

and provides N and P for plant growth during a second cropping period with *Lolium*

*perenne*

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**Running Title:** Sewage sludge chars as slow-release fertilizers

## Abstract

Hydrothermal carbonization and dry pyrolysis transform sewage sludge (SS) into nitrogen- (N) and phosphorus- (P) rich hydrochars (Hyd) and pyrochars (Py), respectively, which may

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act as slow-release fertilizers with carbon (C) sequestration potential. Whereas this has been mostly studied with short-term experiments, this study focussed on the cycling of char-derived N, P and C after aging during a second grass cropping cycle. *Lolium perenne* was grown for 3 months in pots on soil mixed with  $^{13}\text{C}$  and  $^{15}\text{N}$ -enriched SS, Hyd or Py and allowed to age during a first cropping period of ten-month incubation. The  $\delta^{15}\text{N}$  in the plants confirmed that even during the second cropping, N derived from the amendments was plant accessible. Higher uptake of N from Hyd than from Py is explained by the lower biodegradability of the latter. Plant growth during the second cropping period was associated with a decrease of total P in all treatments, but only the soils with Hyd and Py evidenced an increase of Olsen P. Thus, during the second cropping, more insoluble P was mobilized from the carbonized residues than P needed for plant growth. Compared to control soils prepared with and without  $\text{KNO}_3$ , higher biomass production was yielded with the amended soils. Hyd proved to have the highest long-term N mobilization potential. Following the change of  $\delta^{13}\text{C}$  in the soil, we observed that during the second incubation, independently of their aromaticity, all amendments and the native SOM had comparable turnover rates, although the amount of slow-turning organic matter added with the amendment, increased with aromaticity. A rough estimation of the impact of thermal treatment of SS on its C-sequestration potential revealed no major differences for both char types. The higher fertilization capacity of Hyd, however, turns it into a good candidate for soil amendment if long-term fertilization should be combined with a long-term increase of the soil organic C pool.

**Keywords:** biochar, carbon sequestration, long-term fertilization, pot experiments, soil amendments,  $^{13}\text{C}$  and  $^{15}\text{N}$ -labeling

## Highlights

- Medium-term fertilization and C-sequestration potential of chars from sewage sludge were tested
- Hydrochar (Hyd) and pyrochar (Py) provide N and P for plant growth during a second cropping period
- Compared to soils with and without KNO<sub>3</sub>, Hyd and Py increase plant productivity of the second crop
- On a long term scale, native SOM, amended SS, its aged Hyd and Py show comparable turnover rates

## 1. Introduction

In the European Union (EU), the major source of nitrogen (N) and phosphorous (P) input into agricultural soils are mineral fertilizers. Although the use of synthetic fertilizers has dramatically increased food production worldwide, the unintended costs to the environment and human health due to surplus and inefficient applications have also been substantial. Nitrogen and P runoff from farms has contaminated surface and groundwater. Ammonia from fertilized cropland has become a major source of air pollution, whereas emissions of nitrous oxide contribute to the greenhouse effect. These and other negative environmental impacts still lead policymakers to call for further reductions of the use of synthetic fertilizers. In addition, it is estimated that the remaining phosphate rock resources may be exhausted within the next 50 to 100 years (Cordell *et al.*, 2009). Consequently, the search for alternative N and P sources has increased during the recent years. Sewage sludge (SS) may be one of those

alternatives since it is rich in organic N and P, which could be recycled in agriculture (Paneque *et al.*, 2019). According to Eurostat (2019),  $9.36 \times 10^6$  t of SS were produced in the EU during 2015, a considerable part of which is still sent to landfill. However, before applying it to the field, the environmental sustainability of this approach has to be ensured. This implies that heavy metal contents are below the allowed legal threshold. If this prerequisite is fulfilled, pathogenic organisms, as well as potential organic contaminants present in the wastewater still have to be eliminated. Such a hygienization of SS can be achieved for example by a thermal treatment such as hydrothermal carbonization (HTC) or dry pyrolysis.

HTC is performed under pressure at temperatures up to 200-320 °C in the presence of water and produces hydrochars (Hyd) (Libra *et al.*, 2011). Dry pyrolysis occurs in the absence of water and at higher temperatures of 300-700° C. In the following, the products of the latter derived from SS are termed pyrochars (Py). This is based on the high mineral content of this feedstock leading to pyrolysed residues with organic C (Corg) contents below the threshold established by the European Biochar Foundation (Corg > 50%) to be certificated as biochars (EBC, 2019).

During the thermal treatment of SS, its organic N is transformed into heterocyclic N compounds (Paneque *et al.*, 2017). Former studies demonstrated that, during the microbial degradation of charcoal produced from grass at 350°C in the presence of oxygen, at least some of the N of such heterocyclic N structures were mobilized and transformed into plant-available forms (De la Rosa & Knicker, 2011). However, other experiments indicated that this N is less available for plant growth than inorganic N or peptides (Lopez-Martin *et al.*, 2017; Paneque *et al.*, 2019). Based on such observations, it was suggested that N-rich charcoal can act as a slow-release fertilizer (De la Rosa & Knicker, 2011). To test this hypothesis, a pot experiment was performed in which *Lolium perenne* was grown on soils amended with Hyd and Py for 80 days (Paneque *et al.*, 2019). In these experiments, greater plant biomass was

produced on soils amended with Hyd, than with Py or the unamended soil. This was attributed to the presence of easily available inorganic N ( $N_i$ ) (2% of the total N) and amide N in Hyd, whereas no  $N_i$  was detected in Py. It was further demonstrated that although SS derived chars show large total P contents, the plant growth depended mainly on the amount of N.

Studies about the ability of chars to release P into soils or the processes involved in its transformation into available forms, are scarce. Qian and Jiang (2014) reported that during carbonization, P is concentrated and immobilized in the chars. Uchimiya *et al.* (2015) suggested that Py of SS could stabilize pyrophosphate with bridging cations ( $Al^{3+}$ ,  $Fe^{3+}$ , and  $Mg^{2+}$ ) to form stable six-membered ring complexes, or through direct hydrogen bonding. Jin *et al.* (2019) found that after the amendment of soils, chars can slowly release P into the soils. Qian & Jiang (2014) observed that the release of P contained in the chars is promoted at low pH, high environmental temperature and in the presence of coexisting anions. It seems that once applied to soil, the release of P from char is complex and both, biotic and abiotic factors must be taken into account. However, comparable to the fate of N derived from Hyd and Py in soils or the impact of SS derived Hyd or Py on the C sequestration potential of soils, our knowledge about their P-cycling derives mainly from short term studies, focused on the first growing season. Little is understood about the longer term effects of sewage-sludge derived char on the soil C, P and N cycles, and, specifically, the potential to increase SOC and to provide sustained sources of N and P to crops in the second crop season after application.

