Biochemical characterization of hydrochars and pyrochars derived from sewage sludge and their potential as N and P slow release fertilizers and as soil amendment in agriculture

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Memoria que presenta

Dña. **Marina Concepción Paneque Carmona**

para optar al título de Doctora por la Universidad de Sevilla.

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Visado en Sevilla, a 15 de septiembre de 2018

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CERTIFICA: Que la presente Memoria de Investigación titulada "Biochemical characterization of hydrochars and pyrochars derived from sewage sludge and their potential as N and P slow release fertilizers and as soil amendment in agriculture", presentada por Dña. Marina Concepción Paneque Carmona para optar al título de Doctora por la Universidad de Sevilla, ha sido realizada en el Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), y reúne todas las condiciones exigidas a los trabajos de Tesis Doctoral.

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Dr. José Enrique Fernández Luque

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RESUMEN

La cantidad de fertilizantes minerales que actualmente necesita el sector agrícola para mantener su producción no es sostenible. Al mismo tiempo la sociedad actual genera una gran cantidad de residuos orgánicos que representan un problema ambiental a escala global y que deben ser gestionados. En este sentido, el reciclaje se contempla como una de las opciones más sostenibles desde el punto de vista medioambiental y económicamente viables. Se ha mostrado en diversos estudios que el uso de lodos de depuradora en agricultura no solo disminuye la cantidad de este residuo es acumulado en vertederos, sino que además se trata de una fuente de nitrógeno (N) y fósforo (P) que podría contribuir de manera significativa a reducir el uso de fertilizantes inorgánicos. Sin embargo, los nutrientes que contienen los lodos de depuradora pueden ser lixiviados después de que estos hayan sido añadidos al suelo, incluso si estos han sido sometidos a un tratamiento de estabilización previo.

Teniendo en cuenta el problema anterior, investigaciones recientes sobre los procesos de carbonización de residuos orgánicos ricos en N indican que carbonización hidrotermal y la pirólisis (denominadas respectivamente en la presente Tesis Doctoral HTC y Py) podrían producir una enmienda orgánica más estable que aumente el potencial de secuestro de C en el suelo y liberar el N y el P de forma más lenta. Considerando que la estructura química de un producto es clave en su funcionalidad, esta Tesis Doctoral se ha centrado en primer lugar en la caracterización de lodos de depurada tratados mediante pirolisis (Py) y carbonización hidrotermal (HTC). Posteriormente se ha evaluado el potencial estos productos como fertilizantes de liberación lenta de N y P.

La primera fase de este trabajo consistió en la pirolización y HTC de dos lodos de depuradora diferentes. La HTC se realizó a 200 y 260 °C durante 0.5 y 3 horas mientras que la pirólisis se desarrolló a 600 °C durante 1 hora. El producto que resulta de una HTC se denomina comúnmente por la comunidad científica "hydrochar" mientras que el que se obtiene tras una pirólisis se llama "pyrochar". En conjunto, los hydrochars y pyrochars son productos carbonizados (pirolizados) denominados "*chars*".

Para estudiar la composición y propiedades de los lodos y chars así como los cambios que tienen lugar durante los tratamientos térmicos se llevó a cabo una caracterización detallada de las muestras en la que se usaron tanto técnicas analíticas rutinarias como otras más avanzadas, entre las que se incluye la espectroscopia de resonancia magnética nuclear de ^{13}C y ^{15}N en estado sólido.

Los resultados de esta Tesis Doctoral mostraron una reducción en la presencia de grupos carboxilos y O/N alquilos así como un ligero incremento de los grupos

aromáticos tras la HTC mientras que la pirólisis transformó la mayor parte de la materia orgánica en estructuras aromáticas. Además, la HTC preservó parte del N inorgánico (N_i) y estabilizó otra parte en compuestos heterocíclicos aromáticos con substituciones de N. A estas formas heterocíclicas nitrogenadas se las ha denominado "Black Nitrogen" (BN) como sugieren los trabajos científicos previos. En los chars procedentes de la pirólisis, en cambio, todo el N se encontraba en forma de BN. Estos resultados sugirieron que los hydrochars pueden tener propiedades como fertilizante de N a corto y medio plazo mientras que los pyrochars pueden aportar dosis de N más bajas durante un mayor periodo de tiempo y, además, incrementar el potencial de secuestro carbono del suelo.

Para evaluar el impacto potencial de los chars producidos a partir de lodos de depuradora en el crecimiento de los cultivos se realizó un experimento de invernadero en el cual los hydrochars y pyrochars se añadieron como enmienda a un suelo en el que *Lolium perenne* creció durante 80 días. Los hydrochars incrementaron la producción de biomasa total a pesar de que redujeron la tasa de germinación. Los pyrochars, sin embargo, no afectaron ni a la germinación ni a la producción de biomasa de esta planta. El impacto de los hydrochas en el crecimiento de *Lolium* en este experimento se atribuyó a la presencia de N_i y de formas de N orgánicas fácilmente degradables. Estos resultados están en concordancia con las predicciones realizadas previamente, basadas en los datos de caracterización.

El potencial de los lodos carbonizados como fertilizantes de liberación lenta de P se estudió evaluando los cambios del contenido de P en el lodo durante la pirólisis y la HTC. Este trabajo mostró que, a pesar de que ambos tratamiento produjeron un enriquecimiento en P, la cantidad de P disponible disminuyó. Este resultado se debe probablemente a un incremento de los complejos metal/P y a posibles limitaciones físicas. Sin embargo, es necesario realizar una investigación más profunda para corroborar esta hipótesis.

Por otro lado, el experimento de invernadero mencionado en el párrafo anterior también se utilizó para evaluar tanto el comportamiento del P de los lodos tratados como su impacto en el suelo donde se aplicaron. Este estudio mostró que los pyrochars, y en menor medida, los hydrochars producidos a 200°C fueron capaces de incrementar la cantidad de P disponible del suelo, por lo que serían útiles como fertilizantes de P. Además, una parte del P total que originalmente estaba no disponible en los chars pasó a la fracción disponible durante su incubación en el suelo. Este resultado apunta que estos materiales poseen cualidades como fertilizantes de liberación lenta de P.

Para evaluar el potencial de los chars como fertilizantes de liberación lenta de N y para secuestrar carbono se realizó un experimento basado en el uso de materiales enriquecidos en isótopos estables ^{13}C y ^{15}N. Para ello se produjo un lodo de depuradora enriquecido en ^{13}C y ^{15}N, que posteriormente se transformó en hydrochar y pyrochar. Estos chars se añadieron como enmienda un suelo en el que se cultivó *Lolium perenne* durante 10 meses bajo condiciones controladas en invernadero. La recalcitrancia del C y el N orgánico de las enmiendas se estudió monitorizando la distribución del ^{13}C y ^{15}N en el suelo y las plantas a lo largo del tiempo. Los resultados mostraron que el pyrochar es más estable que el hydrochar, coincidiendo con los resultados de caracterización. Por tanto, los pyrochars serían los más apropiados para aumentar el potencial de secuestro de C de los suelos. En relación al potencial como fertilizante de N, la HTC redujo ligeramente la cantidad de N disponible para las plantas en comparación con el lodo no tratado, mientras que el pyrochar mostró una disponibilidad de N mucho menor. Tanto el hydrochar como el lodo no tratado presentaron una mayor disponibilidad de N al principio del experimento, la cual disminuyó con el tiempo. Sin embargo, el pyrochar mostró una disponibilidad de N mucho más baja pero constante a partir del primer mes de incubación. Por otro lado, tanto el pyrochar como el hydrochar fueron capaces de evitar la lixiviación de NO_3- que sí se observó en el lodo no tratado. Este ensayo confirmó que los hydrochars son más adecuados como fertilizantes de liberación lenta a corto y medio plazo. Además, nuestros resultados sugieren que los pyrochars pueden ser útiles para aportar bajas cantidades de N durante periodos prolongados. Sin embargo, es necesario realizar experimentos a largo plazo para poder confirmar esta hipótesis.

ABSTRACT

The current demand for N and P fertilizers to meet the needs of agriculture is not sustainable. On the other hand, the huge amount of organic waste derived from human activity is a current environmental problem that needs to be solved urgently. Therefore, its recycling should be favored over disposal at landfills. Using sewage sludge (SS) as an alternative N and P sources in agriculture can alleviate both issues and may help to reduce our dependence on chemical fertilizers. However, aside of hygienic issues and possible input of organic and inorganic contaminants, nutrient leaching has been observed after SS application to soil, both directly and after stabilization by composting.

Current knowledge indicates that pyrolysis and hydrothermal carbonization (HTC) can convert organic residues into recalcitrant amendments that may add to the C sequestration potential of soils. Bearing further in mind the high N and P content of SS, charring of the latter is likely to result in a product which slowly releases its N and P after its amendment to soils for plant growth. Considering that the chemical structure of a product defines its function, the present PhD thesis first focused on the characterization of pyrolyzed and HTC treated SS. Subsequently its potential to act as slow-release N and P fertilizer and its C sequestration potential was evaluated.

Therefore, the first task of this work was the HTC and pyrolysis of two different SS derived from different steps of the water clean-up process of a rural waste water cleaning plant in Carrión de los Céspedes (Chapter 3). The HTC was performed at 200 and 260 °C with residence times in the reactor of 0.5 and 3 h, whereas pyrolysis was carried out at 600 °C for 1 h.

The changes of the chemical composition of the feedstock during the thermal treatments were studied through a detailed characterization of the products by not only routine analyses but also more advanced techniques such as solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy. It was evidenced that HTC reduced the carboxyl and *O/N* alkyl C contents and slightly increased the proportion of aromatic C, whereas pyrolysis transformed most of the organic matter (OM) into aromatic structures. In addition, HTC preserved part of the formed inorganic N (N_i) and stabilized the remaining N as N-heterocyclic aromatic compounds (Black Nitrogen, BN). In contrast, the pyrochars revealed organic BN. These results suggest that hydrochars have immediate and medium-term N-fertilizer properties, whereas pyrochars seems to be more useful for supplying lower doses of N over a longer time period and for increasing the C sequestration potential of soils.

In order to evaluate the impact of the pyrolyzed and HTC-treated SS on plant growth, the previously produced chars were added to a soil on which *Lolium perenne* grew during a 80-day greenhouse incubation experiment (Chapter 4). Despite the soil amended by hydrochars showed a low germination rate, the 200°C-produced hydrochars exhibited the highest net rate of plant biomass production. In contrast, pyrochars did not alter the germination rate, neither did it increase plant biomass production compared to the control. The positive impact of hydrochars on plant growth in this short-duration experiment was probably caused by the concomitant presence of N_i and easily available organic N forms. In contrast, pyrochars exhibited no short-term N fertilization properties. These results were in line with our previous predictions which were based on the previous characterization.

In a next step, the potential of pyrolyzed and HTC-treated SS as slow-release P fertilizer was evaluated by studying the alterations of the extractability of P of the SS during both thermal treatments were studied (Chapter 5). This work showed that, despite both thermal treatments lead to P enrichment, the extractability of the latter was generally reduced. This was likely due to an increase of metal/P complexes or to physical and chemical sequestration of either inorganic or organic P within the aromatic network. Nevertheless, further research is needed to proof those hypotheses.

The greenhouse experiment was also used to evaluate the behavior and the impact of the chars on P extractability, once they were incorporated in a soil. This study showed that in the pots amended with the pyrochars and the hydrochars produced at 200°, higher amounts of P were extractable from the soil at the end of the experiment than at the beginning. Thus, formerly sequestered and immobilized P-forms must have been mobilized, which suggests their suitability as slow-release P fertilizers.

To study the potential of our products for soil C sequestration and as slow release N fertilizer, materials enriched in stable isotopes of ^{13}C and ^{15}N was carried out (Chapter 6). Therefore, ^{13}C and ^{15}N enriched SS was produced in a fermenter in which ^{13}C-glucose, $^{15}NH_4Cl$ and $K^{15}NO_3$ was added to waste water from a waste water treatment plant and transformed into hydrochar and pyrochar. Subsequently, these amendments were added to a Cambisol on which *Lolium perenne* grew during a 10-months greenhouse incubation experiment. The recalcitrance of the organic C and N in the amendments was studied by monitoring the distribution of ^{15}N and ^{13}C among soil and plants as a function of the incubation time. Our results confirmed that pyrochars are biochemically more stable than hydrochars, which is in agreement with the characterization data. Thus, pyrochars are indeed more appropriate to be used for increasing the C sequestration potential of soils. Regarding the N fertilization potential, HTC provided a slightly lower amount of N for plant growth than the non-treated SS. Pyrochars released the lowest amount of N usable for plant growth. Both the non-treated and HTC-treated SS showed the highest N loss at the beginning of the experiment, which decreased with increasing experiment time. In contrast, pyrochars depicted a low but constant N release only after the first month of experiment. Anyway, both HTC and pyrolysis avoided the NO_3^- leaching observed for the non-treated SS. The results of this experiment demonstrated the suitability of hydrochars as short and medium-term N-fertilizers. However, although the pyrochar was less successful for a short-term fertilization, the slow release of N of this product may be favorable if low-dose but long-term N fertilization is needed.

Summarizing the results of the present study, it was shown that addition of SS-chars to soils, can increase plant biomass production if compared to un-amended soils. However, it is unlikely that SS-chars can completely substitute mineral fertilizers. Anyway, the net balance of this approach is clearly positive due to: i) the remarkable reduction of the N and P losses by avoiding the disposal of this organic waste and ii) the replacement of mineral fertilizers, which are limited and demand high-energy. Thus, the transformation of SS into pyrochar or hydrochar for its use as soil amendment can certainly be seen as an efficient tool to recycle N and P from organic waste.

INDEX

1. INTRODUCTION

1 INTRODUCTION

1.1 Chemical fertilizers

The amount of nitrogen (N) and phosphorus (P) in farmlands greatly determine crop yields. Traditionally, agriculture relied on natural levels of soil nutrients and on the addition of locally available organic matter (Cordell et al., 2009). Chemical N fertilizers were first used in the 19th century. However, it was not until the beginning of the 20th century, with the development of the Haber-Bosch process, that its production and application at high-scale was feasible (Razon et al. 2018). During the same period, inorganic high-grade P fertilizers, derived from mined phosphate rocks, appeared on the market (Cordell et al., 2009).

The extensive application of these fertilizers, along with the introduction of new crop varieties during the so called "Green Revolution" hugely raised crop yields. However, they have been and are still overused which can result in the eutrophication of water bodies, threatening delicate ecosystems, and in an increase of greenhouse gas emission. Thus, N fertilizers have potential impacts on global climate change as well as ozone depletion (Razon et. 2018). Rosmarin et al. (2004) estimated that close to 25% of the 1 billion tons of P mined since 1950 have ended up in water bodies or has been buried in landfills.

Nowadays, the use of these products is more environmentally conscious and more stringent regulated (European Nitrate Directive, Directive 91/676/EEC), which has reduced N surpluses substantially. In addition, the demand for P in most developed countries has stabilized or is decreasing after its use during the first decades of the Green Revolution since it increased the P background levels of many agricultural soils (Cordell et al., 2009). However, intensive agriculture still depends on huge amounts of chemical fertilizers. At present, the annual P need in agriculture of the European Union (EU) is estimated to be 3.85 million tons per year (Toth et al., 2014) whereas the global N fertilization is projected to reach 236×10^6 MT per year in 2050. (Tilman et al., 2001).

Nowadays, fertilizer production is oil-dependent and consumes 1.2% of the annually produced world's total energy, of which 94% is used for the synthesis of ammonia (Razon et al., 2018; IFA, 2009). Considering that world oil-reserves are depleting (Kerschner et al., 2013) and that the remaining phosphate rocks may be exhausted in the next 50-100 years (Cordell et al., 2009), alternative N and P sources are urgently needed.

1.2 Slow-release fertilizers

The release rate of P and N from conventional mineral fertilizers is higher than the respective plant uptake. In addition, P fertilizers can be transformed into non-available forms before the crops use them. Consequently, their nutrient use efficiency is low and ranges between 30-35% for N and between 18-20% for P (Guo et al., 2018). This means not only a waste of energy and materials used for their production but also an important waste of money for farmers. In addition, the non-used fertilizers can either leach or volatilize, which jeopardize the environment. Therefore, there is a great interest and need in developing fertilizers with high nutrient-use efficiency-rates. Various formulations where the nutrients are slowly released or release in a controlled manner have already been proposed (Trenkel, 1997; Rose et al., 2016; Guo et al., 2018).

According to Trenkel (1997), slow- or controlled-release fertilizers are those containing a plant nutrient in a form, which either (a) delays its availability for plant uptake and use after application, or (b) is available to the plant significantly longer than a reference "rapidly available nutrient fertilizer".

According to Rose et al. (2016) most of the present slow-release N fertilizers consist of:

- polymer coatings that delay fertilizer release;
- higher-stability chemical fertilizers that take more time to solubilize;
- granules coated or mixed with specific compounds that inhibit N transformation pathways, such as urease or nitrification inhibitors.

However, the addition of N in the form of N-rich soil organic amendments is also an option. In this case, the N release rate will depend on the degradation rate of the organic N (N_{org}) into available plant-forms.

The development of slow-release P fertilizers barely exists (Landel et al., 2003). Nevertheless, the traditional use of local organic matter (OM) such as manure and human excreta as P source meets with the approach of the N-rich soil organic amendments previously mentioned.

1.3 Organic waste

Human economic and social activities are associated with the generation of huge amounts of waste, which poses not only environmental problems, but has also a negative impact on human health. Therefore, the EU has created a substantial body of regulations to address this problem and turn Europe into a recycling society.

The EU policies which regulate the biodegradable wastes are:

- ✓ The **Sewage Sludge Directive (86/278/EEC)**, which encourages the application of sewage sludge (SS) in agriculture and regulates its use to prevent harmful effects on soil, vegetation, animals and humans. It was initiated more than 30 years ago and currently, the European Commission is considering its revision.

- ✓ The **Landfill Directive (1999/31/EC)**, which obliges the member states to reduce the amount of biodegradable municipal waste that they landfill.

- ✓ The **Waste Framework Directive (2008/98/EC)**, which prioritizes the prevention of waste production, followed by the preparation of waste for reuse and recycling, being waste disposal the least preferable option.

The **Report from the European commission on the implementation of EU waste legislation for the period 2010-2012** (last one published) shows some positive data:

- During this period, approximately 45% of the produced sludge was recycled for agriculture.
- Most member states reported a year-by-year reduction of landfilling of biodegradable waste.
- The 2020-target of recycling 50% of household and similar waste was already reached or in the way to be reached for at least half of the Member States.

However, despite these promising data, the achievements of the different EU countries vary remarkable and some member states still need to work hard to efficiently implement the above-mentioned directives.

These directives are in line with the **EU Action Plan for the Circular Economy**, which supports the transition to a sustainable development based on the principles of a low carbon, resource efficient and competitive economy. This legal framework has promoted the transformation of organic wastes into added-value products usable in agriculture.

1.4 Production of sewage sludge

In the EU, one major source of organic waste is sewage sludge (SS), since the implementation of the Urban Waste Water Treatment Directive 91/271/EEC (CEC, 1991) forced EU countries to improve their wastewater collecting and treatment systems, leading to a steadily increase of its annual production. Despite some member states (i.e. Austria, France, Germany, Sweden) have stabilized or even slightly decreased their SS production over the last years, the EU generates more than 10 million tons of SS (dry solid) every year (Table 1.1).

Table 1.1. Total quantities of sludge production (Thousand tons) in EU countries. Source: Eurostat, 2018 (personal compilation)

	1991	**2006**	**2010**	**2015**
Belgium		127.5	176.3	157.2 *(2012)*
Bulgaria		38	49.8	57.4
Czech Republic	166.9	203.4	196.3	210.2
Denmark			141	141 *(2010)*
Germany	2,956	2,099.9	3,656.60	1,820.60
Estonia		27.6	27.9	34.1 *(2013)*
Ireland		77.7	90	58.4
Greece	48.2*(1990)*	126	151.5 *(2009)*	116.1 *(2014)*
Spain	270	1,065	1,205.10	1082.7 *(2012)*
France	865*(1992)*	1059.8 *(2004)*	966.4	961.5 *(2014)*
Croatia			57.6	25.5
Italy		1056.4 *(2005)*	1,102.70	1102.7 *(2010)*
Cyprus		8.3 *(2005)*	7.1	6.7
Latvia		23.9	21.4	22.8 *(2013)*
Lithuania			51.3	42.9
Luxembourg		15.2	9.7	9.2
Hungary		237.6	184.3	171.8
Malta			1.2	8.4
Netherlands	320 *(1990)*	372.7	551	551.2 *(2014)*
Austria		254.6	262.8	239 *(2014)*
Poland		501.3	895.1	951.5
Portugal	8	189.1 *(2007)*	344.3 *(2009)*	338.8 *(2012)*
Romania		225.6	376.1	411.2
Slovenia		19.5	30.1	29.1
Slovakia		54.78	54.8	56.2
Finland	162	148.8	142.7	141.2 *(2012)*
Sweden	217 *(1990)*	207.1	203.5	197.5
United Kingdom	1,072	1,809	1,419.10	1136.7 *(2012)*
Norway	45.5			
Switzerland		210	210 *(2009)*	194.5 *(2013)*
Albania				91.5
Serbia			303 *(2009)*	14.3
Turkey			1181.9 *(2008)*	850 *(2014)*
Bosnia and Herzegovina		0.8	0.7	1.3 *(2013)*
Total EU	**6130.6**	**10,159.6**	**14,071.40**	**11,233.40**

Sludge derives from the process of **treatment of wastewater**, which consists basically in the following steps (Werther and Ogada, 1999):

- ✓ Firstly, the wastewater passes through racks, screens and grid chambers, where coarse and heavy solid materials are separated.
- ✓ Secondly, readily settable solids and floating materials are removed in sedimentation tanks. The separated material forms the **primary sludge.**
- ✓ A biological treatment phase follows. The activated sludge system is the most common intensive biological treatment of wastewater. During this process the microorganisms convert the colloidal and dissolved OM into various gases which are released into the atmosphere, or incorporated into cell tissues, which subsequently precipitates. The precipitated sludge removed at this stage is called **secondary sludge**.

Some Wastewater Treatment Plants (WWTPs) perform **advance biological treatments** which also **remove N and P dissolved in the wastewater**. This occurs in modified activated sludge systems with anaerobic-aerobic or anaerobic-denitrifying spatial-temporal unit processes. Nitrogen elimination is usually achieved by firstly oxidizing the ammonia to nitrate (nitrification phase) and thereafter converting the nitrate into volatile N (Werther and Ogada, 1999). In contrast, the phosphorous compounds are incorporated into the microorganisms which, thereafter, will be removed through sedimentation (Gebremarian et al., 2011).

These intensive biological treatments achieve a wastewater treatment with reduced space and during a short period of time. In extensive systems microorganisms degrade the colloidal and dissolved OM with low efficiency. They can consist in artificial ponds or in more sophisticated infiltration-percolation or wetland systems. The extensive systems require more space than the intensive one, but are cheaper, use less energy and the operation and maintenance is lower (Rodíguez-Roda et al, 2008). As such, these technologies are often used for small urban agglomerations.

The waste water treatments tend to concentrate **heavy metals, poorly biodegradable trace organic compounds** and potentially **pathogenic organisms** into the SS. However, the heavy metal concentrations in SS have steadily declined in the EU since the mid 80s due to better control of use and discharge of dangerous substances, voluntary agreements and improved industrial practices. In contrast, nanoparticles, pharmaceuticals and personal care products are now new emerging contaminants which must not be ignored (Fijalkowski et al., 2017).

Use of SS in agriculture

In addition to heavy metals and organic compounds, SS contains also valuable **OM** and is rich in nutrients such as **N** and **P**. Thus, the application of this waste on agricultural soils as a fertilizer or as organic soil improver has been promoted by the EU (Sewage Sludge Directive (86/278/EEC).

During 2016, in the EU, the average total N and P contents of sludge recycled for agriculture were 3.6 and 2.8 %, respectively. For Spain those values were 4.5 and 3.6 % (EC, 2008b). The N plant availability in SS ranges between 15-85% compared to the availability of N in inorganic fertilizers and depends on how the sludge was stabilized before its addition to the soil. In contrast, the plant availability of P from SS is less influenced by the treatment process and can account to 50% in most sludge products (EC, 2008b).

Despite the Sludge Directive allows the application of untreated sludge on soil under specific conditions, it is rarely applied and in some member states it is even prohibited (EC, 2008a). The most common **stabilization process** consists in a mesophilic anaerobic digestion, or aerobic digestion. After this, it is treated with polymers and dewatered using filter presses, vacuum filters or centrifuges. Other treatment processes include long-term storage, conditioning with lime, thermal drying and composting (EC, 2008b).

The latter, however, consumes space and increases greenhouse gas emission by releasing CO_2 and volatile N. Alternatively, thermal treatment such as pyrolysis or hydrothermal carbonization (HTC) may be used for efficient hygienization with concomitant stabilizing organic C and N within a relative short process time. Of course, during thermal treatment greenhouse gases are also released, but this emission may be compensated by recycling the produced thermal energy for other energy requiring purposes.

Former solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopic studies indicated that N in N-rich chars can be bioavailable (De la Rosa and Knicker, 2011) but is less bioavailable than inorganic N (López-Martín et al., 2017). Thus, if the biochemical resistant of charred organic residues is not as high as thought but l, these materials can still be degraded, carbonized SS may be applicable as slow-release fertilizers. Thus, a major goal of the present work was to test if this approach is feasible and to elucidate if amendment of charred SS can add to the reduction of the need of mineral P and N.

1.5 Hydrothermal carbonization and dry pyrolysis

These technologies consist in the thermochemical carbonization of biomass at elevated temperatures in the absence or restricted amounts of oxygen. During these processes, liquid and gaseous (by-) products are formed due to the degradation of biomacromolecules, whereas the solid residue considerably differs from the original biomass.

During **pyrolysis**, the biomass is carbonized in a largely inert atmosphere such as N_2 and CO_2 at temperatures between 300 and 700 °C (Lehmann and Joseph, 2015). The resulting product is named pyrochar and can be considered as biochar if it accomplishes with certain characteristics, described below.

Hydrothermal carbonization (HTC) carbonizes the biomass in the presence of water at 180-250 ˚C during 0.5-12 h under autogenous pressure and the solid product is called hydrochar (Libra et al., 2011).

The term '*wet pyrolysis*' also refers to HTC whereas 'dry pyrolysis' is used for pyrolysis.

Pyrolysis can destroy hazardous organic compounds, present in the feedstock but may also produce them by recondensation of volatiles (Sharma et al., 2004). In contrast to organic compounds, metals are not removed and will be enriched in the final char.

The **chemical reactions involved** in both processes include hydrolysis, dehydration, decarboxylation, aromatization and recondensation (Lehmann and Joseph, 2015; Funke and Ziegler, 2010). However, the reactions mechanisms are scarcely understood. Aside from realizing that the chemistry of the substrates defines the properties of the product, it is known that the different reaction media during the dry-pyrolysis and HTC play a defining role. During pyrolysis, the temperature was reported to be the main factor determining the dominating reactions. In contrast, the HTC starts breaking the biomacromolecules into fragments through hydrolysis, which may allow the initiation of completely different chemical pathways than in pyrolysis. Thus, despite pyrochars and hydrochars have some similarities they also differ considerably both in their chemistry and characteristics (Libra et al., 2011). To which extend those differences affect the behavior as soil amendment still has to be explored and will be a part of the present research.

The HTC usually achieves a higher solid yield than pyrolysis. In addition, the nature of the feedstock, process temperature and reaction time are the main factors influencing the **composition and properties of hydrochars and pyrochars** (Lehmann and Joseph, 2015; Libra et al., 2011). Generally, pyrolysis is supposed to increase the porosity and surface area (Bagreev et al., 2001); however this is also highly dependent on the feedstock (De la Rosa et al., 2014). In contrast, hydrochars usually show much lower porosity and surface area values than pyrochars (Bargmann et al., 2014).

