sativum* test and expressed as germination index (GI), according to Zucconi et al. (1981).

4.2.7. Calculations and statistical analyses.

The N mineralization/immobilization rate at the end of incubation experiment was calculated according to the equations developed by Tammeorg et al. (2013):

Net N mineralisation ($\Delta N_{t,F,B}$) in each amendment and biochar treatment was calculated as a difference from the initial and final mineral N content (NO_3^--N + NH_4^+-N) during the incubation as it follows:

$$\Delta N_{t,F,B} = N_{30,F,B} - N_{0,F,B}$$

Where $\Delta N_{t,F,B}$ is the amount of net N mineralisation (mg N Kg^{-1} soil), and $N_{30,F,B}$ and $N_{0,F,B}$ are the mineral N contents at day 30 and day 0, respectively, for a N fertiliser source (F) and each biochar and its application rate (B) (mg N Kg^{-1} soil). F was considered 0 when no N fertiliser (manure or DPA) was added:

$$\Delta N_{t,0,B} = N_{30,0,B} - N_{0,0,B}$$

Subsequently, the effect of biochar application on the net N mineralisation/immobilisation at a given time (t=0 and t=30) was calculated for each N fertiliser source treatment (F) by subtracting the corresponding amount of net N mineralisation in the soil with no added biochar (B=0):

$$\Delta N^{eff}_{t,F,B}=\Delta N_{t,F,B} - \Delta N_{t,F,0} = (N_{30,F,B}-N_{0,F,B}) - (N_{30,F,0}-N_{0,F,0})$$

Where $\Delta N^{eff}_{t,F,B}$ is the net effect of biochar addition on the N mineralization/immobilization in soil (mg N Kg^{-1} soil), F was considered 0 when no N fertiliser (manure or DPA) was added:

$$\Delta N^{eff}_{t,0,B}=\Delta N_{t,0,B} - \Delta N_{t,0,0} = (N_{30,0,B}-N_{0,0,B}) - (N_{30,0,0}-N_{0,0,0})$$

The same equations were used to calculate the P mineralization/immobilization rate at the end of the incubation experiment.

Nitrogen uptake efficiency (NUE) in pot trials was calculated as the amount of N uptake by plants per N supplied per kilogram of fertilised soil (N uptake/N supplied) (Moll et al., 1982).

Statistical analyses of data were performed using the IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). The significance of differences was analysed by one-way analysis of variance (ANOVA) and was defined as p<0.05 by Tukey HSD test.

4.3. Results and discussion

4.3.1. CO_2 evolution in biochar amended soils

Cumulative net respiration (CO_2 emission) was studied in soils amended with two contrasting biochars (OC650 and CM400) and also in combination with organic and mineral fertilisation: manure and DAP. OC650 was selected as a reference biochar, representing a typical lignocellulosic material with a high total carbon and low ash content. On the other hand, CM400 was selected as a biochar produced at low temperature from a pre-treated organic waste, rich in nutrients and relatively low total carbon.

Cumulative soil CO_2 emissions were low in all treatments, ranging from 49 to 130 µg C-CO_2 g^{-1} soil, at the end of the incubation. They all showed a similar trend, characterised by a steady increase during the incubation (Figure 4.1), that is a common behaviour of these thermochemically converted materials (Hamer et al., 2004). Only in the case of soils exclusively amended with biochar, there was a different behaviour for each biochar; whereas OC650 showed a lower respiration than the control soil, in the case of CM400 a significant higher soil respiration was recorded compared to the control. The addition of CM400 biochar caused a short-term increase in soil respiration, which is a direct effect of an increased microbial activity. CM400 can provide energy-rich organic compounds for the microorganisms from the easily decomposable non-aromatic fraction of biochar (Bruun et al., 2012; Jones et al., 2011; Smith et al., 2010). This fraction was not thermochemically altered during the pyrolysis, as reflected by its high volatile content (26.4%) and molar H/C org ratio (1.31) (IBI, 2014) (Figure 3.4 and Table 3.5, respectively, in Chapter 3).

This increase in microbial respiration might have happened in soil amended with OC650 but with a lower intensity, mainly due to the fact that OC650 is a biochar with highly condensed aromatic structures (low volatile content (13.8%) and molar H/C org ratio (0.28)) and therefore thermochemically converted (IBI, 2014). Therefore, the amount of easily available C for the microorganisms was low and it did not have a clear effect on soil respiration (Cross and Sohi, 2011; Luo et al., 2011). In previous works, similar results were found for biochars similar to OC650, produced from hardwood at high temperatures (650ºC) (Lopez-Cano, 2012; Zimmerman et al., 2011).

An abiotic mechanism might be responsible for the apparent decrease in soil respiration in the OC650 treatment. Fornes et al.

(2015) observed that CO_2 can react with alkaline cations present in biochar leading to the formation of carbonates. This mechanism may be present in both biochar (OC650 and CM400) amended soils since both treatments present high alkaline pH (pH 9.4), and contain similar high levels of alkaline cations (K, Na, Ca, and Mg). Thus, biochar can act as an alkaline CO_2 trap, facilitating the fixation of CO_2 as HCO_3^- or even CO_3^{2-}. The inverse mechanism was also observed by Jones et al. (2011) in an acidic soil where dissolved inorganic C was converted to CO_2. This abiotic mechanism may be predominant in OC650 but it should be also present in CM400 biochar-treatment, although its impact may be masked by the high microbial activity.

The physicochemical properties of the biochar also determined their degradation pattern in soil. The differences in aromaticity, ash and volatile contents of both biochars could have also affected differently the microbial populations and even the adsorption of nutrients in the biochar surface, having a distinctive impact on the cumulative net respiration, but these mechanisms are unclear (Liang et al., 2006; Whitman et al., 2015).

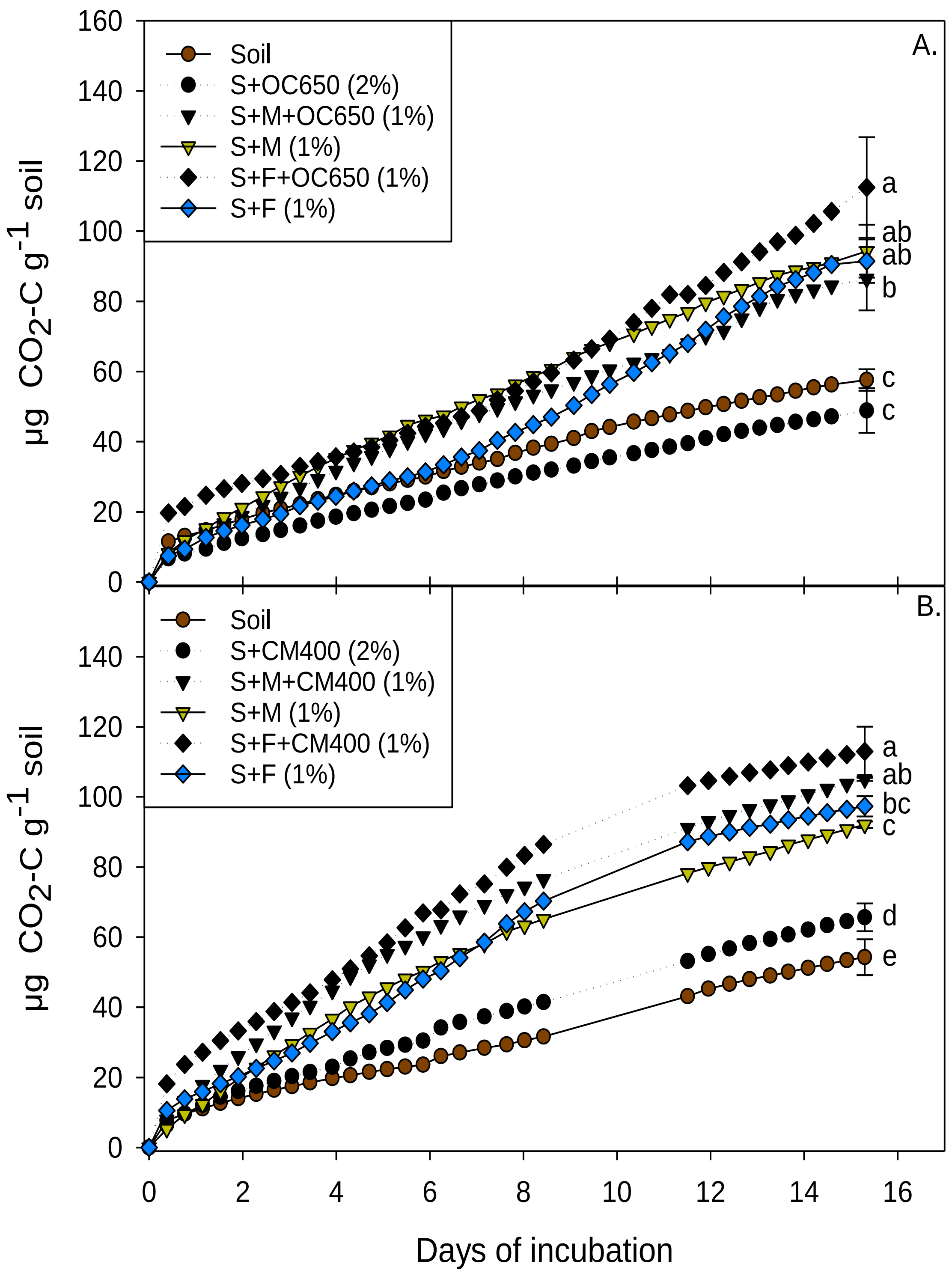

Figure 4.1: Cumulative CO_2-C net respiration during incubation of soil amended with biochars OC650 (A) and CM400 (B) and the presence of manure (M) or mineral fertilisation (F). Error bar represent standard deviation (n=2). Different letters in each plot indicate significant differences at the $p<0.05$ level.

The biochar effect on the N-fertilised treatments was different for each biochar (Figure 4.1). The presence of OC650 did not affect the not show statistical differences. However, in the case of CM400 the combination with manure and mineral fertiliser caused a higher CO_2 evolution than the treatment without biochar.

The effect of adding N fertilisers was also different for each biochar. Whereas the reference biochar, OC650, characterised by a stable lignocellulosic material was not affected by the presence of manure or fertiliser, in the case of CM400, with a high volatile content and H/C org ratio, the presence of an additional source of N and other nutrients increased its microbial degradation in soil.

Figure 4.2 shows the mineralised C for each treatment, expressed as the percentage of C supplied by the amendment. The results ranged from 0.15 to 1.75% of the added C, which represent a low proportion of mineralised C, in accordance to previous result obtained for a broad range of biochars and soil types (Whitman et al., 2015). In the case of the treatment with OC650 alone, there was a negative cumulative C mineralisation (-0.06% of the added C), reflecting the fixation of CO_2 in soil, already explained above. The addition of different N sources to the reference biochar (OC650) caused a similar increase in the percentage of mineralised C, from -0.06 to 0.3%. In the case of CM400, the addition of different N sources showed significant differences in the amount of mineralised C. The addition of manure caused a higher degradation of the added C, probably caused by an enhancement of soil microbial activity. The different characteristics of biochars, mainly due to the different feedstock and pyrolysis temperature, can explain the different C mineralisation patterns for both biochars (Luo et al., 2011). However,

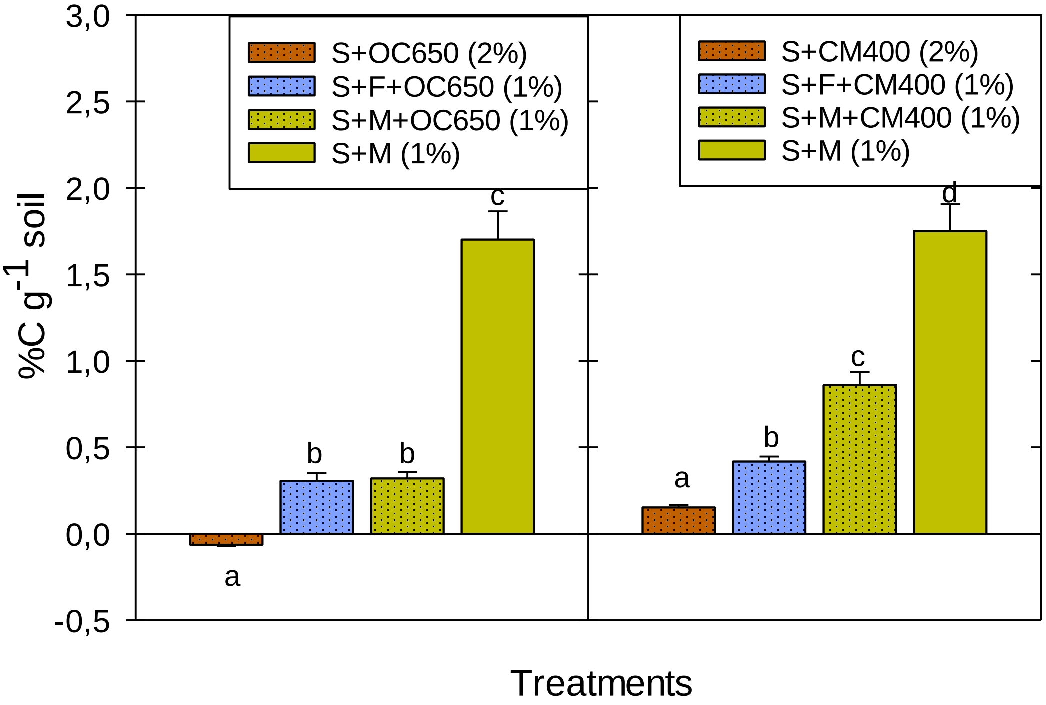

Figure 4.2: Cumulative C mineralised during the incubation, expressed as the percentage of the added C with the amendment, for the studied biochars OC650 and CM400. Error bars represent standard deviation (n=2). Different letters in each plot indicate significant differences at the $p<0.05$ level.

the amount of C released as CO_2 from the soil only represented a small fraction of the C contained in the biochar and does not compromise its potential to sequester C in soil.

4.3.2. N evolution in soil: ammonification and nitrification process.

The N mineralisation dynamics were studied in soils amended with different biochars: OC650, OC450, O400, GH400, GW400, CM400, PM400 and PC400, and in combination with additional N sources (manure and mineral fertilisation). N mineralisation was studied through ammonification and nitrification processes, which were monitored by changes in the concentrations of NH_4^+-N and NO_3^--N during incubation (Figure 4.3).

The addition of biochar alone, without an additional N source, did not significantly affect soil N dynamics in soils. This behaviour was observed for all biochars added at both rates, 1% and 2%, due to the low supply of mineral N by the biochar and its low mineralization rate in soil (chemical recalcitrance).

On the other hand, biochars showed different interactions with N mineralisation dynamics in soil amended with manure and mineral fertilisation (Figure 4.3). The addition of both N sources to the soil caused a significant increase in extractable NH_4^+-N at day 3, as a consequence of the mineralisation of the added organic matter in the case of manure, or the direct supply from DAP.

Previous studies have reported a decrease of N availability in biochar amended soils due to the ammonium retention capacity of biochars (Singh et al., 2010; Steiner et al., 2008). However, the simultaneous addition of biochar with the N sources did not have a clear effect on the amount of NH_4^+-N at day 3, compared to the soil amended with manure (S+M (1%)) or fertiliser alone (S+F (1%)). Only in the case of raw or rich lignocellulosic biochars (OC450, O400 and CM400) there was a significant reduction in the amount of extractable NH_4^+-N, compared to the treatments prepared only with manure (S+ M (1%)) or mineral fertilisation (S+ F (1%)). Biochars prepared from pre-treated organic feedstocks, which contained lower amount of C and higher ash content, were not effective in retaining N in soil and the levels of soil NH_4^+-N were not significantly different from the control.

From day 7 to the end of the incubation, all treatments recorded a sharp reduction of NH_4^+-N and an increase of NO_3^--N, as a consequence of the nitrification process. After 30 days of incubation, all biochar amended soils showed similar NO_3^--N levels than the treatment with only manure (S+M (1%)) but a decrease was observed in treatments with mineral fertilisation (S+B+F (1%)). Especially, the addition of OC650, OC450 or O400 (raw lignocellulosic biochars) originated a similar behaviour regarding the nitrification process in the soil amended with fertiliser (S+F (1%)), characterised by a sharp increase of NO_3^--N between the third and seventh day of incubation. The levels of NO_3^--N increased mainly after the 7th day of incubation. This effect was not so clear in soils amended with manure, where the availability of ammonium depends on the organic matter mineralization.