To fill this knowledge gap, we designed a greenhouse pot experiment in which we followed the turnover of C, N and P derived from aged Hyd and Py of SS in a soil during a second cropping period with *Lolium perenne*. The used soils with the aged chars had been prepared previously by planting *Lolium perenne* for ten months after the initial amendment using  $^{15}N/^{13}C$ -labelled Hyd and Py from SS. With this we wanted to test the hypothesis that 1) SS-derived Hyd and Py provide P and N to plants beyond the first crop cycle and 2) aging of Hyd and Py in soils increases the C-sequestration potential of amended soils.

## 2. Materials and methods

### 2.1. Production of $^{15}\text{N}/^{13}\text{C}$ -enriched sewage sludge, hydrochar and pyrochar

Isotopically enriched SS was produced in a sequencing batch reactor (SBR) of 10 L with wastewater subjected to an enhanced biological phosphorus removal (EBPR) system of the wastewater treatment plant CENTA located close to the small village of Carrión de los Céspedes, approximately 20 km from the city of Seville, Southern Spain. Every 8 hours, part of the residual water in the reactor was renewed to create a flow-through system. Using this approach, it was considered that during waste water treatment, microbial activity in the residual waters is associated with a constant turnover of labile organic fractions into more biochemically stable forms that subsequently form the SS. Thus, for the  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment of the SS we supplied the microbial community with a daily addition of 100 mg of glucose ( $^{13}\text{C}$ , 99% abundance), 15 mg of ammonium chloride ( $^{15}\text{N}$ , 99% of abundance) and 20 mg of potassium nitrate ( $^{15}\text{N}$ , 99% abundance) to the reactor. The generated isotopically enriched SS was periodically removed, dried in an oven (40°C) and stored in sealed bags at 4°C. Analysis of its chemical composition by solid-state nuclear magnetic resonance (NMR) spectroscopy indicated an alkyl C/carboxyl C ratio of 2.5, which is smaller than the respective values determined for the original primary (4.7) and secondary SS (3.5) derived from the same waste water treatment plant (Paneque *et al.*, 2017). This indicates a higher contribution of short-chain alkyl C. Together with the narrow  $\text{C}_{\text{org}}/\text{N}$  of 7 (Table 1), it can be concluded that the isotopically enriched SS contains a higher amount of peptide-like compounds than the primary and secondary SS with  $\text{C}_{\text{org}}/\text{N}$  values of 12 and 8, respectively (Paneque *et al.*, 2017). Most tentatively this is due to a higher contribution of residues from the microbial biomass, which are considered typical ingredients of SS. An aliquot of the dried product was mixed with water (1:20) and subsequently carbonized in a 1 L pressure reactor (Parr reactor 4520

series, IL, USA) at 200 °C for 30 minutes under autogenous pressure (Reza *et al.*, 2014) to yield the  $^{15}\text{N}/^{13}\text{C}$ -enriched Hyd. Respectively, the  $^{15}\text{N}/^{13}\text{C}$ -enriched Py was obtained by dry pyrolysis of the isotopically enriched SS in a closed steel container which was heated in a muffle at 600 °C for 1 h (Paneque *et al.*, 2017). The respective total C and N contents and the obtained  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichments are given in Table 1.

### 2.2. Preparation of the soils with aged and $^{13}\text{C}/^{15}\text{N}$ -enriched amendments

Soils containing aged  $^{15}\text{N}/^{13}\text{C}$ -enriched SS and its Hyd and Py, as well as soil fertilized with  $\text{K}^{15}\text{NO}_3$  and unamended soil were prepared using topsoil treated with each amendment and used to grow *Lolium perenne* for 10 months in a greenhouse to simulate the first growing season of common forage grass. The soil derived from the Ah horizon of a Dystric Cambisol (IUSS Working Group WRB 2014) (N 37° 32' W 6° 15') sampled in the Sierra of Aznalcóllar (Seville). A brief description of the soil is given in (López-Martín *et al.*, 2016). The C and N contents are listed in Table 1. The pH of the soil was 5.7 and addition of the chars did not affect the pH of the mixtures. At the end of the soil preparation period, the soil of the top 4 cm of the three replicates was mixed, homogenized, dried and stored for approximately one year in the dark.

Full details of the aging process are given in the Supplementary Material. In the following soils containing aged  $^{15}\text{N}/^{13}\text{C}$ -enriched SS, Hyd and Py are named S-SS, S-Hyd and S-Py, respectively, whereas the soil fertilized with  $\text{K}^{15}\text{NO}_3$  and the unamended soils are referred to as S- $\text{KNO}_3$  and S-control.

### 2.3. Greenhouse experiment

Three replicates of 35 g of each soil amended with the different treatments were put into 40 mL plant pots (height of 7 cm) at a soil moisture of 60% of the maximal water holding capacity (WHC) into which 10 seeds of *Lolium perenne* (ILURO seeds company, Barcelona,

Spain) were sown. After keeping the pots for 24 hours at 4 °C to induce germination, they were placed into a greenhouse (25°C, 14 h light per day) for 3-months. Their position in the greenhouse was changed regularly to ensure comparable growth and light conditions for the plants in all pots. The irrigation was controlled to maintain soil moisture at 60% of WHC during the entire experiment and to avoid loss of soluble organic matter and nutrients by leaching. No nutrients were added. Germination and survival of plants were measured by counting the number of living plants every 7 to 10 days. Leaf length was determined after 12 and 83 days of incubation, considering only the five largest leaves.

After three months, leaves, soil and roots were separated manually and dried in an oven at 60 °C for 72 hours before further analysis.

#### *2.4. Plant and soil analysis*

The total P (TP) and Olsen P contents were determined in each replicate of the prepared soils with the aged amendments to obtain the amount of P which is potentially available for plant growth during our experiment. Since there was no labelling of the P in the amendments, distinguishing between soil-born P and amendment derived P was not possible. The P concentrations in the soil were measured again at the end of the greenhouse pot experiment (Analytical Service of the IRNAS-CSIC, Seville). In brief, TP was determined after digestion with aqua regia using an inductively coupled plasma-optical emission spectrometer (ICP-OES) 150 spectrophotometer (Varian ICP720-ES, Varian Inc. Palo Alto, CA, USA). In this extract, other elements such as calcium (Ca), aluminium (Al), iron (Fe) and magnesium (Mg) were also determined. The Olsen P was quantified after extraction with sodium carbonate at pH 8.5 (Olsen *et al.*, 1954).

$\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations in the soils were measured in 2.5 g of soil after extraction with 25 mL 1 M KCl solution and measured colorimetrically as described in Benton-Jones (2000) by the Analytical Service of the IRNAS-CSIC, Seville.



### 2.5. Isotope ratio mass spectrometry (IRMS)

Approximately, 1.5 g soil from each pot was ground to a fine powder using a mixer ball mill (Model MM400, Retsch GmbH, Haan, Germany). Plant leaves were cut into small pieces and also milled. Due to the high  $^{15}\text{N}$ -enrichment in the leaves of the plants grown in the presence of  $\text{K}^{15}\text{NO}_3$ , they were diluted with non-enriched leaf biomass (1:12 w:w) prior to their analysis.