According to the **European Biochar Foundation** "Biochar is a heterogeneous substance rich in aromatic carbon and minerals. It is produced by pyrolysis of sustainably obtained biomass under controlled conditions with clean technology and is used for any purpose that does not involve its rapid mineralization to CO_2 and may eventually become a soil amendment." In addition, its organic carbon content should be above 50% and its atomic H/C ratio below 0.7 in order to be considered biochar for this organization. Bearing in mind that this PhD thesis involves pyrolyzed materials which rarely meet these thresholds, the term pyrochar will be use to refer to pyrolyzed materials.

The application of biochar to soil has been proposed as a strategy for restoring C to depleted soils by concomitantly sequestering significant amounts of CO_2. Despite a wide range of literature has been published around this topic, a clear conclusion is still missing (Lehmann and Joseph, 2015). In contrast, the potential of hydrochars to increase the potential of soils as carbon sinks has been scarcely studied (Libra et al., 2011).

Moreover, biochar has been suggested as an useful soil conditioner which enhance plant growth. However, the results of the literature are not consistent since the biochar properties, the soil type and the climate conditions seems to determine the impact of such amendments on the soil properties and on crop yields (Jeffery et. al., 2011; Haider et al., 2017; Jeffery et. al., 2017). The potential of hydrochars to increase crop production has received much less attention, but still, a bunch of works have been published (Bargmann et al., 2013; Thuille et al., 2015; Fang et al, 2015).

Chemistry of charred organic residues

The idea of using biochar to sequester C in soils is based on its assumed high biochemical resistance. The chemical structure of charred organic residues has been traditionally considered a hardly-degradable polycondensed aromatic network. However, new studies found solid data which contradict this idea (Knicker et al., 2005; Hammes et al., 2008) and an alternative model was suggested (Knicker et al., 2008). According to this model, charred organic residues are composed of a heterogeneous mixture of thermally altered biomacromolecules, the alteration degree of which depends upon the chemistry of the feedstock. In any case and in the worst scenario, from the point of view of stability, biochar will always be more stable than the residual biomass that has been used as raw material for its production.

In addition, the presence of elements other than C seems to occur. Accordingly, N-rich residues results in chars with a significant portion of N-heteroaromatic C (Knicker, 2010). Thus, being less graphite-like and containing N, O, P and S substitutions, microbial and chemical attack is more likely than commonly assumed. Some publications already support this theory. De la Rosa and Knicker (2011) demonstrated that at least some of the N incorporated into plant chars was used for plant growth. Moreover, López-Martín et al. (2017) found that N of plant chars was recycled for the built-up of peptides in newly synthesized microbial biomass. However, further research is needed to judge if these results can be extrapolated to chars derived from other feedstocks.

1.6 Fundamentals of the advance techniques used

1.6.1 Solid-state nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a technique based on the interactions of the magnetic properties of nuclei with a strong external magnetic field. This approach allows the identification of single atoms and molecules in various media in solution as well as in solid-state. Depending on the sample and the applied technique information about chemical and physical structure, dynamics, reaction state, and chemical environment of molecules can be obtained.

The following provides a short introduction into the technique. For more details, the interested reader is refered to Knicker (2010):

NMR is a physical phenomenon based upon nuclei with magnetic properties. Such nuclei possess a spin, thus a magnetic moment. Exposed to an external magnetic field, they align themselves either parallel or antiparallel to this field, which forces the nuclear spins onto energy levels the altitude of which is defined by nuclear properties and the strength of the external field. Applying a radiofrequency pulse, which corresponds to a multiple of the energy difference between those levels (resonance condition), spin transitions between the latter are induced. Once the radiofrequency signal is turned off, the distribution of the spins among the energy levels returns to thermal equilibrium, re- emitting radio waves (spin-lattice relaxation), the frequency of which depends upon the magnetic properties and the environment of the nuclei as well as the strength of the external magnetic field.

Thus, nuclei in different chemical and physical environments, for example carbons in carboxylic or aliphatic groups re-emit radio waves with different frequencies, which give rise to distinct resonance lines.

Since NMR can only be performed on nuclei which possess a spin, ^{12}C cannot be used and the experiments have to be performed with ^{13}C. However, if the spin $I > ½$, as it is the case for ^{14}N, the nucleus shows quadrupole properties leading to considerable line broadening. Therefore, the ^{15}N isotope is used. The proton (^{1}H) is the most sensitive nucleus for NMR detection, due to its high natural abundance. For biological studies ^{13}C and ^{15}N are also important, however the presence of these isotopes in nature is with 1 atm% and 0.37 atm% very low.

For soils and biochars, solid-state NMR spectroscopy represents an excellent tool, since it allows the characterization of insoluble heterogeneous organic mixtures, thus of a bulk sample without prior extractions, although the resonance lines are broad and single components are hardly identified.

In general, solid-state NMR spectra of soil organic matter (SOM) and biochars are obtained with the cross polarization magic angle spinning (CPMAS) technique (Wilson, 1987). This technique enhances the ^{13}C and ^{15}N NMR spectra resolution by transferring the magnetization (cross polarizing) from a sensitive spin system such as ^{1}H to a spin system with low natural abundance and low sensitivity like ^{13}C and ^{15}N during the so called Hartmann-Hahn contact. After this transfer, the magnetization of the less abundant nuclei is detected. During the measurement the sample is rotated with high speed around the magic angle of 54.74° in order to average line broadening induced by insufficient averaging of nuclear interaction due to the low molecular motion in solids.

1.6.2 Isotopic ratio mass spectrometry (IRMS)

Stable isotope ratios are typically measured by isotope ratio mass spectrometry (IRMS). Isotopic variations in nature are due to a variety of kinetic and thermodynamic factors and, hence, the measurement of the isotope ratios allows inferring processes (e.g., heterotrophic nitrification), estimating rates (e.g., soil C turnover) and helps understanding the global elemental cycles. In addition, materials with an unusually high or low concentration of an isotope can be used as tracers.

The following introduction into the technique was summarized from Michener R. and Lajtha K. (2007):

A mass spectrometer is an instrument that separates charged atoms or molecules on the basis of their mass-to-charge-ratio, m/z. There are four basic components of isotope ratio mass spectrometry: inlet system, ion source, mass analyzer, and ion detector. Samples are introduced to the mass spectrometer as a gas and are carried in a helium stream through a chromatographic column. If samples are solids, they are combusted prior to entry into the chromatographic column. Then, the sample flows through a changeover valve which discard part of the sample and sends the other part towards the ion source. The ion source consists of a wire of tungsten, rhenium, or thoriated iridium that releases electrons when it is electrically heated under high vacuum (~10−8torr). The electrons are then accelerated and enter in the ionization box, where they impact the sample gas, forming positively charged particles. The resulting ion beam enters in the mass analyzer, where a magnetic field perpendicular to the flight path bends the beams, with the lighter isotope beam bending more than the heavier isotope beam because the radius of curvature is proportional to the square root of the mass-to-charge ratio (√m/z). Specialized Faraday cups are the ion detector. Current-generation isotope ratio mass spectrometers have three or more Faraday cups, positioned to capture specific masses simultaneously.

The isotopic differences between various materials in nature are exceedingly small and it is not convenient to express them as isotopic abundances (mole fractions). Therefore, they are reported relative to internationally accepted standards and expressed in parts per thousand deviations from the corresponding standard by:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) x\ 1000$$

Where R is the ratio of heavy-to-light (typically, but not always, rare-to abundant) isotope, R_{sample} is that ratio in the sample, and $R_{standard}$ is that in the standard.

In tracer studies the isotopes are at high levels, and they are usually expressed in atom%:

$$\text{atom \%} = 100 \text{ x } \left(\frac{R_{sample}}{1+R_{sample}}\right)$$

The natural dynamic of the C and N in soils has been extensively studied by measuring the variation of the natural $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. However, other investigations such as evaluating the efficiency of N fertilizers or the degradation rate of a soil amendment are better achieved with tracer studies.

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2. RATIONAL AND OBJECTIVES

2 RATIONAL AND OBJECTIVES

There is an increasing urge to reduce the use of mineral fertilizers. At the same time, modern society produces a huge amount of organic waste, rich in N and P, which needs to be treated or preferentially recycled as value-added product. Although SS has been identified as having the potential to satisfy both needs, nutrient leaching has been observed after its application to soil both directly and after stabilization. In addition, hygienization as well as destruction of potential organic pollutants have to be performed prior to its application to soils.

Therefore, the present PhD proposes pyrolysis and hydrothermal carbonization (HTC) as a mean to convert SS into a hygienized product which after its application to soil degrades only slowly but still provides its N and P for plant growth. As such, it may have the potential to act as a slow-release fertilizer. This would reduce the amount of organic waste for disposal on the one hand and the dependence on chemical fertilizers as well as nutrient leaching on the other hand.

It is well known that the chemical structure of a product defines its function. However, presently the knowledge about the chemical reactions that occur during the thermal treatment of SS is still scarce. Therefore the main **objectives** of the present work were:

- to bring some light on the thermally induced alteration by an in-detail characterization of the chemical composition and some physical properties of the treated SS produced under different process conditions.
- to elucidate if the thermally altered products allow plant growth and how the latter is affected by the different N and P forms of the chars.
- To obtain some insights about the stability of the organic N forms of the charred SS (Black Nitrogen) in soils after their amendment and to elucidate their potential for C sequestration.

The actual knowledge on the chemistry of N-rich charred residues as well as on its behavior after its addition to soil bases the initial **hypotheses** of this work:

i) During the pyrolyzation and HTC of SS at least part of its N will be integrated into the aromatic network as heterocyclic N which will be mobilized only slowly for plant growth after its amendment to soil.

ii) Although the dynamic and chemistry of P is not comparable to that of the N, we expect a similar behavior for both during the pyrolysis and HTC of at least the organic P fraction.

iii) Once applied to land, these chars will be attacked and degraded by microorganisms, leading to a slow mobilization of its N and P.

iv) Relative to untreated SS, the chars will show a higher biochemical resistance and have the potential to add to the C sequestration potential of soils. As a consequence some of the organic N will also stay in the soil and may be usable for plant growth at a later time.

In order to achieve those objectives the following **goals** were define:

1. The processing conditions (pyrolysis/HTC, temperature, residence time, etc) influence the composition and properties of the final product, which in turn determine its potential use. Consequently, the first goal of this work was **a detailed characterization of the composition and chemical changes occurring during HTC and pyrolysis of SS.** Different temperatures and residence times were used. This characterization was not limited to the organic C fraction but included also the N-forms. **(Chapter 3)**

2. Despite char properties can indicate its potential as soil amendment and nutrient source, its use for plant growth has to be tested. Therefore, the second task of this work was to **evaluate the impact of the hydrochars and pyrochars on the germination and growth of *Lolium perenne* under the controlled conditions of a greenhouse incubation study. (Chapter 4)**

3. The potential of pyrolyzed and HTC-treated SS as slow-release P fertilizer depends on the speciation of this element in the chars as well as on the potential changes of this speciation once the chars are applied to the soil. Therefore, we **studied the alteration of the P contained in the SS during the HTC and pyrolysis processes as well as the possible changes in the P availability of the chars after their soil addition. (Chapter 5)**

4. The biochemical resistance of SS-derived hydrochars and pyrochars determines their degradation rate and thus their potential for C sequestration. However they are also related to their potential as slow release N fertilizers. Therefore, **^{13}C and ^{15}N enriched SS was produced** in the laboratory **and transformed into hydro- and pyrochar, which were amended to soil on which *Lolium perenne grew* during a 10-month greenhouse incubation experiment. The recalcitrance of the organic C and N in the chars was monitored** by determining their stable isotopic ratios in the soil and plants as a function of incubation time. **(Chapter 6)**

3. Hydrothermal carbonization and pyrolysis of sewage sludges:

what happen to carbon and nitrogen?

3 HYDROTHERMAL CARBONIZATION AND PYROLYSIS OF SEWAGE SLUDGES: WHAT HAPPEN TO CARBON AND NITROGEN?

Abstract

Hygienization by thermochemical carbonization may be one option to enable the use of sewage sludge (SS) as soil amendment and nitrogen (N) fertilizer. To evaluate this possibility, SS derived from different water purification processes of a rural waste water treatment plant were hydrothermal carbonized (HTC) at 200 and 260 °C for 0.5 and 3.0 h, and pyrolyzed at 600 °C for 1h. During HTC, temperature rather than residence time affected the chemical alteration. Solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy showed considerably lower aromaticity in the hydrochars than in the pyrochars. Whereas the aromatic network of the hydrochars is dominated by polyfurans and N-heterocyclic aromatic units that of the pyrochars is mainly composed of arene structures. The highest total and inorganic N contents were obtained via HTC at 200 °C for 30 min, thus this material may be applied if both immediate and slow N release is needed.

Keywords

Slow-release N fertilization, hygienization, pyrochar, hydrochar, solid-state NMR spectroscopy

3.1 INTRODUCTION

In the European Union (EU), the major N and P sources in agriculture are synthetic fertilizers although inputs from animal manure remain important, especially in regions of high livestock density. The use of synthetic fertilizers has dramatically increased food production worldwide, but the unintended costs to the environment and human health due to surplus and inefficient application have also been substantial. Thus, alternatives are needed. One of those represents the valorization of organic waste, the amount of which increased exponentially during the last decades. Its estimated annual increase is around 25 million tons (WRAP, 2008).

A major source of organic waste is sewage sludge (SS). Containing high amounts of N, with a median of 3.3 % (Sommers et al., 1977), P and OM, this material offers an excellent feedstock for the production of soil amendments that can reduce the need of synthetic P and N fertilizer (Kern et al., 2008; Theobald et al., 2016). At the same time it may increase the C-sequestration potential of cropped soils. In addition, sludge recycling as fertilizer or organic amendment helps to reduce the amount of organic waste by returning it into the bio-cycle while at the same time soil physical and chemical properties are restored (Goss et al., 2013; Werther et al., 1999). Indeed, the Sewage Sludge Directive (Council Directive 1986/278/EEC) and the working document on sludge (European Commission, 2000) describe the use of SS on soils as beneficial but also seek to encourage a safe use of this material in agriculture and to regulate its application to prevent harmful effects on soil, vegetation, animals and humans (Kelessidis and Stasinakis, 2012). Thus, in order to enable the use of SS as soil application, it first has to be decontaminated by removing organic pollutants and pathogen bacteria. Different treatments can be used for the latter: thermal drying, anaerobic digestion, conditioning with lime, etc. In general, the process of removing pathogen bacteria of SS is called hygienization. Heavy metals content also need to be taken into account. In cases their concentrations are above the allowed thresholds, strategies have to be developed to avoid secondary contamination due to SS application.

The thermochemical carbonization of SS at elevated temperatures in the absence of oxygen has recently been considered to achieve sludge stabilization and hygienization. In the presence of water, this kind of carbonization is called hydrothermal carbonization (HTC), whereas in the absence of water it is known as pyrolysis. During pyrolysis, temperatures between 300-700°C are used (Lehmann and Joseph, 2015). The solid product is considered as biochar, according to the European Biochar Foundation, if it derives from a feedstock approved for this organization, its organic carbon content is above 50% and its atomic H/C ratio is < 0.7 (European Biochar Foundation, 2012). Considering that these thresholds are rarely reached with carbonized SS, in the following we refer to pyrolyzed SS as pyrochar. Hydrochars represent the solid residue after heating biomass together with water at 180-250˚C for 1-12 h under autogenous pressure (Libra et al., 2011). Due to the high humidity of SS, the application of HTC avoids the necessity of an additional drying step. Both types of carbonization include reactions such as dehydration, decarboxylation, aromatization and recondensation (Funke et al., 2010; Libra et al., 2011). However, the different process conditions lead to significant differences of both the chemistry and physical characteristics of the solid products (Libra et al., 2012). Accordingly, hydrochars contain a high amount of furan-type structures. On the other hand, pyrochars produced at high temperatures are composed of an arene-rich core (Falco et al., 2011; Baccile et al., 2009; Baccile et al., 2011).

Despite alterations of the organic C fraction during the pyrolysis and HTC of biomass have already been focus of many investigations (Lehmann and Joseph, 2015; Lu and Berge, 2014; Reza et al., 2014a, 2014b) the knowledge about changes of organic N is still scarce. Previous studies have shown that after incomplete combustion of N-rich plant residues and casein at 350 °C for several minutes a considerable part of their organic N was incorporated into heterocyclic aromatic structures, the so called pyrogenic organic nitrogen (Knicker et al., 2008; Knicker, 2010). Although this nitrogen is less bio-available than mineral fertilizer it was mobilized and used by plants and microbes for the neo-formation of biomass and amides (De la Rosa and Knicker, 2011). Thus, N-rich carbonized OMs may be prime candidates for the development of slow N-release fertilizers, since the mobilization of their N is expected to be sufficiently low to avoid fast N losses due to leaching or nitrification shortly after fertilizer application. A deeper knowledge of the N transformations during the thermochemical carbonization of SS is necessary in order to evaluate its potential to fulfill this function. Wei et al. (Wei et al., 2015) found that ammonia (NH_3) is the main product of protein-N during SS pyrolysis and that the majority of pyridine N originally present in SS tends to be converted into hydrogen cyanide (HCN) at 400-600 °C. Studying HTC as a potential tool to reduce NH_3 and HCN emissions during the pyrolysis of SS-derived residues, Liu et al. (Liu et al., 2017) confirmed the conversion of nitrogen into more stable forms by the application of HTC.

Since chemical composition and physical properties of a soil amendment define its function, the goal of the present research was a detailed characterization of the chemical changes during HTC and pyrolysis of two different SS. This analysis was not limited to the organic C fraction but included also the N-forms. The SS used in this study derived from a rural waste water treatment plant of a community with approximately 3000 inhabitants. They were collected from an extensive and an intensive cleaning process. It is important to bear in mind that physical and chemical properties of the SS depend on the wastewater treatment process by which it was produced and on the characteristics of the treated effluents (Zielinska et al., 2015). For example, the commonly called primary SS is formed from suspended organic material which is separated by sedimentation; whereas secondary sludge constitutes mainly residues of bacteria biomass which is involved in the digestion of the easily metabolized organic fraction of the water. Thus, primary sludge is expected to be biochemically less stable than secondary sludge (Goss et al., 2013). This may also affect the chemical alteration during thermal carbonization, which was applied for hygienization before it can be applied as soil amendment. In order to evaluate the potential of the products as slow-release N fertilizer, their chemical structures was not only analyzed by Infrared (IR-FT) and solid-state ^{13}C NMR spectroscopy but also by solid-state ^{15}N NMR spectroscopy. Preliminary analysis confirmed that heavy metal contents of the products were below allowed thresholds. With the approach used for this study and as known to the authors, we were able to obtain the first solid-state ^{15}N NMR spectra of hydrochars.

3.2 MATERIALS AND METHODS

3.2.1 Sewage sludge

Two different SS were collected at the Experimental Wastewater Treatment plant (CENTA), located in Carrion de los Céspedes, within the province of Seville (Spain). This plant processes the domestic wastewater of the village with over 3000 residents. The experimental nature of this plant allows treating the wastewater by using different intensive and extensive technologies.

The first sample, A_SS, is a primary sludge produced after the settlement of suspended OM and its subsequent anaerobic digestion in a pond. The long residence time of five to ten years, promote stabilization of the remaining OM.

The second sample, T_SS, is a secondary sludge produced in an extended aeration treatment system and later stored in a thickener in order to reduce its water contents. However, due to the long residence time of the sludge in the thickener, part of the organic matter can be further mineralized and humified.

3.2.2 Production of chars

Considering the high amount of water in sewage sludge, we focused on the study of the HTC effect since this technique avoids the necessity of an additional drying step. However, we also produced two samples by using pyrolysis for comparison.

For the production of hydrochars 1 L-stirred pressure reactor (Parr reactor series 4520, IL, USA) equipped with an external resistance heater and internal sensors for pressure and temperature was used and the material was heated at 200 °C and 260 °C for 30 min and 3 h with a heating rate of 3 °C min^{-1}. For each temperature and time, 225 g of SS were mixed with 450 g of distilled water and placed into the reactor. During HTC, the mixture was stirred at a constant speed of 90 rpm. The reactor required approximately 120 min to reach the desired temperatures. The temperatures were maintained at the set-point during the whole reaction time after which the heater was turned off and the heat insulation was removed. A hooked-shaped agitator stirred the sludge water mixture with a rotation velocity of

90 rpm. After cooling for approximately 15 h, the HTC slurry was filtered through fluted filter paper and the solids were dried for 48 h at 60 °C.

For the production of the pyrochars, 200 g of dry SS were placed into a close steel container and heated in a preheated muffle oven at 600 °C for 1 h.

3.2.3 Chemical composition

The dry-weight of the samples after pyrolysis was determined after drying at 105 °C, the ash content after heating at 750 °C for 5 h. The pH values were measured in distilled water (1:2.5, w/v). Hydrogen (H), C and N contents were obtained with an elemental analyzer (Vario EL III, Elementar Analysensysteme, Germany). The amount of oxygen (O) was calculated by difference. For the analysis of plant available N ammonium (NH_4^+) and nitrate (NO_3^-), those N-components were extracted with 0.0125 M $CaCl_2$ (5 ml g^{-1} dry sample) and measured by flow-injection analysis (FIA System, MLE, Germany).

3.2.4 Solid-state ^{13}C and ^{15}N nuclear magnetic resonance spectroscopy

Prior to the solid-state NMR spectroscopy, the SS and the chars were subjected to four successive treatments with 10 % (m/m) hydrofluoric acid (HF) to increase the organic matter concentration by removing minerals and paramagnetics. After discarding the supernatant, the solid residue was rinsed with distilled water until a pH-value > 5 was yielded and finally freeze-dried (Gonçalves et al., 2003).

The solid-state ^{13}C NMR spectra were obtained with a Bruker Avance III HD 400 MHz WB spectrometer, using a triple resonance broadband probe and zirconium rotors of 4 mm OD with KEL-F-caps. The cross polarization (CP) technique was applied during magic-angle spinning (MAS) of the rotor at 14 kHz. A ramped 1H-pulse was used during a contact time of 1 ms to circumvent Hartmann-Hahn mismatches. Pre-experiments confirmed that a pulse delay of 300 ms was long enough to avoid saturation. Between 5.000 and 13.000 scans were accumulated for each sample, and the line broadening was between 50 and 100 Hz. The ^{13}C chemical shift scale was calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). The relative ^{13}C intensity distribution was determined by integrating the following chemical shift regions: alkyl C (0–45 ppm); N-alkyl/methoxyl C (45–60 ppm); O-alkyl C (60–90 ppm); anomeric C (90-110 ppm); aryl C (110-140 ppm); O/N-aryl C (140–160 ppm); carboxyl/amide C (160–220 ppm).

For none of the samples, the atomic H/C ratio was below 0.5 indicating that the protonation degree was sufficiently high for efficient cross polarization. Thus the intensity distribution of the spectra is comparable to the chemical composition of the organic matter of the sample. Note that in highly aromatic samples the region commonly assigned to anomeric C is dominated by resonances from aromatic structures. At a spinning speed of 14 kHz the chemical shift anisotropy is not completely averaged, which causes spinning side bands appearing at both sides of the parent signal at a distance of the spinning speed (here between 250 and 300 ppm and between -50 and 0 ppm). Their intensity was added to the region of the parent signal.

The solid state ^{15}N NMR spectra were obtained using the same instrument but a 7 mm double resonance probe and operating at 40.56 MHz. The contact time was 1 ms. A 90 °H pulse width of 3.5 µs, a pulse delay of 200 ms and a line broadening between 50 and 100 Hz were applied. Between 300.000 and 2 millions of scans were accumulated at a MAS speed of 6 kHz. The chemical shift was standardized to the nitromethane scale (0 ppm) and adjusted with ^{15}N-labelled glycine (-347.6 ppm). The following chemical shift regions were integrated: amide N (-248 to -285 ppm) and pyrrole N (-150 to -248 ppm).

In order to obtain more insights into the chemical alterations occurring during pyrolysis, the recovery of organic C in each C group in the respective char as a function of treatment Cx (hc) was calculated according to equation 1:

$$Cx\ (hc) = \frac{C\ (x) * C\ (t)}{100} \qquad [1]$$

Here, C (t) is the remaining C in the hydrochar in % of the C content of the original SS (Cs), C(x) is the relative ^{13}C intensity of a ^{13}C chemical shift region.

3.2.5 Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra were recorded using a JASCO 4100 spectrometer at wavenumber ranging from 4000 to 400 cm^{-1} and a resolution of 2 cm^{-1}. Potassium bromide pellets containing about 1% (w/w) of each biochar were prepared in a cylindrical piston under high pressure and vacuum. In order to improve the signal-to-noise ratio, 60 scans were registered and accumulated for each recorded spectrum. The spectra were corrected against a spectrum of pure KBr pellet prior to every measurement. All IR data manipulations were performed using JASCO spectra manager software® (Japan).

3.3 RESULTS AND DISCUSSION

3.3.1 Weight loss and alteration of the composition during carbonization

3.3.1.1. Mass loss during charring

Among the applied treatments, pyrolysis at 600 °C resulted in the lowest yields, which accounted for 71 and 65 % of the original dry weight for the pyrochars from A (A_Py_600_1) and T (T_Py_600_1), respectively (Table 3.1). Yields after HTC ranged between 87 and 73%. Whereas an increase of the residence time did not change the yields, augmentation of the temperature from 200 °C to 260 °C resulted in a slight increase of the mass loss. Compared to our recoveries of 87 and 83% for A_HTC_200_0.5 and T_HTC_200_0.5 respectively, Danso-Boateng et al. (2015) reported considerably lower values of 67% of the initial mass after HTC of primary SS at comparable conditions. This may be best explained by the different biochemical stability of the feedstock, which remarks the importance to consider the SS production conditions for the discussion and extrapolation of HTC characteristics.

3.3.1.2. Ash content

Approximately half of the untreated A and T SS is composed of ash (Table 3.1). These results are comparable with those obtained by Zielinska et al. (2015). The ash in the SS sample probably derives from the contribution of street wastewater run offs into the wastewater collecting system and from the sand used during the mechanical pre-treatment of the water-cleaning process. Charring leads to dehydration and loss of OM, which increases the relative content of ash in the respective chars. The highest ash contents were determined for the samples A and T pyrolyzed at 600°C under dry conditions (761 and 726 g kg^{-1} respectively; Table 3.1).