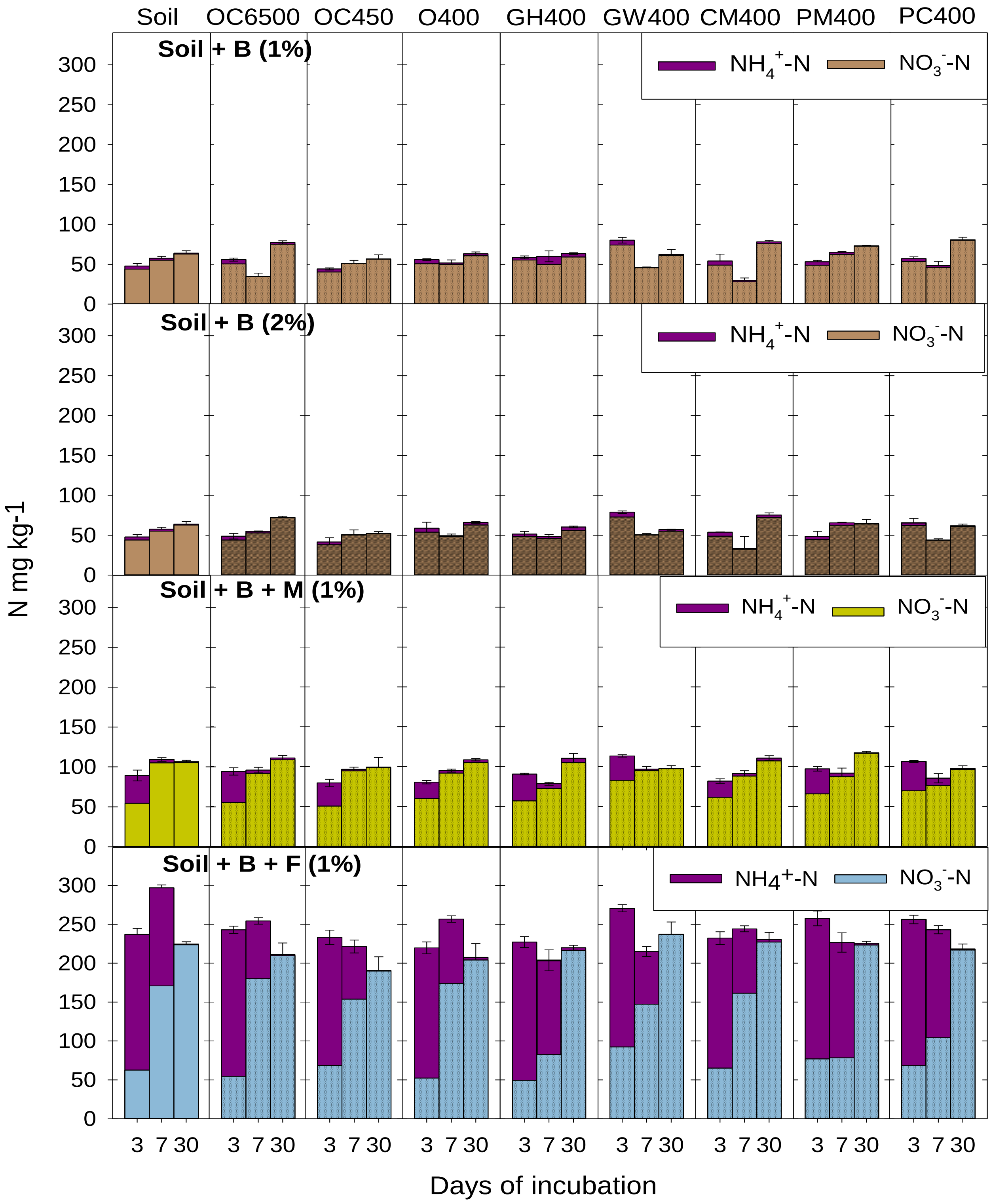

Figure 4.3: Changes in the amounts of mineral N (mg Kg-1 soil as NH4$^+$-N plus NO3$^-$-N) during the incubation of: soil; soil amendment biochars at two doses (S+B (1%) and S+B (2%)) or in combination with manure (S+B+M (1%)) or mineral fertiliser (S+B+F (1%)). Error bars represent standard deviation (n=3).

Figure 4.4 shows the effect of biochar application on the net N mineralisation ($\Delta N>0$) or immobilisation ($\Delta N<0$) rate at the end of the incubation experiment. The positive values were considered as N mineralisation, and therefore it represents an increment of mineral N as NH_4^+-N and NO_3^--N. However, the negative values were considered as a decrease of mineral N, either N immobilisation due to organic N transformation or N losses as volatilisation of NH_3 and N_2O.

The net effect of biochars application on soil mineral N at 1% and 2% was relatively low, because of the lower content of NH_4^+-N and NO_3^--N in biochar, compared with soil. Nevertheless, the biochars from pre-treated organic wastes, which have high ash content (rich in minerals) and low C org, mixed at 1% showed a significant higher mineralisation rate than raw lignocellulosic biochars. This effect could be due to the low fixed C and higher N content of biochars from pre-treated feedstocks, being more easily degradable. However, the addition of OC650 also promoted net N mineralisation despite its low mineral content. This effect was unexpected for hardwood biochars (Bruun et al., 2012; Mandal et al., 2016). On the other hand, the rest of raw lignocellulosic biochars (O400, GH400) and GW400 which did not show any significant effect on soil mineral N, which is in agreement with previous works using this type of biochar (Bruun et al., 2012; Mandal et al., 2016).

Increasing biochar application rates to soil (S+B (2%)), caused an increase in the N immobilisation, which is in agreement with previous incubations studies (Tammeorg et al., 2013) and especially in alkaline soils incubations (Ippolito et al., 2014, 2016). This immobilisation could be attributed to the alkalinity and high adsorption capacity of biochars (Taghizadeh-Toosi et al., 2012) and also to the presence of volatile carbon, which could lead to a N immobilisation by soil microorganisms. This effect was more evident in the treatments with high volatile content and molar H/C org ratio (less thermochemically converted) biochars. Thus, the effect of biochar on soil N mineralisation/immobilisation may differ significantly between biochars according to the feedstock and pyrolysis conditions (Tammeorg et al., 2013), but the increase in biochar application rate may have masked this effect.

Biochars did not show a clear effect on net N mineralisation in soil amended with manure. Thus, it seems that the mineral N produced by ammonification and nitrification processes was controlled by the organic matter mineralisation of manure, rather than the impact of the biochars.

The effect of biochar application on soils amended with mineral fertilisation was affected by the properties of biochars. Raw lignocellulosic biochars showed greater N immobilisation values than biochars obtained from pre-treated feedstocks. These results are in agreement with previous works (Bruun et al., 2012; Mandal et al., 2016; Taghizadeh-Toosi et al., 2012), where different mechanism for biochar-induced mitigation of NH_3 volatilisation were elucidated: NH_3 adsorption in micro pores, NH_4^+ and NO_3^- adsorption in cation exchange sites, oxidation of NH_4^+ to NO_3^-, protonation of NH_3 induced conversion to NH_4^+ and the possible NH_4^+ immobilisation induced by the microbial activity.

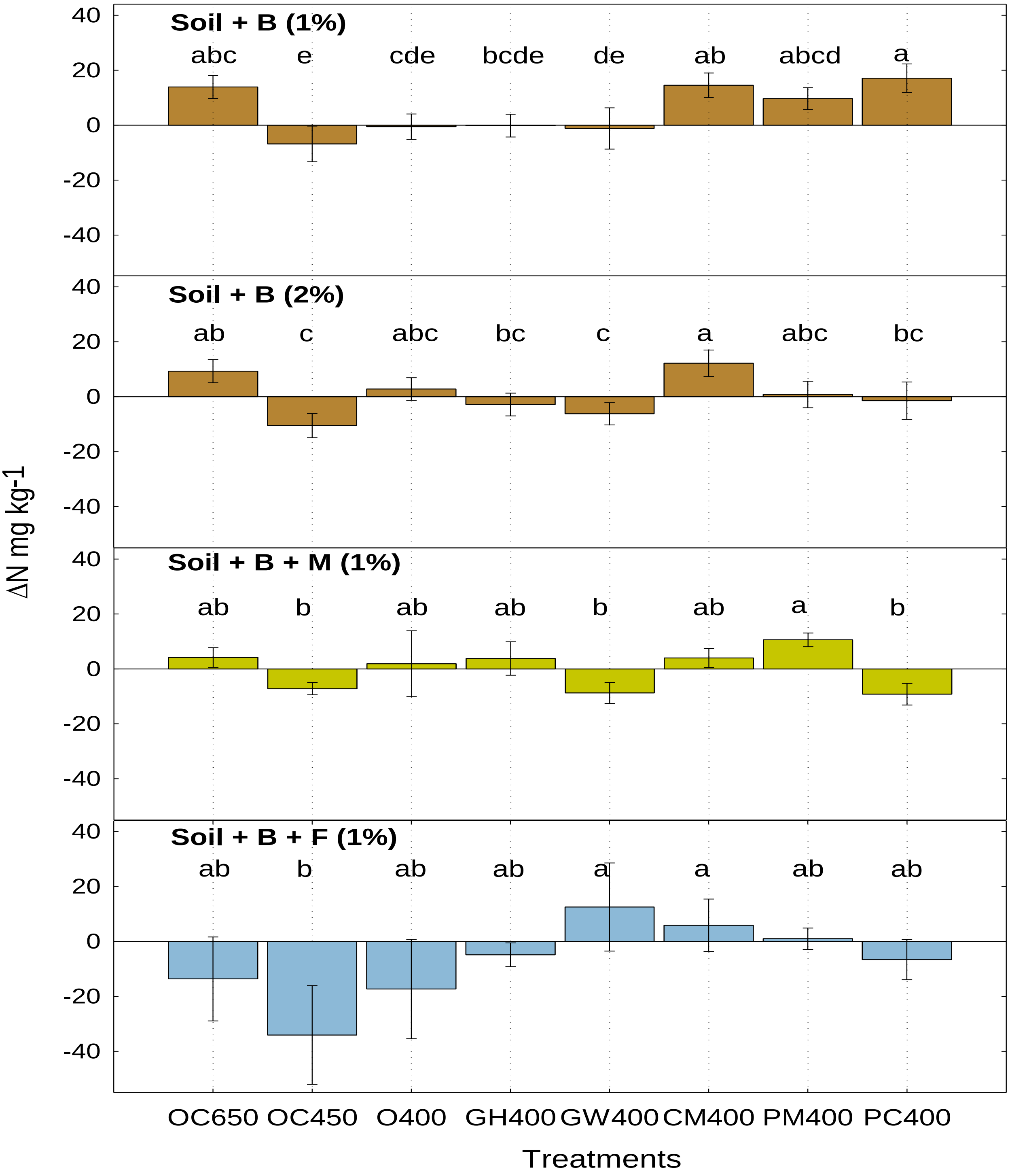

Figure 4.4: Effect of biochars on the soil N mineralisation (ΔN>0) or immobilisation (ΔN<0). OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature. Error bars represent standard deviation (n=3). Different letters in each plot indicate significant differences at the p<0.05 level**.**

4.3.3. Dynamics of available P in soil

An overview of the impact of the different biochars on available P dynamics (water soluble P and P_{OLSEN}) during soil incubation experiments is shown in Figure 4.5.

In general, the soil incubation experiments recorded low amounts of water soluble P, which was expected due to the alkaline nature of soil.

The addition of biochar did not affect the behaviour of P dynamics in the unamended soil (without a N source). A similar trend was found between the biochars at 1 and 2%, due to the low supply of mineral P with the biochar (Table 3.6, Chapter 3). The only exception was PM400, which showed the highest values of soluble P in soil, caused by its greater concentration of P in the biochar.

The water soluble P levels slightly increased after 3 days of incubation in the soils amended with either manure or mineral fertiliser. Manure is a well-known source of soluble and easily available forms of P, organic P and inorganic phosphate compounds, and they represent a traditional source of P for agriculture (MacDonald et al., 2011; Sharpley and Moyer, 2000). Thus, when phosphate from the fertilizer or manure comes in contact with the alkaline soils, various reactions take place making the phosphate less soluble and less available, mostly precipitated as calcium salts. In general, biochar application increased the water soluble P levels after 3 days in the soils amended simultaneously with biochar and the manure (S+B+M (1%)) or the fertilizer (S+B+F (1%)). The addition of biochar generally caused an increase in the amount of available P (P_{OLSEN}). The increase in available P was noticeable even after 30 days of incubation but only small variations were observed during the incubation period. Even in the case of PM400, which caused a significant increase of available P in treatment S+B (2%), there was no significant impact of this biochar when combined with manure or mineral fertilisation (S+B+M (1%), and S+B+F (1%) treatments).

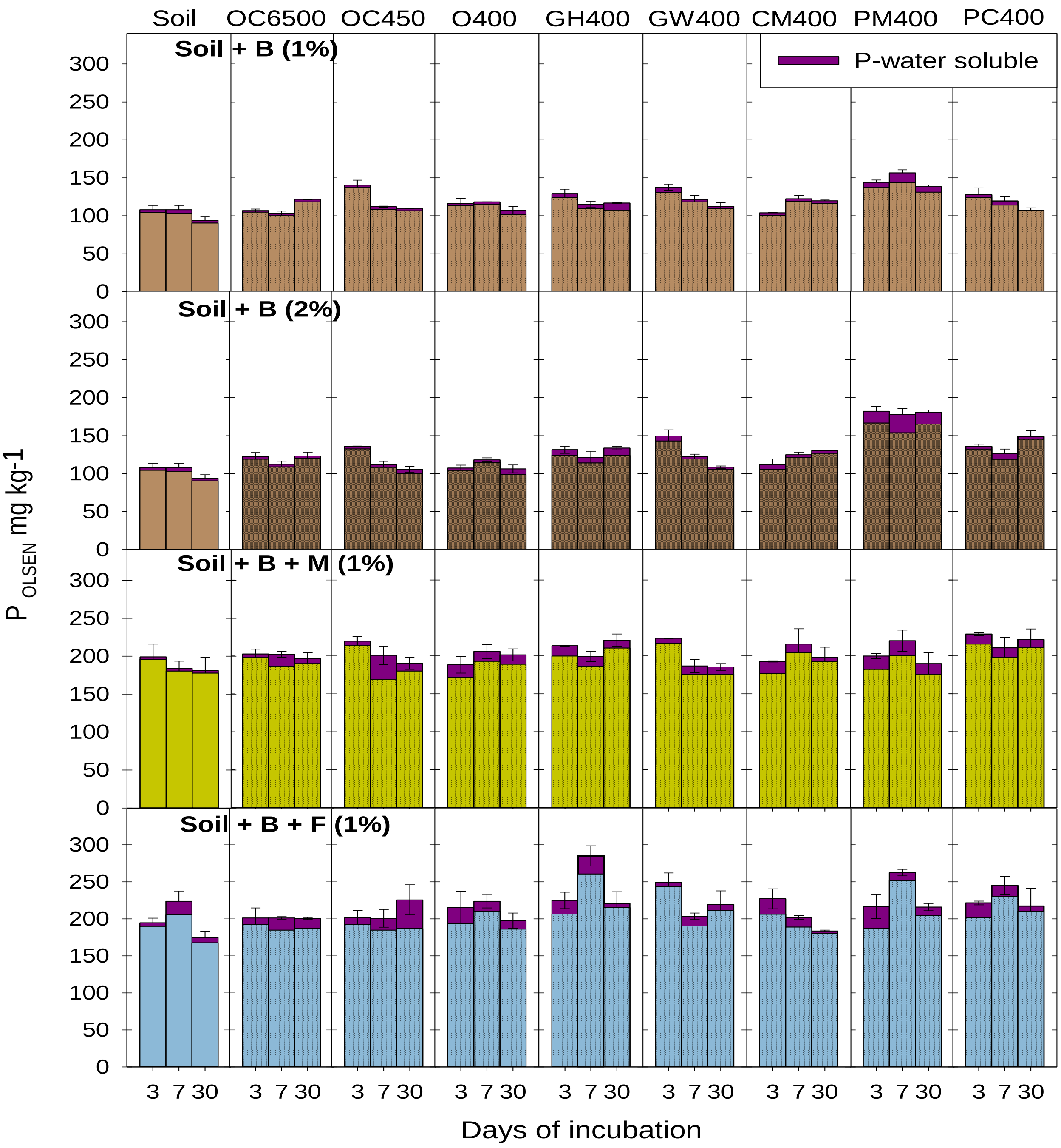

Figure 4.5: Changes in the amounts of water soluble and available P (P_{OLSEN}) (mg Kg-1) during the incubation of: soil; soil amended with biochars at two doses (S+B (1%) and S+B (2%)) or in combination with manure (S+B+M (1%)) or mineral fertiliser (S+B+F (1%)). OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature. Error bars represent standard deviation (n=3).

Figure 4.6 shows the effect of biochar application on the net P mineralisation (ΔP_{OLSEN}>) or immobilisation ($\Delta P_{OLSEN}<0$) rate at the end of incubation experiment. The positive values indicate P mineralisation, since it represents an increment of available P. However, the negative values indicate a decrease of available P, which imply P immobilisation due to the formation of dibasic calcium phosphate dehydrate ($CaHPO_4 \cdot 2H_2O$), octocalcium phosphate $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, or its crystallisation as hydroxyapatite $Ca_5(PO_4)_3(OH)$ in the soil or by other mechanism associated with the biochar.

Biochars usually contain three pools of P, one which is freely soluble, one which is strongly bound to Fe and Al, and the last one which remains organically bound as a residue of the original feedstock and its proportions are dependent on feedstock and pyrolysis conditions (DeLuca et al., 2015). The net effect of biochars at 1% on soil P availability was not significant. At 2% application rate, significant differences were found for biochars obtained from pre-treated organic waste (CM400 and PC400), PM400 and OC650, which showed higher available P values than raw lignocellulosic biochars. Despite the low total P content in OC650, this treatment showed a large P availability at the end of the soil incubation. The bioavailability of P primarily depends on P composition (different chemical forms) in the feedstocks which may affect its subsequent availability after entering the soil (DeLuca et al., 2015; Wang et al., 2012). On the other hand, O400 and OC400 biochar treatments did not show any significant effect on P availability and GH400 and GW400 treatments led to a slight P immobilisation. The difference between OC650, OC450 and O400 biochars could be caused by the fact that organic P present in plant tissues do not volatilise until approximately 700ºC (Knicker, 2007). So the pyrolysis of raw lignocellulosic feedstock can enhance P availability from plant tissue by volatilizing large amounts of C and cleaving organic P bonds, resulting in a residue of soluble P salts associated with the biochar (DeLuca et al., 2015).

Biochars did not show a clear effect on net P mineralisation in soil amended with manure. Thus, it seems that similarly to the case of N, the complex mechanisms of organic matter mineralisation of the manure masked the impact of biochars.

On the other hand, the interaction of biochar with mineral fertilisation showed a different behaviour respect to S+B (2%). Thus, rich lignocellulosic biochars (OC450, O400 and GW400) showed the highest mineralisation values, whereas biochars obtained from pre-treated feedstocks (PM400, PC400 and OC650) recorded slightly lower P mineralisation. To date, however, there is a noted lack of studies evaluating the short-term effect of biochar on P cycling and availability. It seems that a variety of mechanisms through which biochar may directly or indirectly influence the biotic and abiotic components of the P cycle (DeLuca et al., 2015), especially when the phosphorus is added in excess. Thus, the sorption of chelates may have a positive or negative influence on P solubility; due to the favourable surface properties of biochars for sorbing organic molecules. In addition, biochar could act as a promoter of microbial activity and P mineralization, providing greater access to organic and insoluble inorganic pools of P (DeLuca et al., 2015).