The  $^{13}\text{C}$  and  $^{15}\text{N}$  contents of the soils and the harvested plant leaves were measured with a Delta-V advantage isotope ratio mass spectrometer (IRMS) coupled to a Flash 2000 HT combustion elemental microanalyser via a ConFlo IV interface (Thermo Scientific, Bremen, Germany) in duplicates. The analytical error of the elemental microanalyser, reported by the manufacturer, is  $\pm 0.2\%$ . The stable isotope results are expressed in the conventional delta notation ( $\delta$ ), as parts per thousand deviations (‰) from corresponding international standards recognized by the International Atomic Energy Agency (IAEA) [Eq. (1)].

$$\delta (\text{‰}) = \left[ \frac{R_{\text{standard}}}{R_{\text{sample}}} - 1 \right] \times 1000 \quad (1)$$

In [Eq. (1)], R means the ratio of heavy-to-light isotopes.  $R_{\text{sample}}$  is this ratio in the sample and  $R_{\text{standard}}$  is that of the standard. The  $\delta$  of  $^{15}\text{N}$  and  $^{13}\text{C}$  were used to calculate the proportion of the C ( $C_{\text{tsoil}}$ ) and N ( $N_{\text{tsoil}}$ ) of the soil which derived from the C and N of the aged amendments ( $C_{\text{amend}}$  of  $C_{\text{tsoil}}$  (%) and  $N_{\text{amend}}$  of  $N_{\text{tsoil}}$  (%)) by applying a two components isotopic mixing model (Dignac *et al.*, 2005) [Eq. (2)].

$$C_{\text{amend}} \text{ of } C_{\text{tsoil}} (\%) = 100 \times \left[ \frac{\delta^{13}\text{C}_{(\text{amend})} - \delta^{13}\text{C}_{(\text{soil})}}{\delta^{13}\text{C}_{(\text{amend} + \text{soil})} - \delta^{13}\text{C}_{(\text{soil})}} \right] \quad (2)$$

In [Eq. (2)],  $\delta^{13}\text{C}_{(\text{amend})}$  is the  $\delta^{13}\text{C}$  value of the pure isotopically enriched amendments, and  $\delta^{13}\text{C}_{(\text{soil})}$  is the  $\delta^{13}\text{C}$  value of the not-amended soil. In addition,  $\delta^{13}\text{C}_{(\text{amend} + \text{soil})}$  is the  $\delta^{13}\text{C}$  value of the S-KNO<sub>3</sub>, S-SS, S-Hyd and S-Py treatments. The same equation was used for  $N_{\text{amend}}$  in %Nt with the respective  $\delta^{15}\text{N}$ .

The recovery of  $N_{\text{tadd}}$  in the plants was determined with equation 3 [Eq. (3)]. Here,  $\delta^{15}\text{N}_{(\text{plant})}$  is  $\delta^{15}\text{N}$  of the leaves grown on S-KNO<sub>3</sub>, S-SS, S- Hyd and S-Py,  $\delta^{15}\text{N}_{(\text{control})}$  is the  $\delta^{15}\text{N}$  value of the leaves grown on soil without amendment (S-control) and  $\delta^{15}\text{N}_{(\text{amend})}$  is the  $\delta^{15}\text{N}$  value of the respective amendment [Eq. (3)]:

$$N_{\text{add-plant}} \text{ of } N_{\text{tadd}} (\%) = \left[ \frac{\delta^{15}\text{N}_{(\text{plant})} - \delta^{15}\text{N}_{(\text{control})}}{\delta^{15}\text{N}_{(\text{amend})} - \delta^{15}\text{N}_{(\text{control})}} \right] \times 100 \quad (3)$$

Finally, in order to evaluate differences of the  $^{15}\text{N}_{\text{add}}$ -uptake efficiency between plants grown during the last three months of the soil preparation phase and grass produced during the second growing period, the  $\delta^{15}\text{N}$ -enrichment ( $\delta^{15}\text{N}_{\text{norm}}$ ) per mg  $^{15}\text{N}_{\text{add}}$  was calculated according to [Eq. (4)] using the values given in Table 2.

$$\delta^{15}\text{N}_{\text{norm}} (\text{‰ mg}^{-1}) = \frac{\delta^{15}\text{N}_{(\text{plant})} - \delta^{15}\text{N}_{(\text{control})}}{\text{mg } ^{15}\text{N}_{\text{added}}} \quad (4)$$

This approach was used to avoid the accumulation of standard deviations involved in the determination of  $^{15}\text{N}_{\text{add-plant}}$  which limits the detection of small variations.

## 2.6. Statistical analysis

The software SPSS version 17.0 (SPSS, Chicago, IL, USA) was used to analyse data corresponding to the PT and Olsen P, the percentage of Ct and Nt derived from the amendments in the soil, the length of the leaves, the biomass production and the  $\delta^{15}\text{N}_{\text{norm}}$  of leaves from each treatment.

Firstly, the Shapiro-Wilk and the Levene's tests were applied to study the normality and homoscedasticity of the data respectively. Normal distributed variables were analysed by one-way ANOVA (Tukey or Dunnet's test) to test significant differences along the time for each treatment. For comparing only two groups of data, the T-student's test instead of ANOVA was applied. A Kruskal-Wallis' test was used. Differences were considered as significant at  $p \leq 0.05$ .

## 3. Results

### 3.1. Soil

#### 3.1.1. Total and extractable P

At the beginning of the incubation (T1), the used soil mixture with the aged amendments contained significantly higher TP concentrations ( $1447 \text{ g kg}^{-1}$  soil for S-Py,  $1226 \text{ g kg}^{-1}$  for S-Hyd and  $880 \text{ g kg}^{-1}$  for S-SS) than the S-control (Fig. 1A). After three months (T2), those levels were still higher in the amended soils than in S-control, but decreased to  $1220 \text{ g kg}^{-1}$  for

S-Py, to  $1060 \text{ g kg}^{-1}$  for S-Hyd and to  $757 \text{ g kg}^{-1}$  for S-SS. However, only for S-SS, this decrease was significant.

Before starting the pot experiment, higher amounts of Olsen P were extracted from the amended soils than from the control soil (Fig. 1B). The determined amounts were significantly different for the different treatments. The highest content of Olsen P was found for S- Py ( $59 \text{ mg kg}^{-1}$ ), followed by S-SS ( $47 \text{ mg kg}^{-1}$ ) and S-Hyd ( $24 \text{ mg kg}^{-1}$ ).

In spite of plant growth, no major alterations of the concentration of Olsen P were detected for S-control and S-Py, during plant growth. In contrast, the char treated soil S-Hyd showed a significant increase. Bearing in mind that after the three months of incubation, TP decreased in the trail with the amended soils, one can conclude that more insoluble P of the aged carbonized residues was mobilized than the plants needed for their growth.