Table 3.1. Weight recovery after the thermal treatment of primary (A_SS) and secondary (T_SS) sewage sludges, the ash contents, the elemental composition (C, N, O, S, H) and the recovery for the respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively, as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

			Elemental composition				**Recovery with respect to the untreated sample**			
Sample	**Weight Recovery**	**Ash content**	**C**	**N**	**O^b**	**H**	**C**	**N**	**O**	**H**
	% DM^a	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	% DM	% DM	% DM	% DM
A_SS	100	540	228	19	168	34	100	100	100	100
A_HTC_200_0.5	87	611	224	16	108	30	85.1	72.4	55.5	75.2
A_HTC_200_3	87	625	214	14	110	27	81.2	62.9	56.3	68.0
A_HTC_260_0.5	82	656	213	11	82	26	76.9	48.0	40.3	63.2
A_HTC_260_3	82	657	221	12	72	27	79.1	51.6	35.0	63.9
A_Py_600_1	71	761	134	9	82	9	41.6	32.0	34.6	18.6
T_SS	100	507	245	32	167	37	100	100	100.0	100
T_HTC_200_0.5	83	570	233	25	128	30	79.0	64.3	64.1	66.0
T_HTC_200_3	79	612	233	24	89	27	75.1	58.5	42.0	58.0
T_HTC_260_0.5	76	645	225	20	69	26	69.5	46.2	31.1	52.6
T_HTC_260_3	73	661	224	19	57	25	66.7	42.9	24.9	49.7
T_Py_600_1	65	726	168	16	70	9	44.6	32.9	27.3	16.3

[a] DM: dry matter
[b] calculated by difference

3.3.1.3. Elemental composition (C, H, N, O)

The total C content of the non-treated SS is < 250 g kg^{-1}. Whereas between 85 and 67% of the organic C in the SS survived HTC, pyrochars contained only 42 and 45% of the initial amount of C (Table 3.1). This is explained by a more efficient incomplete carbonization during pyrolysis compared to HTC (Libra et al., 2011). Compared to wood-derived biochars which C contents > 500 g kg^{-1}, the low carbon concentration of both, the original SS and the chars may limit their use if only an enhancement of the carbon sequestration potential of soils is wanted. Compared to C yields after charring, the O and H recoveries are lower, which reflects ongoing dehydratation, dehydroxylation, decarboxylation and condensation. This is confirmed in the Van Krevelen diagram (Figure 3.1) which shows a decrease of atomic H/C and O/C ratios as carbonization enhances. Note that all hydrochars depict atomic H/C values > 1. Thus, in average, each C atom is directly bound to at least one H, which opposes a highly condensed aromatic structure (Knicker et al., 2005; Wiedemeier et al., 2015). On the other hand, the pyrochars exhibit values of 0.7 and 0.8, which assigns each 1.5 C to one hydrogen, allowing aromatics condensed structures with maximal six rings in average. These results are within those expected according to bibliography (Sevilla et al., 2011; De la Rosa et al., 2014; Peng et al., 2016).

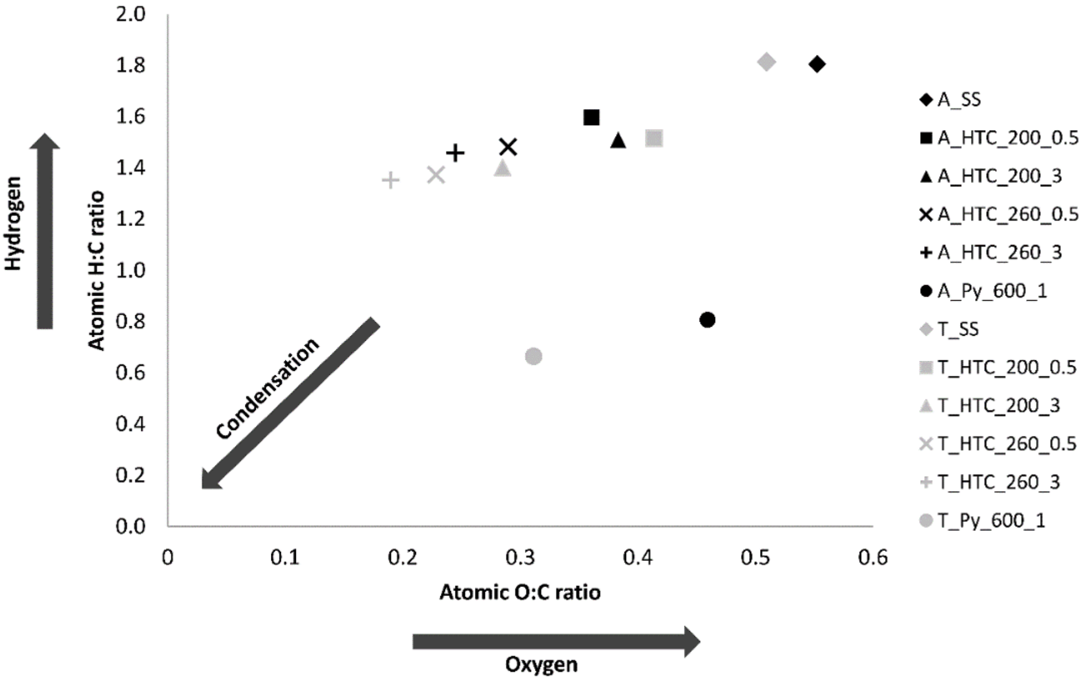

Figure 3.1. van Krevelen diagram of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

The total N content of A_SS and T_SS are 19 and 32 g kg^{-1}, respectively (Table 3.1). These values are within the range reported for this material (Porta et al., 2003). Compared to C, higher losses of N were observed for both hydrochars and pyrochars. However, in all the charred samples the N contents are still > 9 g kg^{-1}, most of which occurs in organic forms (> 97 %) (Table 3.2). Therefore, the organic N sequestered in these chars is likely to affect the N cycling in soils once this material has been applied. The bioavailability of organic N in the chars is influenced by its composition and by the chemical and biological changes it undergoes after its addition to soil, but still, it will be lower than that expected for inorganic N forms (N_i). After all charring treatments, the C_{org}/N ratio (w/w) increased slightly, most tentatively due to deamination and degradation of amino acids and amino sugars, but also by volatilization of NH_3 and nitrous oxides. However, even after inclusion the pyrochars, the C/N ratios do not exceed the value of 20, which is considerably lower than biochars from wood (De la Rosa et al., 2014). Thus, if the organic N in chars occurs in bioavailable forms, both the hydro- and pyrochar can serve as an efficient additional N-source for biomass production provided sufficient bioavailable C is present, too.

The contribution of N_i to total N increased after HTC from 0.5 to 2.6 % and from 0.4 to 2.2 % for A and T hydrochars, respectively. However, after pyrolysis all N_i was lost (Table 3.2). Regarding the different available inorganic nitrogen forms, for both A_SS and T_SS the NH_4-N contents are considerably higher (115 and 123 mg kg^{-1}, respectively) than their NO_2-N and NO_3-N contributions. This trend was also observed by Alvarenga et al. (2015), Adegbidi and Briggs (2003) and Hossain et al. (2011). Increasing NH_4-N levels after HTC at 200 °C are best explained with deamination reactions. Increasing the temperature leads to further transformation and to N losses into the gaseous phase. Here, one has to bear in mind that the liquid phase which was discarded can represent a further considerable sink for inorganic N. Comparable trends, but at a much lower concentration level, are observed for NO_2-N and NO_3-N. The increase of NH_4^+ after HTC is in agreement with the results obtained by Sohn and Ho (1995), who demonstrated that after heating aqueous solutions of amino acids at 180 °C during 2 h, considerable amounts of NH_3 were released. On the other hand, Jin-hong et al. (2014) reported a considerable decrease of available NH_4-N and NO_3-N for SS hydrochars produced at 190 and 260 °C during 1, 6, 12, 18 and 24 h. They may have achieved the lack of N_i in their samples because of intense rinsing of the HTC-chars with distilled water. Thus, post-treatment processes also need to be taken into account to evaluate the fertilizer potential of HTC chars. Here one has to bear in mind that efficient removal of N_i may counteract fertilization purpose since this easily available N form would be accessible already during the early plant growing stage.

Table 3.2. pH, C_{org}/N ratios, and inorganic nitrogen (N_i) contents of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

Sample	**pH**	**C_{org}/N**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**N_i[a] of the total N**
		(w/w)	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%
A_SS	7.4	11.9	115	0.03	0.18	0.5
A_HTC_200_0.5	6.5	14.0	417	0.36	0.59	2.6
A_HTC_200_3	6.5	15.4	349	0.20	0.34	2.5
A_HTC_260_0.5	6.4	19.1	169	0.06	0.15	1.5
A_HTC_260_3	6.6	18.2	227	0.05	0.003	1.9
A_Py_600_1	9.3	15.5	0.2	0.003	-[b]	0.0
T_SS	7.5	7.6	123	0.04	0.57	0.4
T_HTC_200_0.5	6.7	9.3	542	0.76	1.26	2.2
T_HTC_200_3	6.2	9.7	341	0.36	0.44	1.4
T_HTC_260_0.5	6.3	11.4	239	0.09	0.47	1.2
T_HTC_260_3	6.4	11.8	256	0.04	-	1.4
T_Py_600_1	10.0	10.3	0.3	-	-	0.0

a) N_i : sum of NH_4-N,NO_2-N, and NO_3-N in %; b) - : below the detection limit

In contrast to the HTC chars, the pyrochars did not accumulate N_i and consequently, their remaining N must have been sequestered in organic constituents of the char as it was suggested by Knicker et al. (2010). Thus, whereas pyrolysis turns almost the entire liberated N into gaseous compounds HTC leads to deamination and accumulation of NH_4^+. However, some of the N will be also lost through the liquid phase.

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

3.3.2.1. Sewage sludge materials

The solid-state ^{13}C NMR spectra of both A_SS and T_SS are dominated by signals in the chemical shift region of alkyl C (0-45 ppm). Both lipids and peptides can contribute to this region, which comprises 43 for A_SS and 38 % for T_SS of the total ^{13}C signal intensity (Table 3.3). Their carboxyl C and amide C add to the signal at 174 ppm in the chemical shift region between 220 and 160 ppm (Figure 3.2). The O-alkyl C region (90-60 ppm) accounts for 17 and 23 % of the total ^{13}C intensity for A_SS and T_SS, respectively. The latter comprises signals from C2-C5 in carbohydrates, alcohols and ethers. The region between 110 and 90 ppm is commonly assigned to anomeric C (C1) in pentoses and hexoses but can also derive from C2 and C6 in lignin derivatives. However, lignin compounds are of minor importance in SS, which is mainly composed of proteins, carbohydrates, fats and oils (Werther and Ogada, 1999). In addition, A_SS contains slightly more aromatic C (160 to 110 ppm) than T_SS. Considering the low contribution of lignin to SS, most of this signal intensity derives from aromatic amino acids or olefinic structures, which contribute to this chemical shift region, too. Jin-hong et al. (2014) showed also a dominance of the alkyl C region for SS, with a value of 64 %. This is due to the large contribution of fat acids but also peptides typically present in domestic SS (Werther and Ogada, 1999).

Calculating the alkyl C/carboxyl C ratios from the intensity distribution of the ^{13}C NMR spectra as an index for the average chain length of the alkyl residues, values of 4.7 and 3.5 (Table 3.3) are obtained confirming that in our SS samples, lipids comprise a major fraction. This is in line with results by Jin-hong et al. (2014). The higher value of this ratio obtained for A_SS may be due to the anaerobic conditions during its production, which leads to a preferential preservation of long chain paraffinic structures. In addition, the higher N content of T_SS compared to A_SS is consistent with the higher ^{13}C-intensity in the region of N-alkyl C spectrum. This points to a higher proportion of proteinaceous material for the former than for the latter, which contributes to decrease the alkyl C/carboxyl C ratio. Carbohydrates are commonly preferentially degraded during thermal treatments, leading to the relative enrichment of all other compound classes. Based on this, Baldock et al. (1997) introduced the ratio alkyl C/O-alkyl C as an index for the degradation degree. Calculating this ratio, a value of 2.5 was obtained for A_SS and 1.7 for T_SS (Table 3.3), confirming that the material of the anaerobic pond (A) is more humified, thus biochemically stabilized, than that derived from the "thickener pond" (T).

The solid-state ^{15}N NMR spectra of both A_SS and T_SS are dominated by a signal at -260 ppm in the chemical shift region of amide N (from -248 to -285 ppm), Figure 3.3. This signal contributes with 69 and 74 % to the total ^{15}N intensity of the spectra of A_SS and T_SS, respectively (Table 3.5). Most of this nitrogen occurs as peptides and, to a lower amount, as amides in amino sugars. Note that some amide structures are also contributing to the shoulder between -230 and -248 ppm. The respective *N*-alkyl C of these compounds gives rise to signals in the chemical shift region between 60 and 45 ppm of the ^{13}C NMR spectra. This signal contributes with 13 and 14 % to the total ^{13}C intensity of the spectra of A_SS and E_SS, respectively (Table 3.3). Although lignin is not considered as a major component of sewage sludge, its methoxyl C would contribute to the signal of this region, too. The peak at

-346 ppm, which appears in both A_SS and T_SS ^{15}N NMR spectra, corresponds to free aliphatic amino groups of amino acids or amino sugars, but the low signal-to-noise ratios of the spectra does not allow an unbiased differentiation of such a signal from the noise. Regarding pyrrole compounds (from -150 to -248 ppm) A_SS shows a higher contribution, with a 22 %, than T_SS with a 17 % (Table 3.5). However, in contrast to Liu et al. (2017) who applied X-ray photoelectron spectroscopy (XPS), no indications for the presence of pyridine N or nitrile N was evidenced. In studies about the nitrite fixation to humic material, their chemical shifts were found between -65 to -80 ppm and -90 to -140 ppm, respectively (Thorn et al., 2016).

3.3.2.1. Hydrochars and pyrochars

After HTC, the contribution of the chemical shift region of alkyl C to the total ^{13}C intensity increased for the A samples from 43 (A_SS) to 55 % (260 °C, 30 min) and for the T set from 38 (T_SS) up to 52 % (260 °C, 3 h) (Table 3.3). An augmentation in this region was also observed by Jin-hong et al. (2014) after HTC of SS at 190 and 260 °C for 1 h. Table 3.4, showing the recovery of C in the different C groups after HTC treatment, indicates that there was no net-synthesis or degradation of lipids, but a relative enrichment due to the loss of other compounds. Thus, lipids were not affected by HTC, which is in line with previous works of Popov et al. (2016) and Shin et al. (2012). The latter demonstrated that three kinds of fatty acids were stable under subcritical water conditions at temperatures of 300 °C or below.

As expected, HTC resulted in decarboxylation which is revealed by the diminishment of the recovery of C as carboxyl C. Whereas O- and N-alkyl C were degraded, as HTC charring temperature and time increased, aromatic C was formed, most likely by dehydration and cyclization of carbohydrates and peptideous material. This is indicated by the increase of C which occurs as aryl C and O- and N-aryl C for both, the “A” and “T” hydrochars (Table 3.4). The latter suggests the formation of furans and N-heterocyclic aromatic structures. Jin-hong et al. (2014) reported comparable results for SS hydrochars. Considering that O/N-aryl C constitutes a third of the aromatic C in the hydrochars, a predominantly polyfuran core with additional N-heterocyclic aromatic units instead of a benzoidal graphenic network (arene structures) has to be assumed (Baccile et al., 2009). As expected, an increase in the alkyl C/O-alkyl C ratio occurred after HTC. This increase was more affected by temperature than by time with HTC. The highest values were obtained for hydrochars produced at 260 °C for 3 h, with 17.8 and 16.9 for “A” and “T” hydrochars, respectively (Table 3.3).

Table 3.3. Intensity distribution (%) of the solid-state ^{13}C NMR spectra of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1 h (_Py__600_1).

	Carboxyl/ Amide C	***O/N-* Aryl C**	**Aryl C**		***O*-Alkyl C**	***N*-Alkyl/ Methoxyl C**	**Alkyl C**	**Alkyl C/ Carboxyl C**	**Alkyl C/ O-Alkyl C**
ppm	**220-160**	**160-140**	**140-110**	**110-90**	**90-60**	**60-45**	**45-0**		
A_SS	9.1	2.8	10.4	4.3	17.3	12.9	43.1	4.7	2.5
A_HTC_200_0.5	6.3	5.0	16.3	3.6	12.0	9.6	47.2	7.5	3.9
A_HTC_200_3	6.4	5.6	18.1	2.5	8.9	7.5	51.1	8.0	5.7
A_HTC_260_0.5	5.4	5.9	20.3	2.2	4.2	6.7	55.4	10.3	13.2
A_HTC_260_3	4.8	6.1	22.0	2.1	3.1	6.7	55.2	11.5	17.8
A_Py_600_1	6.0	10.8	68.5	4.2	3.3	1.8	5.4	0.9	1.6
T_SS	10.7	1.9	7.5	4.9	22.6	14.3	37.6	3.5	1.7
T_HTC_200_0.5	10.1	5.0	19.3	2.8	8.4	9.2	45.2	4.5	5.4
T_HTC_200_3	7.4	6.1	24.1	2.7	5.1	6.1	48.6	6.6	9.5
T_HTC_260_0.5	7.3	6.5	25.2	1.9	3.3	4.8	51.0	7.0	15.5
T_HTC_260_3	5.0	6.1	25.2	2.8	3.1	5.7	52.3	10.5	16.9
T_Py_600_1	5.8	12.1	68.6	4.9	2.8	1.3	4.5	0.8	1.6

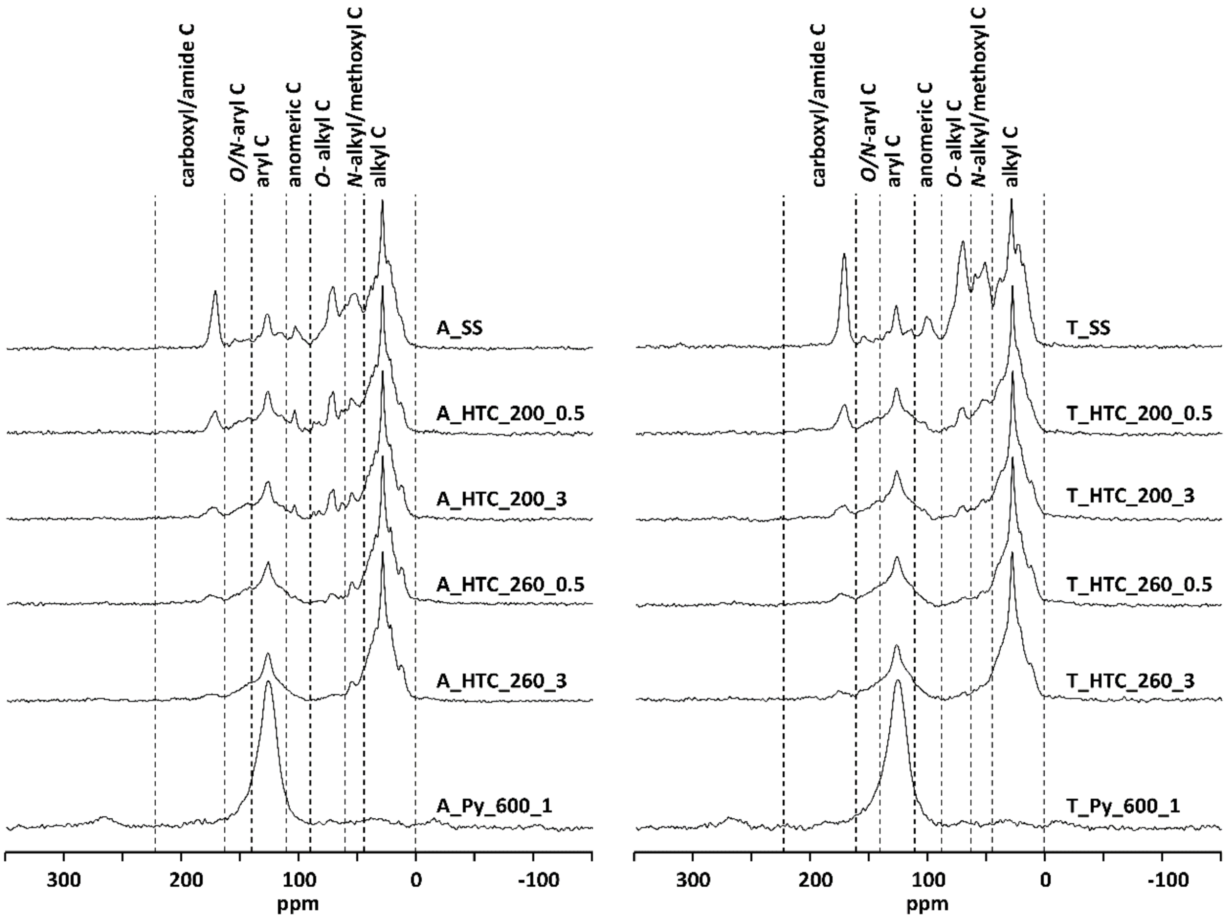

Figure 3.2. Solid-state ^{13}C NMR spectra of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

Table 3.4. C-recovery of the different C groups of primary (A_SS) and secondary (T_SS) sewage sludges as a function of charring conditions (% of C present in the original sewage sludge).

	Carboxyl/ amide C	***O*/ *N*- Aryl C**	**Aryl C**		***O*-Alkyl C**	***N* Alkyl/ Methoxyl C**	**Alkyl C**
ppm	**220-160**	**160-140**	**140-110**	**110-90**	**90-60**	**60-45**	**45-0**
A_SS	9	3	10	4	17	13	43
A_HTC_200_0.5	5	4	14	3	10	8	40
A_HTC_200_3	5	5	15	2	7	6	42
A_HTC_260_0.5	4	5	16	2	3	5	43
A_HTC_260_3	4	5	17	2	3	5	44
A_Py_600_1	3	5	29	2	1	1	2
T_SS	11	2	8	5	23	14	38
T_HTC_200_0.5	8	4	15	2	7	7	36
T_HTC_200_3	6	5	18	2	4	5	37
T_HTC_260_0.5	5	5	18	1	2	3	35
T_HTC_260_3	3	4	17	2	2	4	35
T_Py_600_1	3	5	31	2	1	1	2

Pyrolysis resulted in more drastic chemical changes, which are expressed in an almost complete transformation of organic matter into aromatic structures. In the spectra of A_Py_600_1 and T_Py_600_1, the aryl C region between 140 and 110 ppm accounts for 69 % of the total ^{13}C intensity (Table 3.3) and the O- and N-aryl C region for only 11 to 12 %, respectively. This can be taken as an indication that benzoic rings rather than furans dominate the aromatic network.

Table 3.5. Relative distribution of the ^{15}N intensity between the chemical shift region -150 to -285 ppm of the solid-state ^{15}N NMR spectra of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

	Pyrrole N	**Amide N**
ppm	**-150 to -248**	**-248 to -285**
A_SS	22.4	77.6
A_HTC_200_0.5	38.7	61.3
A_HTC_200_3	50.9	49.1
A_HTC_260_0.5	65.3	34.7
A_HTC_260_3	60.3	39.7
A_Py_600_1	75.4	24.6
T_SS	17.2	82.8
T_HTC_200_0.5	30.5	69.5
T_HTC_200_3	56.3	43.7
T_HTC_260_0.5	64.7	35.3
T_HTC_260_3	57	43
T_Py_600_1	58.2	41.8

The ^{15}N NMR spectra of the hydrochars confirm the formation of N-heterocyclic aromatics during HTC already at 200 °C by a clear shoulder in the region of pyrrole-type N (-150 and -248 ppm). Structures such as indoles, imidazole and pyrroles are contributing to the intensity in this region. Protonated pyridine-type N may be a further component giving rise to intensity around this chemical shift but considering the pH of the

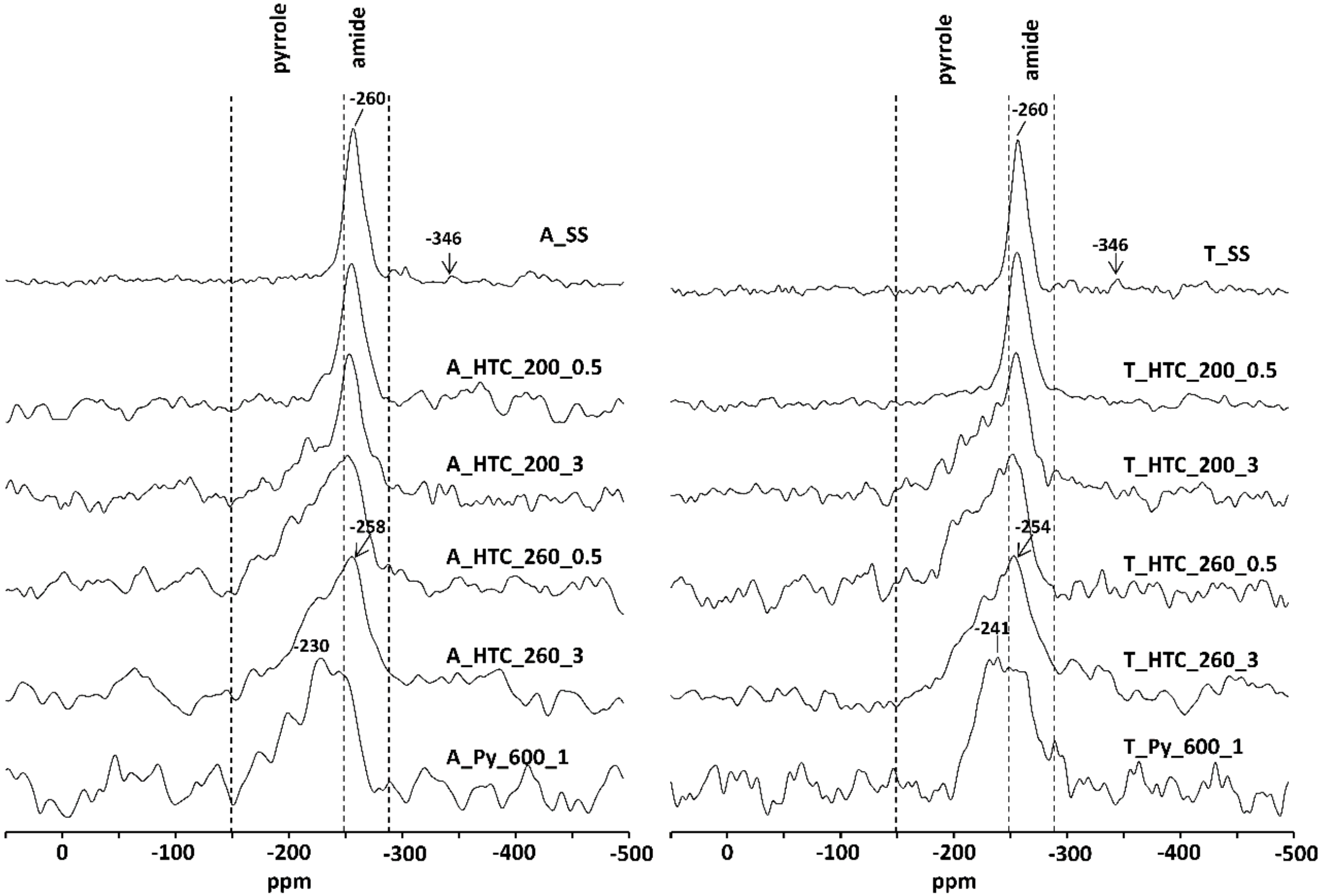

Figure 3.3. Solid-state ^{15}N NMR spectra of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1h (_Py__600_1).

samples, such structures are unlikely. However, unprotonated pyridine-type N may contribute to the weak shoulder around -70 ppm, but the low signal-to-noise ratios of the spectra does not allow an unbiased differentiation of such a signal from the noise. In contrast to the observations derived from ^{13}C NMR spectroscopy, the solid-state ^{15}N NMR spectra indicate chemical changes, expressed as an augmentation of the pyrrole-type N, not only with increasing temperature but also with residence time. The change is also expressed in a shift of the peak of the main signal from -260 ppm to -254 ppm. Note that pyrrole N has its chemical shift around -230 ppm and indole N around -245 ppm. The largest contribution of pyrrole-type N is evidenced in the samples obtained after heating at 260 °C for 30 min, with a 65 % for A_HTC_260_0.5 and T_HTC_260_0.5 (Table 3.5). The ^{15}N intensity in the chemical shift region of amide N (-248 to -285 ppm) underwent a drastic decrease after HTC. This region dominates only the spectra of hydrochars produced at the mildest HTC conditions. Here, it has to be taken into account that carbazole N has its chemical shift around -262 ppm. Benzamide-type N has its resonance frequency at -275 ppm. Thus, such compounds are likely to contribute to the amide N region as well. Regarding dry pyrolysis, it resulted in a dominance of pyrrole-type N with a contribution of 75 and 58 % for A_600_1 and T_600_1, respectively (Table 3.5). Here, the main peak occurs at -240 ppm.