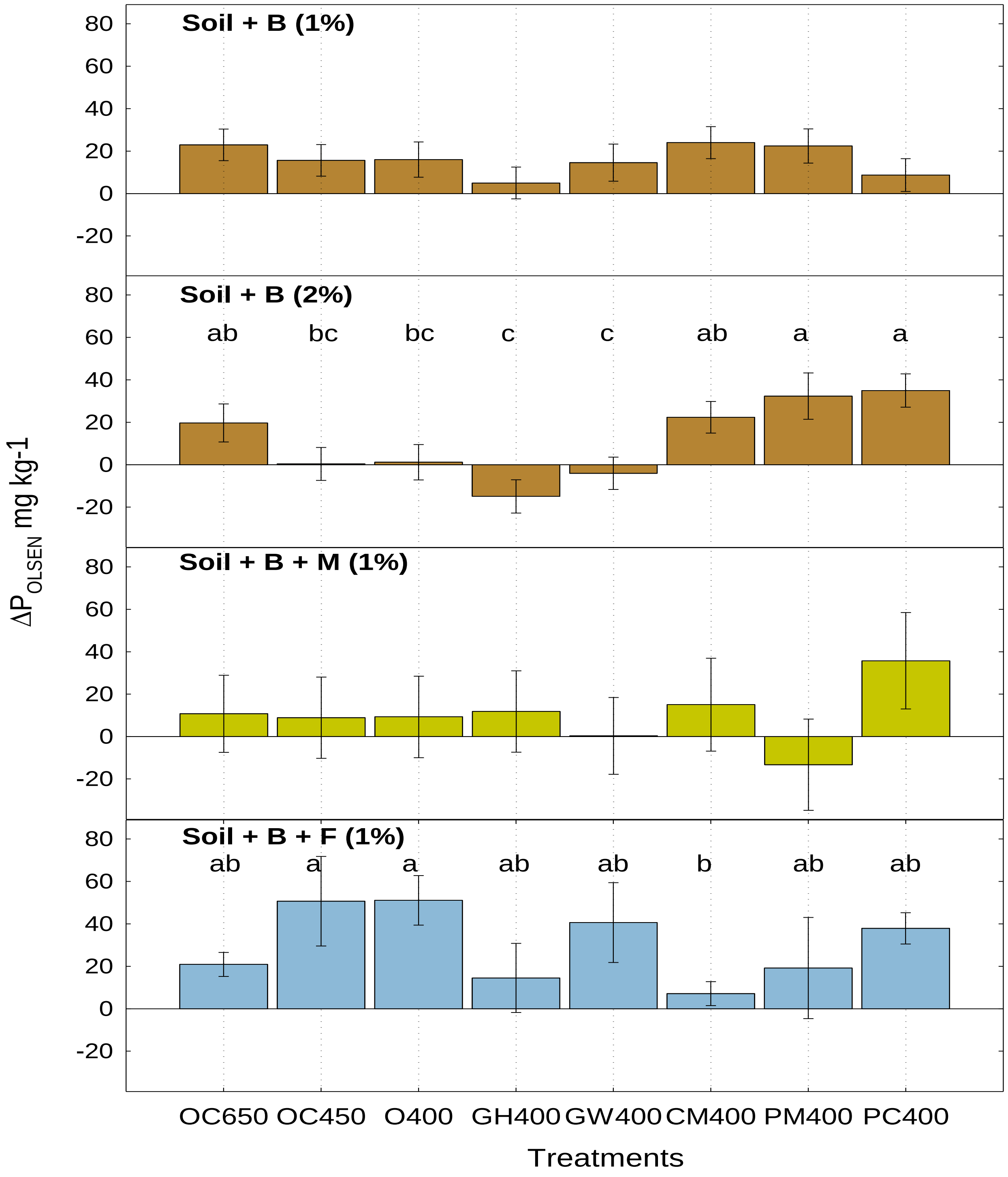

Figure 4.6: Effect of biochars on the soil P mineralisation ($\Delta P>0$) or immobilisation ($\Delta P<0$). OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature. Error bars represent standard deviation (n=3). Different letters in each plot indicate significant differences at the $p<0.05$ level.

4.3.4. Dynamics of available micro nutrients in soil

Micro nutrients also play an important role in the mineral nutrition for plant growth. Although their contents in some raw lignocellulosic biochars are rather low (Table 3.6, Chapter 3), biochars prepared from urban wastes (GW400, CM400, and PC400) and from manure (PM400) present high mineral contents, and may have agronomic relevance. The availability of these micronutrients in the biochar itself and after soil application was evaluated by the changes in the DTPA-extractable fraction.

Table 4.3 shows DTPA-extractable micronutrient (Fe, Cu, Mn, Zn and B) concentrations in biochars. In general, the amount of DTPA extractable elements represented less than 10% of the total concentration (Table 4.4) reflecting the low mobility of these elements in the biochar itself.

The soil dynamics of DTPA-available micronutrients Fe, Cu, Mn, Zn and B during the incubation are shown in Figures 4.7, 4.8, 4.9, 4.10 and 4.11, respectively.

In general, the addition of biochar did not increase the amount of DTPA extractable micronutrients, with the only exception of the concentration of Mn (Figure 4.9). Increasing biochar application rate to the soil did not affect DTPA-available content of micronutrients in spite of the great differences between mineral concentrations in the different biochars, especially in the case of biochars prepared from pre-treated organic wastes, such as CM400, PC400, GW400 and PM400, characterised by a high content in Fe, Cu, Mn and Zn (Chapter 3, Table 3.6).

Table 4.3: DTPA-extractable micronutrients (Fe, Cu, Mn, Zn and B) concentrations in biochars. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**Fe**	**Cu**	**Mn**	**Zn**	**B**
			(mg kg^{-1})		
OC650	5.1 *(0.0)*	0.7 *(0.0)*	59.3 *(1.4)*	11.9 *(0.1)*	0.24 *(0.01)*
OC450	0.9 *(0.0)*	0.2 *(0.1)*	24.4 *(0.0)*	1.5 *(0.0)*	0.45 *(0.00)*
O400	0.1 *(0.1)*	0.4 *(0.1)*	1.6 *(1.0)*	5.6 *(0.6)*	0.39 *(0.33)*
GH400	0.1 *(0.0)*	0.5 *(0.3)*	15.2 *(1.4)*	2.0 *(0.1)*	0.82 *(0.04)*
GW400	2.9 *(0.5)*	0.7 *(0.0)*	437.0 *(1.6)*	5.4 *(0.1)*	2.12 *(0.00)*
CM400	106.9 *(1.6)*	1.4 *(0.0)*	4.4 *(0.1)*	3.9 *(0.0)*	0.82 *(0.01)*
PM400	4.2 *(0.4)*	3.3 *(0.3)*	45.8 *(0.5)*	21.5 *(0.4)*	5.58 *(0.33)*
PC400	18.9 *(0.6)*	2.7 *(0.0)*	16.1 *(0.2)*	9.1 *(0.4)*	2.07 *(0.01)*

Standard deviation in brackets (n=3).

The DTPA-available Fe and Mn concentrations decreased in the soil alone and the soil amended with organic or mineral fertilisation (Figure 4.7 and 4.9, respectively). The decreases in available Fe and Mn were likely due to mineral forms changing from more to less available over time (Ippolito et al., 2014). In general, the impact of biochar on these nutrients was not relevant, except in the case of PM400 and PC400, that showed a slight immobilisation of Fe and Mn in the soil alone and the amended soil, compared to the rest of biochars.

Table 4.4: Percentage of DTPA-extractable micronutrients (Fe, Cu, Mn, Zn and B) respect to total concentration in biochars. OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**Fe**	**Cu**	**Mn**	**Zn**	**B**
			%		
OC650	0.9	7.1	12.8	39.5	1.5
OC450	0.6	4.4	16.0	17.9	6.6
O400	0.0	2.2	3.6	7.5	2.9
GH400	0.0	2.8	22.3	5.2	3.4
GW400	0.0	1.7	24.2	2.7	5.5
CM400	1.1	1.5	2.0	1.1	2.8
PM400	0.1	3.0	12.5	4.8	8.7
PC400	0.2	2.8	5.7	2.8	7.1

In the case of Mn, there was a large variability in the impact of biochar addition, especially in the case of GH400 and GW400, which recorded the highest DTPA-extractable Mn concentration in the soil after incubation.

Biochar addition did not show any effect on Cu availability, even at the highest application rate. In alkaline soils, it is known that Cu forms strong associations with Fe and Al (hydr)oxides and thus may limit its availability (Figure 4.8). The DTPA extractable concentrations of Cu were as expected for this type of soil, in concordance to previous works (Ippolito et al., 2014). DTPA-extractable Cu concentrations in biochars showed a low solubility of this element, despite the high total concentrations in some of the biochars, such as CM400, PM400 and PC400. The addition of manure caused an increase in Cu availability, which can be almost entirely ascribed to the manure addition. Previous work in alkaline soils showed a similar short-term impact on Cu (Ippolito et al., 2014, 2016).

The pattern of DTPA-extractable Zn in soil was similar to that observed for Cu, due to a low available Zn caused by strong zinc sorption by $CaCO_3$ that is typical of alkaline soils. There were no significant effects on Zn availability, due to the addition of biochar at

1 and 2% application rate (Figure 4.10), with the exception of PM400 that showed a short-term immobilisation of Zn at day 3, despite its high DTPA-extractable Zn concentration in the biochar. The addition of manure raised the Zn availability regardless of the biochar applied.

B was slightly immobilised during the incubation due to the calcium carbonate present in soil, which acts as a sink for B in calcareous soils (Goldberg and Forster, 1991) (Figure 4.11). The addition of biochars OC450, O400, GW400, CM400 and PM400 to the soil, at 1 and 2% application rate, avoided the short-term B immobilisation. However, OC650 showed a similar behaviour than the control soil, while GH400 and PC400 showed a slight B availability at the end of incubation. These results indicated that B availability in soil was not directly related to the DTPA-extractable concentrations in biochars.

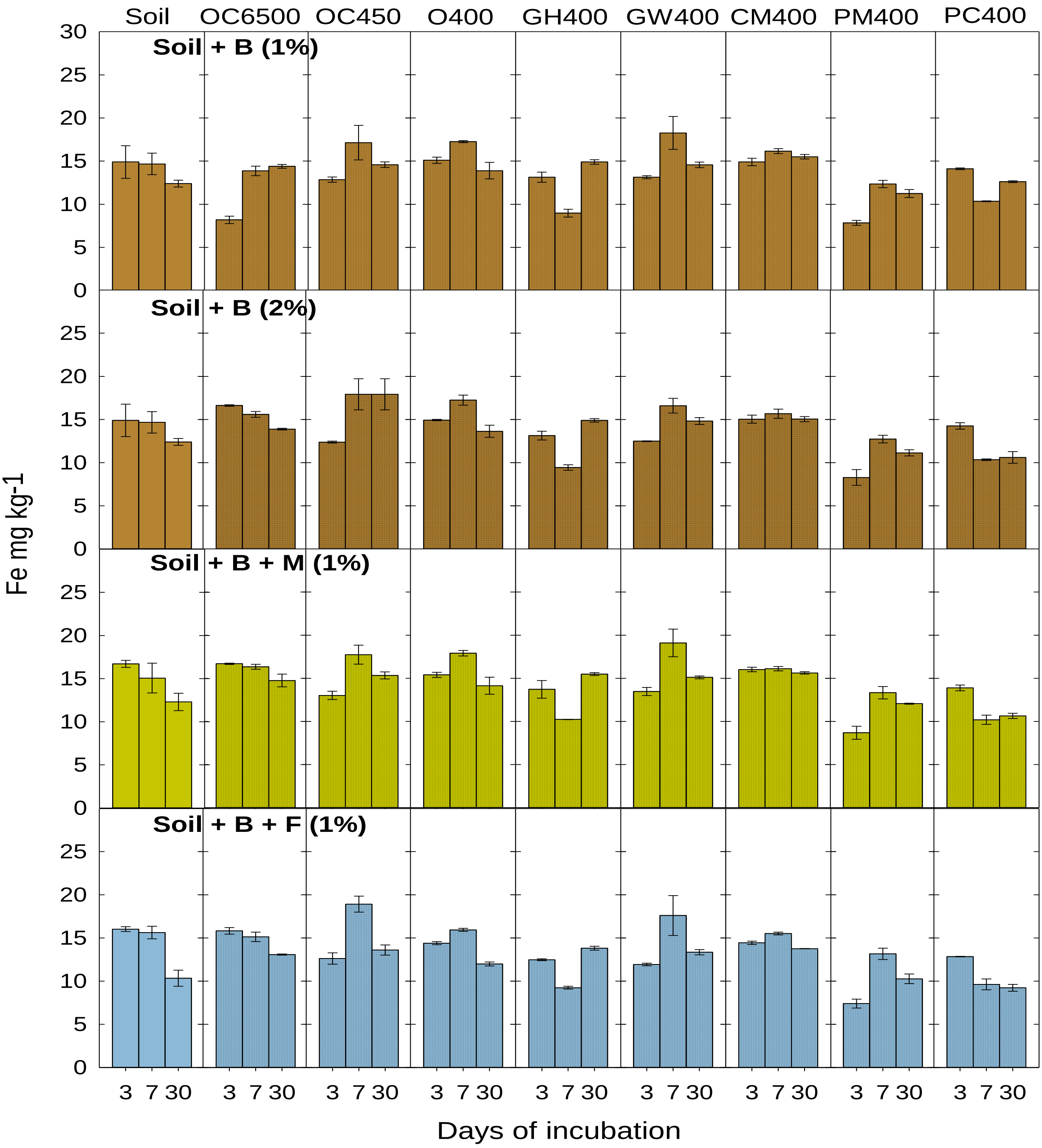

Figure 4.7: Concentration of DTPA-extractable Fe in soil during the incubation. The different treatments included: a control (soil without any amendment), soil with biochars at two doses (S+B (1%) and S+B (2%)), soil with manure (S+M (1%)) or mineral fertiliser (S+F (1%)) or soil with biochar in combination with manure (S+B+M (1%)) or with mineral fertiliser (S+B+F (1%)). Biochars included: OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature. Error bars represent standard deviation (n=3).

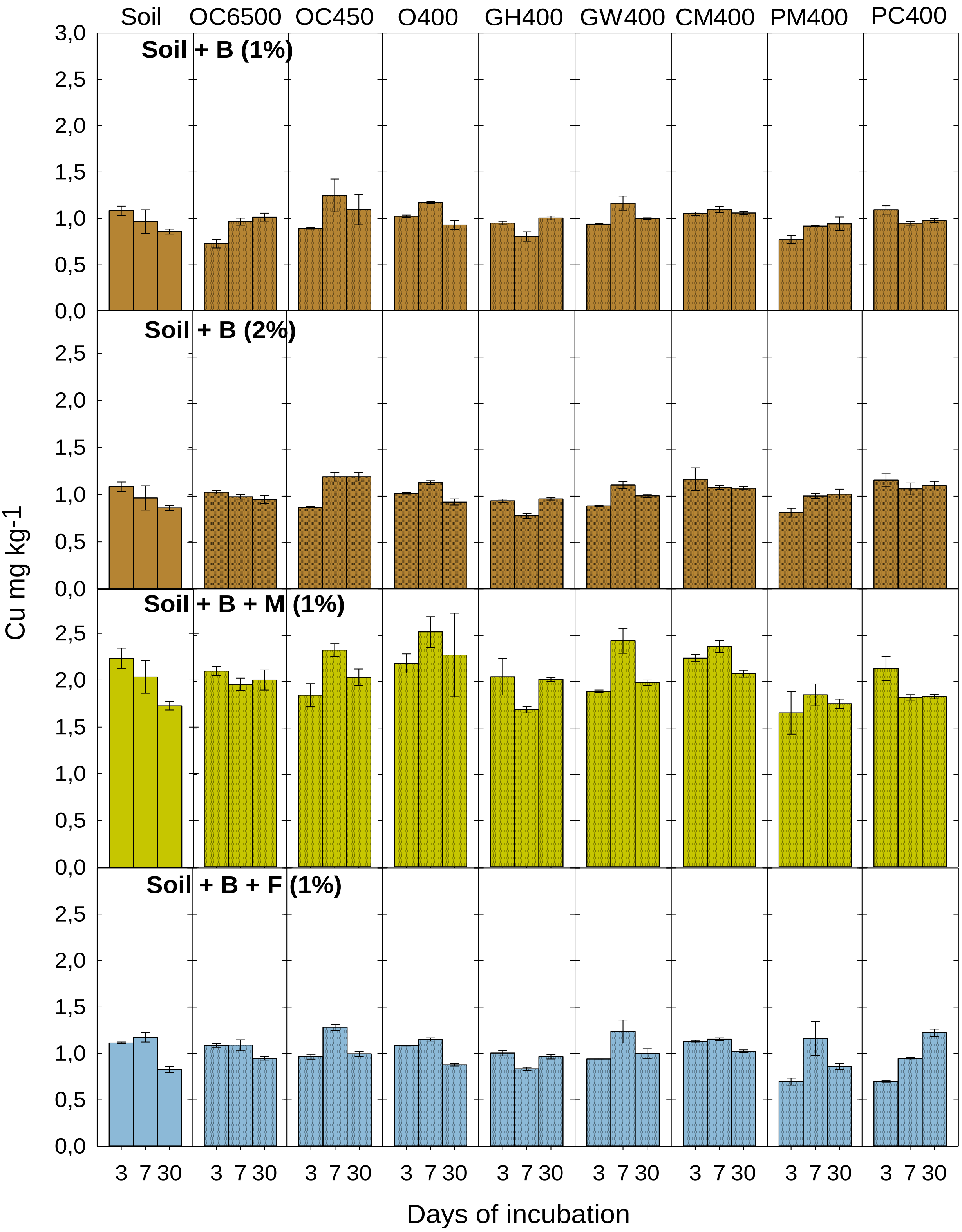

Figure 4.8: Concentration of DTPA-extractable Cu in soil during the incubation. The different treatments included were described in Figure 4.7. Error bars represent standard deviation (n=3).