### 3.1.2. $\text{NO}_3^-$ and $\text{NH}_4^+$

The  $\text{NH}_4^+$  levels, of both the unamended and amended soils, were between  $6$  and  $8 \text{ mg kg}^{-1}$  at the beginning of the incubation, (in the following referred to as T1), but decreased until the end of the incubation (referred to as T2) to values  $< 2 \text{ mg kg}^{-1}$  (Table 3). In the soils containing aged carbonized SS, the  $\text{NH}_4^+$  concentrations were even below the detection limit. At T1, the  $\text{NO}_3^-$  concentrations in the soil were low and ranged between  $3.3$ - $0.6 \text{ mg kg}^{-1}$ , even for the soil prepared by addition of  $\text{KNO}_3$ . The latter indicates that during the 10 months of soil preparation, most of the  $\text{KNO}_3$ -derived N applied to the soil was already used up for plant growth, incorporated into microbial residues or soil organic matter or lost by volatilization. Comparably to  $\text{NH}_4^+$ , those concentrations decreased further during plant growth, confirming that accumulation of mobilized N did not occur.

### 3.1.3. C and N traceability of the amendments during the greenhouse experiment

At T1, only 26% of  $C_t$  added with the amendment ( $C_{t_{add}}$ ) was recovered as amendment-derived C ( $C_{amend}$ ) in S-SS, confirming the expected low biochemical stability of untreated SS. Thermal treatment increased the recovery of  $C_{t_{add}}$  in S-Hyd and S-Py to 43% and 93%, respectively, at T1 (Table 4). Note, although Py was produced at high temperatures, 7% of its carbon were still mineralized during the soil preparation period. Until the end of the experiment, those numbers changed only slightly to 22% and 84% for S-SS and S-Py, respectively. Calculating the relative contribution of  $C_{amend}$  to  $C_{t_{soil}}$  at T1 revealed that S-SS, S-Hyd and S-Py, 8.8%, 12.6% and 20.7% of  $C_{t_{soil}}$  in S-Py originated from the aged amendment (Fig. 2A). Until the end, those numbers did not change significantly, suggesting that the organic C of the soil and the aged amendments degraded at comparable rates. Thus, relative to the organic carbon in the original soil, no preferential preservation or degradation occurred for the charred material.

A different picture is revealed with respect to the N derived from the amendments ( $N_{amend}$ ) in the soil (Fig. 2B). Although only significant for S-Hyd and S-KNO<sub>3</sub>, the relative contribution of  $N_{amend}$  tended to decrease during the pot experiment in all treatments. The low recovery of 3% of  $N_{t_{add}}$  of KNO<sub>3</sub> at the start of the incubation is related to a high loss, most tentatively due to denitrification occurring already during the soil preparation phase (Table 4). For the soils amended with SS and Hyd, the  $N_{t_{add}}$  losses during the preparation phase and the second growing phase are in the same range as observed for the reduction of  $C_{t_{add}}$ . However, Py lost considerably more  $N_{t_{add}}$  than  $C_{t_{add}}$  during both phases, suggesting a preferential degradation of its N-containing fraction.

### 3.2. Plant growth and biomass production

During the studied cropping, thus through the second growing period, after 11 days, the number of living plants showed no major differences between the treatments (Table 5), although the number of living plants slightly decreased in S-Hyd after 50 days of incubation. In addition, the plants exhibited no significant difference with respect to root biomass production (Table 6) or leave lengths (Fig. 3A) after 14 and 83 days. However, compared to the pot with S-control, significantly higher leave biomass production was achieved with S-SS, S-Hyd and S-Py (Fig. 3B). The treatment with pure  $\text{KNO}_3$  had no impact on this parameter, which is best explained by the low  $\text{N}_i$  content in the substrate at the end of the experiment (Tab. 3).

Aboveground fresh biomass production with S-SS, S-Hyd and S-Py was between 0.49 and 0.54 g per pot, whereas that of S- $\text{KNO}_3$  and control was 0.37 g per pot. Comparably, the dried biomass production showed the same tendency. Note that N contents and the carbon-to-nitrogen ratios (C/N (w:w)) of the plant residues of the different treatments are comparable to that of the control pots ( $\text{N}_{t_{\text{plant}}} = 7.3 \text{ g kg}^{-1}$ , C/N = 52). Since those values are in the range of those commonly found for hay (Table 7), it can be assumed that we didn't have high surplus or deficiency of N in the soils during our experiment.

### 3.3. Use of $\text{N}_{t_{\text{add}}}$ by *Lolium perenne*

The recoveries of  $\text{N}_{t_{\text{add}}}$  in the plant residues ( $\text{N}_{t_{\text{add-plant}}}$ ) at T2 demonstrate that during the second growth period, at least some of the  $\text{N}_{t_{\text{add}}}$  was still plant-available (Table 7). They correspond to 0.2 to 0.5 % of  $\text{N}_{t_{\text{add}}}$  in all plants except those grown on S-Py (0.04%). The contribution of  $\text{N}_{t_{\text{add}}}$  to the total N of the plants ( $\text{N}_{t_{\text{plant}}}$ ) is highest for those produced on S-SS

(13%) followed by those grown on S-Hyd (12%). Lower values of 7% and 2% were obtained for the plants from soils with  $\text{KNO}_3$  and aged Py, respectively.

The  $\delta^{15}\text{N}$ -enrichment is highest for plants growing during the second cropping period on the soil previously fertilized with  $\text{KNO}_3$  (Fig. 4), followed by those grown on S-SS and S-Hyd (Fig. 4). A considerably lower  $\delta^{15}\text{N}$ -enrichment was achieved in the soil containing aged Py ( $\delta^{15}\text{N}_{\text{norm}} = 43 \text{ ‰ mg}^{-1} \text{ }^{15}\text{N}_{\text{add}}$ ). Note that for this treatment,  $\delta^{15}\text{N}_{\text{norm}}$  is comparable to that determined for the plant residues grown during the last three months of the soil preparation phase indicating a low but more or less constant release of  $^{15}\text{N}$  from Py over a longer period of time. Compared to the plant residues produced during the last three months of soil preparation phase, the  $\delta^{15}\text{N}$ -enrichment in the plants from S-Hyd increased but did not change for those from S-SS. These inconsistent behaviours are most likely associated with difference in the quality and the biochemical recalcitrance of the N-compounds of the respective amendment.

## 4. Discussion

### 4.1. The impact of the amendment on soil P

Our experiment confirmed that addition of SS and its carbonized residues can enhance TP contents in soils not only directly after the amendment (Christel *et al.*, 2014), but also during a second cropping period. However, prolonged plant growth lowers the TP levels (Fig. 1). By keeping the soil humidity at 60% of WHC we prevented leaching during the irrigation of the pots. Therefore, we conclude that P was successfully recycled within the plant-soil system for the built-up of new plant biomass.

Previous works have found that pyrolysis of P-containing feedstock leads not only to a relative enrichment of P-forms with increasing temperature (Yuan *et al.*, 2016) but also to a

decrease of its extractability (Christel *et al.*, 2014; Dai *et al.*, 2015; Huang & Tang, 2015). Huang & Tang (2015) suggested that in SS, P-forms are dominated by organic P, orthophosphate and long-chain polyphosphates. Whereas pyrolysis increases the amount of pyrophosphate and short chain polyphosphates, hydrothermal treatment results in the production of only inorganic orthophosphate (Huang & Tang, 2015). It was suggested that the latter reacts with Al, Mn, Fe or Ca to form insoluble P-forms which are trapped within the organic network of hydrochars (Heilmann *et al.*, 2014). A comparable mechanism was suggested for the stabilization of P in pyrochars (Frišták *et al.*, 2018). Considering that both in the soil at the beginning and the end of the incubation, we found higher Ca and Al concentrations in the S-Hyd and S-Py than in the control soil (Table 8), some of the TP that could not be extracted as Olsen P may still have been stabilized by this mechanism.