3.3.3 Fourier transform-infrared spectroscopy

All FT-IR spectra (Figure 3.4) of the original SS, hydrochars and pyrochars exhibit several peaks around 3700-3600 cm^{-1}, which are attributable to vibration of OH groups in the mineral matter (Hossain et al., 2011; Bernier et al., 2013). The peaks appearing from 3600 cm^{-1} to 3300 cm^{-1} are assigned to stretching OH bond in water, carboxyl and hydroxyl groups (Bernier et al., 2013). All spectra show these two bands but their intensities decrease after the HTC and dry pyrolysis processes. In addition, all spectra exhibit an intense signal at 1030 cm^{-1} which can be caused by Si-O-Si vibrations (Zielinska et al., 2015; Peng et al., 2016) or by C-O vibrations in polysaccharides. Silicate may derive from street run-offs which also enter the waste water systems or from residues of the sand used during the mechanical pre-treatment of the water-cleaning process. The increase of this band with temperature supports such an assignment to Si-O-Si vibration since partial combustion of organic matter during heating leads to a relative enrichment of mineral matter of the sample. The FT-IR spectra of the untreated SS (A_SS and T_SS) indicate a pattern, which can be assigned to microbial biomass. This is composed of distinct aliphatic methylene bands at 2925 and 2855 cm^{-1}, the amide band I (C=O vibration) at 1655-1648 cm^{-1}, the amide band II (N-H vibration) at 1539 cm^{-1}, the amide band III (C-N vibration) at 1230 cm^{-1} and the stretch of phosphodiesters at 1080 cm^{-1} (Smidt and Parravicini, 2009). However, the contribution of the Si-O stretching in the 1100-1000 cm^{-1} region cannot be overlooked. Additionally, one has to bear in mind that the amide I and III bands can be overlapped by the C=O vibration of the carboxylates and the C-O vibration of carboxylic acids, respectively. In the carbonized samples they may also contain contributions from pyrans and furans derived from heat-altered carbohydrates. In addition, the peak around 797-785 cm^{-1} which is visible in all spectra is attributable to out of plane NH wagging in amides and/or bending of the aromatic ring C-H bonds (Bernier et al., 2013). The bands appearing below 600 cm^{-1} are probably due to M-X stretching vibrations in both organic and inorganic halogens compounds (M-metal, X-halogen) (Hossain et al., 2011).

Table 3.6. Peak positions in the FT-IR spectra in the current study and proposed assignments.

Peak position (cm^{-1})	**Proposed assignment**
3700-3600	OH groups in the mineral matter
3600-3300	OH bond in water, carboxyl and hydroxyl groups
2925	aliphatic methylene band
2855	aliphatic methylene band
1655-1648	amide band I (C=O vibration)
1600	aromatic C=C vibrations
1539	amide band II (N-H vibration)
1230	amide band III (C-N vibration)
1080	strech of phosphodiesters
1030	Si-O-Si vibrations
797-785	out of plane NH wagging in amides and/or bending of the aromatic ring C-H bonds
below 600	M-X streching vibrations in both organic and inorganic halogens compounds (M-metal, X-halogen)

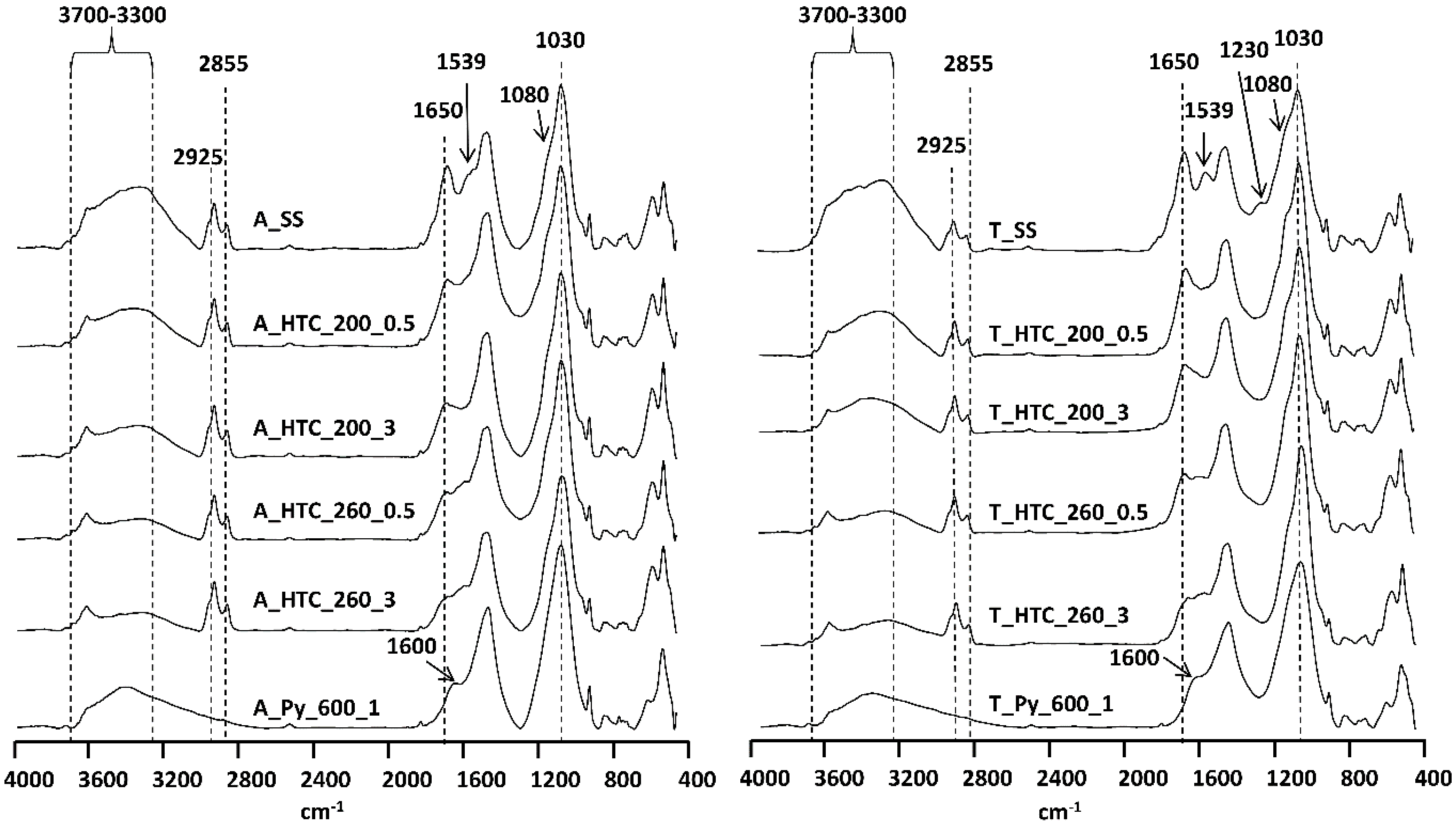

Figure 3.4. FT-IR spectra of primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars produced at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 hours (_0.5, _3, respectively), as well as the pyrochars produced at 600 °C for 1 h (_Py__600_1).

As already observed by solid-state NMR spectroscopy, HTC produced milder alterations of the FT-IR pattern as dry pyrolysis. Thus, the spectra of the hydrochars show the same peaks than those of the non-treated SS except for the lack of the amide II and III signals in the former. This is in line with the decrease of the amide N intensity in the ^{15}N NMR spectra as a result of the pyrolyzation process. In addition, the decrease of the FT-IR band corresponding to the OH vibrations derived from organic matter is in accordance with the loss of ^{13}C intensity in the O-alkyl C region. In addition, a new peak at 1600 cm^{-1} assigned to aromatic C=C vibrations appears in the spectra of the pyrochars. This may be interpreted that the higher temperatures resulted in a preferential accumulation of arene structures as it was also indicated by the respective solid-state ^{13}C NMR spectra. It is worth to notice that all inorganic signals are still present after dry pyrolysis whereas those assigned to aliphatic methylene (2925 and 2855 cm^{-1}), phosphodiester (1080 cm^{-1}) and amide I, II and III disappeared.

3.3.4 Summary of the main chemical alterations caused by thermochemical carbonization of sewage sludge samples

It is accepted that the degradation of biomass primarily initiates with a hydrolysis step at approximately 200˚C during HTC, due to the increased vapor pressure (Funke et al., 2010; Reza et al., 2014b). Considering the main components of SS, hydrolysis will likely produce the cleavage of amide bond of proteins and the ether bond of carbohydrates, resulting in peptides, amino acids and monomeric sugars. Part of those compounds are dissolved in the reaction water and removed after HTC, contributing to the observed decline of total organic matter and of O- and N-alkyl C, carboxyl C but also of amide N after the HTC treatment. However, highly reactive products with low molecular weight are also formed which are likely to recondensate to form insoluble products which can precipitate. A further process represents dehydration, which removes water from the organic molecules leading to formation of aromatic structures. This was also observable in our experiment by the increase of C groups assigned to the ^{13}C chemical shift region between 160 and 110 ppm. Relating the C content adding to the intensity in the O- and N-alkyl C region (90-45 ppm) with that contributing to the aromatic C region (160-110 ppm) reveals high and negative correlation with R^2=0.95 for “A” samples and R^2=0.97 for “T” samples. The loss of carboxyl C during HTC confirms the occurrence of decarboxylation processes, although the detailed reaction mechanisms are largely unknown (Funke et al., 2010).

3.3.5 Potential as source of N for agriculture

The total N content has to be considered for both fast and slow-release N fertilization purposes. However, N_i contents are mostly important from a fast fertilization point of view. Total nitrogen losses increased with temperature and were higher for pyrolysis than for HTC. However, the organic N resisting in the chars occurs in forms which are expected to have higher biochemical recalcitrance than the N forms in the original feedstock. This is an observation which has to be considered if biochars/pyrochar and hydrochars are produced from N-rich feedstocks with the intention to use them as slow release fertilizer. Careful calculations are necessary if the thermally induced N-losses can compensate the N-losses occurring after direct application of the feedstock without thermal pretreatment. In the case of the latter, fast N-losses are expected if the N-mobilization from SS is faster than N-immobilization due to N-uptake by the growing plants. The highest total and inorganic N contents were obtained by using HTC at 200 °C for 30 min so such HTC chars will be preferable if fast fertilization at the beginning of the growing period is necessary. However, a key property of a slow-release N fertilizer is its N sequestration either by incorporation of N into compounds with low microbial accessibility or by N adsorption on the surface of the chars. Thus, the thermal transformation of peptides into N-containing heterocyclic aromatic compounds may be of higher value if such products are needed.

A further consideration lays in the observation recently made by Bargmann et al. (2014). They observed a higher increase of the microbial activity in soil amended with hydrochars than with biochars, which was explained with a higher content of biochemically labile compounds in the first if compared to the latter. Microbial growth is commonly connected to N-immobilization into newly synthetized microbial biomass, which represents an important sink for decreasing mineral-N concentrations in soils.

Besides the valuable retention and slow release of plant nutrients like nitrogen, it has to be taken into account that the HTC process may also form a variety of potentially harmful benzenic, phenolic and furanic volatiles along with various aldehydes and ketones (Becker et al., 2013). These compounds formed in feedstock- and temperature-specific patterns are also found in the liquid and the solid phase of HTC chars. This problem may be handled by different post-treatments which remove these undesirable substances. As reported by Schulze et al. (2016) readily available and fast-cycling carbon fractions can efficiently be separated from hydrochars by washing and fermentation.

Regarding the organic amendment potential of SS chars, carbon contents are below 250 g kg-1, which does not justify to define this type of char as biochar (Meyer et al., 2017; European Biochar Foundation, 2012) and which reflects its minor role in increasing the soil organic carbon content. However, other soil properties which have not been considered in this study may be improved. Compared to the analyzed HTC chars, our pyrochars may have a higher impact on increasing the carbon sequestration potential of soils due to their higher aromaticity and their lower content of oxygen or nitrogen substituted aromatic C.

Besides the sequestration of carbon, nitrogen and other nutrients, in particular of phosphate in the case of chars derived from SS, laboratory studies of Dicke et al. (2014) demonstrated the potential of HTC chars to reduce N_2O emissions.

3.4 CONCLUSIONS

Our study showed that peptide-like N of SS is transferred into heterocyclic aromatic N during heat treatment, although the efficiency of this transformation is different for pyrolysis and HTC. Former studies on N-rich charcoal confirmed that the N bound in this so-called black nitrogen can be used for plant growth as a kind of slow-N-release fertilizer (De la Rosa and Knicker, 2011). However, the different contribution of various N-classes to the total N in hydrochars and pyrochars from SS, will affect their N fertilization capability. Based on the higher N_i content, the hydrochars produced at 200 °C for 30 min may be the most appropriate if both immediate and medium term N-fertilization is required, whereas the pyrochar may be useful if smaller N doses are needed over a longer time range. However, one has to bear in mind that pyrolysis results in high N loss due to volatilization. On the other hand, this N loss may be outbalanced by the sequestration of the remaining N in heterocyclic forms, which increases the pool of organic N with medium-term biochemical recalcitrance. Nevertheless, both HTC and pyrolysis represent efficient tools for hygienization of SS.

Considering that SS is an organic waste, the N and P of which are lost if deposited on landfills or used for combustion as energy source, the transformation of this material into pyrochar or hydrochar for their use as soil amendment can be seen as an efficient tool to recycle N and P from organic waste.

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4. Hydrothermal carbonization and pyrolysis of sewage sludges:

effects on Lolium perenne germination and growth

4 HYDROTHERMAL CARBONIZATION AND PYROLYSIS OF SEWAGE SLUDGES: EFFECTS ON *LOLIUM PERENNE* GERMINATION AND GROWTH

Abstract

The pyrolysis and hydrothermal carbonization (HTC) of sewage sludge (SS) result in products free of pathogens, with the potential of increasing crops yields. However, more research is needed to determine the most beneficial production conditions for agricultural purposes. With this work, we evaluate the impact of pyrolysis-treated (600°C, 1h) and HTC-treated (200 °C, 260 °C; 0.5 h, 1h) SS on the germination, survival and growth of *Lolium perenne* during an 80-days greenhouse experiment. The hydrochars and pyrochars were applied on a Calcic Cambisol with doses of 5 and 25 t ha^{-1}. Our results showed that the addition of pyrochars to the Cambisol had neither impact on *Lolium* germination and survival rates, nor on its biomass production. In comparison to the control, higher or equal biomass was yielded from hydrochar-amended pots, even though some hydrochars decreased plant germination and survival rates. Among all the evaluated char properties, only the organic and inorganic nitrogen (N) contents of the chars, along with their organic C values, correlated with increased total and shoot biomass production. Our work shows that low-temperature HTC of SS results in a soil amendment with N-fertilization potential whereas chars produced from HTC with higher temperatures and pyrolysis do not exhibit the same suitability.

Keywords

Hydrochar, pyrochar, nitrogen, fertilizer, greenhouse experiment, biosolids

4.1 INTRODUCTION

The revised Waste Framework Directive (Council Directive 2008/98/EC) promotes prevention and minimization of organic waste as well as their reuse and recycling. This can be supported by its transformation into added-value products for use in agriculture. Traditionally, this is achieved by digestion and composting (Kelessidis and Stasinakis, 2012).

Sewage sludge (SS) is a nutrient-rich organic waste, which is produced in increasing amounts. More than 10 million tons (dry weight) are produced annually in Europe (Millieu Ltd., WRc and RPA, 2010). Recycling of SS to agriculture can return nitrogen (N), phosphorus (P), other plant nutrients and organic matter to the soil and may help to reduce the dependency on fossil fuel-consuming synthetic N fertilizer and non-renewable P sources. However, its application poses some environmental risks such as nutrient leaching, reduced soil biodiversity, increased greenhouse gas emissions (Millieu Ltd., WRc and RPA, 2010), and health risks if not pre-treated properly. One possibility for hygienization of SS before its application to soil represents composting. However this process consumes not only space and time but also releases greenhouse gases such as CO_2 and volatile N. An alternative may be a thermal treatment of SS through pyrolysis or hydrothermal carbonization (HTC). These technologies allow efficient hygienization while concomitantly stabilizing organic C and N within a relative short process time. Of course, during thermal treatment greenhouse gases are also released, but this emission may be compensated by recycling the produced thermal energy for other energy requiring purposes. Both pyrolysis and HTC carbonize biomass in low-oxygen environments. Temperatures between 300 and 700 °C are typically used during dry pyrolysis. Hydrochars are typically produced at temperatures between 180 and 250 °C in the presence of water, which creates autogenous pressure. The advantage of transforming SS into hydrochar rather than pyrochar lies in lower energy costs due to lower process temperatures and the fact that pre-process drying of the feedstock is not necessary. On the other hand they are considered to be biochemically less stable than biochar (Lehmann and Joseph, 2015; Libra et al., 2011).

Commonly, SS is characterized by a high ash content yielding in pyrolyzed products with organic matter contents which are too low to meet the requirements of the International Biochar Initiative or the European Biochar Certificate (International Biochar Initiative, 2015; European Biochar Foundation, 2016) to be called biochars (Bachmann et al., 2016). Therefore, we will refer to pyrolyzed SS as pyrochar.

Despite the potential of pyrochars and hydrochars to increase the amount of stable carbon stored in soils, its use in agriculture will only be economically feasible if they provide additional benefits such as increasing crop production. Whereas pyrolysis of green waste and wood commonly results in products with a high porosity (De la Rosa et al., 2014; Bachmann et al., 2016) the pyrolysis of SS turns into carbonized residues with fertilizing potential (Chan et al., 2007; Chan et al., 2008; Paneque et al., 2017; Frišták et al., 2018).

Solid-state 15N nuclear magnetic resonance (NMR) studies confirmed that most of the organic N in pyro- and hydrochars from SS occurs as heterocyclic N, which represents an integrated part of the aromatic network of the chars (Paneque et al., 2017). Bearing in mind that this so-called black nitrogen (BN) (Knicker, 2010) is less bioavailable than inorganic N, a big advantage of applying such fertilizers lies in the fact that N losses due to leaching can be reduced. However, this concept only works if the organic N is sufficiently bioavailable for maintaining plant health and growth. Thus, both, the biochemical resistance and the biodegradability of BN, determine the potential of N-rich chars as fertilizer.

Previous studies indicated that BN is less biochemically recalcitrant as commonly assumed and that this N can be used for the build-up of new plant biomass (De la Rosa et al., 2011). A recent study demonstrated that Fusarium oxysporum is involved in the biochemical degradation of SS-derived pyrochar (De la Rosa et al., 2018). However, the biochemical degradability of BN in charred residues is still lower than that observed for peptides found in unburnt plant residues (Lopez-Martin et al., 2017). Thus, the observed co-occurrence of some quickly accessible inorganic N-forms in hydrochars (Paneque et al., 2017) may be advantageous for overcoming a possible N-deficit, which may restrict plant growth, particularly during the early stages of plant development.

Pyrolysis and HTC of SS decreases P mobility (Huang and Tang, 2016). However, Frišták et al. (2018) revealed higher P contents in plants growing on pyrolyzed-SS amended soils than in those of the control, which demonstrates that at least part of the P was bioavailable. These data point out to the potential of HTC/pyrolysis treated SS to act both as slow-release P and N product.

These promising results are counteracted by the fact that phytotoxic compounds may be formed during thermal treatments. Indeed, negative impacts on germination and seedling growth have been observed in other studies (Jandl et al., 2013; Busch et al., 2012; Bargmann et al., 2013; Thuille et al., 2015). They may be eliminated by well-designed pyrolysis conditions (Buss and Masek, 2014) and washing treatments (Bargmann et al., 2013). However, the knowledge about the most suitable conditions for converting SS into thermally treated products suitable for agriculture is still scarce.

Bearing this in mind, the goal of the present work is to fill those knowledge gaps by complementing a former investigation on the chemical transformation of organic C and N forms during HTC and pyrolysis of two different SS (Paneque et al., 2107) with a 80-day greenhouse experiment. The focus of those experiments was to obtain insights on the impact of the application of the respective hydrochars and pyrochars on germination, survival and biomass production of Lolium perenne. In addition, the char properties were related to the growth of Lolium.

4.2 MATERIALS AND METHODS

4.2.1 Characteristics of the sample material

Two different sewage sludges (SS) were collected at the Experimental Wastewater Treatment plant (CENTA), located in Carrion de los Céspedes, near Seville, Southern Spain. The first sample, further called "A_SS", is a primary sludge produced by the settlement of suspended organic matter in a pond. The second sample, assigned "T_SS", is a secondary sludge accumulated in an extended aeration treatment system and later stored in a thickener, in order to reduce its water content. In addition, "W" pyrochar was yielded from urban green waste, grape pomace, wood, *Miscanthus* and other suitable biomass. Paneque et al. (2017) found that the material collected from the pond (A_SS) was more humified, thus biochemically stabilized, than that derived from the thickener (T_SS). The total N content of A_SS and T_SS were 19 and 32 g kg^{-1}, respectively (Table 4.1) and occurred mainly as peptides and in amino sugars (Paneque et al., 2017). Regarding heavy metal content, only Zn for A_SS and Cd for both A_SS and T_SS slightly exceeded the thresholds established in the Working document on sludge (European Comission, 2000) (data not shown).

Hydrochars were produced from the primary (A) and secondary (T) SS at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively) as described by Paneque et al. (2017) in a 1L stirred pressure reactor (Parr reactor series 4520, IL, USA). The reaction water was removed through a filter and the material was rinsed with distilled water until the rinsing water turned clear.

To obtain the A and T pyrochars, further called A_Py and T_Py, 200 g of dry SS were pyrolyzed in a close steel container in a preheated muffle oven at 600 °C for 1h. W_Py carries the European Biochar Certificate and was produced at 600 °C for 20 min by Swiss Biochar, Lausanne, Switzerland.

According to the International Biochar Initiative (IBI, 2015), the organic C content of the hydro- and pyrochars (Table 4.1) allows their classification as class 3 biochar, however, only the pyrochars fulfill the second requirement of having an atomic H/C ratio < 0.7. A detailed characterization of the organic matter composition is given in Paneque et al. (2017). Due to the high N content of the source material all chars contained considerable amounts of N between 1 and 3%, most of which (>97%) occurred in an organic form (Table 4.1). In contrast to the HTC chars, the pyrochars did not concentrate inorganic N (Ni). Total phosphorus (P_T) and potassium (K_T) contents were determined after digestion with aqua regia (1:3 v/v conc. HNO_3/HCl) in a microwave oven (Microwave Laboratory Station Mileston ETHOS 900, Milestone s.r.l., Sorisole, Italy) by inductively coupled plasma-optical emission spectrometer (ICP-OES) spectrophotometer Varian ICP720-ES (Table 4.1). The pH of the samples was measured in distilled water (1:10, w/v) (Table 1).

The heavy metals content of the chars tended to increase compared to the non-treated SS, however only Cd and Zn exceeded the limits (European Comission, 2000), similar to the non-treated SS. The concentrations of selective volatile organic compounds in process liquid (5-HMF, 2-furfural, phenol, catechol, cresol, and resorcinol) was measured using a modified ICS 3000 Dionex (Thermo Scientific) with a UV detector (wavelength 280 nm) and Knaur Eurosphere II (C 18) column. A 15% acetonitrile (85% DI water) was used as mobile phase in the IC. Column temperature was set at 23°C and flow rate was 1.0 ml min^{-1}. No presence of these molecules was found in A_SS, T_SS or in their chars.

Table 4.1. The pH, C_{org}, N_{org}, N_i N, C_{org}/N_T, P_T and K_T contents of primary (A_SS) and secondary (T_SS) sewage sludges and the respective hydrochars produced at 200°C (_HTC_200) and 260°C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively), as well as the pyrochars (_Py). Paneque et al. (2017)

	pH	**C_{org}** g kg^{-1}	**N_T** g kg^{-1}	**N_{org}** g kg^{-1}	**N_i[a] of N_T** %	**C_{org}/N_T** (w/w)	**P_T** g kg^{-1}	**K_T** g kg^{-1}
A_SS	7.4	228	19.2	18.9	0.5	11.9	12.3	6.8
A_HTC_200_0.5	6.5	224	16.0	15.6	2.6	14.0	13.9	7.3
A_ HTC_200_3	6.5	214	13.9	13.7	2.5	15.4	14.5	7.6
A_ HTC_260_0.5	6.4	213	11.2	10.8	1.5	19.1	15.4	8.0
A_ HTC_260_3	6.6	221	12.1	11.8	1.9	18.2	14.9	7.9
A_Py	9.3	134	8.6	9.0	0.0	15.5	17.0	9.5
T_SS	7.5	245	32.3	31.9	0.4	7.6	16.7	6.1
T_ HTC_200_0.5	6.7	233	25.0	24.5	2.2	9.3	20.0	6.8
T_ HTC_200_3	6.2	233	23.9	23.7	1.4	9.7	20.8	7.1
T_ HTC_260_0.5	6.3	225	19.7	19.8	1.2	11.4	21.6	7.3
T_ HTC_260_3	6.4	224	19.0	18.7	1.4	11.8	21.6	7.6
T_Py	10.0	168	16.3	16.0	0.0	10.3	25.2	9.2
W_Py	9.3	829	1.80	n.a.[b]	n.a.	922	0.7	4.5
Soil	8.4	10	1	n.a.	n.a.	7	0.4	1.7

a) N_i : inorganic nitrogen (sum of NH_4-N,NO_2-N, and NO_3-N in %); b) n.a.: not available

4.2.2 Greenhouse incubation experiment

For the incubation experiment, 250 mL-plastic containers (16 cm height) were perforated and filled with 250 g of dried fine earth (< 2 mm) from the Ah horizon of a sandy loamy Calcic Cambisol (IUSS Working Group WRB, 2007) mixed with amounts equivalent to 5 and 25 t ha^{-1} of each char (0.8 and 4% w/w, respectively) and topped with 25 certified grass seeds (*Lolium perenne*-ILURO seeds company, Spain). The soil derived from the experimental station "La Hampa" of the Instituto de Recursos Naturales y Agrobiología de Sevilla, in the Guadalquivir River Valley (SW Spain; 37° 21.32′ N, 6° 4.07′ W), Coria del Río, Seville. After sampling, the soil was dried at 40 °C for 48 h, and sieved (<2 mm). Small branches, fresh mosses and plant remains, as well as roots were removed manually. The soil material contains 21 g C kg^{-1} of which 10 g kg^{-1} was attributed to organic carbon and 1 g N kg^{-1}. Its pH in water was 8.5 and its water holding capacity (WHC), according to Veihmeyer and Hendrickson (1949), was 24%.

For each treatment four replicates were prepared (n = 4). Additionally, 6 controls without any char amendment but with plants were included (n = 6).). However, of those 6 only 4 were used for the final analysis. Greenhouse conditions were similar as previously described by De la Rosa et al. (2014). Briefly, soil moisture was adjusted to 60% of the maximum WHC, the pots were placed in a greenhouse at 25 ±2 °C/17 ±2 °C (day/night) maintaining a 14 h light day^{-1} cycle with the support of growing lights (120 µE m^{-2} s^{-1} of photosynthetically active radiation) for 80 days. Average relative humidity of the air in the green-house was maintained during the experiment in the range 60 ±10%. The position of the pots was changed three times per week to assure comparable light and growing conditions. No nutrient solution was added. Although the pots were placed on saucers to collect possible excess water, there was no leaching after watering the pots. The same amount of water was added to each pot three times per week, which was 145 L m^{-2} at the end of the experiment. This is equivalent to 662 L m^{-2} per year and is similar to the natural average annual precipitation in the region around the experimental station.