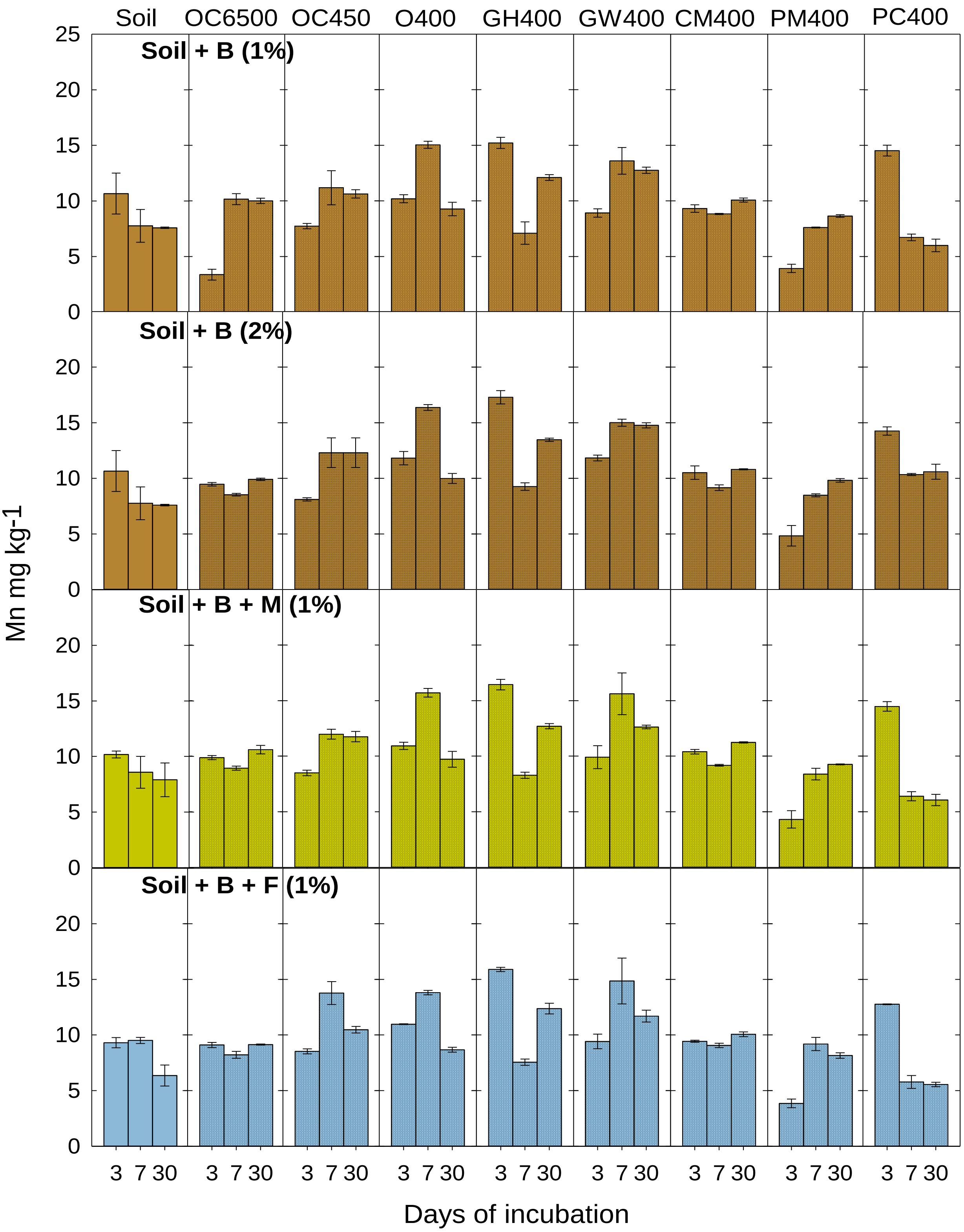

Figure 4.9: Concentration of DTPA-extractable Mn in soil during the incubation. The different treatments included were described in Figure 4.7. Error bars represent standard deviation (n=3).

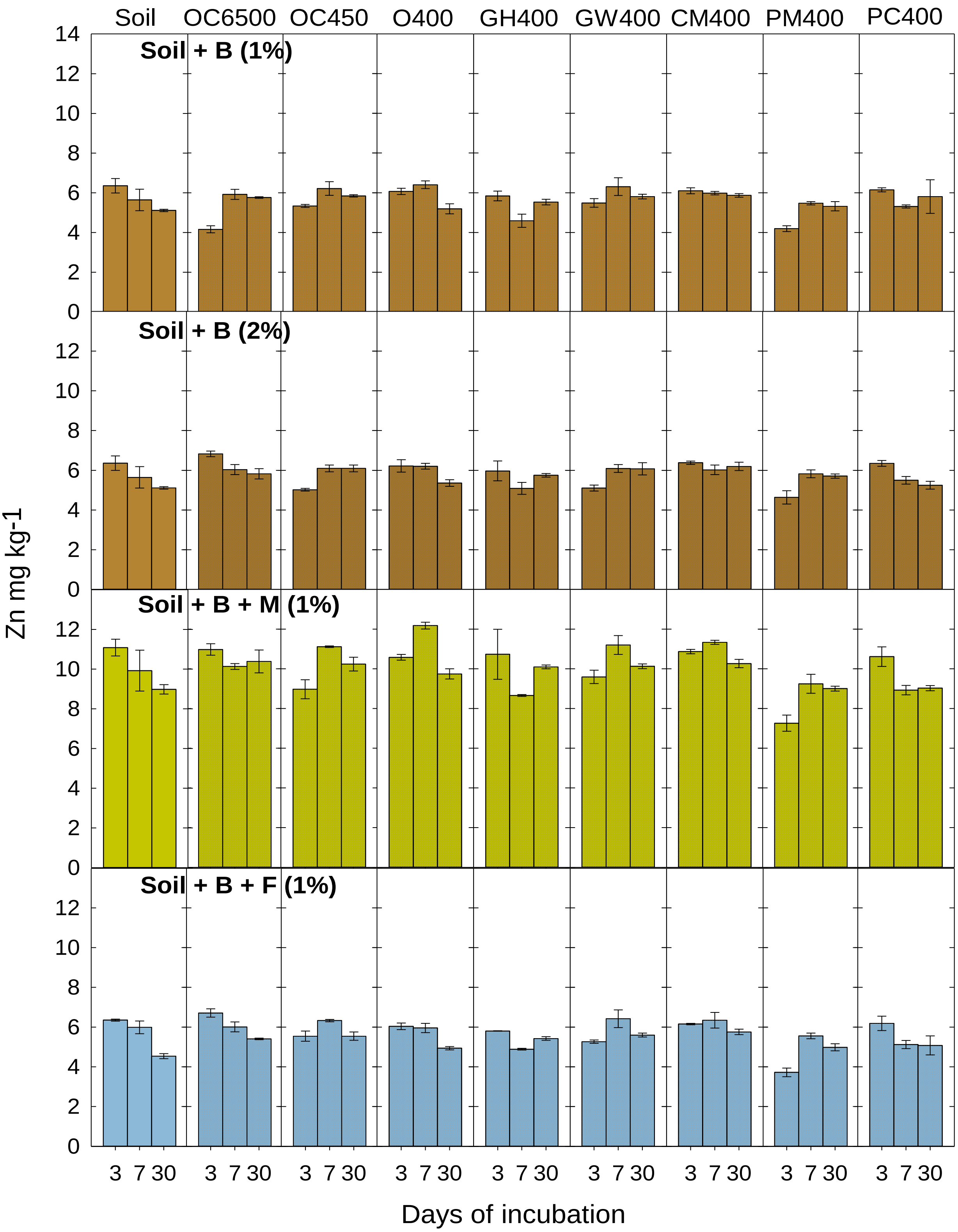

Figure 4.10: Concentration of DTPA-extractable Zn in soil during the incubation. The different treatments included were described in Figure 4.7. Error bars represent standard deviation (n=3).

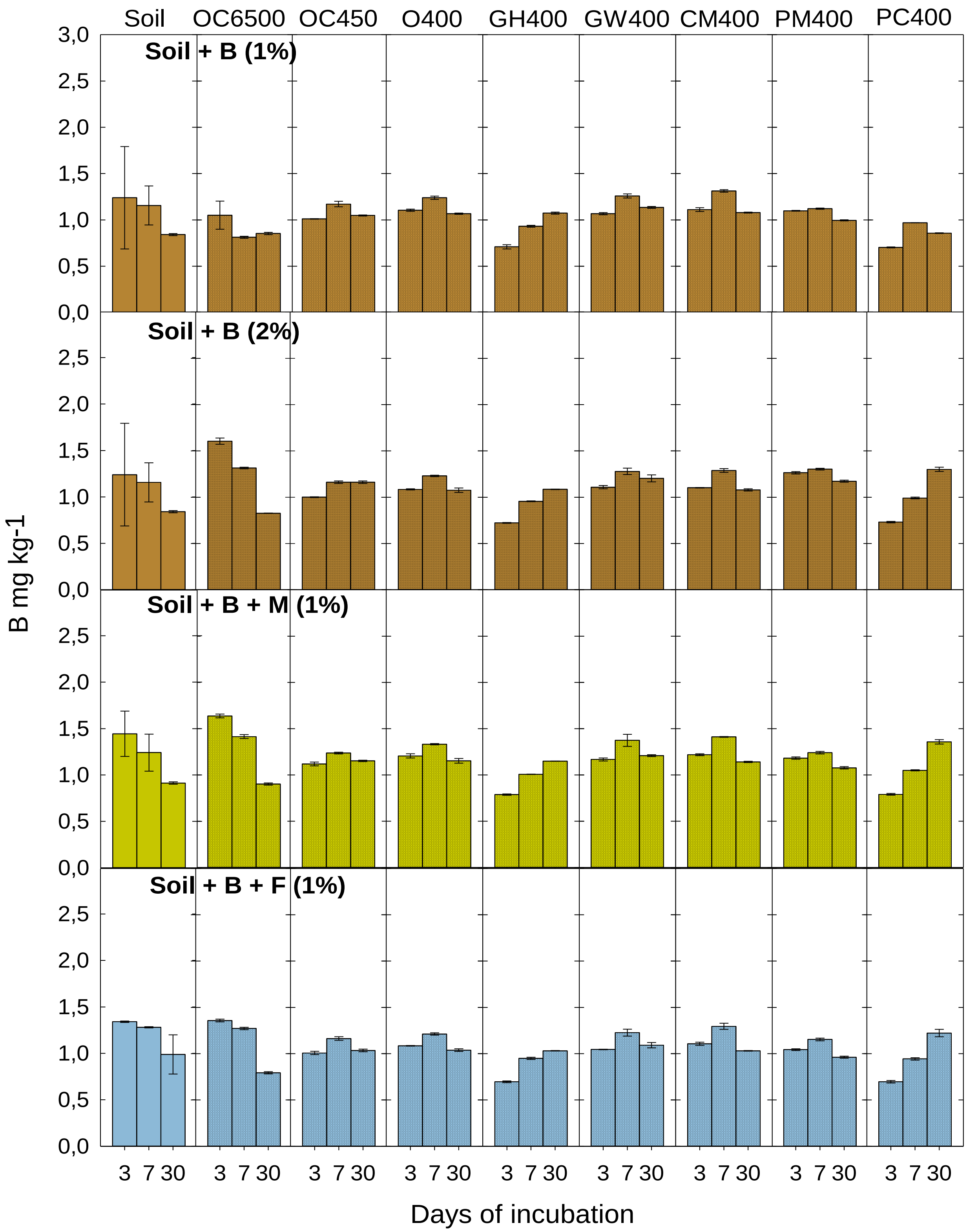

Figure 4.11: Concentration of DTPA-extractable B in soil during the incubation. The different treatments included were described in Figure 4.7. Error bars represent standard deviation (n=3).

4.3.5. Dynamics of DTPA-extractable heavy metals in soil

The presence of heavy metals in organic wastes represent an important limitation for their use in agriculture. Pyrolysis of organic wastes lead to a concentration of the heavy metals, originally present in the feedstocks, into the ash fraction of the biochars (Table 3.7, Chapter 3).

Some of the biochars studied in Chapter 3 had a high concentration of heavy metals. For instance, CM400 biochar was classified as "*not allowed as organic amendment*", and PM400, PC400, GW400 and O400 were classified as "*class B"* according to the Spanish legislation on organic amendments (BOE, 2013).

Despite the regulatory classification and the legal limitations for their use in agriculture, all the biochars obtained were studied as soil amendment to assess the fate of heavy metals in soil. The DTPA-extractable heavy metals (Cd, Cr, Ni and Pb) concentrations in biochars is presented in Table 4.5. The extractable fraction of the heavy metals was in general very low and only represented a small fraction of the total heavy metal concentration in the biochar, below 5% in the case of Ni and 16% in Pb (Table 4.6). Figures 4.12 and 4.13 show the dynamics of the DTPA extractable Ni and Pb during soil incubation of the different biochars in soil (amended and unamended). The concentrations of Cr and Cd were below the detection limits.

The addition of biochar caused a slight solubilisation of Ni and Pb in all the treatments, *i.e.* with only biochars (S+B (1%) or (2%)) or in combination with organic amendment (S+B+M (1%) and fertiliser (S+B+F (1%), compared to the treatments with organic amendment

Table 4.5: DTPA-extractable Cd, Cr, Ni and Pb concentrations in biochars. Biochars included: OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**Cd**	**Cr**	**Ni**	**Pb**
		(mg kg^{-1})		
OC650	<0.01	<0.01	0.44 *(0.03)*	0.1 *(0.0)*
OC450	<0.01	<0.01	0.07 *(0.00)*	0.1 *(0.0)*
O400	<0.01	<0.01	0.32 *(0.25)*	2.8 *(2.0)*
GH400	0.02 *(0.00)*	<0.01	0.17 *(0.01)*	0.6 *(0.1)*
GW400	0.05 *(0.01)*	0.08 *(0.00)*	0.19 *(0.01)*	1.9 *(0.0)*
CM400	0.11 *(0.02)*	0.04 *(0.00)*	0.29 *(0.01)*	21.7 *(0.0)*
PM400	<0.01	<0.01	0.04 *(0.00)*	0.2 *(0.0)*
PC400	0.01 *(0.00)*	<0.01	0.32 *(0.01)*	5.0 *(0.3)*

Standard deviation in brackets (n=3).

(S+M (1%)) and fertiliser alone (S+F (1%)). However, these differences were not significant in the case of the control soil. Therefore, the addition of organic amendment or mineral fertiliser had a very limited effect on the DTPA-extractable content of both elements and the recorded increase can be almost entirely ascribed to the addition of biochar. In general, the DTPA-extractable concentrations of Ni in soils were very low. Biochars that typically caused the larger Ni increases were O400, GW400 and CM400,

Table 4.6: Percentage of DTPA-extractable heavy metals (Cd, Cr, Ni and Pb) respect to the total concentration in biochars. Biochars included: OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature.

Biochar	**Cd**	**Cr**	**Ni**	**Pb**
	%			
OC650	0.0	0.0	4.8	8.3
OC450	0.0	0.0	2.1	9.1
O400	0.0	0.0	1.2	15.6
GH400	0.0	0.0	1.1	10.3
GW400	6.3	0.1	0.5	2.9
CM400	7.9	0.0	0.4	10.2
PM400	0.0	0.0	0.5	4.2
PC400	0.7	0.0	0.7	5.0

containing high levels of total heavy metals in the feedstock. In addition, CM400 (the biochar with the highest DTPA-extracted Pb) caused a 2-fold increase in available Pb with respect to the soil. However, the treatments with PC400 and PM400 biochars recorded lower Ni and Pb concentrations than the control.