Having a closer look at the concentrations of Olsen P before and after the second growth period, it becomes evident that in contrast to the control soils and S-SS, in the char amended soils more non-extractable P was mobilized than was needed for plant growth. Thus, although SS amendment demonstrated a high potential as a P-fertilizer, it is likely that without additional fertilization, plant-available P concentrations will constantly decrease with each cropping period. It seems that charring sequesters some of the P in its residues in forms that can be slowly but constantly mobilized during plant growth. Bearing in mind that the majority of P used in agriculture derives from mined phosphate rock, which is a non-renewable resource, recycling P from SS with low heavy metal contents via its transformation into Hyd or Py certainly represents a sustainable alternative approach with a positive long-term impact to common fertilization practices.

#### *4.1.1. Fate of the N derived from the amendments*

In spite of high addition of  $N_i$  with  $KNO_3$  and the amendment of SS and Hyd, almost comparable  $N_i$  contents were observed after the soil preparation phase (Table 3). Bearing in



mind that keeping the WHC at 60% prevents leaching of water, we must assume that aside from being incorporated into microbial and plant biomass, a considerable part of the  $N_i$  added with those amendments were lost most likely by volatilization. Note that this N loss is in line with the decreasing  $N_{t_{soil}}$  content of S-KNO<sub>3</sub> during the second growing period and the fact that here only 3 and 2% of  $N_{t_{add}}$  was recovered at the beginning and the end of the pot experiment. Loss of  $N_{t_{add}}$  was also evidenced for the soils with aged chars, but it was certainly much lower, which allows the conclusion that part of the N-structures in the char were microbially attacked and thus degraded. Comparable conclusion were obtained in earlier studies with pyrogenic organic matter produced in the presence of oxygen (De la Rosa & Knicker, 2011; Lopez-Martin *et al.*, 2017). Having a look at the proportion of  $N_{amend}$  to  $N_{t_{soil}}$  during our experiment (Fig. 2B), the degradation of this  $N_{amend}$  occurred either at the same rate as or slightly faster than the native soil N.

#### 4.2. Potential of hydrochars and pyrochars from sewage sludge to increase the soil C sink

Our study revealed that, one year after soil amendment, irrespective of the amendment, the relative contribution of  $C_{amend}$  to  $C_{t_{soil}}$ , did not change with incubation time (Fig. 2A). This confirms that, at this stage of the humification process, native SOM and amendment had comparable turnover times. A different situation is evidenced for the soil preparation period. As expected, a considerably faster turnover of  $C_{add}$  from SS than of native soil C caused a decrease of  $C_{amend}$  from 11% to 9% lowering the recovery of  $C_{t_{add}}$  to 26% (Table 4, Fig. 2A). Our results suggest that after the decomposition of the labile fraction of SS, its more stable organic material (OM) exhibits a turnover rate comparable to that of mature native SOM.

For S-Hyd, the relative contribution of  $C_{amend}$  to  $C_{t_{soil}}$  remained relatively constant, both throughout the soil preparation phase and the second cropping period, confirming that the

HTC treatment increased the biochemical recalcitrance of the feedstock to turnover rates comparable to the native SOM. At the end of the experiment, half of  $C_{t_{add}}$  of Hyd was recovered which corresponds to twice the percentage of the  $C_{t_{add}}$ -recovery with SS (Table 4). This confirms that in spite of the mild production conditions, Hyd can increase the pool size of the slow turning SOM pool.

Pyrochars are commonly considered to exhibit a high biochemical recalcitrance (Malghani *et al.*, 2013). Compared with the other amendments of our study, this view is supported by the obtained results. Only 7 % of its  $C_{t_{add}}$  was lost during its aging in the soil which went along with an enhancement of its relative contribution to  $C_{t_{soil}}$  until the end of the soil preparation phase. However, this increase did not continue during the second growing period, pointing towards a change of the microbial accessibility of its carbon. It seems that microbial reworking and aging decreased the biochemical recalcitrance of the highly aromatic material. This decrease is related to the loss of the potential of being selectively preserved with respect to humified SOM.

One objective of using charred organic residues as soil amendments is increasing the amount of C sequestered in soils in order to reduce the emission of greenhouse gases (Jung *et al.*, 2019). Using the recoveries of  $C_{t_{add}}$  at the end of the soil preparation phase as a rough estimate for the potential of an amendment to increase the soil carbon pool with slow turnover, SS can contribute with 20 to 30% of its Ct to this fraction. HTC treatment doubles this number, whereas pyrolysis at 600°C increases it to 80 to 90%. However, carbon is also released during the thermal treatment. A former study evidenced that the application of HTC on SS as it was applied in the present investigation leads to a C-loss of approximately 15-20%, whereas between 50 and 60 % of the carbon in the feedstock were reported to be released during dry pyrolysis at 600°C (Paneque *et al.*, 2017). Taking those numbers, it can be estimated that for either Hyd or Py only 30 and 45% of the carbon of the original feedstock remained in the soil as aged amendment. Considering the higher energy need for the

production of Py, the application of Hyd as soil additive may be a more efficient tool for C-sequestration than Py amendment, if neither energy nor by-products emitted during pyrolysis can be reused.

#### 4.3. Impact of the amendment on plant development

In general, the plants showed similar germination and plant survival rates in all the treatments (Table 4/5). Thus our amendments neither promoted nor decreased to a larger extent the health of the plants compared to the control. Only at the end of the incubation, the S-Hyd treatment slightly decreased the number of living plants. In fact, Hyd may decrease plant germination due to the presence of phytotoxic volatile compounds (Bargmann *et al.*, 2013). However, these deleterious compounds are usually easily degraded which may question their real impact. Thus, more research is needed to unveil the reason of this observation.

Among the different experiments, chars and SS amended pots produced more leave biomass than the control and S-KNO<sub>3</sub> pots (Fig. 3B). This suggest that the amendments preserved their fertilisation potential even after aging. Comparable results have been previously reported elsewhere (Rajkovich *et al.*, 2012; Bargmann *et al.*, 2014; Dong *et al.*, 2017; Tan *et al.*, 2018). This is not necessarily due to fertilization with N and P, since deficiency of those nutrients is not evident in any of the trials. At least, comparable C/N ratios were obtained for the plants of all treatments, even for those growing on S-Py. Here, only 2% of their N derived from the amendment (Table 7). The higher biomass production could also have been caused by the addition of other micro-nutrients with the organic amendments. Likewise, improvement of other soil parameters such as soil density, water holding capacity or soil microbiota due to the presence of the amendments may also have contributed to the increase of biomass production.