The number of living grass shoots was counted after 5, 9, 13, 18, 20, 25, 30, 60 and 80 days. The germination rate was determined from measurements after 20 days of incubation, since it represented the time with the highest number of living plants. The survival rate was calculated by using the 80 count-day data. In addition, the shoots were cut and then left to regrow after 18, 33, 47, 61 days. The final harvest was after 80 days of incubation. The harvested shoot biomass was dried (48 h at 40 °C) and weighed in order to determine the shoot biomass production. After the experiment, the roots were manually separated from the soil, rinsed with distilled water, dried (48 h at 40 °C) and weighed to determine the root biomass.

4.2.3 Statistical analysis

All measured variables were submitted to the same statistical analysis by using SPSS version 17.0 (SPSS, Chicago, IL, USA). Shapiro-Wilk and Levene tests were used to test for normality and homoscedasticity of data, respectively. Transformations were applied to meet model assumptions when necessary. A t-test was used to identify significant effects between the control and each treatment. The same test was used to evaluate the application dose effect within each char. In addition, an analysis of variance was performed followed by a comparison of means (Tukey's test) to test for significant differences between chars, independently of the dose applied. Effects were considered as significant at $p \leq 0.05$.

The R version 3.4.1 was used to conduct the non-metric multidimensional scaling (NMDS) ordination. Samples variation was represented by an ordination using a Euclidean distance matrix. The 'envfit' function in the "vegan" package (Oksanen et al., 2015) was used to draw vectors representing chars properties with a statistical effect on *Lolium perenne* response onto the NMDS ordination. The SigmaPlot version 13.0 was used to plot the previously obtained NMDS data.

4.3 RESULTS

4.3.1 Germination and survival rates

Generally, pyrochars added to the Calcic Cambisol did not affect the germination or survival rate of *Lolium perenne*. Only W_Py applied at 25 t ha^{-1} decreased both parameters (Figures 4.1 and 4.2). Hydrochars derived from the "T" sewage sludge showed lower germination and survival rates than the control. In contrary, most of the tested A hydrochars revealed no major impact on the germination or survival rate. In addition, no differences were found when comparing the different production conditions or application doses between each other.

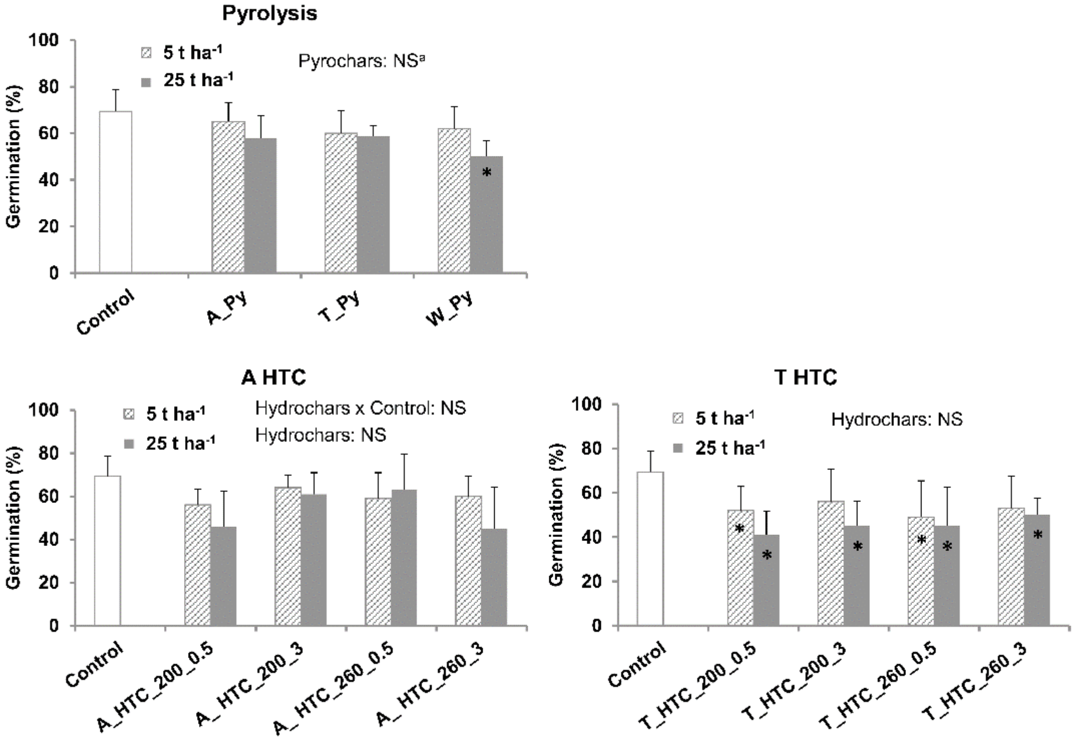

Figure 4.1. Germination rate (%) of *Lolium perenne* in the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1}. Hydrochars were produced from primary (A_) and secondary (T_) sewage sludges at 200°C (_HTC_200) and 260°C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively). Pyrochars were produced from primary (A_) and secondary (T_) sewage sludges (_Py). a) NS: no significant. Asterisks inside the bars show significant differences to the control according to *t*-test.

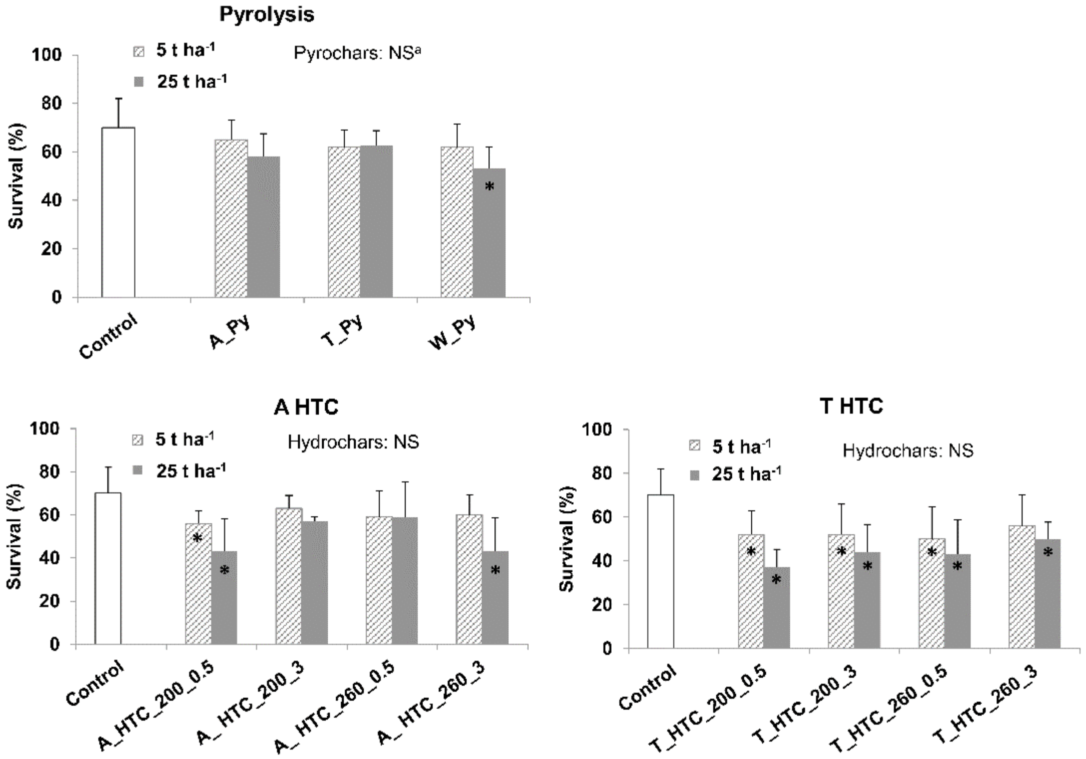

Figure 4.2. Survival rate (%) of *Lolium perenne* in the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1}. Hydrochars were produced from primary (A_) and secondary (T_) sewage sludges at 200°C (_HTC_200) and 260°C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively). Pyrochars were produced from primary (A_) and secondary (T_) sewage sludges (_Py). a) NS: no significant. Asterisks inside the bars show significant differences to the control according to *t*-test.

4.3.2 Biomass production

The biomass production was determined as the sum of the shoot and the root biomass. The use of pyrochars had no effect on biomass production of *Lolium perenne* (Figure 4.3). The total biomass production after hydrochar application was always higher or equal to the control (Figure 4.4). Hydrochar addition increased *Lolium* biomass production except hydrochar produced at 260 °C for 3 h (Figure 4.3). Note that the greatest biomass was obtained with the hydrochars produced at the lowest temperature for both A and T hydrochars, A_HTC_200 and T_HTC_200, respectively. Residence time seems to have a lower impact than temperature on biomass production. Increasing the application rate did not significantly alter the total biomass production per pot (Figure 4.4), but affected the strength of the developing plants. With higher application rate, greater biomass per plant was achieved.

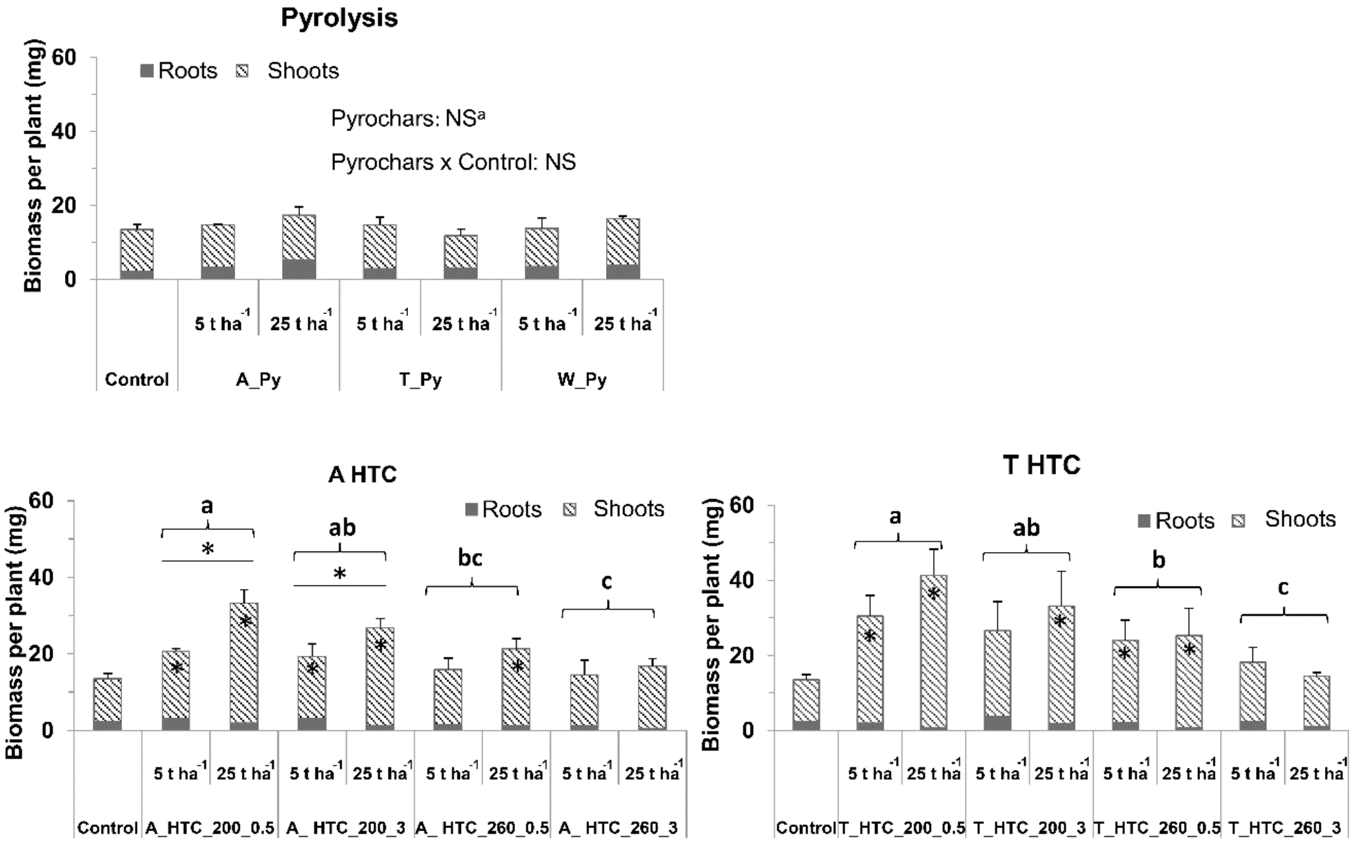

Figure 4.3. Biomass production per plant (mg per plant) of *Lolium perenne* for the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1}. Hydrochars were produced from primary (A_) and secondary (T_) sewage sludges at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively). Pyrochars were produced from primary (A_) and secondary (T_) sewage sludges (_Py). a) NS: no significant. Asterisks inside the bars show significant differences to the control. The above-line asterisks indicate significant differences between doses. The letters show significant differences between treatments.

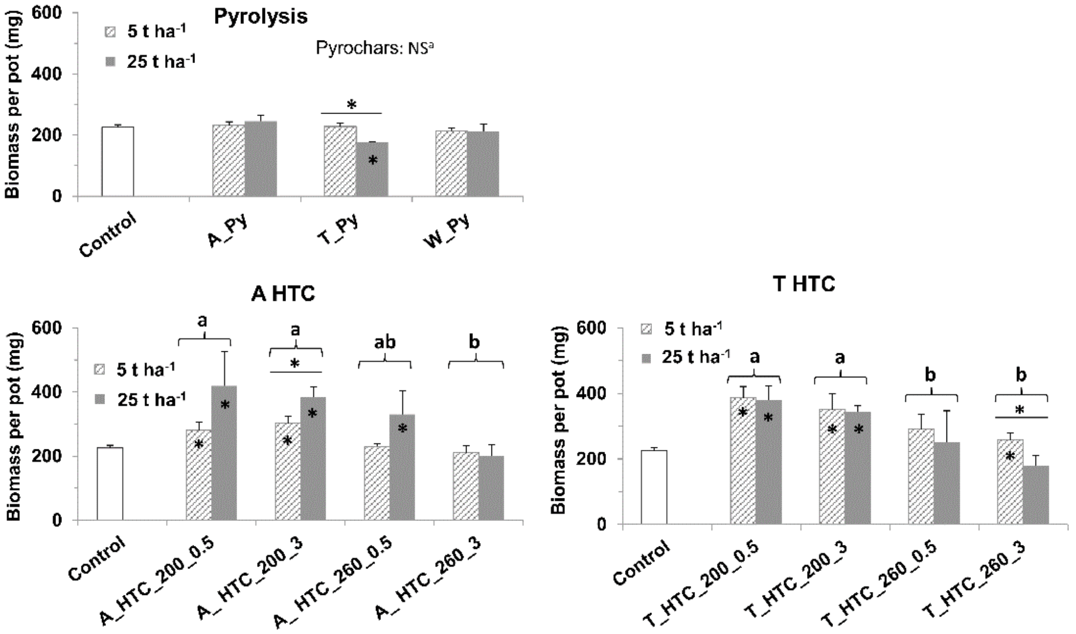

Figure 4.4. Biomass production per pot (mg per pot) of *Lolium perenne* for the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1}. Hydrochars were produced from primary (A_) and secondary (T_) sewage sludges at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively). Pyrochars were produced from primary (A_) and secondary (T_) sewage sludges (_Py). a) NS: no significant. Asterisks inside the bars show significant differences to the control according to t-test. The above-line asterisks indicate significant differences between doses according to t-test. The letters show significant differences between treatments according to Tukey's test.

4.3.3 Root-to-shoot ratios

Pyrochars addition significantly increased the root-to-shoot (R:S) ratio of Lolium perenne whereas the presence of A and T hydrochars resulted in lower or equal values than the control (Figure 4.5). Within hydrochars, production conditions did not affect this parameter; however R:S ratio tended to decrease with increasing rate of hydrochar application. These changes were mainly due to a higher root biomass in the case of pyrochars and a higher shoot biomass in the case of hydrochars (Figure 4.3).

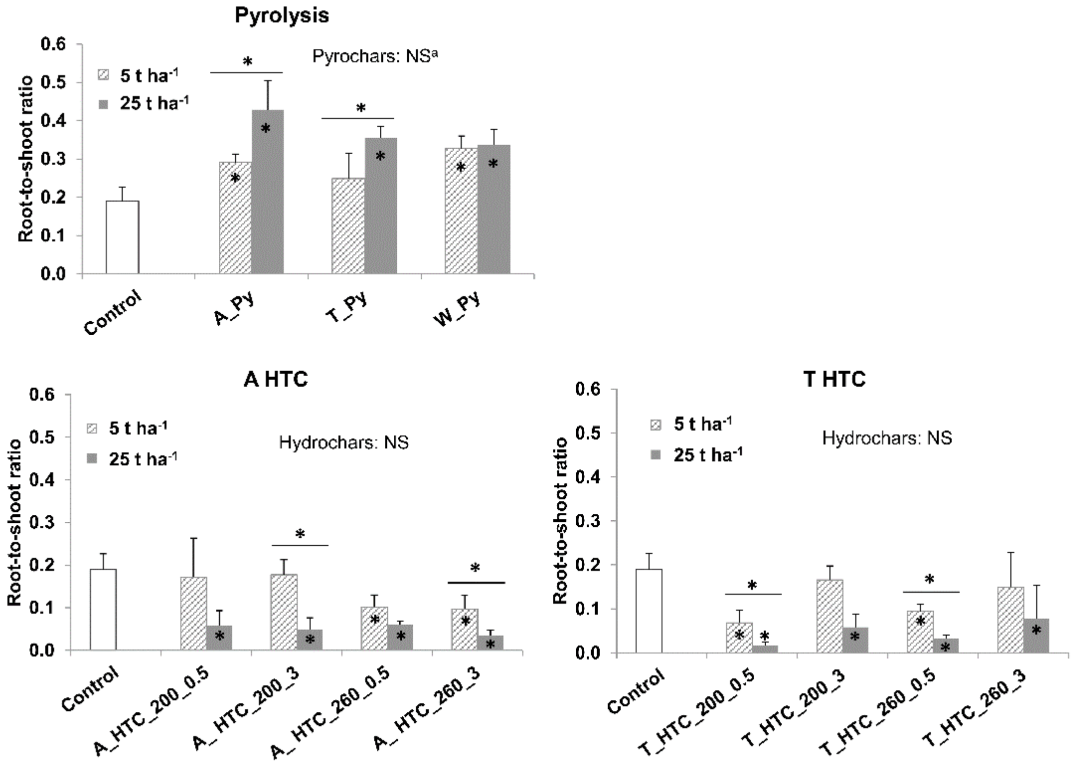

Figure 4.5. Root-to-shoot ratio of *Lolium perenne* for the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1}. Hydrochars were produced from primary (A_) and secondary (T_) sewage sludges at 200 °C (_HTC_200) and 260 °C (_HTC_260) for 0.5 and 3 h (_0.5, _3, respectively). Pyrochars were produced from primary (A_) and secondary (T_) sewage sludges (_Py). a) NS: no significant. Asterisks inside the bars show significant differences with the control according to *t*-test. The above-line asterisks indicate significant differences between doses according to *t*-test.

4.3.4 Relationship between plant response and chars properties

The NMDS graph showed that carbonization type had an overall effect on all parameters since pyrochars were separated from hydrochars (Figure 4.6). Pyrochars were characterized by higher R:S ratios together with higher root biomass and, in a lower extent, by higher germination and survival rates than hydrochars. In contrast, hydrochars were characterized by larger shoot and total biomass per plant. Regarding the feedstock type, T hydrochars showed larger shoot and total plant biomass than A hydrochars. In addition, total biomass per plant and shoot biomass significantly correlated with increased N_i and N_{org} contents of the chars. To a lower extent, total biomass per plant and shoot biomass also correlated with increased C_{org} levels of the chars. The p values of the above mentioned correlations were less or equal to 0.001. The C_{org}/N ratio, P_T, and K_T parameters showed p values higher than 0.001 and hence were not considered significant variables.

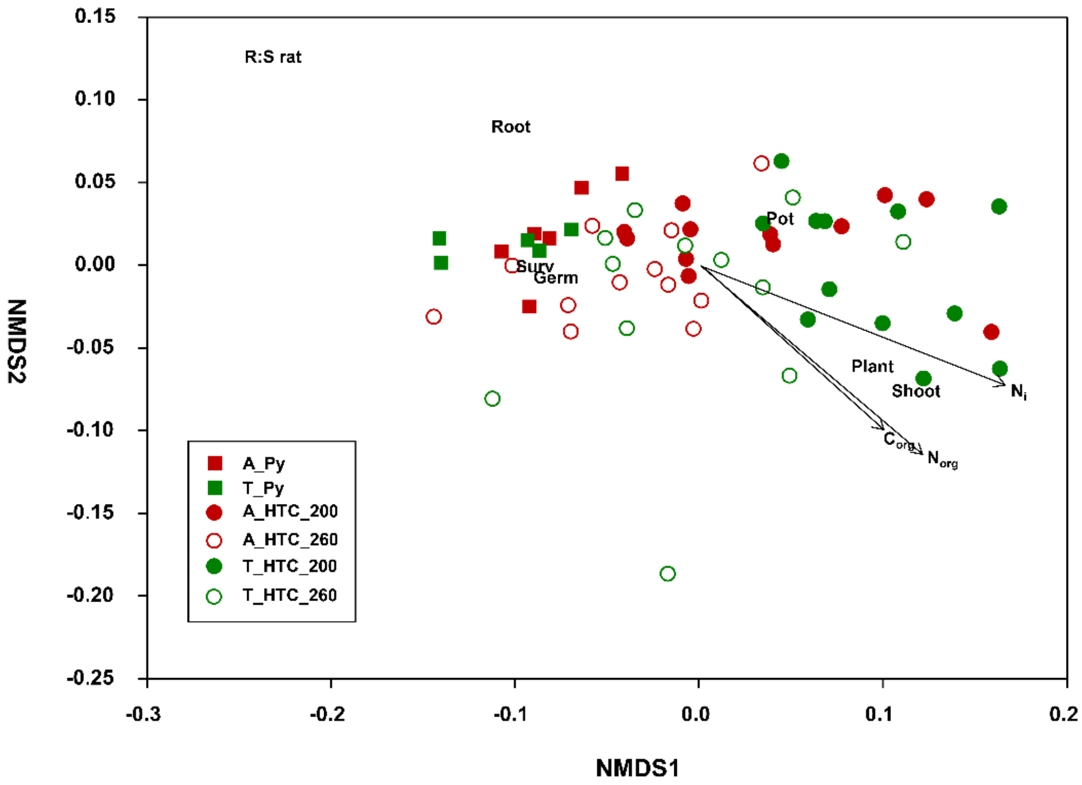

Figure 4.6. NMDS ordination indicates how the different thermally treated SS impact on *Lolium*-measured parameters. R:S rat: Root-to shoot ratio; Roots: roots biomass per plant; Surv: survival rate; Germ: germination rate; Pot: total biomass per pot; Plant: total biomass per plant; Shoot: shoot biomass per plant. Char properties significantly fitting onto NMDS are shown as vectors ($p<0.001$). N_i: inorganic nitrogen; N_{org}: organic nitrogen; C_{org}: organic carbon. Goodness of fit was 0.05.

4.4 DISCUSSION

4.4.1 Germination and survival rates

Our data did not allow an unbiased assignment of factors causing the observed differences in germination and survival rates between hydrochars and pyrochars. A decrease in germination after addition of hydrochars has also been observed by others (Jandl et al., 2012; Busch et al., 2012; Bargmann et al., 2013; Thuille et al., 2015) and was attributed to phytotoxic volatile organic compounds adsorbed on the surface of the hydrochar. Bargmann et al. (2013) demonstrated that these compounds were mostly water soluble and could be removed by washing the hydrochars with distillate water but soluble nutrients are expected to be at least partially lost during this process. However, analysis of the chars prior to incubation did not indicate the presence of HMF, furfural, resorcin, catechol, phenol and kresol. Thus, either other non-measured phytotoxic compounds were present in our hydrochars or other factors are responsible. Previous works have also found diverse germination responses among hydrochars produced from different feedstocks (Jandl et al., 2012; Bargmann et al., 2013). Recently, the fermentation of hydrochars in an anaerobic biogas reactor has been proposed as an alternative method to eliminate toxic compounds since it avoids the loss of nutrients (Mumme et al., 2014; Lanza et al; 2018).

Dry pyrolysis performed at temperatures greater than 500 °C in appropriate pyrolysis reactors reduces the presence of volatile compounds, including polycyclic aromatic compounds and other phytotoxic (De la Rosa et al., 2016). Thus, the possible negative impact due to soil amendment with pyrochars on germination rates should be less prominent than with HTC chars. Previous works have shown both neutral and positive effects of pyrochars (Free et al., 2010; Busch et al., 2012; De la Rosa et al., 2014; Thuille et al., 2015). This is in agreement with our results indicating no major impact of pyrochar addition except for W_Py applied at 25 t ha^{-1}.

4.4.2 Biomass production and chars properties

Despite some hydrochars decreased the survival rate of *Lolium perenne*, the total biomass production per pot was always higher or equal in the hydrochar amended pots, compared to the control. This is best explained by the size of the remaining plants, since hydrochars tended to increased biomass production per plant. However, our pyrochars had no effect on biomass production. These results are in contrast to studies by Thuille et al. (2015) who obtained higher shoot biomass production of winter wheat in the presence of corn silage-derived pyrochar than in presence of corn silage-derived hydrochar. However, here it has to be taken into account that in the former study inorganic nitrogen fertilizer was added.

Nitrogen availability is most likely also responsible for our observation, as it is indicated by the positive correlation between N_i and N_{org} contents of the chars with the total and shoot biomass production per plant. Accordingly, the pyrochars showed the lowest N_{org} contents along with absence of N_i which is in line with no increase of *Lolium* biomass production. The hydrochars produced at 260 °C exhibited higher N_{org} than the pyrochars and contained some N_i which slightly affected plant biomass production. However, amendment of the hydrochars produced at 200 °C, which contained the highest concentrations of N_{org} and N_i, yielded in the highest amount of biomass. Those results strongly indicate that biomass production is more related to the availability of N or other nutrients than to physical properties of the char. Note that we are not using wood biochars but chars with very low porosity and that the N content of our soil is extremely low. Most of this nitrogen occurs in organic form. Thus, adsorption of N_i from the soil onto the char surface is unlikely. As a consequence, the processes limiting bioavailability of N in our systems is determined by the biochemical degradability of black nitrogen, rather than by adsorption of Ni. Fang et al. (2015) also found that greenwaste-derived hydrochars produced at 200°C showed higher yields than those produced at higher temperatures.

In contrast to N, P_T and K_T contents of the chars revealed no correlation with growth parameters. Either the soil contained sufficient plant available P and K already prior to char application or the P and K of the chars are less plant available. In both cases, our results indicate that other factors than P_T and K_T contents have a stronger impact on plant growth. The correlation of C_{org} contents of the chars with the total and shoot biomass production may be due to an improvement of some soil physical properties like a decrease in soil density, an increase of the soil water holding capacity or providing an habitat for soil microorganisms. Note that hydrochars exhibited higher C_{org} contents and thus higher biomass production than pyrochars. Since the latter are expected to exhibit a higher biochemical stability than hydrochars, one has to keep in mind that increasing the potential of soils to act as a C sink via addition of charcoal does not necessarily coincide with enhancement of biomass production.

4.4.3 Root-to-shoot ratios and char properties

Although pyrochars addition had no impact on *Lolium* biomass production, their presence increased their R:S ratios, compared to the control. Considering that increased R:S ratios have been observed when growth is limited by N or P supply (Andrews et al., 1999), our observation may be related to the absence of N_i in our pyrochars. This deficiency may have been increased by adsorption of NO_3^- and NH_4^+ from the soil solution to the char surface as it was suggested to occur for some biochars (Spokas et al., 2012; Nelissen et al., 2012).