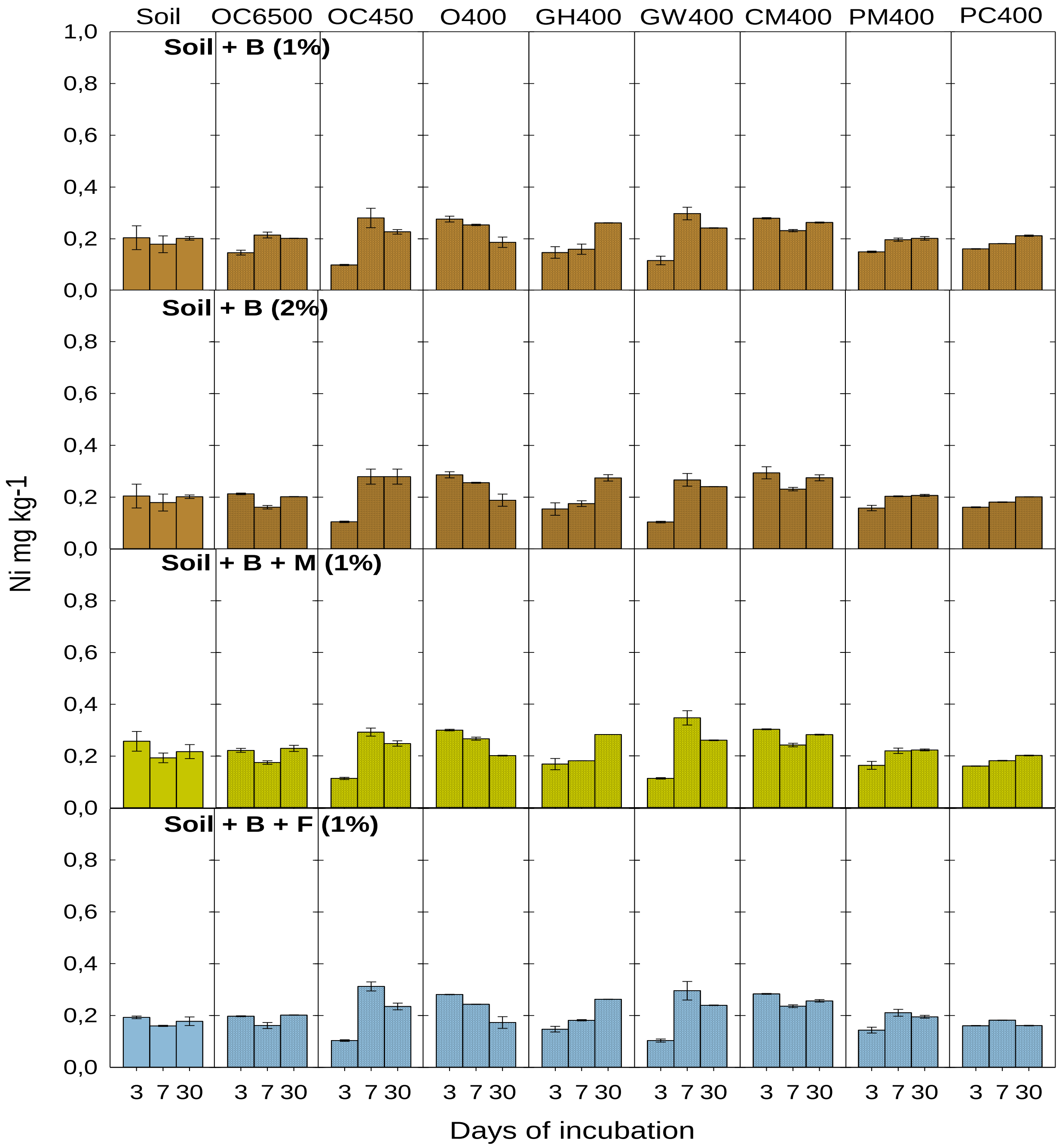

Figure 4.12: Concentrations of DTPA-extractable Ni in soil during the incubation The different treatments included: a control (soil without any amendment), soil with biochars at two doses (S+B (1%) and S+B (2%)), soil with manure (S+M (1%)) or mineral fertiliser (S+F (1%)) or soil with biochar in combination with manure (S+B+M (1%)) or with mineral fertiliser (S+B+F (1%)). Biochars included: OC and O: Holm oak waste, GH: Greenhouse waste; CM: CellMatt; PM: Pig manure; PC: Press cake, at each temperature. Error bars represent standard deviation (n=3).

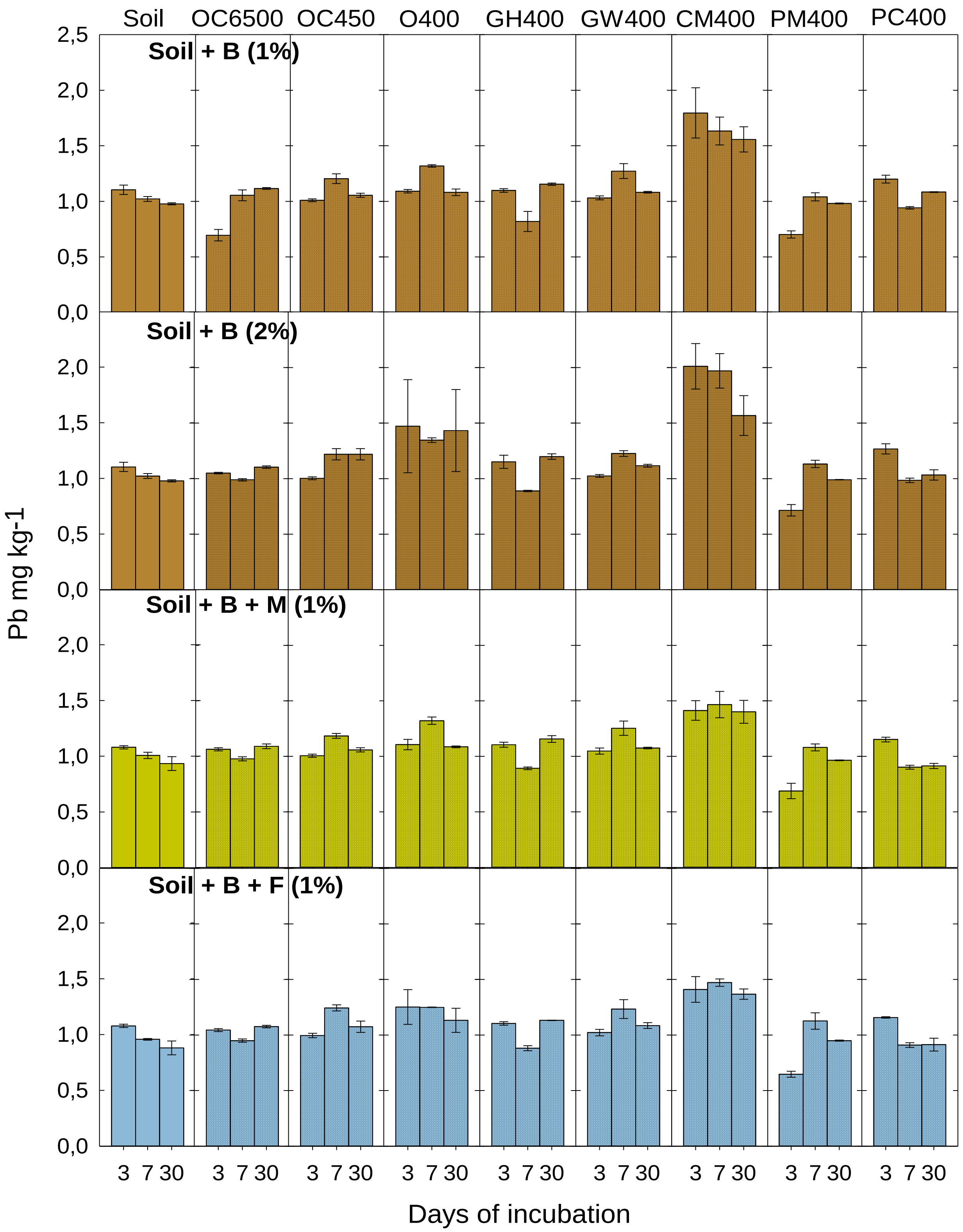

Figure 4.13: Concentration of DTPA- extractable Pb in soil during the incubation. The different treatments included were described in Figure 4.12. Error bars represent standard deviation (n=3).

4.3.6. Effect of biochar on plant growth

4.3.6.1. Phytotoxicity

The phytotoxicity of the materials was evaluated by the germination index (GI) of the biochars alone and by the germination success (G) measured in soils amended with biochars (Table 4.7).

All biochars showed lower GI than the control, except in the case of OC450, which had no impact in the GI. CM400 showed the lowest GI value (61%), which involves low phytotoxicity symptoms according to (Zucconi et al., 1981). A possible cause of the low GI rate in CM400 is the presence of heavy metals and especially DTPA-extractable Pb, which inhibitory effect had been reported previously (Munzuroglu and Geckil, 2002). In addition, O400, GH400, GW400 and PC400 biochars could have toxic soluble and volatile organic compounds, which accumulated through re-condensation of gases during the charring process, decreasing the GI (Fornes et al., 2015).

The germination success was measured through seedling emergence, at day 12 after sowing. Biochar type had significant effects on lettuce emergence in soil. Generally, the percentage of germinated seeds increased in all cases with respect to the control soil (100%), except GW400, which was significantly lower (83%).

Conversely to the phytotoxicity test performed directly on biochars, CM400 mixed with soil recorded the highest germination (133%), due to the buffer capacity of the soil and the dilution of pollutants.

4.3.6.2. Irrigation water use

Biochar addition affected the amount of water added of soil. Soils amended with urban and pre-treated biochars (GW400, CM400 and PC400) required, in general, lower amounts of water than the control during the growing experiment (Table 4.7). GH400 was the biochar that required the lowest amount of water (2137 g). In contrast, raw lignocellulosic biochars (OC450 and O400) did not show a significant difference with the control. The physicochemical properties of biochar may explain the differences in the amount of water required by the crop during the experiment, as revealed by the statistically significant correlation found between the amount of water added and several physicochemical properties of biochar affecting water retention on amended soils, such as WHC ($r=-0.65$; $p<0.01$). Our results contradict the findings of Jeffery et al. (2015), where no significant effects of biochar application on soil water retention were found".

4.3.6.3. Plant growth and nutritional status

The influence of biochar added to the soil on plant growth was assessed through total biomass, root-to-shoot ratio, leaf SPAD and chlorophyll fluorescence, as indirect measurements in the plant nutritional status (Table 4.7).

Biochar addition significantly affected the total dry plant biomass (shoot and root systems): OC450, PC400 and GW400 addition caused an increase of 10%, 7.7% and 4.6% respectively, compared to the control, while for the rest of biochar treatments showed reductions of 2.0%, 2.7% and 4.6% for O400, CM400 and GH400, respectively. However, in the case of the root system (root biomass), GH400 and CM400 registered a significant increase in root biomass of 9.8% and 4.8%, respectively, whereas raw lignocellulosic biochars generally did not show any significant differences with the control. A previous study with wood biochars and sunflower crop did not find any statistically significant effect on the root production (Alburquerque et al., 2014). Thus, the impact on the root system may be affected by the origin and properties of the biochar.

The root-shoot ratio was also evaluated. In general, changes in the root-shoot ratio depend on a number of factors. Thus, root-shoot ratio increases with nutrient deficiency, moisture stress, elevated concentrations of organic or inorganic (heavy metals) compounds with toxic effect, or under elevated CO_2, but decreases in strong light (Atwell et al., 1999; Lehmann et al., 2011; Wilson, 1988). GH400 treatment showed the highest root-shoot ratio, which differed significantly respect to the rest of the treatments. According to the results, the increase in root-shoot ratio in GH400 treatment was not caused by a toxic effect of heavy metals. A nutrient deficiency was also discarded in this treatment based on the measurements of chlorophyll fluorescence and leaf SPAD that did not show any differences between treatments. A possible effect of elevated CO_2 concentration and changes in light was also discarded since the experiment was fully randomised. Thus, an increase in the root-shoot ratio would indicate that plant was probably growing under less favourable conditions due to lower water availability.

Statistically significant interactions were found between plant growth characteristics and biochar characteristics indicating that growth responses were dependent upon biochar properties. Thus, WHC of biochars were strongly correlated to several growth characteristics according to Pearson's correlations: biomass (r=-0.48;

$p<0.01$); shoot system ($r=-0.56$; $p<0.01$); root-shoot ratio ($r=-0.66$; $p<0.01$). These findings suggest that water in GH400 pots were less accessible by plants. Wu et al. (2008) observed that in order to diminish consumption and increase absorption of water, plants in dry conditions often decrease their growth rate and biomass production, and contribute more biomass to roots, so that they could maintain a higher root-shoot ratio. In addition, a net increase in root growth and an increase in the root-shoot ratio by plants growing in conditions of low water availability may therefore be interpreted as an important adaptive feature (Sharp and Davies, 1979).

Previous works also reported that changes in the WHC and bulk density of the soil, caused by biochar addition, could affect root hair and pore-water dynamics that are difficult to measure (Prendergast-Miller et al., 2014). The impact on the root system may be affected by the origin and properties of the biochar, but is unclear. Lehmann et al. (2015) showed an increased in the root-shoot ratio while other pot studies recorded mostly reductions in root-shoot ratios (Lehmann et al., 2011).

The optimum nutritional status of lettuces during the growth experiment was indirectly corroborated by measurements of

Table 4.7: Influence of biochar type on plant variables measured during the growth and at the harvest of lettuce crop.

Biochar	**GI^1**	**G^2**	**Σ water consumed^3**	**Root biomass**	**Shoot biomass**	**Total biomass**	**Root-shoot ratio**	**Leaf SPAD**	**Chlorophyll fluorescence**
	%		**g**		**g**				**(Fv/Fm)^4**
Soil	-	-	2304^{a}	0.82	3.85^{ab}	4.67^{ab}	0.21^{b}	27,8	0.825
			(46)	(0.15)	(0.60)	*(0.73)*	*(0.02)*	*(2,4)*	*(0.007)*
OC450	101^{a}	117^{ab}	2302^{a}	0.89	4.24^{a}	5.14^{a}	0.21^{b}	27,2	0.826
	(1)	*(10)*	*(55)*	(0.19)	(0.21)	*(0.30)*	*(0.03)*	*(1,9)*	*(0.008)*
O400	88^{ab}	117^{ab}	2317^{a}	0.75	3.79^{ab}	4.54^{ab}	0.20^{b}	24,9	0.827
	(6)	*(16)*	*(42)*	(0.10)	(0.33)	*(0.35)*	*(0.03)*	*(1.2)*	(0.003)
GH400	69^{ab}	108^{ab}	2137^{c}	0.90	3.18^{b}	4.08^{b}	0.29^{a}	26,8	0.829
	(5)	*(23)*	*(43)*	(0.11)	(0.28)	*(0.31)*	*(0.04)*	*(1.1)*	*(0.009)*
GW400	85^{ab}	83^{b}	2281^{ab}	0.80	4.08^{a}	4.88^{a}	0.20^{b}	26,7	0.825
	(9)	*(19)*	*(60)*	(0.09)	(0.24)	*(0.21)*	*(0.03)*	*(2.6)*	*(0.008)*
CM400	61^{b}	133^{a}	2222^{bc}	0.86	3.71^{ab}	4.58^{ab}	0.20^{b}	27,2	0.826
	(7)	*(10)*	*(45)*	(0.11)	(0.29)	*(0.36)*	*(0.2)*	*(1.2)*	*(0.006)*
PC400	85^{ab}	100^{ab}	2288^{ab}	0.83	4.20^{a}	5.03^{a}	0.23^{b}	28,3	0.824
	(5)	*(19)*	*(49)*	(0.07)	(0.33)	*(0.34)*	*(0.02)*	*(2.5)*	*(0.014)*

GI^1: Germination Index on *Lepidium sativum* (control: distilled water); G^2: Germination rate at day 12 on lettuce (control: soil); Σ water consumed^3: Sum of water (g) consumed during the growth;(Fv/Fm)^4: is an indicator of the quantum yield of photosystem II; standard deviation in brackets (n=6); different letters indicate significant differences at $p<0.05$.

chlorophyll fluorescence and leaf SPAD at day 52. The fluorescence parameter Fv/Fm, which decreases when plants experience stress (Maxwell and Johnson, 2000), did not showed significant differences between treatments. In addition, leaf SPAD values did not show any significant differences during the growth. These results confirmed the observation made where all treatments had a healthy green leaf colour independent of the type of biochar applied.

4.3.6.4. Effect of biochar types on N use efficiency

The N concentration in the shoot biomass, the N uptake by the shoot system and the NUE are shown in Table 4.8. Total N content in the shoot system was affected by biochar type. GH400 increased the N concentration, while OC400 decreased the N concentration. The different behaviour of the biochars was caused by a mass concentration effect related to the amount of shoot biomass in each treatment. So, through N uptake by the plant this effect was corrected and no significant differences were found between treatments.

The NUE represent the N uptake respect to the applied N. All biochars had a similar behaviour and there were no significant differences in NUE respect to the control. However, there were significant differences between biochars. GH400 showed the lowest

(63.1%), whereas PC400 showed the highest (75.0%) NUE values. In the case of GH400, the low NUE could be due to the lower water availability in the soil, reducing N absorption by the plant. On the other hand, the higher NUE in PC400 treatment could be due to a high mineral content in the soil added with this biochar, which could contribute to the fertilisation of the plant.

Table 4.8: Influence of biochar type on N plant shoot concentration at the harvest of lettuce crops.

Biochar	**N**	**N uptake**	**NUE^1**
	mg g^{-1}	**mg N kg^{-1} soil**	**%**
Soil	7.98^{ab} *(0.65)*	28.7 *(4.9)*	67.5^{ab} *(11.5)*
OC450	7.59^{b} *(0.62)*	30.2 *(3.5)*	71.4^{ab} *(3.5)*
O400	8.02^{ab} *(0.62)*	28.1 *(2.1)*	66.4^{ab} *(4.9)*
GH400	9.07^{a} *(0.73)*	26.7 *(2.2)*	63.1^{b} *(5.3)*
GW400	8.11^{ab} *(0.69)*	30.9 *(2.1)*	73.0^{ab} *(5.0)*
CM400	8.42^{ab} *(0.63)*	29.2 *(1.7)*	69.1^{ab} *(4.0)*
PC400	8.08^{ab} *(0.6)*	31.7 *(3.5)*	75.0^{a} *(8.3)*

NUE: N uptake/N supplied; standard deviation in brackets (n=6); different letters indicate significant differences at $p<0.05$.

4.3.6.5. Heavy metals in the plant shoot system

The uptake of the most important heavy metals (Cd, Cr, Ni and Pb) by lettuces was measured in the different biochar treatments (Table 4.9). In general, the amounts of heavy metals in lettuce were found to be very low. However, the amount of heavy metals absorbed by the lettuces was higher than expected from the DTPA-extractable data at the end of the incubations. Thus, the crop was able to extract higher amounts from the biochars and from the soil than predicted from the DTPA analysis.

The Cr levels did not show significant differences in DTPA-extracted in plant, despite the fact that the Cr concentration showed a two-fold increase in CM400 lettuces, which was in accordance to the high levels of this metal in the biochar. On the contrary, the concentrations of Pb did not show differences among treatments despite the high DTPA-extractable Pb in CM400 biochar, but the results are in agreement with the evolution of the DTPA-extractable concentrations observed during the incubation (Figure 4.13). The Ni extracted by lettuces was low, but slightly higher in O400 treatment, which was probably due to the levels of Ni in the original biochar (possible contamination during the pyrolysis process by the use of Ni-

Cr steel in construction of pyrolysis rectors (Camps-Arbestain et al., 2015)).

Maximum levels for Cd and Pb in fresh vegetable established by the European Commission are 0.05 and 0.30 mg Kg^{-1} of plant (wet weight), respectively (EuropeanCommission, 2011, 2014). Thus, Cd levels, which were below the detection limit (0.02 mg Kg^{-1}), and Pb levels, which were detected in the rage from 0.07 to 0.16 mg/kg of plant (wet weight) were below the limits for leaf vegetables.