Finally, the root biomass was comparable among the treatments (Table 5), supporting the lack of nutrient deficiency, which had forced the plants to increase their root development. Alternatively, the small size of the pots may have prevented normal root growth.

#### 4.4. Potential of the amendments to act as slow-release N fertilizer

At the beginning of the second cropping period, the contribution of  $N_{t_{add}}$  to  $N_{t_{soil}}$  of S-KNO<sub>3</sub>, was already very low with respect to S-SS or the soils with aged chars (Fig. 2B). This is relevant because it underlines the short availability of mineral fertilizers in soils and explains the low  $N_{t_{add-plant}}$  to  $N_{t_{plant}}$  in the plants harvested from S-KNO<sub>3</sub> at the end of the experiment (Table 7). Assuming that N availability was not a limiting plant-growth factor, it can be concluded that the amount of  $N_{t_{add-plant}}$  was controlled by the portion of available  $N_{t_{add}}$  in the soil rather than by N deficiency or the lack of alternative N sources. As a consequence, the amount of  $N_{t_{add-plant}}$  must be related to the rate of N mobilization during amendment turnover.

This consideration explains the low incorporation of  $N_{t_{add}}$  derived from the slowly degrading Py into the plants harvested at the end of the experiment. Higher amounts N-uptake rates were observed for  $N_{t_{add}}$  from Hyd. Paneque *et al.* (2019), Tan *et al.* (2018) and Liu *et al.* (2018) also found better assimilation of N by plants when the biochar was produced at lower, thus milder temperature conditions. Thuille *et al.* (2015) observed that in the presence of Hyd, plants were able to use N from Hyd instead of N from synthetic fertilizers.

On the other hand, the comparable contribution of  $N_{t_{add-plant}}$  to  $N_{t_{plant}}$  in the plants grown on S-Hyd and S-SS can be misleading. Here one has to bear in mind that although the same amount of amendment was used, twice the amount of N added with Hyd was applied with SS. Consequently, relative to SS, Hyd exceeds the potential to provide N for plant growth by a factor 2. This is interesting to mention, since it is commonly assumed that peptide structures in organic residues are easily available for microbial reworking and N mobilization. However,

those structures are likely to have been already attacked during the soil preparation phase, leaving only more biochemically recalcitrant N-containing remains in the soil. Alternatively, incorporation of SS-derived N into microbial biomass or matured SOM during its turnover may have decreased its remobilization potential.

As a consequence, we assume that among the tested amendments, Hyd provides the best N mobilization potential during a second growing period. However, to which extent this is also valid for even longer time frames has still to be tested.

## 5. Conclusions

The present research confirmed that both Hyd and Py derived from SS can act as slow-release P fertilizer, since even during the second cropping period after their addition to the soils, part of their insoluble P, produced during their carbonization, was transformed into extractable, thus plant-available P. With respect to N, Hyd provided more plant available N than Py during the second cropping experiment. However, it may be assumed that although at a slow rate, Py may release N over a longer time range than Hyd. The high medium-term fertilization potential of Hyd combined with the observation that Py and Hyd provide a comparable proportion of the organic C of their feedstock to the slow SOM pool suggest that application of Hyd may be more efficient than Py for increasing soil resilience on a long term scale.

Considering the fact that in spite of different aromaticity, comparable C turnover was evidence for the aged amendments, it may be concluded further that at an advanced aging state, aromaticity loses its importance for their degradability. Most likely, factors and mechanisms, commonly controlling the turnover of humified native SOM determine and control their future biochemical stability.

### **Author's contribution**

The experimental part, the data analysis and writing of this manuscript was performed by the first author BLS. MP provided the soil samples and was involved in the design and performance of the experiment. JAGP assisted with the measurement and interpretation of the stable isotope data. HK developed the original idea, led the project and together with MP, JMR and MJL, supervised the experiment and contributed to the discussion and interpretation of the data. All authors were active in correcting and editing the manuscript.

### **Conflicts of interest**

There have not been any conflicts of interest to declare.

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### **Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## FIGURE CAPTIONS

**Figure 1.** Total P (TP) (A) and Olsen P (B) concentrations in the soils without (S-control) and with the aged amendment of sewage sludge (S-SS), hydrochar (S-Hyd) or pyrochar (S-Py) before (T1) and after the second growing season (T2). The error bars show the mean  $\pm$  standard error (n=3). The letters indicate significant differences between the treatments at T1 and T2 (Tukey,  $p \leq 0,05$ ). Besides, \* indicates significant differences between T1 and T2 for S-SS (A) and for S-SS and S-Hyd (B) (T-student,  $p \leq 0,05$ ).

**Figure 2.** Percentage of total soil C (A) derived from S-SS, S-Hyd and S-Py treatments and percentage of total soil N (B) derived from S-KNO<sub>3</sub>, S-SS, S-Hyd and S-Py, in the soils with aged amendment, before (T1) and after the second growing period (T2). The error bars show the mean  $\pm$  standard error (n=3). NS (A) indices no significant differences between T1 and T2 for each treatment (T-student,  $p \leq 0,05$ ). In addition, \* (B) indicates significant differences between T1 and T2 for KNO<sub>3</sub> and Hyd (T-student,  $p \leq 0,05$ ).

**Figure 3.** Length of the leaves (A) in plants grown on the soils without (S-control) and with aged amendments (S-KNO<sub>3</sub>, S-SS, S-Hyd or S-Py) at 14 (14 d) and 83 days (83 d) of the incubation during the second growing period and the respective fresh and dried biomasses productions (B). The error bars show the mean  $\pm$  standard error (n=3). NS (A) indices no significant differences were found between the treatments at 12 d (ANOVA,  $p \leq 0,05$ ) and 83 d s(Kruskal-Wallis,  $p \leq 0,05$ ) for length of the leaves.

The letters (B) indicate significant differences between treatments with fresh and dried biomass (Tukey,  $p \leq 0,05$ ).

**Figure 4.** The  $^{15}\text{N}$ -enrichement ( $\delta^{15}\text{N}_{\text{norm}}$ ) ( $\text{‰ mg}^{-1}$ ) normalized to  $\text{mg } ^{15}\text{N}_{\text{add}}$  in the leaves grown on soils with aged amendments (S-KNO<sub>3</sub>, S-SS, S-Hyd and S-Py) and harvested at end of the experiment incubation (2<sup>nd</sup>). For comparison,  $\delta^{15}\text{N}_{\text{norm}}$  was also determined for plant residues grown and harvested during the last three months of the preparation phase of the used soil (1<sup>st</sup>) The error bars show the mean  $\pm$  standard error (n=3). The letters indicate significant differences between the treatments at for the 1<sup>st</sup> and 2<sup>nd</sup> cropping (Tukey,  $p \leq 0,05$ ). Besides, \* indicate significant differences between the 1<sup>st</sup> and 2<sup>nd</sup> cropping for Hyd (T-student,  $p \leq 0,05$ ).