Hydrochars applied at 25 t ha^{-1} showed lower R:S ratios than the control whereas the 5 t ha^{-1} application rate produced no significant impact or a lower decrease of this parameter. In general, when nutrient availability increases, plants can develop their aboveground vegetation in detriment of their subsoil part because less effort is required to acquire nutrients (Ågren and Franklin, 2003). This is in agreement with our results, since char amendment with 25 t ha^{-1} delivered higher nutrients levels than addition of 5 t ha^{-1}. Comparably, Thuille et al. (2015) observed a very short root system of winter wheat in the presence of a corn silage derived hydrochar, whereas with the respective pyrochar, root development was comparable to that of the plants grown without corn silage treatment.

4.4.4 Fertilization potential of thermally treated SS

Our work indicates that low-temperature HTC of SS results in a product with N-fertilization potential whereas chars yielded from HTC with higher temperatures and pyrolysis do not exhibit the same suitability. This potential depends on the amount of N_i contained in the chars together with the bioavailability of their organic N. Whereas N_i provides fast and immediate N fertilization, the degradation rate of the organic N compounds determines the slow-release N fertilization potential of the amendments. Paneque et al. (2017) showed that organic N in SS occurred mainly in peptide-like structures, part of which may be easily degradable. During both HTC and pyrolysis this compounds were partially transformed into N-heterocyclic aromatic entities, which are less microbiologically accessible. This transformation was more efficient for pyrolysis than for HTC which suggested a quicker degradation of the organic N in hydrochars than in pyrochars. Therefore, not only the total nutrient content values but also their speciation should be considered since their bioavailability from chars alters with processing conditions.

In addition, it also has to be considered that our hydrochars and pyrochars may exhibit a different crop response depending on the crop type as well as on the soil properties and the climatic conditions (Sakrabani et al., 2017).

4.5 CONCLUSIONS

Although the problem of reduced germination after HTC application still has to be solved, our study indicates a higher potential of SS-derived hydrochars as soil amendment with immediate fertilizing effect than the respective pyrochars. Bearing in mind that plant growth parameters correlated better with N than with P and K contents, the higher impact of hydrochars on plant growth is best explained by the concomitant presence of inorganic N and easily available organic N forms. However, further experiments have to show if the disadvantage of less microbially accessible N in pyrochars may turn into an advantage for crops which need low but constant N fertilization.

From an energetic and thus economic point of view, HTC seems to be a better choice than pyrolysis since drying of the SS prior to its thermal treatment can be avoided. Lower temperatures and shorter residence times are needed which considerably reduce the costs for energy. Our studies reveal further that for the production of a slow-release fertilizer from SS, HTC at 200 °C is more suitable than 260 °C, which allows even less energy consumption. In addition, the composition of the preserved N seems to be favorable for plant growth. Thus, aside from being advantageous with respect to economic considerations, the application of lower temperatures during HTC is also of environmental interest since the use of mineral fertilizer can be reduced and the release of greenhouse gases into the atmosphere occurring during disposal of untreated SS can be avoided.

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5. Hydrochar and pyrochar derived from sewage sludge can increase the available phosphorus in amended soils:

A greenhouse study.

5 HYDROCHAR AND PYROCHAR DERIVED FROM SEWAGE SLUDGE CAN INCREASE THE AVAILABLE PHOSPHORUS IN AMENDED SOILS. A GREENHOUSE STUDY.

ABSTRACT

Sewage sludge (SS), containing considerable amounts of phosphorus, may be recyclable as P-fertilizer after its hygienization by means of pyrolysis and hydrothermal carbonization (HTC). However, neither the structure nor the plant-availability of the different P forms occurring in heat-treated SS are well identified. Therefore, this work studies the alteration of P extractability after pyrolysis-treated (600 °C, 1 h) and HTC-treated (200 °C, 260 °C; 0.5 h, 3 h) SS. To test the plant availability of P of the SS chars, a 80-days pot experiment was conducted in an greenhouse during which the SS chars were added to a calcic Cambisol on which *Lolium perenne* was allowed to grow. Our results showed that both thermal treatments lead to P enrichment in the chars, although its extractability was generally reduced. After the addition of those chars to the soil the pyrochars and the 200 °C-produced hydrochars increased the amount of soil extractable P (Olsen-P). At the end of the greenhouse experiment, more extractable P was yielded than initially added, although the plants must have used P for their plant growth. Thus, part of the non-extractable P of our amendments must have turned into extractable forms during the incubation. Since pH effects can be excluded, this observation points towards liberation of P-forms which were either physically or chemically sequestered in the aromatic network of the char or bound in organic forms. Based on our results we suggest that the use of pyrochars and, to a lower extent, the 200 °C-produced hydrochars, produced from SS may work as slow-release P-fertilizers.

5.1 INTRODUCTION

The annual phosphorus (P) input need of EU's agriculture was estimated to reach 3.85 million tons per year (Tóth et al., 2014). Most of this need is covered by inorganic P fertilizers which derive mainly from mined phosphate rocks, a non-renewable resource that will be exhausted in the next 50-100 years (Cordell et al., 2009). Thus, alternative P sources are urgently needed.

One of those may represent P-rich organic residues. Almost all of P which is eaten by humans is excreted and ends up in wastewater treatment plants (WWTPs) (Cordell et al., 2009). A part of this P remains soluble in the water after the cleaning process and cannot be recovered whereas another part is fixed in the sewage sludge (SS) and could be recycled for agriculture. Considering that in Europe every year more than 10 million tons of SS are generated and that its P content is around 28 g kg^{-1} as average (Millieu Ltd., WRc and RPA, 2010), this residue may indeed be of help for the reduction of the current dependence on phosphate rocks.

However, the sludge applied on land has to meet with the established standards regarding pollutants but also has to be treated in order to destroy the pathogens within it and to reduce its fermentability. In this sense, pyrolysis and hydrothermal carbonization (HTC) have recently been proposed as alternative options to convert SS into a value-added product which can be used as soil amendment or fertilizer. Compared to composting it is expected that such heat treatments result in a more efficient C stabilization, which may contribute to a decrease of the greenhouse gas emissions associated to this waste (Lehmann et al., 2006; Millieu Ltd., WRc and RPA, 2010). However, bearing in mind that thermal treatment is not only stabilizing organic matter, but can also affect the nutrient contents and their plant availability, the suitability of this approach still needs to be investigated in more detail.

Approximately 50% of the P in most SS is plant available (Millieu Ltd., WRc and RPA, 2010). Phosphorus in SS occurs mainly in the form of orthophosphate, condensed phosphate (e.g pyrophosphate and polyphosphate) and organically bound phosphate (Qian and Jiang, 2014; Huang and Tang, 2015). Moreover, these molecules can be present in different chemical states such as cation complexes, surface minerals, incorporated into other mineral phases, or precipitated as P-containing minerals (Huang and Tang, 2016). In general, both pyrolysis and HTC have been observed to reduce the availability of P, regardless of the feedstock (Wang et al., 2015; Dai et al., 2015). In addition, this immobilization depends upon the production conditions and enhances as reaction temperature increases, both for HTC and pyrolysis (Christel et al., 2014; Bruun et al., 2017; Idowu et al., 2017).

It seems that pyrolyzation of SS transforms the organic P and maybe part of the orthophosphate into condensed phosphate (Qian and Jiang, 2014). In addition, the P may be sequestered physically within the aromatic network formed during the heat induced organic matter condensation (Hung and Tang, 2016). An increase of the complexation of P with metals has been observed during both pyrolysis and HTC which would further promote its immobilization. Huang and Tang (2016) observed that the (hydro) thermal treatment of SS increased the presence of relatively stable Ca- and Fe-associated P species. However, SS is a diverse material and hence Ca and Fe are not the predominant metals in all cases.

The present knowledge regarding the potential of HTC-treated SS as a source of P for plants is scarce (Melo et al., 2018). However, there are indications that pyrolyzed-SS can efficiently supply P to the crops (Wang et al., 2012; Frišták et al., 2017; Faria et al., 2017), in some cases even to a higher extent than phosphate rock fertilizers (Wang et al., 2015). The apparent contradiction between these results and the observed immobilization of P may be related to changes of the P availability once the SS-chars are applied to the soil. In order to test this possibility we investigated the alteration of the availability of P captured in two SS converted under different HTC conditions and the behavior of this element once it is applied to a sandy loamy soil during a 80-day greenhouse experiment with *Lolium perenne*. For comparison, two pyrolyzed-SS and a greenwaste-pyrochar were included. With this approach we intended to clarify which technology is most suitable for the production of char with efficient P-fertilizer properties.

5.2 MATERIALS AND METHODS

5.2.1 Sewage sludge collection, treatment and characterization

Two sorts of sewage sludge (SS) samples were collected at the Experimental Wastewater Treatment plant (CENTA), located in Carrion de los Céspedes (SW, Spain). They consisted of a primary sludge produced by the settlement of suspended organic matter in a pond, called A_SS, and a secondary sludge produced in an extended aeration treatment system and finally stored in a thickener which reduces its water content, this will be called T_SS. A brief description of the material is given in Paneque et al. (2017)

The SS samples were subjected to two contrasting thermochemical carbonization processes. First, the A_SS and T_SS samples were hydrothermally carbonized (HTC) in a 1 L-stirred pressure reactor (Parr reactor series 4520, IL, USA) at 200°C and 260˚C for 30 min and 3 h with a heating rate of 3 °C min^{-1}. The mixture was stirred during the whole reaction time at 90 rounds per minute. After turning off the reactor and cooling down for 15 h, the HTC slurry was filtered through fluted filter paper and the solids were dried for 48 h at 60°C.

In addition, the samples A_Py and T_Py were obtained after pyrolyzing 200 g of dry SS in a close steel container in a preheated muffle oven at 600°C for 1h (Paneque et al., 2017). The third pyrochar, named W_Py, was bought from the company Swiss

Biochar and derived from urban greenwaste, grape pomace, wood, miscanthus and other suitable biomasses, which were pyrolyzed at 600°C for 20 min.

The pH and electrical conductivity (EC) of the chars were measured in distilled water (1:10, w/v). Total phosphorus (P_T) contents were determined by digestion with aqua regia (1:3 v/v conc. HNO_3/HCl) in a microwave oven (Microwave Laboratory Station Mileston ETHOS 900, Milestone s.r.l., Sorisole, Italy). All the extracts were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES) spectrophotometer (Varian ICP720-ES). Available P (extr-P) was determined by extraction with sodium carbonate at pH 8.5 (Olsen et al., 1954). Labile P fraction was calculated as follows:

$$Labile\ P\ fraction\ (\%) = \frac{Extr - P}{P_T} * 100$$

5.2.2 Greenhouse incubation experiment

A detailed description of this experiment is given in chapter 4. Briefly, for the greenhouse incubation experiment 250 g of a calcareous sandy loamy soil classified as Calcic Cambisol (IUSS Working Group WRB, 2007) was mixed with an amount of char equivalent to 5 and 25 t ha^{-1} (0.8 and 4 % w/w, respectively). The mixtures were placed in plastic containers and topped with 25 certified seeds of *Lolium perenne*. For each treatment four replicates were prepared (n = 4). Additionally, 6 controls without any char amendment but with plants were prepared (n = 6). After adjusting the soil humidity to 60% of the maximum water holding capacity (WHC), the pots were placed in a greenhouse at 25 °C and 14 h light day^{-1} for 80 days. No mineral nutrient solution was added. The samples were irrigated regularly during the whole experiment.

At the end of the experiment, pH and the electric conductivity (EC) of the control and amended soils were measured using a Crison 40 pH-meter and a conductivity meter Crison Basic 20 (Crison, Spain) respectively. The standard soil:water ratio of 1:2.5 w/v was used. Available P (extr-P) was determined by extraction with sodium carbonate at pH 8.5 (Olsen et al., 1954).

The amount of soil extr-P at the end of the experiment deriving from the char (extr-$P_{char\text{-}end}$) was calculated by subtracting the amount of extr-P of each treatment from the amount of extr-P of the control. The difference between the extr-$P_{char\text{-}end}$ and the extr-P added to the soil with each char and dose at the beginning of the experiment (extr-$P_{char\text{-}added}$) was calculated and hereafter it will be called $P_{diff.}$ The amount of P released per kg of char was determined by considering the P_{diff} data and the corresponding amount of char added with each dose.

5.2.3 Statistical analysis

Data corresponding to pH and EC are presented as mean values ± standard error of triplicate measurements. The impact of the different chars and application doses on soil extr-P at the end of the experiment as well as the differences on P_{diff} and on the P release kg^{-1} char where identified by using a Kruskal–Wallis followed by Mann–Whitney U tests since these data did not meet ANOVA assumptions. The Shapiro-Wilk and Levene tests were used to test for normality and homoscedasticity of data, respectively. The level of significance used was 0.05. All statistical analyses were carried out using SPSS version 17.0 (SPSS, Chicago, IL, USA).

5.3 RESULTS

5.3.1 Characterization of non-treated SS and the produced chars

5.3.1.1. pH and electrical conductivity (EC)

The pH of the non-treated SS, A_SS and T_SS was 7.4 and 7.5, respectively. Hydrochars produced from both A_SS and T_SS were slightly acidic, with values ranging between 6.2 and 6.7; whereas the pyrochars showed pH values between 9.3 and 10 (Table 5.1). This is in agreement with previous results, which found lower pH values for hydrochars than for pyrochars derived from the same feedstock (Bargmann et al., 2013; Bargmann et al., 2014; Takaya et al., 2016).

The non-treated SS, A_SS and T_SS showed similar EC values, with 15 and 14.5 mS cm^{-1}, respectively. The EC of hydrochars ranged from 25.5 to 3 mS cm^{-1} whereas that for pyrochars was between 9.3 and 1.1 mS cm^{-1} (Table 5.1). Thus, pyrolysis decreased EC whereas the impact of HTC on EC depended on the SS type. The T hydrochars showed considerably higher EC values than T_SS whereas the EC of A hydrochars was always equal or lower than those for A_SS. This suggests different characteristic of A_SS and T_SS. Note that in general, this parameter decreased when the temperature and residence time used for char production increased. Wang et al. (2012) obtained comparable results for pyrolysis.

Table 5 .1. pH, EC, P_T, P recovery and extr-P values for primary (A_SS) and secondary (T_SS) sewage sludges and for their respective hydrochars and pyrochars

	pH	**EC**^{a}	**P_T**^{b}	**P recovery**	**Extr-P**^{c}
		mS cm^{-1}	g kg^{-1}	(%)	mg kg^{-1}
A_SS	7.4	15.0	12.3	100	510
A_HTC_200_0.5	6.5	15.0	13.9	97.9	183
A_ HTC_200_3	6.5	12.8	14.5	90.4	139
A_ HTC_260_0.5	6.4	3.0	15.4	87.8	181
A_ HTC_260_3	6.6	8.5	14.9	79.1	145
T_SS	7.5	14.5	16.7	100	651
T_ HTC_200_0.5	6.7	25.5	20.0	99.7	278
T_ HTC_200_3	6.2	24.8	20.8	82.3	234
T_ HTC_260_0.5	6.3	18.3	21.6	78.5	200
T_ HTC_260_3	6.4	21.3	21.6	73.3	70
A_Py	9.3	6.3	17.0	81.2	1017
T_Py	10.0	9.3	25.2	75.9	668
W_Py	9.3	1.1	0.7	-	51

[a] Electrical Conductivity
[b] Total phosphorus
[c] Extractable phosphorus

5.3.1.2. Phosphorus characterization of non-treated SS

The non-treated SS showed P_T contents of 12 and 17 g kg^{-1} for A_SS and T_SS, respectively (Table 5.1). The availability of this P_T was around 4% since the extractable P of A_SS and T_SS was 510 and 651 mg kg^{-1}, respectively (Table 5.1; Figure 5.1). Both the P_T contents and the percentages of available P are below the average values found for SS produced in Europe (Millieu Ltd., WRc and RPA, 2010). The amount of extr-P was also low compared to the average SS produced in Spain (Spanish Government, 2009). This is likely due to the fact that, in contrast to common SS, our sludges derive from a wastewater treatment plant (WWTP) which processes the domestic waste water of a small village with at most 3000 residents, whereas most of the common WWTPs process the wastewater of larger populations. In addition, considering that aluminum (Al) and calcium (Ca) are the predominant metals of A_SS and T_SS (Table 5.2), Ca and Al phosphates are likely to dominate in our samples since the type of metals-phosphate complexes are highly influenced by the composition of the feedstock. These results are in agreement with Wang et al. (2012).

Considering these data, the potential of our SS as a source of P for soils is likely lower than that of the average SS and this has to be taken into account when it comes to the extrapolation of the results.

Table 5.2. Calcium (Ca), magnesium (Mg), iron (Fe) and aluminum (Al) contents of the primary (A_SS) and secondary (T_SS) sewage sludges and of their respective hydrochars and pyrochars

	Ca	**Mg**	**Fe**	**Al**
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
A_SS	73.3	8.6	15.8	36.5
A_HTC_200_0.5	80.7	9.0	18.5	42.0
A_ HTC_200_3	84.4	9.3	19.4	44.0
A_ HTC_260_0.5	87.4	10.0	19.7	45.7
A_ HTC_260_3	86.5	9.6	19.1	44.2
T_SS	62.2	9.2	19.1	32.2
T_ HTC_200_0.5	72.8	10.1	23.1	39.6
T_ HTC_200_3	76.1	10.9	24.0	41.0
T_ HTC_260_0.5	78.5	11.6	24.4	42.2
T_ HTC_260_3	78.7	11.7	26.1	42.3
A_Py	100.0	10.9	21.7	50.1
T_Py	92.5	13.6	28.2	48.5

5.3.1.3. Effect of the thermal treatments on P contents of SS

The data of the recovered P show that most of the P contained in the non-treated SS remained after both pyrolysis and HTC (Table 5.1). Recovery P values ranged from 99 to 76%. Within hydrochars, this parameter decreased as temperature and residence time increased. In addition, the "A" chars showed slightly higher values than "T" chars, which is in accordance with the higher yields of the former compared to the latter (data not shown). Paneque et al. (2017) found that the losses of carbon (C), nitrogen (N) and hydrogen (H) were always higher than the P losses for the same chars. This explains the observed increase of P_T contents after both thermal treatments with a relative enrichment of this element. Considering that the losses of C, N and H were higher during pyrolysis than during HTC, the P_T contents were higher in the pyrochars than in the hydrochars derived from the same SS. In addition, the process water of the HTC must contain part of the water soluble P. Thus, the A_Py and T_Py pyrochars exhibited P_T contents of 17 and 25 g kg^{-1} respectively; whereas their hydrochars showed lower P_T values (Table 5.1). The high preservation and enrichment of P after both pyrolysis and HTC of SS have been previously reported (Wang et al., 2012; Wang et al., 2017; Frišták et al., 2018).

Despite both thermal treatments preserved most of the P of the non-treated SS, the availability of this element decreased after HTC. Thus, the extr-P values of the hydrochars were between 278 and 70 mg kg^{-1} (Table 5.1). Pyrolysis, however, did not demonstrate a consistent effect since A_SS and A_Py showed comparable extr-P values whereas T_Py exhibited double the amount of extr-P than T_SS. This points out to different P compounds in A_SS and T_SS and hence to a different speciation/complexation during pyrolysis. The W_Py had the lowest extr-P value due to its low P_T content.

The observed decrease of the P availability after HTC resulted, logically, in a lower portion of labile P, comprising between 1.4 and 0.3% (Figure 5.1). In general, both the labile P fraction of the hydrochars and their extr-P contents decreased with intensifying the production conditions. Regarding the pyrochars, less labile P was found in T_Py than in T_SS whereas A_Py showed a higher labile P fraction than A_SS. This supports the above-mention conclusion of different P compounds in starting A_SS and T_SS, which then leads to different speciation/complexation during pyrolysis.

In general, these results indicate that the P immobilization was higher for HTC than for pyrolysis. However, the underlying mechanisms for this behavior still have to be explored. Huang and Tang (2016) observed an immobilization of P after both HTC and pyrolysis which was attributed to an increase of metal/P complexes. This is likely to have happened in our chars, too, since a relative enrichment of Ca, Mg, Fe and Al occurred after both thermal treatments (Table 5.2). However, physical constraints such as embedment of P species into the char structure may also play a role. Huang and Tang (2016) found that different Al and Ca phosphates as well as phytic acid were extracted by H_2O or $NaHCO_3$ in non-treated SS but they could only be extracted by NaOH or HCl in the chars. The mechanisms which explain the results of A_Py, however, must be further investigated.

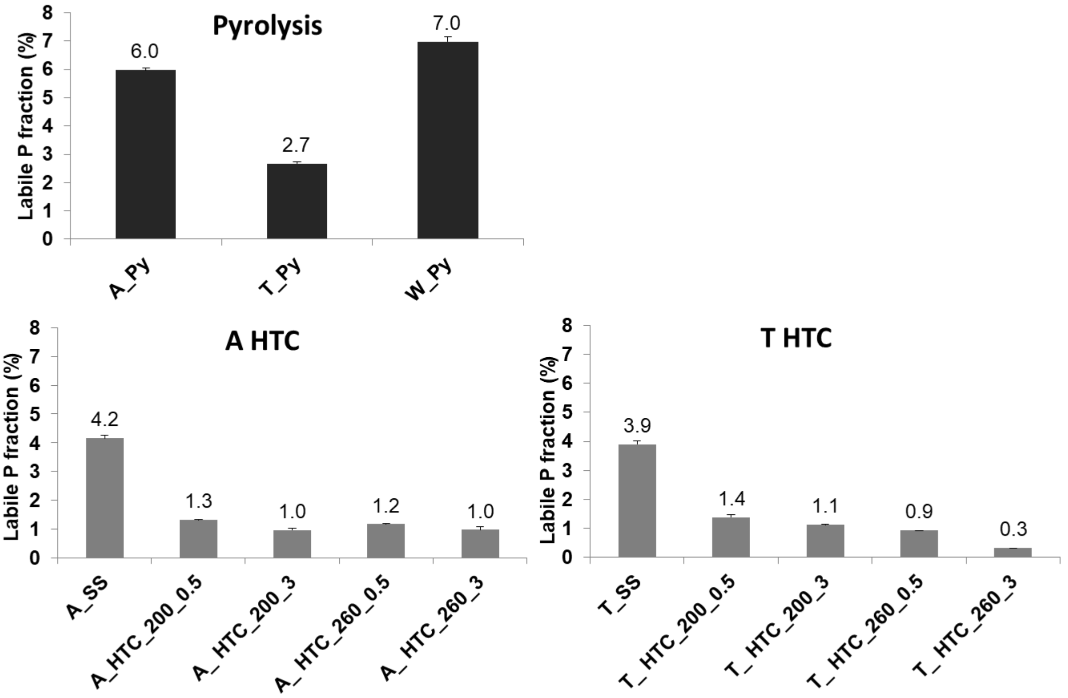

Figure 5.1. Labile fractions of P extracted by the Olsen method of the primary (A_SS) and secondary (T_SS) sewage sludges and their respective hydrochars and pyrochars.

Summarizing our results, the higher P_T and extr-P contents of the SS-derived pyrochars compared to the hydrochars suggest that pyrolysis may be more suitable than HTC for producing SS-derived chars useful as P-fertilizer.

5.3.2 Greenhouse experiment

5.3.2.1. pH

The Calcic Cambisol used as soil matrix for the incubation experiment exhibits a pH of 8.4 (Table 5.3), which is within the range expected for such soils in the sampled region (Paneque et al., 2016). Most likely due to the lower pH of the hydrochar, its addition resulted in decreased soil pH values at the end of the experiment. The extent of this effect depended on the dose applied. Amendment of 5 t ha^{-1} lowered the pH values of the soil between 0.3 and 0.5 units while 25 t ha^{-1} decreased this parameter by 0.8 and 1.4 units. However, plant growth may have contributed to the pH decrease.

In spite of the high pH of the pyrochars, its addition at 5 t ha^{-1} caused minor to no changes on soil pH, whereas A_Py and T_Py lowered the soil alkalinity when applied at 25 t ha^{-1}. The presence of roots exudates, which acidify the soil, together with their inherent buffering capacity may explain these results. Bruun et al. (2017) also found minor changes after the addition of pyrochar to a low clay acidic soil. The impact on soil pH of both hydrochars and pyrochars at 5 t ha^{-1} is slight and may not have a beneficial effect on soil nutrients availability. However, the soil pH at the highest application dose was closer to neutrality and hence nutrients availability may have been favored.

Table 5.3. Soil pH and EC of the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha^{-1} at the end of the greenhouse experiment.

	pH		**EC** μS cm^{-1}	
Control	8.4 ± *0.02*		100.6 ± 2.0	
Amended soils	**5 t ha^{-1}**	**25 t ha^{-1}**	**5 t ha^{-1}**	**25 t ha^{-1}**
A_HTC_200_0.5	8.0 ± *0.03*	7.4 ± *0.07*	116.4 ± *7.7*	280 ± *18*
A_ HTC_200_3	8.2 ± *0.03*	7.0 ± *0.02*	112.6 ± *7.5*	380.3 ± *40*
A_ HTC_260_0.5	8.2 ± *0.05*	7.5 ± *0.09*	111.0 ± *14*	231.8 ± *11*
A_ HTC_260_3	8.0 ± *0.12*	7.3 ± *0.09*	103.5 ± *1.9*	256.5 ± *38*
T_ HTC_200_0.5	8.0 ± *0.11*	7.3 ± *0.06*	111.9 ± *2.2*	644.3 ± *22*
T_ HTC_200_3	8.2 ± *0.05*	7.0 ± *0.04*	109.0 ± *1.2*	524.8 ± *11*
T_ HTC_260_0.5	8.2 ± *0.03*	7.5 ± *0.04*	117.0 ± *2.4*	554.0 ± *27*
T_ HTC_260_3	8.1 ± *0.07*	7.6 ± *0.05*	117.3 ± *5.4*	378.5 ± *8.1*
A_Py	8.2 ± *0.04*	7.3 ± *0.09*	115.4 ± *2.1*	236.0 ± *15*
T_Py	8.2 ± *0.04*	7.8 ± *0.03*	129.5 ± *3.9*	223.3 ± *9*
W_Py	8.4 ± *0.04*	8.5 ± *0.07*	92.4 ± *0.1*	108.5 ± *5.2*

5.3.2.2. Electrical conductivity (EC)

The Calcic Cambisol used for the incubation experiment exhibited an EC value of 101 μS cm^{-1} (Table 5.3), which is in the range of this soil-type of the region. Both hydrochars and pyrochars added at 5 t ha^{-1} produced minor to no changes on soil EC. However, hydrochars applied at 25 t ha^{-1} did increase the EC of the soil in an extent which depended on the EC value of the char. Thus, T hydrochars applied at 25 t ha^{-1} ranged from 378 to 644 μS cm^{-1}, whereas for A hydrochars this parameter was between 231 and 380 μS cm^{-1}. The A_Py and T_Py addition increased soil EC to a lower extent than hydrochars, which is in accordance with their lower EC values, whereas W_Py had no impact on this parameter.

Most of the crops grow up properly in soils with EC values between 100 and 1000 μS cm^{-1}. However, soils with more than 2000 μS cm^{-1} are considered saline (Soil survey division staff, 1993). Despite the EC of the hydrochars and pyrochars exceeded the above mentioned limits, their application at 5 and 25 t ha^{-1} is not expected to affect the crop since all the treatments showed soil EC values below 2000 µS cm^{-1}.