Table 4.9: Heavy metals in plant shoot at the harvest of lettuce crop (wet weight).

Biochar	**Cd**	**Cr**	**Ni**	**Pb**
		(mg kg^{-1})		
Soil	0.02 *(0.00)*	0.03 *(0.01)*	0.03 *(0.01)*	0.07 *(0.01)*
OC450	0.02 *(0.01)*	0.01 *(0.01)*	0.01 *(0.01)*	0.09 *(0.01)*
O400	0.02 *(0.01)*	0.04 *(0.04)*	0.07 *(0.02)*	0.11 *(0.01)*
GH400	0.02 *(0.01)*	0.01 *(0.02)*	0.01 *(0.02)*	0.16 *(0.01)*
GW400	0.02 *(0.01)*	0.01 *(0.02)*	0.01 *(0.02)*	0.12 *(0.01)*
CM400	0.02 *(0.01)*	0.06 *(0.02)*	0.06 *(0.02)*	0.07 *(0.01)*
PC400	0.02 *(0.01)*	0.02 *(0.02)*	0.02 *(0.01)*	0.13 *(0.01)*

Standard deviation in brackets (n=6).

4.4. Conclusions

The type and origin of feedstock only had a minor impact on the response of biochar in soil and its interaction with the most important nutrient and C cycles. The main differences among biochars were originated by the presence of relatively higher content of ash in biochars prepared from pre-treated organic wastes, compared to biochars from raw lignocellulosic biomass. The presence of ashes in biochars prepared from urban and pre-treated organic wastes caused an increase in the availability of N and P in soil, compared to raw lignocellulosic biochar. However, the latter were more efficient in the interaction with mineral fertilisation, causing a decrease in soil mineral N availability (reducing the risk of potential N losses by lixiviation) and an increase in soil available P.

All tested biochars exhibited favourable properties as soil amendments and no phytotoxic effects or negative impacts on soil nutrient dynamics were observed during the soil incubation experiments. The physicochemical properties of biochars, mainly their WHC, affected the retention of water in soil. Thus, biochars with higher WHC needed lower amount of water to maintain the moisture levels. However, the availability of this water to the plant is uncertain.

The agricultural use of the biochars included in this study can be only limited by the presence of heavy metals in some of the biochars prepared from feedstocks of urban origins.

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Chapter 5: Biochar improves N cycling during composting of olive mill wastes and sheep manure

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5.1. Abstract

The use of biochar has been revealed to have beneficial effects during the composting of manures and other N-rich materials by reducing N losses and enhancing the rate of the process. However, the impact of biochar has not been explored in other complex organic matrices with low N nitrogen that may hinder the composting process. The main novelty of this work was to study the impact of a small amount of biochar (4%) on the composting process of olive mill wastes, which are characterised by a recalcitrant lignocellulosic composition with reduced nitrogen (N) availability. Two treatments: (i) control (olive mill waste 46% + sheep manure 54%, dry weight) and (ii) the same mixture treated with biochar (4%), were composted during 31 weeks.

The incorporation of a small amount of biochar improved N cycling by increasing NO_3^--N content, indicating a higher nitrifying activity, and reducing N losses by 15% without affecting the amount of N_2O released. The use of biochar as an additive for composting could improve the value of olive mill waste composts by reducing N losses and increasing N availability in lignocellulosic and N-poor materials.

Keywords: charcoal, greenhouse gases, lignocellulose, methane, nitrous oxide.

5.2. Introduction

Biochar is a carbonaceous product obtained by pyrolysis of organic materials that can be added to soils to sequester carbon (C) and reduce gaseous emissions (Lehmann and Joseph, 2015). Other benefits of the use of biochar as a soil amendment are associated to enhancements of soil functions and fertility, being highly dependent on soil characteristics and biochar type (Jeffery et al., 2015) . Biochar is known to impact soil nutrient cycling, by enhancing retention and release of nutrients (Gul et al., 2015). In particular, biochar may interact with soil N transformations and increase N utilization efficiency and bioavailability in agricultural soils, by enhancing N retention and reducing soil N leaching (Zheng et al., 2013).

The use of biochar for the composting of organic wastes has been recently proposed as a strategy to improve the process by enhancing aeration (due to its high porosity) and increasing water-holding capacity and sorption of available C compounds (Jindo et al., 2012; Steiner et al., 2015). These physicochemical impacts may lead to a stimulation of the microbial activity and changes in the microbial communities (Jindo et al., 2012). Moreover, biochar has proved to be effective in enhancing the process and the quality of the final compost (Steiner et al., 2015). The use of biochar in composting could also represent an opportunity to ensure a sustainable use of the resources, by integrating biochar production into the waste management scheme of the farm. The production of biochar from lignocellulosic wastes generated at the farm (tree pruning and/or other green wastes) would allow the local recycling of their resources (Sohi et al., 2015).

Different composting experiments have shown that biochar increased the organic matter degradation rate during composting (Sánchez-García et al., 2015) and increased the agronomical value of the compost reducing N-losses during the process and enhancing the absorption of NH_3 and NH_4^+ (Chowdhury et al., 2014; Hua et al., 2009). The impact of biochar on composting has been studied mainly in N-rich materials such as manures (Steiner et al., 2010; Wang et al., 2013). However, there are no studies investigating the impact of biochar on more recalcitrant organic wastes such as TPOMW, characterised by a rich lignocellulosic composition and low N availability, which slows down the TPOMW composting process.

Two-phase olive mill waste (TPOMW) is one of the main by-products generated by the olive oil processing industry (Roig et al., 2006). The high amount of TPOMW produced every year causes serious environmental problems. However, TPOMW can represent a valuable soil organic amendment after its aerobic stabilisation through composting (Chowdhury et al., 2013; Roig et al., 2006). For this reason, methods to optimise TPOMW composting are being extensively studied. TPOMW is a semi-solid material with a high water content, which limits aeration during composting (Alburquerque et al., 2009; Serranía et al., 2010). On the other hand, the composting process of TPOMW is usually long due to its recalcitrant nature (high lignocellulose content), the presence of low and high molecular weight phenolic substances and low nitrogen content (< 2%), which slows down its microbial degradation. Therefore, TPOMW is generally co-composted with animal manure to optimise the composting process by improving aeration conditions (bulking agent) and balancing the C/N ratio. TPOMW composting is generally characterised by a scarce nitrification degree and by N losses, which can reach up to 45% of the initial N content and reduce the agronomic value of the compost produced (Alburquerque et al., 2009; Cayuela et al., 2006; Serranía et al., 2010).

This work is aimed at improving the composting process of TPOMW by adding biochar to a mixture of TPOMW and sheep manure, paying special attention to its interaction with the N cycle, the nutritional quality and its influence on gas emissions (CO_2, CH_4, N_2O, CO and SH_2).

Thus, our hypotheses were that the addition of biochar: (i) accelerates the composting process through stimulating microbial degradation, (ii) enhances the efficient use of N by minimising its losses during composting, and (iii) improves the nutritional value of the final compost.

5.3. Material and methods

5.3.1. Starting materials.

TPOMW, sheep manure and biochar were used for the composting experiment. Sheep manure was used as bulking agent to improve the physical properties of the mixture and as N source to adjust the unbalanced initial C/N ratio of TPOMW. Both TPOMW and sheep manure were supplied by the organic farm “Casa Pareja” (Murcia, Spain).

The biochar was made from holm oak wood by slow pyrolysis at 650°C, at atmospheric pressure, being the residence time of about 15 hours. The biochar (particle size <10 mm) had a high concentration of total organic C (67%) and a high degree of aromatic condensation (H/C org molar ratio=0.32). It was produced by Proininso Inc. (Málaga, Spain) within the EU project FERTIPLUS (www.fertiplus.eu). The main characteristics of the starting materials are summarized in Table 5.1.

5.3.2. Composting process.

Two composting piles were prepared using the following dry weight proportions:

- Mixture OS: TPOMW (46%) + sheep manure (54%).
- Mixture OS+B: was prepared by adding biochar (4%) to the OS mixture.

The mixtures, with an initial weight of about 1500 kg each, were composted in trapezoidal piles of 1.5 m high with a 2x3 m base in an outdoor composting plant. The composting process was monitored

Table 5.1. Main characteristics of the starting materials: TPOMW, sheep manure and biochar.

Starting materials	**Moisture**	**pH**	**EC**	**OM**	**C/N**	**C**	**N**	**P**	**K**	**Ca**	**Mg**	**NH_4^+-N**	**NO_3^+-N**
	(%)		**(dS m^{-1})**	**(%)**		**(%)**			**(%)**			**(mg kg^{-1})**	
TPOMW	67.2 *(0.3)*	5.2^a *(0.03)*	5.7^a *(0.08)*	93.8 *(0.04)*	36.5 *(0.48)*	49.8 *(0.48)*	1.36 *(0.03)*	0.12 *(0.01)*	2.12 *(0.15)*	0.29 *(0.02)*	0.08 *(0.01)*	nd	3.90^b *(0.08)*
Sheep Manure	42.0 *(0.7)*	9.1^a *(0.01)*	10.4^a *(0.01)*	59.5 *(0.65)*	11.7 *(0.09)*	28.5 *(0.09)*	2.43 *(0.01)*	0.38 *(0.03)*	3.28 *(0.33)*	5.11 *(0.52)*	0.85 *(0.09)*	1195 *(25.6)*	92.09^b *(2.0)*
Biochar	29.7 *(1.7)*	9.3^b *(0.13)*	0.6^b *(0.04)*	77.3 *(0.37)*	80.16 *(1.02)*	67.3 *(1.02)*	0.84 *(0.03)*	0.19 *(0.01)*	0.72 *(0.02)*	5.25 *(0.10)*	0.33 *(0.00)*	3.20 *(0.01)*	0.61^b *(0.00)*

[a] Water extract 1:10 w:v 25°C; [b] water extract 1:20 w:v 25°C; EC: Electrical Conductivity; OM: Organic Matter; C: total organic Carbon; nd: not detectable; standard deviation in brackets (n=2).

during 31 weeks. The aeration system used was by the pile turning (windrow). The piles were turned every two or three weeks during the thermophilic phase (Figure 5.1), and water was added to maintain the moisture level within the range 40–55%. After two months of composting, the piles were covered with a polypropylene woven geotextile (permeable to gaseous exchange) in order to avoid the excessive water loss produced by wind and evaporation.

The temperature was measured every three days during the thermophilic phase. The composting piles were sampled at weeks 0, 6, 12, 16, 22 (during the thermophilic phase) and 31 (at the end of maturation phase). Each collected sample was mixed, homogenised and subdivided into two sub-samples. One was frozen (-18ºC) for the measurement of ammonium by extraction and the other was air dried and ground to 0.5 mm for physicochemical analysis.

5.3.3. Gas emission measurements.

A static closed chamber technique was used to measure CO_2, CH_4, N_2O, CO and SH_2 fluxes from the surface of the composting piles (Sánchez-Monedero et al., 2010; Sommer et al., 2004).

Gas samples were taken every two weeks, during the thermophilic phase of composting and at the end of the maturation phase, from four aluminium closed chambers (volume: 0.009 m^3, area: 0.038 m^2) placed on the top of the pile at four different locations. The chambers were pushed 5 cm into the composting pile and gas samples were taken within the headspace at 0, 10, 20 and 30 min using disposable syringes. Gas samples were transferred to 12mL glass vials (Exetainer, Labco, UK) previously purged with He and evacuated, and were analysed by gas chromatography (VARIAN CP-4900 Micro-GC Palo Alto, CA, USA) to measure CH_4, N_2O and CO_2 concentration. CO and SH_2 fluxes were measured using a portable NDIR analyser (Dräger X-am 7000, Dräger Safety, Lübeck, Germany) directly from the static chambers at the same intervals.

Gas emission fluxes were calculated by fitting the experimental data to a second-order polynomial equation (gas concentration vs. time). The flux at time 0 was calculated by taking derivatives of the second-order polynomial (Hao et al., 2001) and was expressed as grams of C or N per unit of area (m^2) and per unit of time (d). All emission fluxes results represent the mean of four points measured for each pile and the standard error is shown by errors bars.

5.3.4. Analytical methods.

Air dried samples were used for the determination of the following parameters: electrical conductivity (EC) and pH were measured in a 1:10 (w/v) aqueous extract. Moisture content was obtained by drying at 105°C for 24h. Organic matter (OM)/ash content was determined by a muffle furnace at 550 °C (TMECC, 2002). Total nitrogen (N) and total organic carbon (C) were analysed by automatic elemental analysis (LECO CHNS-932, USA). Macro-, micro-nutrients and heavy metal concentrations were measured after microwave HNO_3/H_2O_2 digestion by Inductively Coupled Plasma spectroscopy (ICP-OES) (ICAP 6500 DUO THERMO, England). The content of NO_3- and NO_2- were measured in a 1:20 (w/v) aqueous extract and determined by ion chromatography (DIONEX ICS-2100, Sunnyvale, CA, USA). Phytotoxicity was assayed by the *Lepidium sativum* test and expressed as germination index (GI), according to Zucconi et al. (1981). Frozen samples were used for determination of NH_4^+ by extraction with 2M KCl at 1:10 (w/v) and subjected to a colorimetric method based on Berthelot's reaction (Sommer et al., 1992).

Chemical analyses are expressed on an oven-dry basis (105ºC, 24h) and represent the mean of at least duplicate analyses.

5.3.5. Calculations and statistical analyses.

The losses of organic matter were calculated according to the equation:

$$\text{OM-loss (\%)} = 100\text{-}100[(X_1\ OM_2)/(X_2\ OM_1)]$$

where X_1 and X_2 are the initial and final ash concentrations, and OM_1 and OM_2 are the initial and final OM concentrations (Cayuela et al., 2006). OM-losses were fitted to a first order kinetic model using SigmaPlot 12.0 software (Systat Software Inc., San Jose, CA, USA) according to the following equation: OM-loss=C_0 (1-e^{-kt}); where "C_0" represent the maximum degradation (% of initial OM content), "k" the rate constant (days^{-1}) and "t" is the composting time (days).

The losses of total N were calculated with the equation:

$$\text{N-loss (\%)} = 100\text{-}100[(X_1\ N_2)/(X_2\ N_1)];$$

where N_1 and N_2 are the initial and final total N.

Statistical analyses of data were performed using the IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). The significance of differences between treatments for gas emissions (CO_2, CH_4 and N_2O) was determined by repeated measures ANOVA with treatment (OS and OS+B) as independent variables and time as the within subject factors. As the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied.

5.4. Results and discussion

5.4.1. Impact of biochar on the composting process and organic matter degradation.

The composting process showed a long thermophilic phase in both treatments (22 weeks; average T 47ºC; max T 68ºC in control pile and average T 45ºC; max T 60ºC in biochar pile). A long thermophilic-active phase is typical for this organic matrix because TPOMW is rich in recalcitrant compounds, which are not easily degraded by microorganisms and hence lengthened the composting process (Alburquerque et al., 2009; Cayuela et al., 2004). However, the addition of biochar favoured the activation of the composting process, showing an initial faster temperature increase and maintaining lower temperatures than the control in the last stage of the active phase (Figure 5.1a).

As composting progressed, both piles showed a rapid increase of the pH until values above 9 from week 6th onwards (Figure 5.2a). This is common during TPOMW composting and it has been found to be due to the decarboxylation of organic anions during the aerobic decomposition of TPOMW (Cayuela et al., 2008). During the maturation stage, the biochar-treated pile showed a lower pH, which was associated to a higher nitrification (Sánchez-Monedero et al., 2001) as discussed below.

Although the C/N ratio decreased in both piles and followed a similar trend (Figure 5.2b), the overall decrease of the C/N ratio (when initial and final values were compared) was larger in the biochar treated pile, which declined 7 units compared to the control with only a 4 units change.

The mineralisation of both mixtures (Figure 5.2c) was characterised by an initial phase of fast OM degradation, leading to the highest values of OM-losses. After that, a second period (which overlaps with the end of the thermophilic phase) was characterised by a slowing down in the OM losses, which reflected a progressive stabilization of the mixtures. The mineralization rate values were quite similar in both treatments according to the parameters of kinetic equations shown in Table 5.2, and these results were within the range obtained for this kind of mixture: 0.14-1.33 (% OM d^{-1}) (Alburquerque et al., 2009; Serranía et al., 2010). Therefore, the biochar addition did not have a significant impact on the overall OM degradation.