## TABLES

**Table 1.** Total carbon (Ct), total nitrogen (Nt), abundance of  $^{15}\text{N}$  and  $^{13}\text{C}$ , their atomic H:C and C<sub>org</sub>/Nt (w/w) ratio of the  $^{15}\text{N}$  and  $^{13}\text{C}$  enriched sewage sludge (SS), hydrochar (Hyd) and pyrochar (Py) (Paneque, 2018).

	Ct (g kg <sup>-1</sup> )	$^{13}\text{C}$ (atm%)	Nt (g kg <sup>-1</sup> )	$^{15}\text{N}$ (atm%)	$\text{NO}_3^-$ (mg kg <sup>-1</sup> )	$\text{NH}_4^+$ (mg kg <sup>-1</sup> )	N <sub>i</sub> (% of Nt)	H/C (atm)	C <sub>org</sub> /N (w/w)
SS	290.1	2.8	41.6	1.0	15.5	1510	3.7	1.7	7.0
Hyd	238.5	1.9	19.4	0.6	3.0	401	2.1	1.5	12.3
Py	186.5	2.2	23.8	0.7	4.3	<0.2	0.0	0.6	7.8
Soil	38.7	1.1	3.4	0.37	1.9	27.5	0.9	n.d.*	11.4

\*n.d. not determined

**Table 2.** Amount (mg per pot and kg ha<sup>-1</sup>) of total carbon (C<sub>tadd</sub>), N (N<sub>tadd</sub>), <sup>13</sup>C (<sup>13</sup>C<sub>add</sub>), <sup>15</sup>N (<sup>15</sup>N<sub>add</sub>), ammonium (NH<sub>4</sub><sup>+</sup><sub>add</sub>) and nitrate (NO<sub>3</sub><sup>-</sup><sub>add</sub>) added with SS, Hyd, Py and KNO<sub>3</sub> to the pot for preparing the soil with aged amendments. The conversion of mg per pot in kg ha<sup>-1</sup> is based on the inner pot diameter (6 cm) at the soil surface (28.27 cm<sup>2</sup>).

	C <sub>tadd</sub>	C <sub>tadd</sub>	<sup>13</sup> C <sub>add</sub>	N <sub>tadd</sub>	N <sub>tadd</sub>	<sup>15</sup> N <sub>tadd</sub>	NH <sub>4</sub> <sup>+</sup> <sub>add</sub>	NH <sub>4</sub> <sup>+</sup> <sub>add</sub>	NO <sub>3</sub> <sup>-</sup> <sub>add</sub>	NO <sub>3</sub> <sup>-</sup> <sub>add</sub>
	(mg)	(kg ha <sup>-1</sup> )	(mg)	(mg)	(kg ha <sup>-1</sup> )	(mg)	(mg)	(kg ha <sup>-1</sup> )	(mg)	(kg ha <sup>-1</sup> )
K <sup>15</sup> NO <sub>3</sub>	-	-	-	48.5	172	6.2	-	-	48.5	172
SS	581.8	2058	16.5	83.2	294	0.8	3.0	11	0.031	0.11
Hyd	476.9	1687	9	38.8	137	0.2	0.8	3	0.006	0.02
Py	372.9	1319	8.2	47.6	168	0.3	0.0	0	0.009	0.03

**Table 3.** Total nitrogen (Nt) Ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and inorganic N (N<sub>i</sub>) concentrations in the soils without (S-control) and with amendments (S-KNO<sub>3</sub>, S-SS, S-Hyd and S-Py) at the beginning (T1) and the end of the second cropping period (T2). The values for Nt, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations in the respective soil at the start of the soil preparation (T0) were calculated from Table 2.

	Nt <sub>soil</sub> (g kg <sup>-1</sup> )			NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )			NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )			N <sub>i</sub> (mg kg <sup>-1</sup> )		
	T0*	T1	T2	T0*	T1	T2	T0*	T1	T2	T0*	T1	T2
S-control	3.4	3.0 ± 0.2	3.3 ± 0.4	27.5	7.1 ± 0.5	2.0 ± 0.8	1.9	0.7 ± 0.3	1.5 ± 0	29.4	7.8	3.5
S-KNO <sub>3</sub>	3.9	3.3 ± 0.2	2.6 ± 0.2	27.5	6.0 ± 0.9	1.3 ± 0.5	608	3.3 ± 2.2	1.1 ± 0.2	635.5	9.3	2.4
S-SS	4.4	3.7 ± 0.1	3.4 ± 0.2	65.3	7.9 ± 0.3	0.1 ± 0.2	2.2	1.2 ± 0.3	0.7 ± 0.1	67.5	9.1	0.8
S-Hyd	3.8	3.7 ± 0.3	3.2 ± 0.1	37.5	6.7 ± 0.1	b.d <sup>#</sup>	1.9	0.6 ± 0.2	0.4 ± 0.4	39.4	7.3	0.4
S-Py	3.9	3.8 ± 0.4	3.3 ± 0.2	27.5	7.4 ± 0.4	b.d	1.9	1.6 ± 0.5	0.4 ± 0.2	29.4	9	0.4

\*calculated values

<sup>#</sup> below detection limit

Table 4: Recovery of  $C_{\text{amend}}$  and  $N_{\text{amend}}$  (%) of total added carbon and nitrogen ( $C_{\text{tadd}}$ ,  $N_{\text{tadd}}$ ) in the soils amended with  $\text{KNO}_3$  (S- $\text{KNO}_3$ ), sewage sludge (S-SS), its hydrochar (S-Hyd) and its pyrochar (S-Hyd) at the beginning (T1) and the end (T2) of the second growing period with *Lolium perenne*. Values were calculated from Figure 2 and the respective carbon ( $C_{\text{tsoil}}$ ) and N content ( $N_{\text{tsoil}}$ , Table 3) of the samples:

	$C_{\text{tsoil}}$ ( $\text{g kg}^{-1}$ )			$C_{\text{amend}}$ (% $C_{\text{tsoil}}$ )	$C_{\text{amend}}$ (% of $C_{\text{tadd}}$ )		$N_{\text{amend}}$ (% of $N_{\text{tadd}}$ )	
	T0*	T1	T2	T0*	T1	T2	T1	T2
S- $\text{KNO}_3$	39	37 ± 4	41 ± 3	-			3	2
S-SS	45	43 ± 1	43 ± 3	17	26	22	18	13
S-Hyd	44	41 ± 2	41 ± 2	14	43	43	64	47
S-Py	42	42 ± 3	40 ± 2	11	93	84	66	45

\* calculated from Table 1 and 2



**Table 5.** Number of living plants grown on the soil without (S-control) and with amendments (S-KNO<sub>3</sub>, S-SS, S-Hyd or S-Py) during the second growing season in the greenhouse.