5.3.2.3. Extractable phosphorus (Extr-P)

In general, most of the chars were able to increased soil extr-P; however, the impact of SS-derived pyrochars was higher than that of the hydrochars (Figure 5.2). This result was expected since the hydrochars showed lower labile-P fractions than their respective pyrochars (Figure 5.1). The extr- P content of the control soil was 13 mg kg^{-1} at the end of the incubation. The A_Py and T_Py pyrochars applied at 5 t ha^{-1} increased the soil extr-P up to 59 and 49 mg kg^{-1} respectively. These values were between 3 and 4 times higher at the highest application rate. However, the addition of W_Py to the soil did not affect this parameter at any dose, which is explained by the low total and extr-P contents of this pyrochar. The behavior of W_Py compared to A_Py and T_Py is in accordance with the low nutrient value of greenwaste pyrochars (Chan et al., 2007).

Within hydrochars, the impact on soil extr-P reduces when production condition increases. Thus, the hydrochars obtained at 200°C after 30 min charring raised soil extr-P the most whereas those produced at 260°C for 3h had no impact on this parameter. Both A and T hydrochars showed comparable behaviors in this sense. However, T hydrochar addition resulted in slightly higher soil extr-P values than the amendment of the A hydrochars, which is due to the higher PT and extr-P of the former compared to the latter. In addition, an augmentation of the application rate from 5 to 25 t ha^{-1} increased the soil extr-P values both for hydrochars and pyrochars.

These results confirm the results obtained after the characterization P indicating that pyrolysis is apparently more appropriate than HTC to convert SS into a P fertilizer.

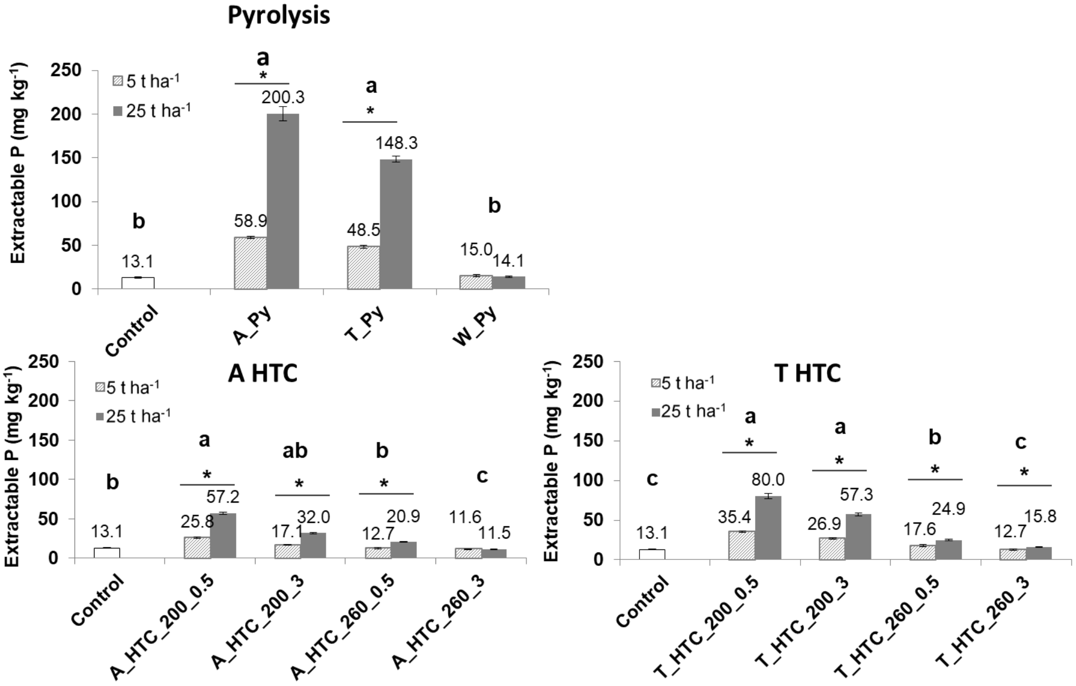

Figure 5.2. Extractable P in the control (soil without amendment), hydrochars and pyrochars amended pots at 5 and 25 t ha-1 at the end of the greenhouse experiment. The above-line asterisks indicate significant differences between doses. The letters show significant differences between treatments

5.3.2.4. Phosphorus release

The P_{diff} represents the difference between the amount of soil extr-P at the end of the experiment corresponding to the char and the extr-P added to the soil with each char and dose at the beginning of the experiment (Figure 5.3 A). The pots amended with the hydrochars produced at 260°C and the W_Py pyrochar showed almost no or even negative P_{diff}. Thus, it can be assumed that almost all of extr-P within the chars was used by the plants during the experiment. In contrast, the hydrochars produced at 200°C and SS-derive pyrochars showed positive P_{diff} values, ranging between 35 and 0.3 mg kg^{-1} for the former and 146 and 30 mg kg^{-1} for the latter. This means that the amount of soil extr-P was higher at the end of the experiment than at the beginning and hence that part of the originally non-extractable P must have turned into extractable forms during the incubation. Thus, changes in P availability seems to occur once thermally treated SS are applied to soil, which has to be considered for evaluating their potential as a P source for plant growth. A similar behavior was observed for pyrochars derived from digested (Brunn et al., 2017) and from pig slurry (Christel et al., 2014).

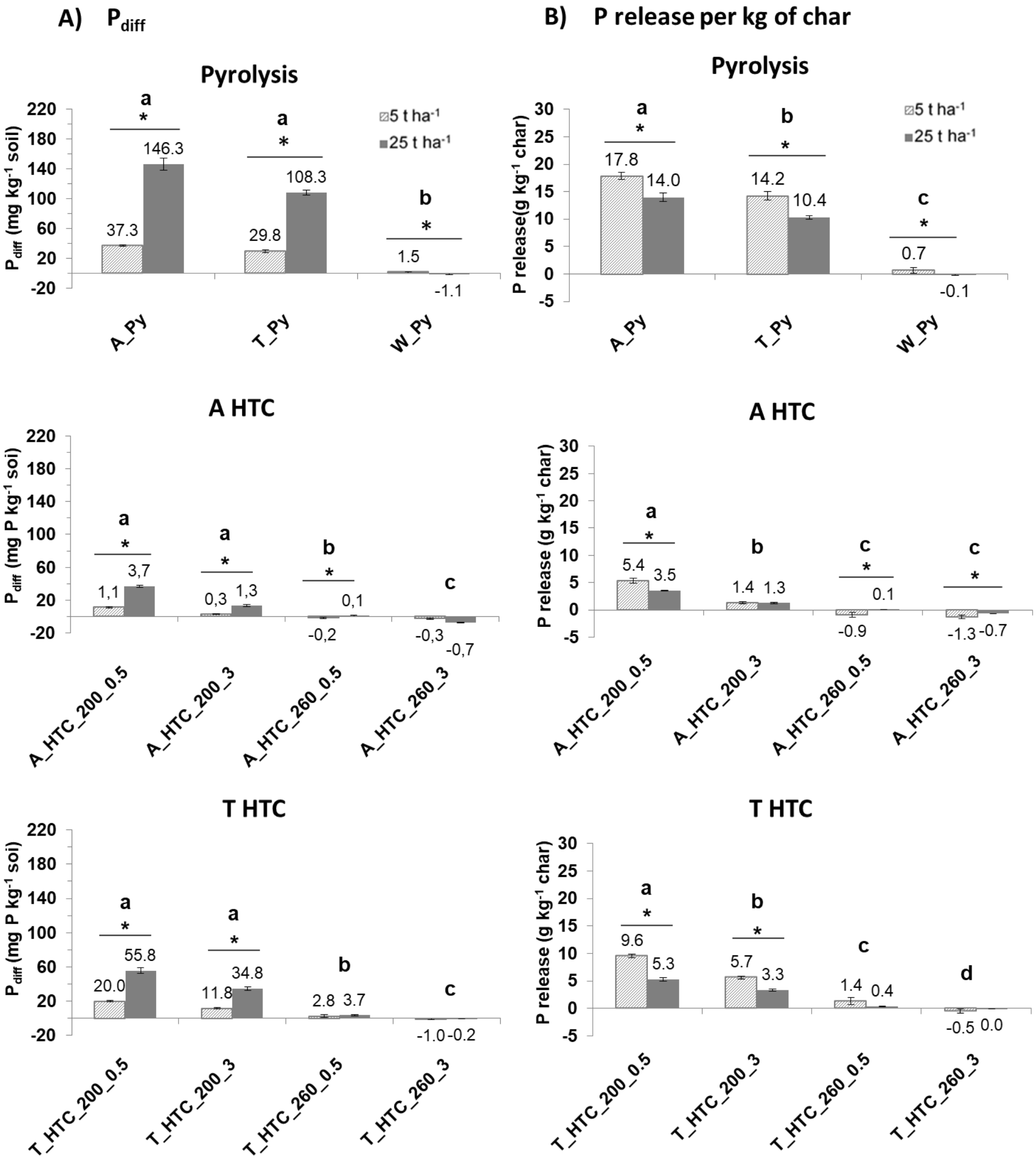

Figure 5.3. Amount of P release by the different hydrochars and pyrochars when applied at 5 and 25 t ha^{-1} at the end of the greenhouse experiment. A) Phosphorus difference between the amount of soil extr-P at the end of the experiment and the respective amount at the beginning; B) Phosphorus release per kg of char at the end of the experiment. The above-line asterisks indicate significant differences between doses. The letters show significant differences between treatments.

In order to obtain more insights into the potential of these chars to release P, the amount of P released per kg of char was calculated (Figure 5.3 B). In accordance with the higher P_{diff} in pyrochars compared to hydrochars, the P release per kg of char was also higher for pyrochars than for hydrochars. Thus, the A_Py and T_Py pyrochars released between 18 and 10 g of P kg^{-1}, whereas the amount of mobilized P of the hydrochars produced at 200°C was between 10 and 1.3 g kg^{-1}. Note that the T hydrochars showed a higher P release than the A ones, which is best explained with the higher P_T contents of the former compare to the latter.

Generally, increasing the application dose from 5 to 25 t ha^{-1} raised the P_{diff} values (Figure 5.3 A). This is a logical result since a higher amount of P_T per pot was added. In contrast, the amount of P release per kg of char is not expected to depend on the application dose. However, in our experiments, this parameter decreased when the application rate increased (Figure 5.3 B). Commonly, such an observation is explained with changing pH. The phosphorus in the soil is at maximum availability when soil pH is between 6 and 7.5. Considering that the 25 t ha^{-1} amended pots showed soil pH values closer to this range than the 5 t ha^{-1} amended ones, the pH seems to have only a low impact on our results. Alternatively, our results may be related to an equilibrium between dissolved P and adsorbed/precipitated forms in the soil. Thus, at 25 t ha^{-1} saturation of the dissolved P fraction may have occurred.

5.4 Summary and general discussion

Previous characterization studies indicated that an immobilization of P occurs after both HTC and pyrolysis of SS (Huang and Tang, 2016). Except for A_Py, this is in agreement with our results since a decreased of the labile P fraction was observed. However, both the pyrochars and the hydrochars produced at 200°C were able to increase the amount of soil ext-P compared to the control. This increase was due to the amount of extr-P within the chars but also to transformation of originally non-extractable P of the chars into extractable forms during the incubation. This explains the apparent contradiction between the observed immobilization of P after both HTC and pyrolysis determined through characterization techniques (Huang and Tang, 2016) and works which demonstrate that SS-derived pyrochars can act as an efficient source of P for plants (Faria et al., 2017; Frišták et al., 2017).

The results obtained in this work should be carefully extrapolated. Differences among different SS types have been observed both in this work and in literature (Huang et al., 2016). In the same way, the application of the same chars to different soils may result in a different P behavior (Brunn et al., 2017; Xu et al., 2014). However, as previously mentioned, the two SS used in this study were especially low in P compared to the average in the EU. Thus, the potential of thermally treated SS as a source of P for soils is expected to be generally higher.

5.5 CONCLUSIONS

Both dry-pyrolysis and, in a lower extent, low-temperature HTC seems to be useful techniques to produce treated-SS with P fertilizing properties. The initial immobilization of P after both thermal treatments may reduce P losses by leaching. In addition, the subsequent P release once the chars were applied to the soil indicates their suitability as slow-release P fertilizers.

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6. Degradability of organic C and N of a ^{13}C and ^{15}N enriched hydrochar and a pyrochar derive from sewage sludge in a soil and the availability of their N for plant growth

6 DEGRADABILITY OF ORGANIC C AND N OF A ^{13}C AND ^{15}N ENRICHED HYDROCHAR AND A PYROCHAR DERIVE FROM SEWAGE SLUDGE IN A SOIL AND THE AVAILABILITY OF THEIR N FOR PLANT GROWTH

ABSTRACT

In order to study the biodegradability of pyrochars and hydrochars in amended soils, sewage sludge (SS) enriched in ^{13}C and ^{15}N were obtained under controlled conditions in an fermenter after adding K $^{15}NO_3$ (^{15}N, 99 atm%), $^{15}NH_4Cl$ (^{15}N, 99 atm%) and ^{13}C-glucose (^{13}C, 99 atm%) to waste water from a water treatment plant located in "Carrion de los Cespedes", within a rural area at about 20 Km from Seville city. This material was subjected to pyrolysis at 600°C for 1 h and hydrothermal carbonization (HTC) at 200 °C for 30 min, yielding in ^{15}N and ^{13}C enriched pyrochars and hydrochars. They were added to a calcic Cambisol on which *Lolium perenne* was grown during 10 months under the controlled conditions of a greenhouse. The distribution of ^{15}N and ^{13}C among the soil and plants was monitored after 1 and 3 months as well as at the end of the experiment. Our results confirmed that both N and C in the pyrochar were biochemically more stable than those of the hydrochar. During the experiment, the hydrochar showed a slightly lower amount of plant available N than the non-treated SS. However, the hydrochar exhibited higher plant available N than the pyrochar. Whereas, both the non-treated SS and the hydrochar released most of their N at the beginning of the experiment a low but constant amount of N was mobilized for plants growth from the pyrochar only after the first incubation month. This work confirmed the suitability of SS-derived hydrochars for short and medium-term N-fertilizing purposes and suggest that SS-derived pyrochars could work as a low-dose and long-term N fertilizer.

6.1 INTRODUCTION

Paneque et al. (2017) showed a considerably lower aromaticity of SS-derived hydrochars than of the respective pyrochars (chapter 3). Based on this observation, a lower biochemical stability of the hydrochars, thus a lower C-sequestration potential, is expected. Comparably, due to the higher content of heterocyclic N in the pyrochars relative to the hydrochar, the N of the first is commonly associated with a lower biochemical availability.

The greenhouse experiment developed in chapter 4 of this PhD showed that the 200°C-produced hydrochars exhibited the highest plant biomass production rate whereas the respective pyrochars were comparable to the control. The analysis of the growth parameters of *Lolium perenne* indicated furhter that the N_i and N_{org} contents of the amendments are the main responsible for improved plant growth. Considering that the presence of N_i and easily available organic N forms is most likely the reason for the better plant production on hydrochar than on pyrochar amended soils, the fate and bioavailability of heterocyclic N in pyrochar is of particular interest for its role as slow-release fertilizer.

A common way to study OM turn-over processes in soils represents the use of the stable isotopes ^{13}C and ^{15}N as tracer (Michener and Lajtha, 2007). The addition of isotopically enriched organic residues to a soil matrix during an incubation experiment allows studying the partitioning of the tracer among the different soil compartments, its uptake by plants or its loss due to mineralization and volatilization by determining the changes of the isotopic ratios along the incubation time. In the following study, this approach was used to obtain more information about the biochemical degradability of hydrochars and pyrochars from SS after their addition to soil. Therefore, a ^{13}C and ^{15}N enriched SS produced by adding ^{13}C enriched glucose and ^{15}N-enriched ammonium to wastewater passing a bioreactor was thermally treated by hydrothermal carbonization (HTC) and pyrolysis. The respective hydrochar and pyrochar were added to a soil matrix on which *Lolium perenne* was grown during a 10-months greenhouse incubation experiment. The distribution of ^{15}N and ^{13}C among soil and plants was monitored through periodic analyses by using a isotopic ratio mass spectrometer (IRMS).

6.2 MATERIALS AND METHODS

6.2.1 Production of ^{13}C and ^{15}N enriched SS

The labelled SS was produced in the Experimental Wastewater Treatment plant of CENTA (http://www.centa.es/), located in Carrion de los Cespedes (Seville). This plant processes the domestic wastewater of the village with over 3000 residents. Part of this wastewater was deviated from the established cleaning process and treated in two 10 L sequencing bath reactors. Each bath replicates an enhanced biological phosphorus removal (EBPR) system at pilot scale. The EBPR is a modified activated sludge system composed of anaerobic-aerobic temporal unit processes, through which microorganisms and wastewater are recycled.

Daily, 100 mg of glucose (^{13}C, 99% abundance), 15 mg of ammonium chloride (^{15}N, 99% abundance) and 20 mg of potassium nitrate (^{15}N, 99% abundance) were added to the pilot-units for the buildup of new microbial biomass. Considering that microbial residues comprise a major fraction of SS, we were able to obtain a SS with a ^{15}N and ^{13}C abundance of 2.8 and 1.0 %, respectively. The amount of the daily dose at which the enriched compounds had to be applied to ensure the detectability of the isotopic tracers in the plants and the soil during the incubation was determined in pre-tests. The alteration of the enrichment degree after the HTC and the pyrolysis treatment of this SS was also considered. The reactors were working from 9 months in order to obtain the necessary amount of enriched material.

6.2.2 Production of ^{13}C and ^{15}N enriched chars

For the production of the hydrochar, the isotopically enriched SS was mixed with water (1:20) and heated at 200°C for 30 min in a 1 L-stirred pressure reactor (Parr reactor series 4520, IL, USA) equipped with an external resistance heater and internal sensors for pressure and temperature as described in chapter 3. A final amount of 63.7 g of hydrochar, named Hyd, was obtained.

For the production of the pyrochar, 40 g of dry SS were placed into a close steel container and heated in a preheated muffle oven at 600 °C for 1 h. This process was done twice and a total amount of 44.6 g of pyrochar, named Py, was obtained.

6.2.3 Characterization analyses

Hydrogen (H), C and N contents were measured per triplicate with an elemental analyzer Leco Truspec CHNS micro. The solid-state ^{13}C NMR spectra were obtained with a Bruker Avance III HD 400 MHz WB spectrometer, using the parameter described in chapter 3. Between 1800 and 9300 scans were accumulated for each sample. For the determination of the chemical composition the following chemical shift regions were integrated: alkyl C (0–45 ppm); N-alkyl/methoxyl C (45–60 ppm); O-alkyl C (60–90 ppm); anomeric C (90–110 ppm); aryl C (110–140 ppm); O/N-aryl C (140–160 ppm); carboxyl/amide C (160–220 ppm).

6.2.4 Greenhouse experiment

A 10-month greenhouse experiment was conducted with the following treatments: only soil (control), soil amended with the three labeled materials (SS, Hyd, Py) and soil with $K^{15}NO_3$ (KNO_3). The soil matrix used for the greenhouse incubation experiment derived from the Ah horizon of a Cambisol (IUSS WorkingGroup WRB 2014) (N 37° 32′ W 6° 15′) from the Sierra de Aznalcóllar, southern Spain. A brief characterization of the soil is given by Lopez-Martin et al. (2015). After removing visible root and plant residues, the soil was sieved through a 2-mm sieve and oven-dried at 40 °C. The soil contained 38.7 g C kg^{-1} of which all was attributed to organic carbon and 3.4 g N kg^{-1} of which 27.5 mg corresponded to NH_4^+ and 1.87 mg to NO_3^-. Its pH in water is 5.7 and its water holding capacity (WHC) is 22%.

The used pots were 250 ml-plastic containers (16 cm height) with a hole at the bottom part. For each treatment 9 replicates were prepared (n = 9). The control pots were filled with 230 g of soil. For the SS, Hyd and Py treatments 190 g of soil was covered with a 40 g amendment:soil mixture (5%, w:w). The enrichment degree of the amendments and the amount of C_T, N_T, ^{13}C and ^{15}N added per pot is shown in table 6.1. Finally, 25 certified grass seeds *(Lolium perenne*-ILURO seeds company, Spain) were sowed in each pot and soil moisture was adjusted to 60% of the maximum water holding capacity (WHC). The pots were placed into trays and put into a greenhouse at 25 °C under natural solar light conditions. No nutrient solution was added during the experiment. The WHC was checked three times per week and adjusted by weighing the pots and adding the mass difference as declorated water. The position of the pots on the tray was changed after adjusting the WHC to assure comparable light and growing conditions.

Table 6.1. Relative atomic abundance of the stable isotopes in the amendments and the amount of C_T, N_T, ^{13}C and ^{15}N added per pot.

	A* (%^{13}C)	**Amount of added C_T** (mg per pot)	**Amount of added ^{13}C** (mg per pot)	**A** (%^{15}N)	**Amount of N_T added** (mg per pot)	**Amount of ^{15}N added** (mg per pot)
SS	2.8	581.8	16.5	1.0	83.2	0.8
Hyd	1.9	476.9	9	0.6	38.8	0.2
Py	2.2	372.9	8.2	0.7	47.6	0.3

*relative atomic abundance

After 1, 3 and 10 months of incubation, three replicates of each treatment were destructively sampled. For each pot, the shoots were cut, dried (72 h at 60 °C) and weighed. The first 3-4 cm of the soil column were separated from the rest and named topsoil. The remaining soil was called subsoil. The roots were manually separated from both the top- and the subsoil, rinsed with distilled water, dried (72 h at 60 °C) and weighed. The soil nitrate (NO_3^-) and ammonium (NH_4^+) contents were measured both in the top- and subsoil. The total C, N, ^{13}C and ^{15}N contents of both the top- and subsoil as well as of the plant shoots were measured with a Flash 2000 HT combustion elemental microanalyzer and a Flash HT Plus elemental analyzer coupled to a Delta-V advantage isotopic ratio mass spectrometer (IRMS) via ConFlo IV interfase (Thermo Scientific, Bremen, Germany), with an analytical measurement error of ± 0.2‰.

The proportion of total C derived from the amendments ($\%C_{amend}$) in the top- and subsoil for each amendment and sampling time was calculated by using a two component isotopic mixing model (Bernoux et al., 1988):

$$\%C_{amend} = \frac{\delta 13\mathrm{C(amend{+}soil)} - \delta 13\mathrm{C(soil)}}{\delta 13\mathrm{C(amend)} - \delta 13\mathrm{C(soil)}} \qquad (1)$$

where the δ^{13}C(amend+soil) represents the δ^{13}C value of a SS, Hyd or Py amended pot at a specific sampling; the δ^{13}C(soil) represents the δ^{13}C value of the non-amended; and the δ^{13}C(amend) represents the δ^{13}C value of the SS, Hyd or Py.

The proportion of total N derived from the amendments ($\%N_{amend}$) in the top- and subsoil for each amendment and sampling time was calculated by substituting the δ^{13}C values for δ^{15}N values in the equation (1).

In addition, the amount of N derived from the amendments uptake by the plants was studied by applying the following enrichment factor (EF), modified from Michener and Lajtha, 2007:

$$\mathrm{EF} = \frac{\delta 15\mathrm{N(amend)} - \delta 15\mathrm{N(control)}}{mg\ 15Nadded * days\ of\ incubation}$$

Here, δ^{15}N(amend) represents the δ^{15}N value of the shoots growth in presence of the SS, Hyd or Py at a specific sampling; the δ^{15}N(control) represents and the δ^{15}N value of the shoots growth only with soil at a specific sampling.

6.2.5 Statistical analysis

Data corresponding to the percentage of total C and N derived from the amendments in the soil were submitted to Shapiro-Wilk and Levene tests to test for normality and homoscedasticity, respectively. After that, an analysis of variance (one-way ANOVA) followed by a comparison of means (Tukey's test) were performed to test for significant differences along the time for each char. Differences were considered as significant at $p \leq 0.05$. Data were analyzed using SPSS version 17.0 (SPSS, Chicago, IL, USA).

6.3 RESULTS AND DISCUSSION

6.3.1 Characterization of the amendments

The total C content of the non-treated SS was 290.9 g kg^{-1} whereas that of the Hyd and Py was 238.5 and 186.5 g kg^{-1} respectively (Table 6.2). The SS and Hyd exhibited an atomic H:C ratio of 1.7 and 1.5 which indicates low aromaticity. The lower H:C ratio of 0.5 for Py, on the other hand, is in line with a high content of aromatic structures in this sample of which every second C is protonated.

Table 6.2. Carbon (C), N, H:C$_{at}$ ratio and C$_{org}$/N ratio of the soil, sewage sludge (SS) and its hydrochar (Hyd) and pyrochar (Py).

	C *g kg^{-1}*	**N** *g kg^{-1}*	**H:C** atomic ratio	**C$_{org}$/N**
Soil	3.8	3.4		11.4
SS	290.9	41.6	1.7	7.0
Hyd	238.5	19.4	1.5	12.3
Py	186.5	23.8	0.6	7.8

The solid-state ^{13}C NMR spectra are in accordance with the atomic H:C ratios (Figure 6.1). The C$_{org}$ in SS occurred mainly as peptides (*N*-alkyl C, amide C and part of the alkyl C) and carbohydrates (*O*-alkyl C) followed by lipids, which account for approximately 10%. Aromatic compounds contribute with less than 8% (*O*/*N*- aryl C and aryl C) to the total C$_{org}$ (Table 6.3). After HTC, the proportion of aromatic C increased to 20% of the total C$_{org}$ in detriment of carbohydrates and peptides whereas pyrolysis transformed almost all of the OM into aromatic structures.

Table 6.3. Intensity distribution (%) in the solid-state ^{13}C NMR spectra of the sewage sludge (SS), its hydrochar (Hyd) and its pyrochar (Py).

	Carboxyl / Amide C	***O/N*** **-Aryl C**	**Aryl C**		***O-*** **Alkyl C**	***N*****-Alkyl/ Methoxyl C**	**Alkyl C**
ppm	**225-160**	**160-140**	**140-110**	**110-90**	**90-60**	**60-45**	**45-0**
SS	13.9	2.0	5.9	5.8	25.2	13.98	33.6
Hyd	8.6	4.6	16.0	4.4	12.5	11.17	42.9
Py	4.2	11.5	63.0	6.8	3.9	1.92	8.7

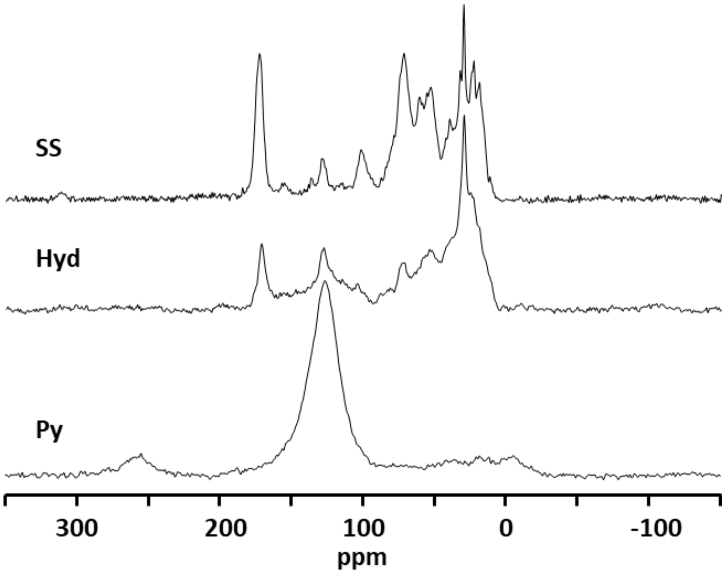

Figure 6.1. Solid-state ^{13}C NMR spectra of the sewage sludge (SS), its hydrochar (Hyd) and its pyrochar (Py)

The N content of the non-treated SS was 41.6 g kg^{-1} (Table 6.2). The Hyd and Py exhibited lower values, with 19.4 and 23.8 g kg^{-1}, respectively. The higher loss of N during pyrolysis than during HTC is not common but Bargmann et al. (2014a) reported the same behavior in HTC-treated and pyrolyzed spent brewer's grains. Paneque et al (2017) showed that most of the N in non-treated and thermally treated SS occurs in organic forms, although, some Ni is expected. They showed further that peptides and, to a lower amount, amides and amino sugars, which are the main N_{org} forms in SS are transformed into to N-heterocyclic aromatics during thermal treatment. During pyrolysis considerably more heterocyclic N was formed than during HTC: The non-treated SS and its derived hydrochar and pyrochar exhibited C_{org}/N ratios of 7.0, 12.3 and 7.8 respectively (Table 6.2) indicating that indeed a considerable amount of N was incorporated into the charred organic fraction. Note that the C_{org}/N values of the isotopically enriched SS and chars are lower than those observed for the SS obtained from ponds in Paneque et al. (2017). This is best explained with the fact that the enriched material was produced in the laboratory and was mostly composed of microbial residues whereas the pond material contained additional ingredients supplied with the wastewater. However, comparing the NMR spectra of the chars from the pond SS with those derived from the waste water reactor, one can assume that in spite of this difference the latter can be taken as being representative of chars derived from SS.