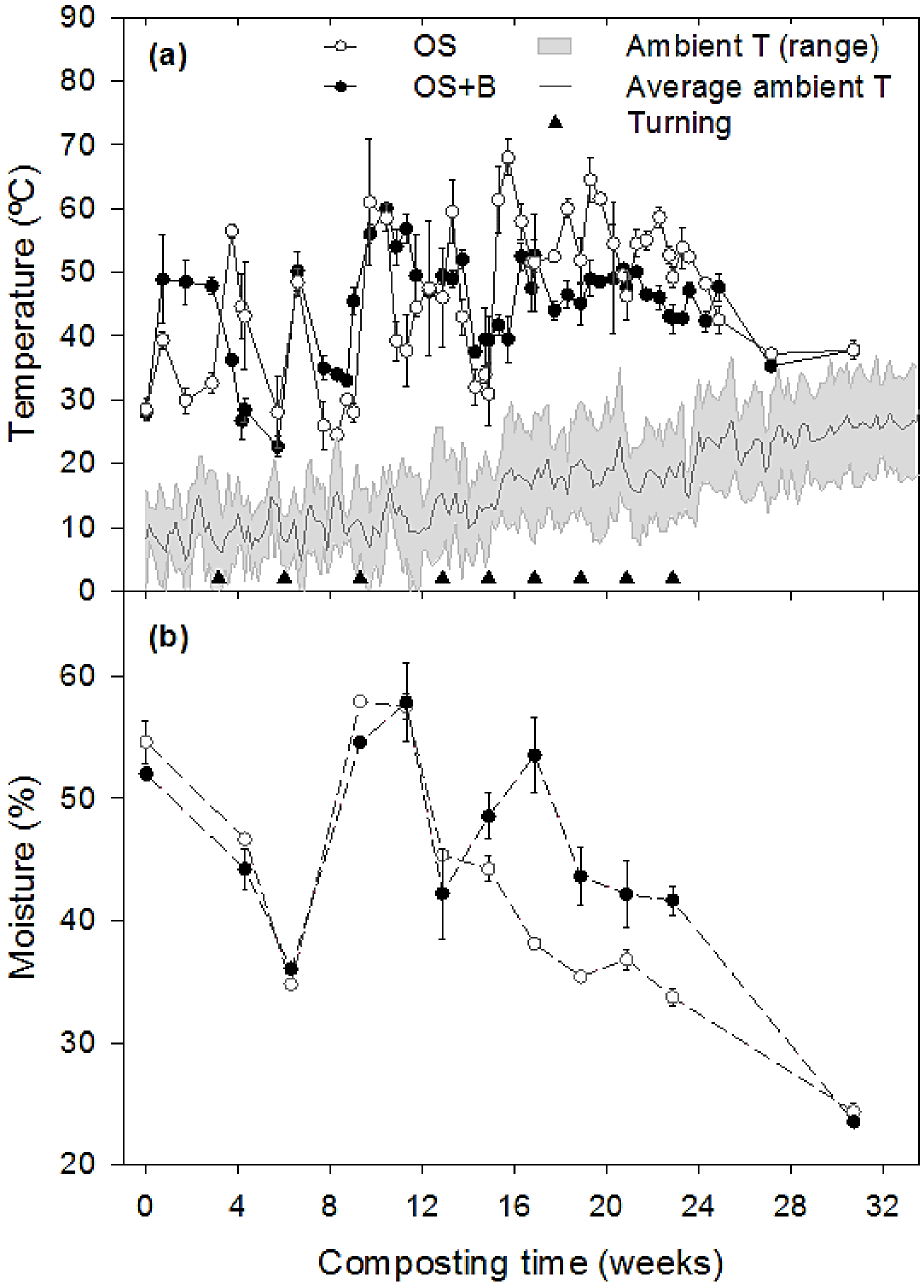

Figure 5.1: Temperature (a) and moisture (b) during the composting of OS (TPOMW + Sheep manure) and OS+B (TPOMW + Sheep manure + Biochar).

Table 5.2: Kinetic models fitted to experimental data of OM-losses. OS (TPOMW + Sheep manure) and OS+B (TPOMW + Sheep manure + Biochar).

Treatment	**C_0 (% OM)**	***k* (d^{-1})**	**C_0 x *k* (% OM d^{-1})**	**RMS**	**F**
OS	48.4 (3.4)	0.013 (0.02)	0.630	5.57	266.9*
OS+B	45.8 (1.4)	0.0148 (0.001)	0.678	1.37	1001.2*

C_0: represent the maximum degradation (% of initial OM content); *k*: rate constant ($days^{-1}$); RMS: residual mean square; F: Factor. Significant at $p<0.001$. Standard deviation in brackets (n=2).

During the thermophilic phase, the CO_2 fluxes (Figure 5.2d) corresponded well with the temperature and moisture profiles in each pile, showing the highest emissions when temperatures were above 40ºC and moisture around 45% (Figure 5.1a and 5.1b). In general, the CO_2 and CH_4 emissions were low, below 400 g CO_2-C m^{-2} d^{-1} and 900 mg.CH_4-C m^{-2} d^{-1}, respectively. These emission rates were in accordance with previous studies on TPOMW composting (Sánchez-Monedero et al., 2010) and indicated a low degradation rate of TPOMW mainly owing to the high lignin content and the low level of N in the organic matrix (Alburquerque et al., 2009; Cayuela et al., 2008).

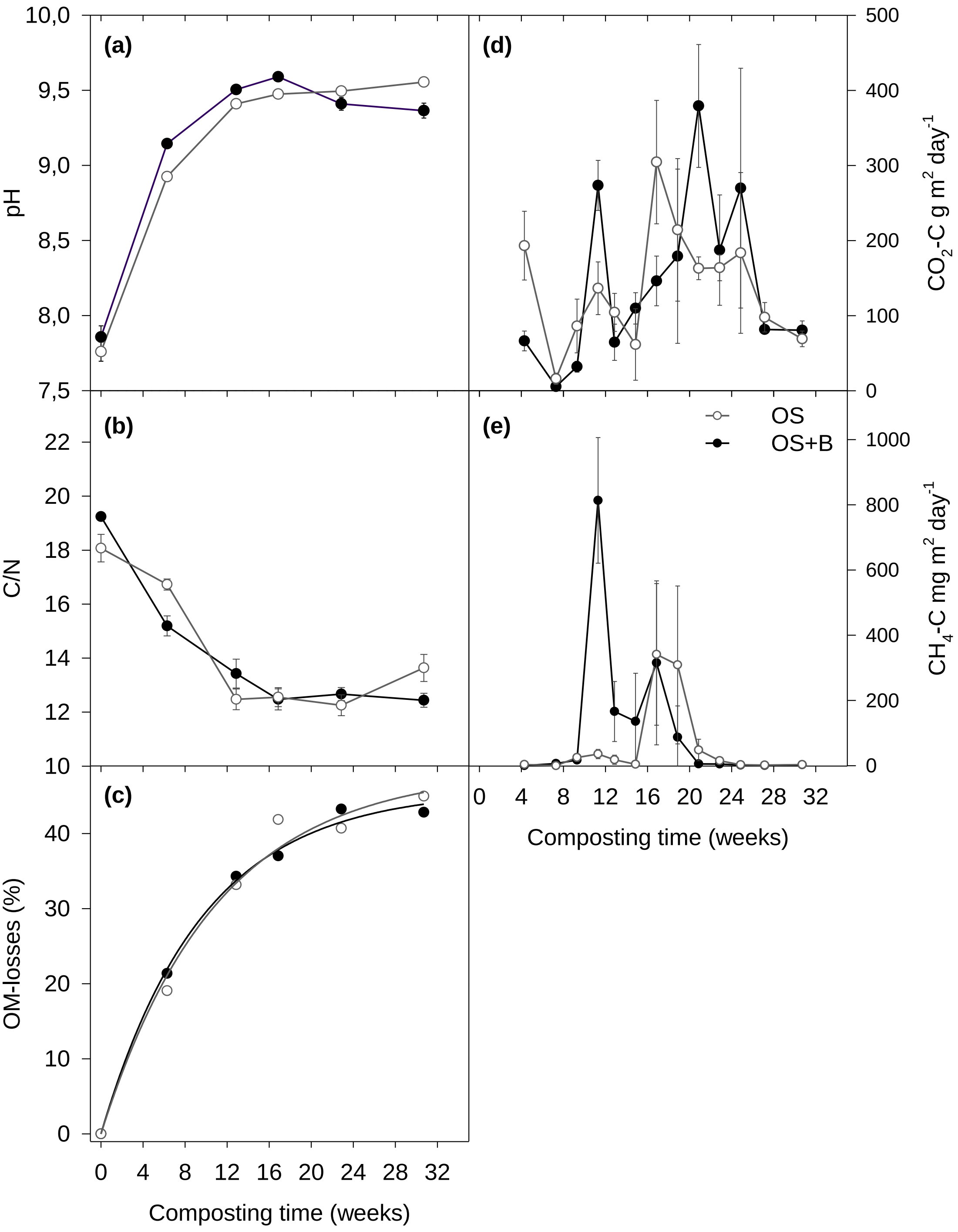

Figure 5.2: Evolution of pH (a), C/N (b), OM-losses lines represent curve-fitting to experimental data (c), emission rates of CO$_2$-C (d), and CH$_4$-C (e) measured during the composting of OS (TPOMW + Sheep manure) and OS+B (TPOMW + Sheep manure + Biochar). Error bars mark standard deviation (n=2), except to CO$_2$-C and CH$_4$-C where mark standard error (n=4).

The OS+B mixture registered a peak of CH_4 emissions (814 mg CH_4-C m^{-2} d^{-1}) at week 11, corresponding to a peak of temperature and CO_2 emissions (274 g CO_2-C m^{-2} d^{-1}). All these changes reflected an intense microbial activity during that period where the available sources of organic compounds have been degraded, lowering the oxygen levels in the pile and promoting CH_4 emission (Sánchez et al., 2015). Since week 21st, CO_2 and CH_4 emission rates decreased in both treatments to around 80 g CO_2-C m^{-2} d^{-1} and 3 mg CH_4-C m^{-2} d^{-1}, reflecting the organic matter stabilization achieved during the maturation phase (Sánchez-Monedero et al., 2010; Steiner et al., 2015). Considering the overall composting process, biochar addition did not exert a significant impact on emissions of both CO_2 and CH_4 gases (P=0.130 and P=0.078, respectively).

CO and SH_2 emissions were not detected during the composting process in any of the composting piles. As stated by Sánchez et al. (2015), these gases only represent a minor presence during composting of lignocellulosic waste composting, probably because they require high levels of anaerobiosis and, for SH_2, the presence of abundant S-containing proteins.

5.4.2. Impact of biochar on nitrogen transformations.

Figure 5.3 shows the changes in mineral N forms (NH_4^+ and NO_3^-), N_2O and N-losses during the composting process.

Biochar addition had a considerable impact on mineral N dynamics. The initial NH_4^+ concentration (mainly derived from sheep manure) decreased in both treatments. After that, OS+B pile kept low NH_4^+ concentrations (around 120 NH_4^+-N mg kg^{-1}) while OS showed an increase, reaching a peak of 758 NH_4^+-N mg kg^{-1}. This reflects the organic nitrogen ammonification as a consequence of OM degradation and coincided with a period of intense microbial activity as reflected by the higher temperatures recorded in the OS pile from week 16 to 24th (Figure 5.1a).

Biochar has been recently found to decelerate the ammonification process in soil, with a build-up of organic N, while promoted soil ammonia-oxidizer populations and accelerated gross nitrification rates (Prommer et al., 2014). However, the impact of biochar on N transformations has been scarcely studied during composting. Our results support the previous findings, since low concentrations of NH_4^+ were observed during the whole process, whereas high concentrations of NO_3^- were detected at the last stage of the process in pile OS+B.

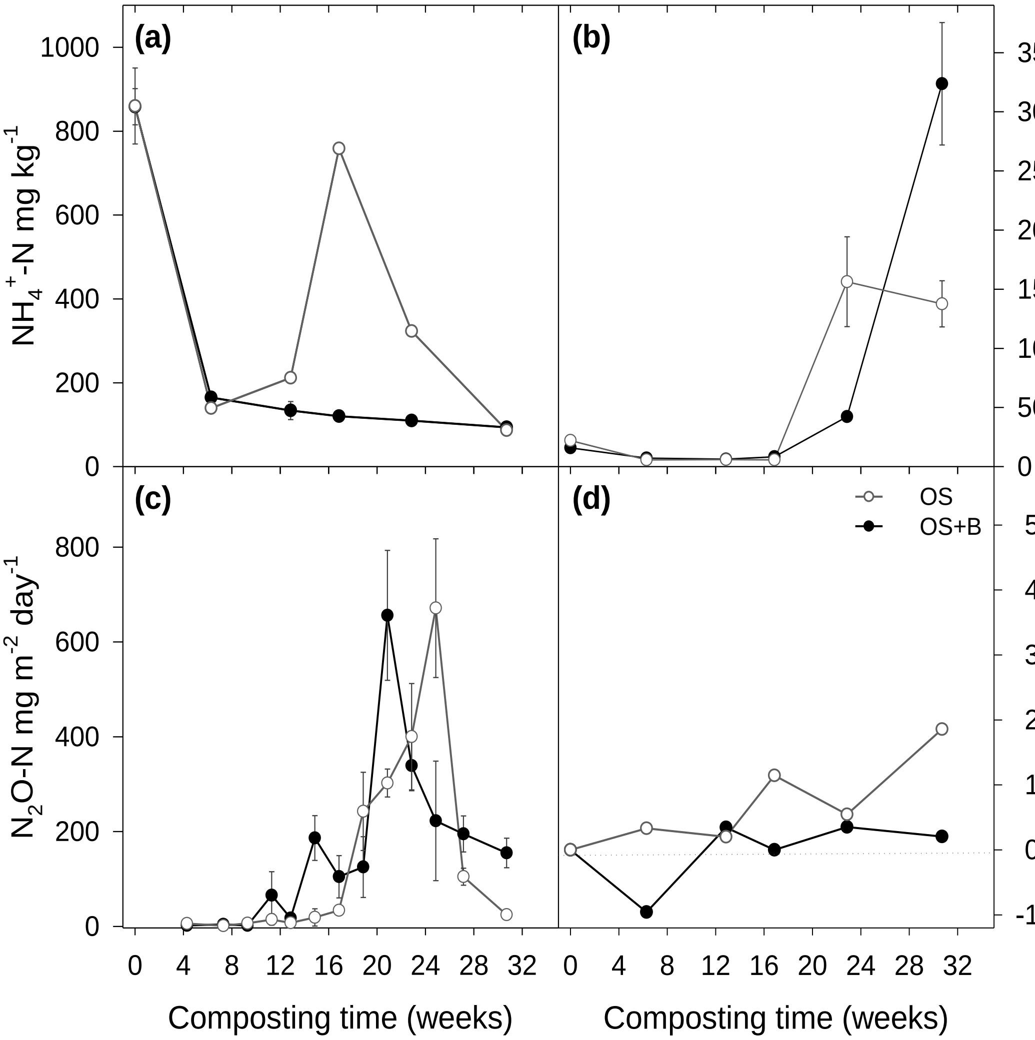

Figure 5.3: Evolution of NH_4^+-N (a), NO_3^--N (b), N_2O-N (c), and N-losses (d) during composting of OS (TPOMW + Sheep manure) and OS+B (TPOMW + Sheep manure + Biochar). Error bars mark standard deviation (n=2), except to N_2O-N, where mark standard error (n=4).

Both piles showed an increase in NO_3^- concentrations during the maturation phase, when temperatures were close to the ambient temperature (Figure 5.3b). However, the addition of biochar resulted in greater amounts of NO_3^- and lower pH. This supports the hypothesis that biochar promotes conditions that improve nitrifying activity. Zhang and Sun (2014) also found that biochar enhanced nitrification, and suggested that biochar creates a favourable microenvironment for nitrifying bacteria.

The intensity of the nitrification process in composting matrices like TPOMW is limited by its low N concentration and, more importantly, to its low N availability originated by the protection of N within the lignocellulosic structure. Previous studies found lignin to be one of the most important plant polymers controlling N mineralisation during the decomposition of plant-derived residues, with a negative relationship between lignin and the rate of gross nitrogen mineralization (Gómez-Muñoz et al., 2011). During TPOMW composting, nitrification is mainly limited by the amount of NH_4^+ available to nitrifying bacteria and by the NH_3 losses through volatilization (Sánchez-Monedero et al., 2001; Steiner et al., 2015).

During the composting process both piles had similar N_2O total emissions, ranging from 0 to 700 mg N_2O-N m^{-2} d^{-1} (Figure 5.3c). These emissions were lower than those registered in other composting matrices containing animal manures, such as pig manure, up to 2818 mg N_2O-N m^{-2} d^{-1}, or cattle manure, up to 1800 mg N_2O-N m^{-2} d^{-1} (Sánchez et al., 2015; Steiner et al., 2010; Wang et al., 2013). Low N_2O fluxes are typical of TPOMW (< 3 mg N_2O-N m^{-2} d^{-1}) due to the low N availability and slow degradation rate (Sánchez-Monedero et al., 2010; Serranía et al., 2010). The biochar pile cooled down (below 50ºC) and showed the N_2O peak 4 weeks before the control pile (Figure 5.3c). Biochar addition did not significantly increase N_2O emissions (P=0.665) in spite of the higher nitrification observed compared to control.

Figure 5.3d shows N-losses for both treatments during the composting process. At the end of the process, total N losses represented 2% of the initial N content of OS+B waste mixture (0.4 kg of N per ton of the starting mixtures of organic wastes), while in the case of the waste mixture prepared without biochar (OS), total N losses increased up to 18% of the initial N content (3.6 kg of N were lost for each ton of treated waste) (Figure 5.3d). Therefore, a low amount of biochar (4%) was enough to enhance an efficient transformation of N during composting, favouring nitrification and decreasing N losses by 3.2 N kg Ton^{-1} treated wastes. As a result, N was retained in the compost and improved the agronomic value of the end product. One possible mechanism for N retention could be the capacity of biochar to adsorb NH_4^+ (Hua et al., 2009; Steiner et al., 2010) or even NH_3 (Gai et al., 2014; Hina et al., 2015; Taghizadeh-Toosi et al., 2012), which could reduce N loss via volatilization and promote nitrification.

5.4.3. Biochar effect on compost quality.

The quality of the composts was assessed in terms of their nutritional value. Table 5.3 shows the total and water soluble concentrations of macro, micronutrient and heavy metal in the composts. Thus, biochar addition did not significantly affect concentration of neither nutrients nor heavy metals (total and water soluble forms) except for total N and NO_3^- concentrations, which was twice higher than in the control pile.

A recent study by Kammann et al. (2015) found that biochar is able to capture and retain NO_3^- during composting. The captured NO_3^- was found to be largely protected against leaching and partially plant-

Table 3. Main characteristics of composts: OS: TPOMW + Sheep manure and OS+B: TPOMW + Sheep manure + Biochar.