6.3.2 Greenhouse incubation experiment

6.3.2.1. Fate of the C derived from the amendments during the incubation experiment

The percentage of C derived from isotopically enriched SS (C_{SS}) decreased after 1 month of incubation (Figure 6.2), which points to a faster degradation of C_{SS} if compared to the soil organic carbon (SOC) during this time. Most likely, this is due to the fact that SOC represented already well humified material, whereas C_{SS} still contained considerable amounts of easily degradable residues. The relative contribution of C derived from Hyd (C_{Hyd}) decreased, too, but to a lower extent than C_{SS}. Thuille et al. (2015) found comparable results after a 39-day greenhouse experiment with soils amended with a silage-derived hydrochar or with the non-treated silage. Between the first and the 10th incubation month, the relative proportion of C_{SS} and of C_{Hyd} in the soil did not change, suggesting that either none of the C pool (SOC and C_{amend}) decomposed further or, which is more likely, that both C pools degraded with a similar rate.

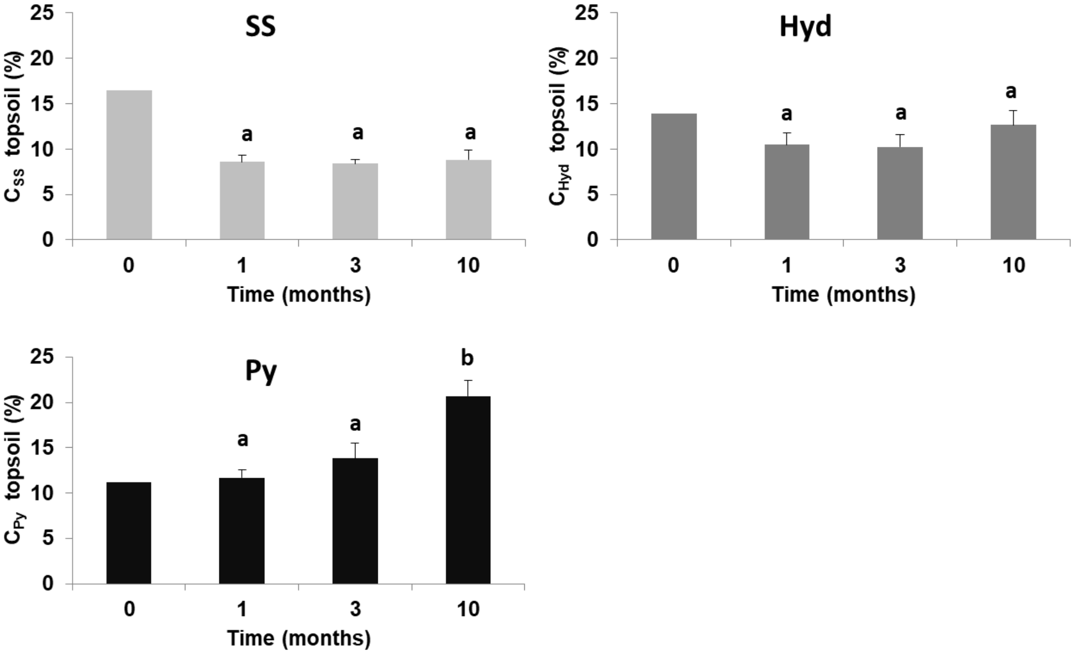

Figure 6.2. Percentage of the total C derived from the sewage sludge (C_{SS}), hydrochar (C_{Hyd}) and pyrochar (C_{Py}) in the topsoil at the different sampling times.

The proportion of C derived from Py (C_{Py}) did not change during the first incubation month. This suggests that C_{Py} and SOC were degraded at a similar speed. However, the percentage of C_{Py} increased between the 3rd and 10th month of incubation, allowing the conclusion that SOC suffered a preferential degradation relative to C_{Py}. This would support a higher biochemical recalcitrance of pyrogenic organic matter relative to the SOC of the used soils.

With respect to the subsoil, the relative contribution of C_{amend} to the total C_{org} was lower than 0.5% for all treatments (Figure 6.3) revealing that leaching of the soil amendment or its degradation residues was negligible.

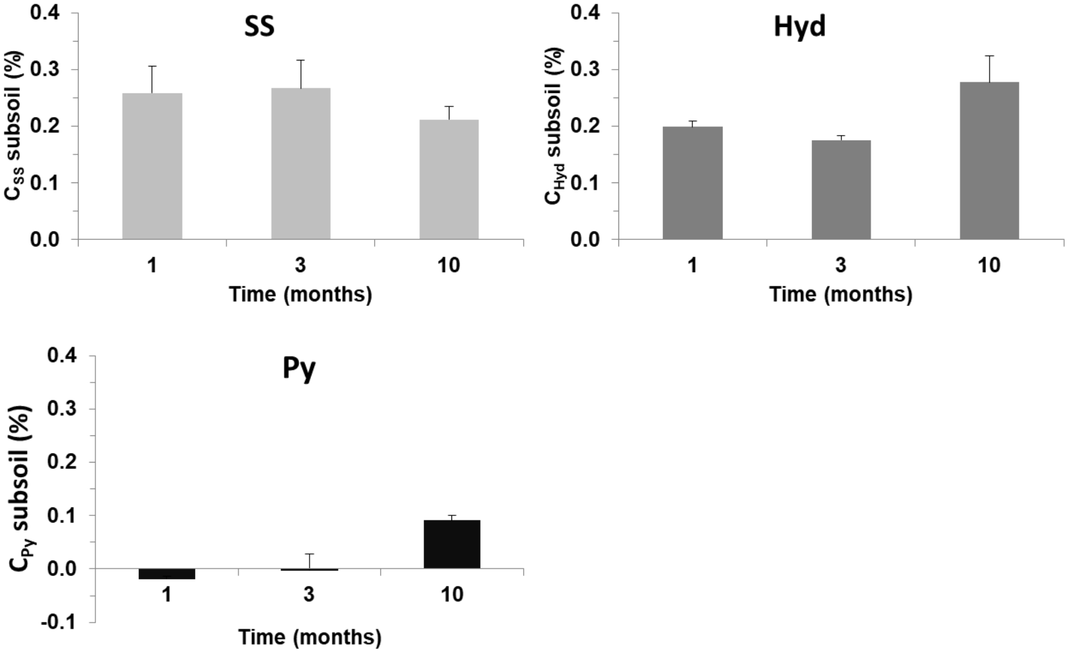

Figure 6.3. Percentage of the total C derived from the SS (C_{SS}), its hydrochar (C_{Hyd}) and pyrochar (C_{Py}) in subsoil at the different sampling times.

6.3.2.2. Soil nitrogen evolution during the incubation experiment

- Nitrate (NO_3^-)

During the first 3 months, the SS-amended pots exhibited between two and five times more topsoil NO_3^- concentrations than the control (Figure 6.4). This is most likely due to NO_3^- addition with the amendment. Despite that SS was only applied to the topsoil, the subsoil showed between 3 and 4 times more NO_3^- than the control. This is explained by a leaching of this compound promoted by irrigation. The lack of NO_3^- in both the top and subsoil after the 10th month of incubation is in line with the uptake of this nutrient by plants and microorganisms. However, loss due to volatilization cannot be excluded.

Both addition of Hyd and Py decreased the topsoil NO_3^- contents compared to the control after the first incubation month, which is in accordance with Bargmann et al. (2014a; 2014b). They observed that hydrochars promoted microbial immobilization whereas biochars seemed to increase N adsorption to particle. In addition, Haider et al. (2017) provide evidence that a "nitrate capture" may occur frequently after biochar addition. To which extend this can explain our results, however, has to be approached in future studies. After 3 and 10 months of incubation, NO_3^- was found neither in the Hyd and Py treatments nor in the control.

In addition, these treatments had no impact on NO_3^- content of the subsoil at any incubation time.

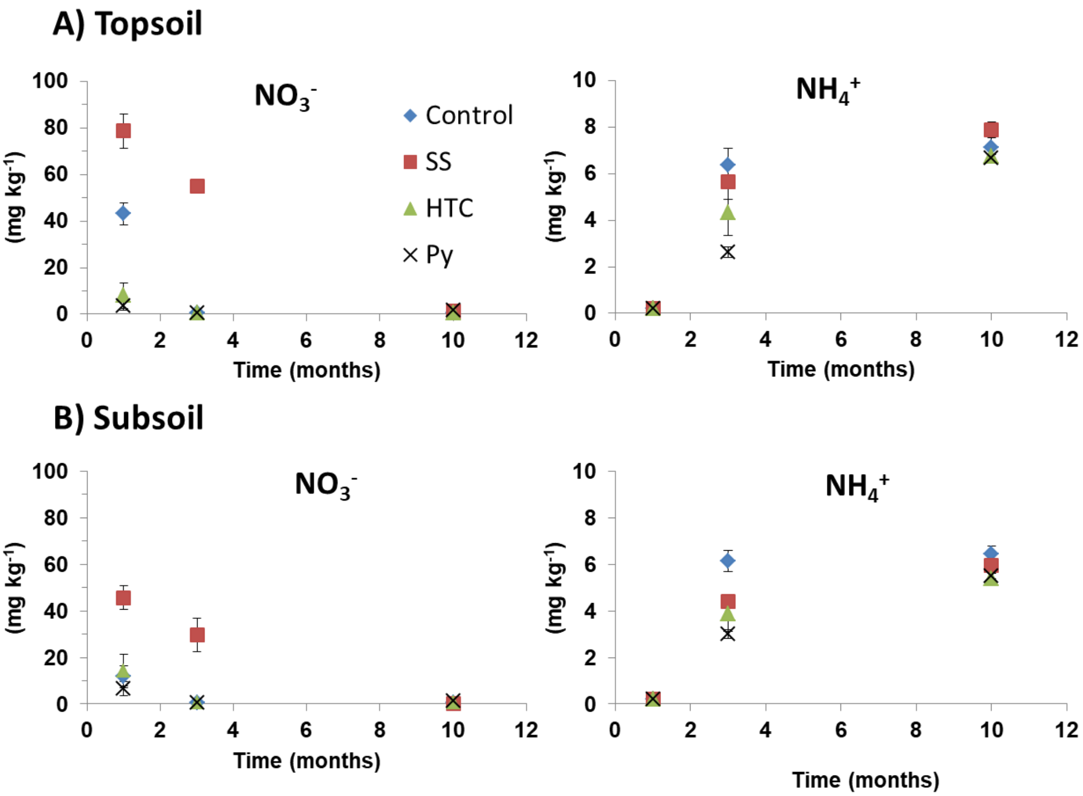

Figure 6.4. Top- and subsoil contents of nitrate (NO_3^-) and ammonium (NH_4^+) in the control, SS, Hyd and Py amended pots at the different sampling times.

- *Ammonium (NH_4^+)*

None of the treatments contained mentionable NH_4^+ concentrations, neither in the top- nor in the subsoil after the 1st incubation month (Figure 6.4). However, after 3 and 10 months their abundance increased to 2.6 and 7.9 mg kg^{-1}, which is in line with the degradation and desamination of organic N compounds. The lower NH_4^+ contents in the Py-amended pots than in the control after 3 months, may be due to adsorption of the nutrient onto the biochar surface and/or to an increase in the NH_4^+ consumption rates (Nelissen et al., 2012). After 10 months, all the amended pots and the control exhibited similar NH_4^+ contents

6.3.2.3. Fate of the N contained in the amendments during the incubation experiment

- *Changes after 1 month*

The relative contribution of N derived from SS (N_{SS}) to the total N of the topsoil decreased after 1 month of incubation (Figure 6.5), indicating a preferential use of N_{SS} with respect to soil N. Although C_{SS} was also reduced during this time, the decrease of the N was stronger, which is related to the fact that the uptake and volatilization of NO_3^- occurs independently from the C pool.

The proportion of N derived from Hyd (N_{Hyd}) decreased to a lower extent than that of SS, which points to a lower N availability of the former compared to the latter during this time. This is likely due to the lack of N_i in Hyd together with a slightly higher content of heterocyclic N compounds. The degradation rate of the C_{Hyd} and the N_{Hyd} pools seems to be similar in this case.

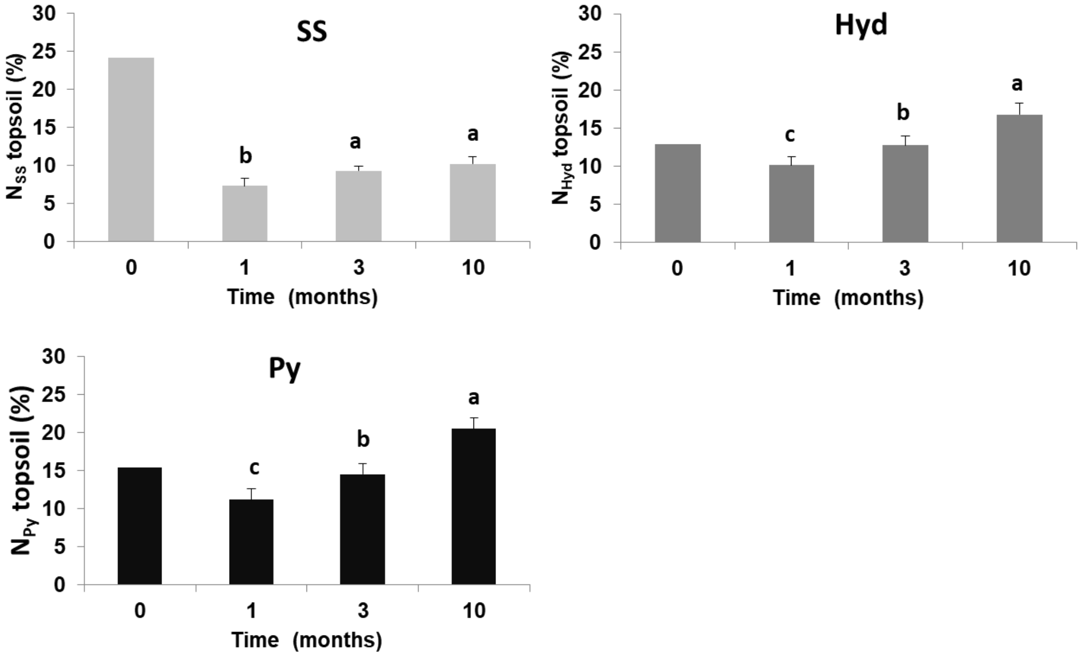

Figure 6.5. Percentage of the total N derived from the SS (N_{SS}), hydrochar (N_{Hyd}) and pyrochar (N_{Py}) in topsoil at the different sampling times.

Despite the percentage of C_{Py} was not altered after 1 month of incubation, the proportion of N derived from the pyrochar (N_{Py}) decreased during this time. This result needs to be confirmed with further analyses before going into a deeper discussion.

Bearing in mind that the amount of N derived from the amendments (N_{amend}) in the subsoil was less than 1% in all cases (Figure 6.6), the N loss is best explained with removal by N-uptake in plants and volatilization. The design of this experiment does not allow distinguishing between the pristine N_{amend} (still non-degraded) and the N_{amend} already degraded and immobilized by soil microorganisms. Considering that the N_{amend} decreased during this first month, immobilization does not seem to play an important role.

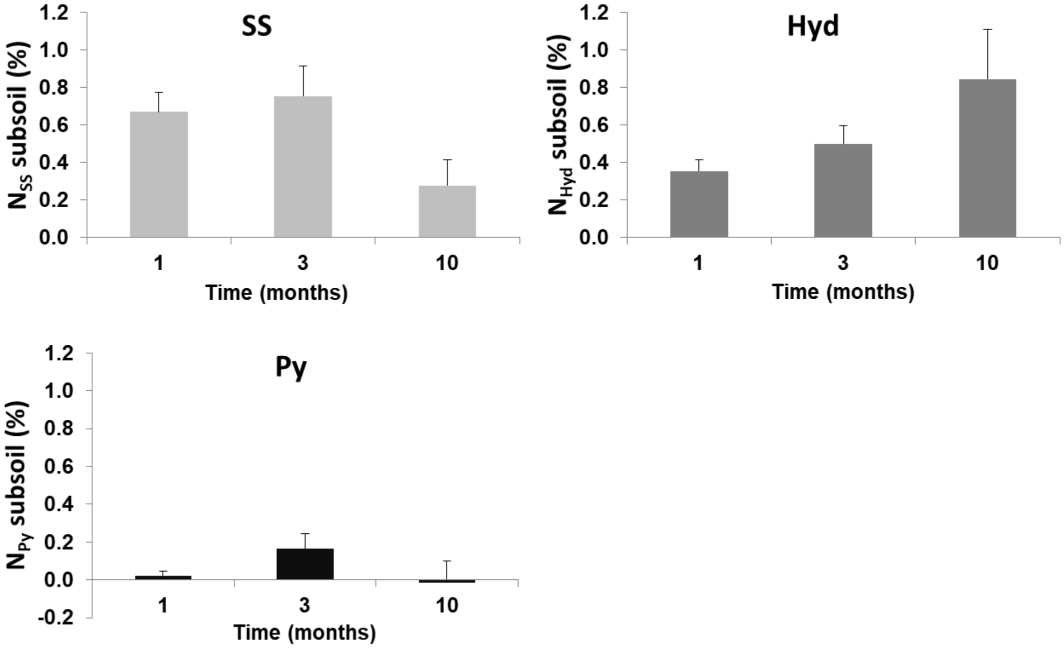

Figure 6.6. Percentage of the total N derived from the SS (N_{SS}), hydrochar (N_{Hyd}) and pyrochar (N_{Py}) in subsoil at the different sampling times.

- *Changes between the 1st and the 10th month of incubation*

The proportion of N_{SS} of the total N in the soil slightly increased between the first and 10th incubation month, suggesting that soil N was preferentially lost compared to N_{SS} during this time (Figure 6.5). Thus after degradation of the more labile N-containing compounds of SS during the first month, the remaining organic N_{SS} exhibited a higher biochemical recalcitrance than soil N. The fact that, in contrast to N_{SS}, the percentage of C_{SS} did not increase during this time, may indicate that N containing components had a higher biochemical recalcitrance than those without N. Alternatively, our observation may be interpreted with deamination and subsequent recycling of the released N for the build-up of new microbial biomass whereas the remaining C-skeleton was metabolized to CO_2.

The proportion of N_{Hyd} of the total N in the soil exhibited a greater increase than that of the N_{SS} during the same time, which points towards a higher biochemical stability of the first. This indicates that the N-containing compounds remaining in the Hyd after the first incubation month were more recalcitrant than those remaining in the SS. As in the SS treatment, the percentage of C_{Hyd} of the total C in the soil did not increase during this time, suggesting a higher degradability of N-containing organic compounds than of those without N in the hydrochars.

The Py exhibited the greatest increment of the proportion of N_{Py} of the total N in the soil compared to the other two amendments. The proportion of C_{Py} to the total C of the soil also increased during this time whereas that of the SS and Hyd did not change, which confirms a higher biochemical recalcitrance of this material compared to the others. This is in accordance with the results obtained by solid-state ^{13}C NMR spectroscopy showing that Py exhibits the highest aromaticity and that most of its N is bound in heterocyclic aromatic structures.

6.3.2.4. Nitrogen uptake behavior of the different treatments

The ^{15}N-enrichment factor (EF) of the plant shoots with increasing experiment time are depicted in Figure 6.7. The plants grown on SS-amended soils showed the highest shoot EF after 15 days of incubation. This parameter decreased with increasing experiment time, suggesting that at the beginning of the experiment the amount of plant available N_{SS} was highest and decreased with time. Note that although soil N was used in higher extent than N_{SS} after the first incubation month, the N_{SS} was also used for plant growth throughout the experiment. Accordingly, the shoots harvested after 10 months of incubation exhibited a low but still positive EF value.

The shoot EF of plants grown on soils amended with Hyd followed the same trend. However, the shoot EF values were lower in all cases, suggesting that less N_{Hyd} was plant-available. This is in accordance with the isotopic distribution in the soils, since the proportion of N_{Hyd} to the total N in the soils increased in a higher extent than that of the N_{SS}. In addition, Hyd contained less N_T than SS and negligible N_i. However, as in the experiment with SS, the amendment of Hyd effectively supplied N usable for plant growth during the whole experiment.

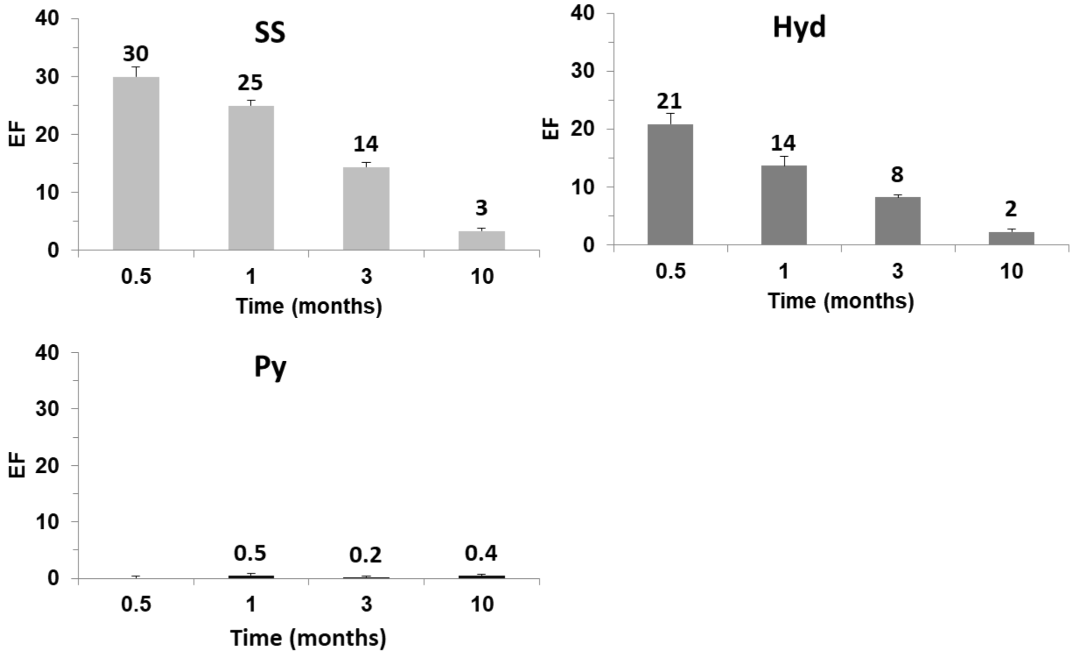

Figure 6.7. Enrichment factor (EF) of the plant shoots for each treatment at the different harvests.

The plants grown on Py-amended soil showed no enrichment after the first harvest and very low EF values in subsequent harvests. This indicates that the N_{Py} was not available during the first 15 days and that a slow transformation of non-available N into available forms started only after 15 days of incubation but continued until the end of the experiment. Thus, degradation of the heterocyclic aromatic N compounds must have occurred. Since the EF factor in the harvested plants remained comparable until the end of the experiment, we assume a constant degradation rate throughout the whole experiment. The low plant N availability of this amendment is in accordance with the fact that in Py most of N occurs in heterocyclic aromatic N structures and with the observation of the relative abundance of N_{PY} and C_{Py} in the soil between the 1st and 10th month of incubation. However, the low EF value of the plants harvested after 1 month of incubation is in contrast with the observed decrease of the proportion of N_{Py} in the soil during the same time. Thus, further analyses are necessary.

6.4 CONCLUSIONS

This study confirms that pyrolysis achieved a more effective stabilization of SS than HTC. However, HTC was able to reduce the amount of vey labile compounds that otherwise would be degraded within the first month. Despite the amount of N uptake from Hyd was lower than that of SS, this amendment effectively worked as a source of N for plants during the whole incubation time. In addition, the NO_3^- leaching observed for the non-treated SS was avoided. Due to the greater recalcitrance of SS-derived pyrochars, the amount of plant available N was much lower than that of the SS and Hyd, indicating a low suitability of this material to be used as fast-release N fertilizer. However, the analysis of samples after 18 and 24 months of incubation, which are still in process, may reveal its suitability as long-term fertilizer.

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7. CONCLUSIONS

7 CONCLUSIONS

Our study showed that transferring SS into hydrochars cannot only provide N_i for immediate use for plant growth but also sequesters a considerable proportion of its N as BN in which N is bound in N-heterocyclic aromatic structures. In contrast, the pyrochars revealed only organic BN (Chapter 3). Those studies are in contrast to former believes that in biochars, N occurs predominately as N_i which is sequestered in the porous structure. Thus, we can conclude that pyrolysis and HTC can indeed immobilized N in the organic network of the products which points towards the possibility to efficiently valorize this organic waste as a slow release fertilizer. Indeed, the incubation studies with ^{15}N and ^{13}C enriched hydrochars and pyrochars, monitoring their fate in soils during a growing period of *Lolium perenne* confirmed the higher biochemical recalcitrance of BN, if compared to N in unheated SS (Chapter 6).

In the present incubation studies, the hydrochar produced at 200° C for 30 min turned out to be the most appropriate if both immediate and medium term N-fertilization are required (Chapter 4 and 6). However, the observed slow degradability of BN, may turn pyrochar into a more efficient soil amendment, if small N doses are needed over a longer time range. Based on our results, we hypothesize that N of BN may still be present and transformed into a plant-available form during the second and even latter growing period. This would be advantageous in particular in extensively managed grasslands in remote regions. The proof of this hypothesis was beyond the scope of the present work, but certainly deserves further considerations in future research.

Regarding the use of thermal treatments as a form to stabilize organic matter, it has to be born in mind that pyrolysis results in high N loss due to volatilization. On the other hand, this N loss may be outbalanced by the sequestration of the remaining N in heterocyclic forms, which increases the pool of organic N with medium-term biochemical recalcitrance.

Despite, both HTC and pyrolysis represent efficient tools for hygienization of SS it is unlikely that SS-chars could completely substitute the mineral fertilizers. Anyway, the net balance of this approach is clearly positive due to: i) the remarkable reduction of the N and P losses by avoiding the disposal of this organic waste and ii) the replacement of mineral fertilizers, which are limited and demand high-energy. The fact that greenhouse gas emissions may be reduced represents a further advantage. However, from an agronomic point of view the reduction in the germination rates of HTC amended soils still needs to be solved (Chapter 4).

On the other hand, further research is needed to evaluate to which extent a broad application is economically feasible. HTC seems to be a better choice than pyrolysis from an energetic and thus economic point of view. HTC avoids the needed for drying the SS prior the pyrolysis and lower temperatures are applied, which considerably reduce the costs for energy. Aside from being advantageous with respect to economic considerations, our study revealed that HTC at 200 °C was more suitable than 260 °C for plant growth (Chapter 4).