	EC[a]	pH[a]	C/N	NH_4^+/NO_3^-	IG	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn	Ni	Pb	Cr
	(dS m^{-1})				%	%						(mg kg^{-1})						
Total concentration																		
OS	7.17	9.56	13.6	0.6	64.9	2.40	0.38	3.60	5.63	0.96	0.4	2585	41.2	163.1	71.8	18.4	4.0	26.8
	(0.81)	*(0.01)*	*(0.5)*		*(6.8)*	*(0.09)*	*(0.00)*	*(0.05)*	*(0.54)*	*(0.07)*	*(0.0)*	*(82)*	*(0.2)*	*(8.1)*	*(0.8)*	*(1.8)*	*(0.2)*	*(3.5)*
OS+B	8.55	9.37	12.4	0.3	68.2	2.69	0.39	4.04	5.15	0.83	0.4	2591	42.8	186.1	71.4	20.7	4.1	33.6
	(0.06)	*(0.05)*	*(0.3)*		*(6.0)*	*(0.00)*	*(0.09)*	*(0.81)*	*(1.31)*	*(0.20)*	*(0.1)*	*(644)*	*(8.4)*	*(46.3)*	*(17.7)*	*(0.0)*	*(0.8)*	*(8.6)*
Water soluble concentration																		
OS							0.05	3.55	0.13	0.03	0.4	172	8.5	6.5	8.5	0.9	0.3	0.2
							(0.00)	*(0.00)*	*(0.00)*	*(0.00)*	*(0.0)*	*(5)*	*(0.3)*	*(0.2)*	*(0.2)*	*(0.2)*	*(0.2)*	*(0.0)*
OS+B							0.05	3.97	0.15	0.04	0.4	163	11.9	8.5	9.5	1.2	0.6	0.4
							(0.00)	*(0.08)*	*(0.01)*	*(0.00)*	*(0.0)*	*(15)*	*(0.9)*	*(0.6)*	*(0.7)*	*(0.5)*	*(0.4)*	*(0.2)*

[a] Water extract 1:10 w:w 25°C; EC: Electrical Conductivity; IG: Germination Index ; TC: Total Concentration; WSC: Water Soluble Concentration; Standard deviation in brackets (n=2).

available, although the mechanisms for NO_3^- capture in biochar are still unknown.

Both mature composts reached a similar C/N ratio (< 15), which is adequate to be incorporated into the soil. The nitrification (NH_4^+/NO_3^- ratio ≤0.6) and GI (>60%) indexes indicated that the composts had reached a reasonable degree of maturity (Bernal et al., 1998; TMECC, 2002). In accordance with the guidelines of European Commission (2001) both composts were classified as class 1 according to the heavy metal total concentration and satisfied the sanitation requirements (>55ºC at least during 2 weeks and 5 turnings). As a result, both composts were suitable for agricultural uses and they could represent a viable option to recycle nutrients without environmental risks.

5.5. Conclusions

This study demonstrated that a small proportion of biochar (4% dry weight) during composting of lignocellulosic N-poor materials had a significant effect on N transformations. Biochar favoured net nitrification, doubling the mineral N content, and minimised N losses. The addition of biochar increased the concentration of NO_3^- in the mature composts from 137.7 to 323.7 mg/kg and reduced N loss by

3.2 kg of N per ton of treated wastes. However, biochar did not increase the amount of N_2O, CH_4 and CO_2 released to the atmosphere, neither nutrient nor heavy metal total concentrations (total and water-soluble forms) were significantly affected.

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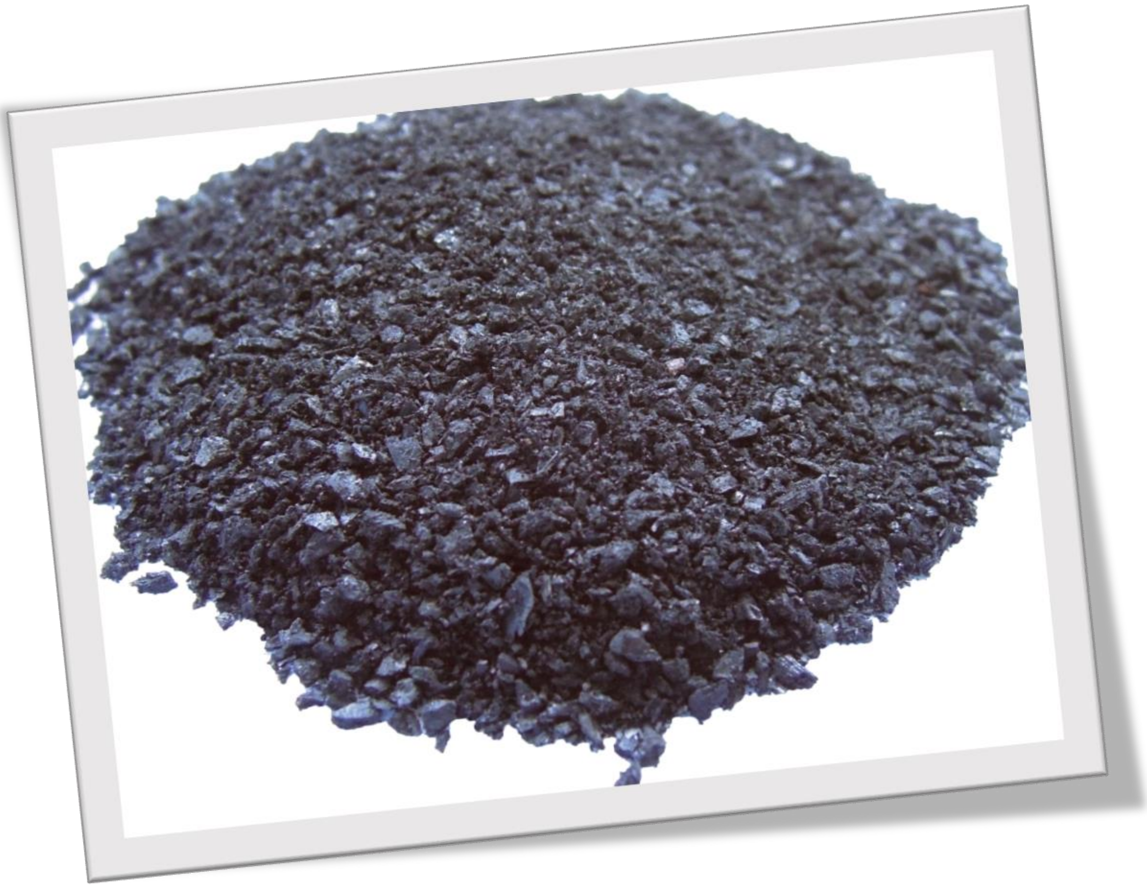

Chapter 6: General discussion and conclusions

6.1. General discussion

The integration of biochar into agricultural practices can represent a new strategy to C sequestration, GHG emission reductions and renewable energy generation (Roberts et al., 2010). It can also allow closing the nutrients cycle, by returning exported nutrients to agricultural soils (Lehmann and Joseph, 2015). All these agronomical and environmental benefits, associated to the agricultural use of biochar, have attracted the attention of researchers around the world. However, the acceptance by farmers of this agricultural practice is limited by the cost of biochar and the availability of sustainable feedstocks for the pyrolysis process.

A wide range of feedstocks can be used to produce biochar. Most of the research performed in this area has been based on the use of clean wood biomass for the production of biochar. However, there is an increasing interest for the use of alternative and sustainable feedstocks such as the case of organic wastes coming from urban and agricultural origins, which could replace the traditional use of wood. The revised legislative proposal on waste (EuropeanCommission, 2015) aims at reducing the environmental impact from landfilling of wastes (including agricultural residues, household, commercial and industrial wastes) and to ensure their valorisation, reuse or recycling. The pyrolysis of these organic wastes to produce biochar, have gained considerable interest worldwide as an alternative recycling option.

In this thesis, the suitability of different organic wastes as feedstock for the production of biochar has been explored. This suitability was assessed by studying the agronomical characteristics of biochars from waste biomass and by performing an agricultural and environmental evaluation of biochars as soil amendment or as composting additive. The fate of heavy metals associated to the use of biochar as soil amendment was also investigated, especially in the case of organic wastes of urban origin, where the presence of pollutants may represent a limitation for their agricultural use.

6.1.1. Agronomical characteristics of biochars

The origin of feedstock and the pyrolysis conditions are known to define the physicochemical properties of biochar, and consequently their opportunities and constraints (IBI, 2014; Lehmann and Joseph, 2015). In chapter 3 a wide variety of biochars were produced by slow pyrolysis according to two different technologies (a Pyromat reactor and a mono retort reactor) and two pyrolysis temperatures (at 400 and 600°C) from six different feedstocks.

The temperature of pyrolysis had a remarkable impact on molar ratios H/C org and O/C org, which have been proposed as an index of aromaticity of biochars and also show the degree of thermochemical alteration (Enders et al., 2012; IBI, 2014). A molar H/C org ratio below 0.7 indicates that the biochars have been "thermochemically converted" (IBI, 2014) and present typical properties of biochars, like a high proportion of recalcitrant C, whereas biochars with H/C ratio above 0.7 (O400, GW400, CM400 and PM400) may be "thermochemically altered". They are not considered to be thermochemically "converted" because they have lower proportion of fused aromatic ring structures. Apparently, biochars at 400°C will be more easily biodegradables and reactive in soil become therefore an interesting option to study.

Beyond the pyrolysis conditions, the feedstock origin, either agricultural or urban waste and its lignocellulosic composition were the main factors driving the physicochemical properties of biochars.

The feedstock origin and the lignocellulosic composition determined the elemental composition, ash, fixed C and volatile matter content and the properties of biochar. Two groups of biochars with contrasting physicochemical properties were obtained depending on the feedstock origin. The pyrolysis of raw lignocellulosic biomass (O_{FS} and GH_{FS}) gave rise to biochars with similar physicochemical properties, characterised by percentages of C org, ash, fixed C and volatile matter, aromaticity and stability degree. However, the use of urban and pre-treated wastes as feedstocks (GW_{FS}, CM_{FS} and PC_{FS}) produced biochars with lower organic C, similar volatile content, and a lower concentration of fixed C. The increase of pyrolysis HTT in lignocellulosic biochars led to an increase in the concentration of C org and the aromatization of C. However, the trend was opposite for biochars from pre-treated wastes, with lower C org concentration in biochars at higher HTT.

According to the IBI guidelines (IBI, 2014), biochars can be categorised according to their C org content. The biochars in this study can be classified as follows: O and GH in class 1; OC, GW, CM and PM in class 2, and PC in class 3. Thus, only raw lignocellulosic feedstocks gave rise to the top quality class 1 biochars.

The agronomical characterisation stablished a group of wood and raw lignocellulosic biochar characterised by a general low amount of macro and micro nutrients and another group of urban waste and manure biochar with a higher amount of nutrients. In general, the N content was low in all biochars, but slightly higher in rich-ash biochars from agricultural and urban waste than in oak biochars. Thus, PM biochars had the largest amount of N that can be due to the high protein and mineral N concentration in the feedstock (Tsai et al., 2012), whereas in the rest of biochars N components were incorporated into the biochar structure during the pyrolysis process (Knicker, 2010). These differences in the N composition would have consequences for the N availability in soil (Knicker, 2007). In general, all biochars showed reduction of N concentration with increasing temperature. In the case of Fe, Cu, Mn and Zn, the levels of these elements in the biochars were driven by their concentrations in the original feedstocks. In general, the concentration of nutrients was enriched with the increasing pyrolysis temperature, especially in raw lignocellulosic biochars, due to a concentration effect, as was previously observed by Enders et al. (2012).

The original feedstock also affected the water holding capacity of biochar. This is an important characteristic of biochar that can improve the agronomic quality of soils suffering from water scarcity

(Masiello et al., 2015). Biochars with a high C org content were able to retain higher amounts of water. Therefore the raw lignocellulosic biochars, especially GH and O biochars prepared at high temperatures (Gray et al., 2014), showed an increase in this capacity.

6.1.2. Agronomical evaluation of biochar as soil amendment

Despite the differences observed in physicochemical properties of biochars obtained from different feedstocks, their response in the soil was quite similar for all of them, showing a low impact with the most important nutrient cycles (C, N and P). The main differences among biochars were originated by the presence of relatively higher content of ash in biochars prepared from urban and pre-treated organic wastes, compared to biochars from raw lignocellulosic biomass. Finally, when biochars were added to the soil during the incubation experiments, negative impacts on the dynamics of nutrients or phytotoxic effects were not observed, even with biochars from urban and pre-treated organic wastes that could have higher toxic soluble and volatile organic compounds. Moreover, soils amended with urban and pre-treated organic biochars (GW400, CM400 and PC400) required, lower amounts of water than the control during the growing experiment but the availability of this water to the plant is uncertain. Hence, the agricultural use of these biochars can be only limited by the presence of heavy metals.

6.1.3. Agronomical evaluation of biochar as compost additive

The use of biochar in composting can also represent an opportunity to ensure a sustainable use of the resources, by integrating biochar production into the waste management scheme of the farm. Chapter 5 focussed on the use of biochar as additive during composting of TPOMW and sheep manure.

A number of benefits associated to the use of biochar as additive in composting have been recently reported (Steiner et al., 2015). The main advantages are the enhancement of the composting process, a reduction of the N losses associated to the process and a reduction of greenhouse gas emissions, especially CH_4 and N_2O. The composting experiment was performed at pilot scale in an organic farm “Casa Pareja” (Murcia, Spanish southeast). A small addition of biochar (4%) improved N cycling by increasing NO_3^--N content, indicating a higher nitrifying activity, and reducing N losses by 15% without affecting the amount of N_2O released.

The improvement of N cycling is especially important in the case of TPOMW composting since these wastes are characterised by a low N concentration and, more importantly, a low N availability originated by the recalcitrant structure originated by the large amounts of lignocellulosic compounds. In spite of the low application rate of biochar, there was a considerable impact on N transformations and at the end, biochar addition showed a significant effect for total N and NO_3^- concentrations, which was twice higher than in the control pile. However, biochar addition did not significantly affect concentration of neither other nutrients nor heavy metals. As a consequence, the biochar improved the agronomic value of the end product because a higher amount of N was retained in the compost.

These results open a new opportunity for the integration of biochar into local waste management practices at farm level. The use of locally available lignocellulosic residues, such as olive tree prunings may represent a valuable option to obtain biochar and incorporate it in the composting activities taking place in the farm.

6.1.4. Potential limitations of the agricultural use of biochar.

Heavy metals

The use of organic wastes as feedstock for the production of biochar may lead to an enrichment in the concentration of heavy metals, already present in the wastes. The pyrolysis causes a loss of organic matter that leads to an enrichment in heavy metal concentration as a consequence of a mass concentration effect. For this reason, the study of the fate of heavy metals is of especial relevance.

The concentrations of heavy metals in biochar were driven by the origin of the feedstock. Agricultural wastes (O_{FS} and GH_{FS}) presented low levels of heavy metals, whereas urban wastes and pre-treated wastes (GW_{FS}, CM_{FS} and PC_{FS}) presented high heavy metal concentration due to the urban origin and /or the concentration effect underwent during the pre-treatment.

In all cases the concentration of heavy metals in the biochars were low, and only in the case of CM biochars, GW600 and PC600, these levels were above the thresholds established by the EBC, a guideline on the production of biochar developed by the European biochar Foundation (EBC, 2012). In the case of O and GH biochars the contamination with Cr and Ni was evident, as a consequence of leaking from the reactors (Camps-Arbestain et al., 2015).

Despite these concentrations, the heavy metals present in biochar are usually immobilised due to precipitation and the alkaline pH (Lu et al., 2015; Méndez et al., 2012). As reported in Table 4.5

and 4.6, the amount of DTPA-extractable heavy metals in the biochar was very low, and in general, the levels of available heavy metals represented less than 10% of the total amount present in the biochar, confirming the immobilisation of these elements in the structure of the biochar.

The low availability of the heavy metals was confirmed in the soil incubation experiments, where the levels of DTPA-extractable heavy metals in soils were very low. Thus, Cr and Cd were below the detection limits (0.02 mg Kg^{-1}), and Ni and Pb were below 0.30 and 1.97 mg Kg^{-1}, respectively, which is again in agreement with the immobilisation capacity of biochar (in Figures 4.12 and 4.13).

The mobility of heavy metals from biochar is known to be affected by the presence of plants that can alter the physicochemical and biological conditions of the soil, affecting the solubility of heavy metals and favouring their bioavailability (Houben, 2013). In our experiments, the levels of heavy metals uptaken by the plants were below the detection limit in the case of Cd, and in the case of Cr, Ni and Pb these levels were lower than the maximum allowed concentration for edible plants (Cd and Pb) (EuropeanCommission, 2011; EuropeanCommission, 2014).

Despite the potential limitations because of the presence of heavy metals in the organic wastes and the concentration effect underwent during the thermochemical conversion, the availability of heavy metals after biochar soil application seems to be very low. However, biochars from urban origin need to be treated with caution, since their use might be limited in some cases because of their high heavy metals concentration.

6.2. General conclusions

1. A series of biochars with suitable properties as soil amendment was prepared from organic wastes of agricultural and urban origins, with the only exception of biochars from urban wastes prepared at 600°C and CM biochars.

2. The physicochemical properties of biochars were driven by the characteristics of feedstocks and the pyrolysis temperature. Raw lignocellulosic feedstocks originated biochars with the lowest ash concentrations and highest fixed C. On the other hand, the use of pre-treated organic wastes as feedstocks produced biochar with the highest ash and nutrient concentrations. The use of high temperatures increased the ash content and increased the losses of N and O containing functional groups.

3. The concentration effect from organic matter loss and/or the contamination from the pyrolysis reactor increased the concentration of heavy metals in biochar respect to the original feedstock. Thus, especial attention must be paid in the selection of organic wastes of urban origin as feedstocks, since the presence of heavy metals can represent a limitation for their use in agricultural practices.

4. The different origin and physicochemical properties of biochars (C org and ash content) had a low impact on the most important nutrient cycles (C, N and P), being the responses in soil quite similar. No phytotoxic effects or negative impacts on soil nutrient dynamics were found.

5. The presence of heavy metals in some of the biochars prepared from feedstocks of urban origin can limit their use as soil amendments (according to biochar current guidelines). However, an experiment with lettuce showed that the heavy metals uptaken by plants were lower than the maximum allowed for edible plants.

6. Biochar acted as an effective additive for olive mill waste composting and favoured the initial activation of the composting process but did not have a clear impact on CO_2, CH_4, and N_2O emissions.

7. The addition of 4% of biochar decreased N losses and doubled the mineral N content of olive mill waste compost, increasing its nutritional value. Biochar did not affect the concentration of heavy metals in compost.

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