<b>Number of living plants</b>											
<b>Days</b>	6	11	18	27	36	43	50	57	64	76	85
<b>S-Control</b>	6.0±2.6	9.0±1.2	11.0±1.2	9.0±1.2	9.0±1.2	10.0±0.6	10.0±0.6	10.0±0.6	10.0±0.6	10.0±1.0	10.0±1.5
<b>S-KNO<sub>3</sub></b>	6.0±3.2	10.0±2.5	11.0±2.7	10.0±0.6	10.0±1.5	10.0±2.0	10.0±1.5	11.0±2.7	11.0±2.5	11.0±2.0	11.0±2.1
<b>S-SS</b>	10.0±1.5	10.0±2.0	11.0±2.7	11.0±1.2	11.0±2.3	11.0±2.3	11.0±2.3	11.0±1.7	11.0±1.5	11.0±1.5	11.0±1.5
<b>S-Hyd</b>	9.0±2.6	9.0±1.8	10.0±1.7	10.0±1.7	10.0±1.0	10.0±1.0	9.3±1.5	9.0±1.0	9.0±1.0	8.0±1.5	8.0±1.5
<b>S-Py</b>	8.0±3.2	10.0±2.7	10.0±1.2	11.0±1.5	10.0±1.2	10.0±1.7	10.0±1.7	10.0±2.1	10.0±2.1	11.0±1.5	11.0±1.5

Values in the columns are means ± standard deviation (n=3).

**Table 6.** Root biomass production of *Lolium perenne* grown on soils without (S-control) and with amendments (S-KNO<sub>3</sub>, S-KNO<sub>3</sub>, S-SS, S-Hyd or S-Py) during a second cropping period after soil amendment.

<b>Root biomass production (g)</b>	
<b>S-Control</b>	1.5 ± 0.2
<b>S-KNO<sub>3</sub></b>	1.4 ± 0.1
<b>S-SS</b>	1.5 ± 0.3
<b>S-Hyd</b>	1.3 ± 0.2
<b>S-Py</b>	1.5 ± 0.2

Values in the columns are means ± standard deviation (n=3).

**Table 7:** Recovery of added N ( $N_{t_{add}}$ ) in the plants grown on the soils amended with  $KNO_3$ , sewage sludge and its hydro- and pyrochar (S- $KNO_3$ , S-SS, S-Hyd, S-Py) during a the second growing period of 3 months. The  $N_{t_{add}}$  in the plants ( $N_{t_{add-plant}}$ ) was calculated using the stable  $^{15}N/^{14}N$  ratios of the plants, their N content ( $N_{t_{plant}}$ ), dry weight and the amount of  $N_{t_{add}}$  provided at the beginning of the soil preparation.

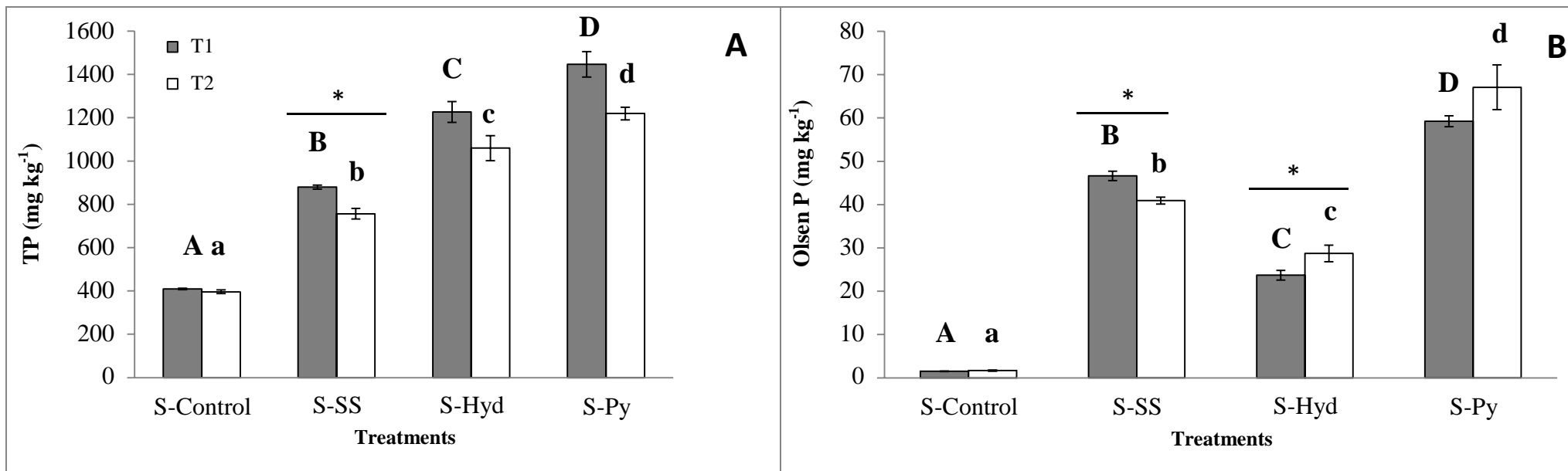
	$N_{t_{plant}}$ ( $g\ kg^{-1}$ )	C/N (w: w)	$N_{t_{add-plant}}$ $/N_{t_{plant}}$ (%)	$N_{t_{add-plant}}$ % of $N_{t_{add}}$	$N_{t_{add-plant}/plant}$ dry mass ( $mg\ g^{-1}$ )	Plant dry mass $/N_{t_{add}}$ (g/g)
S- $KNO_3$	7. $\pm$ 7 0.9	52	7.7 $\pm$ 1. 0	0.26	0.6	3.1
S-SS	7. $\pm$ 7 1.2	55	13. $\pm$ 4 0. 5	0.23	1.0	2.8
S- Hyd	7. $\pm$ 6 0.9	53	12. $\pm$ 2 0. 2	0.50	0.9	5.4
S-Py	6. $\pm$ 9 0.5	55	1.8 $\pm$ 0. 5	0.04	0.1	4.2

**Table 8.** Al, Ca, Fe and Mg concentrations in the soils without (S-control) and with amendments (S-SS, S-Hyd or S-Py) at the end of the first (T1) and second growing season (T2).

Treatments		T1				
T2		Al	Ca	Fe	Mg	
Al	Ca	Fe	Mg			
		(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )
(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )
<b>S-Control</b>		23.9 ± 2.2	2.3 ± 0.1	41.3 ± 3.1	2.8 ± 0.1	
		15.7 ± 0.2	2.2 ± 0.1	44.4 ± 1.8	3.0 ± 0.1	
<b>S-SS</b>		36.8 ± 3.7	3.7 ± 0.1	40.7 ± 2.0	3.2 ± 0.1	
		15.9 ± 0.1	3.5 ± 0.2	41.6 ± 0.7	3.1 ± 0.1	
<b>S-Hyd</b>		24.2 ± 2.8	4.7 ± 0.4	41.3 ± 0.9	3.1 ± 0.1	
		23.7 ± 6.8	4.3 ± 0.3	42.2 ± 0.8	3.3 ± 0.2	
<b>S-Py</b>		56.6 ± 7.9	5.2 ± 0.2	39.7 ± 0.5	3.5 ± 0.2	
		30.8 ± 2.5	4.7 ± 0.1	43.3 ± 2.3	3.5 ± 0.1	

Values in the columns are means ± standard deviation (n=3).

Figure 1



**Figure 2**

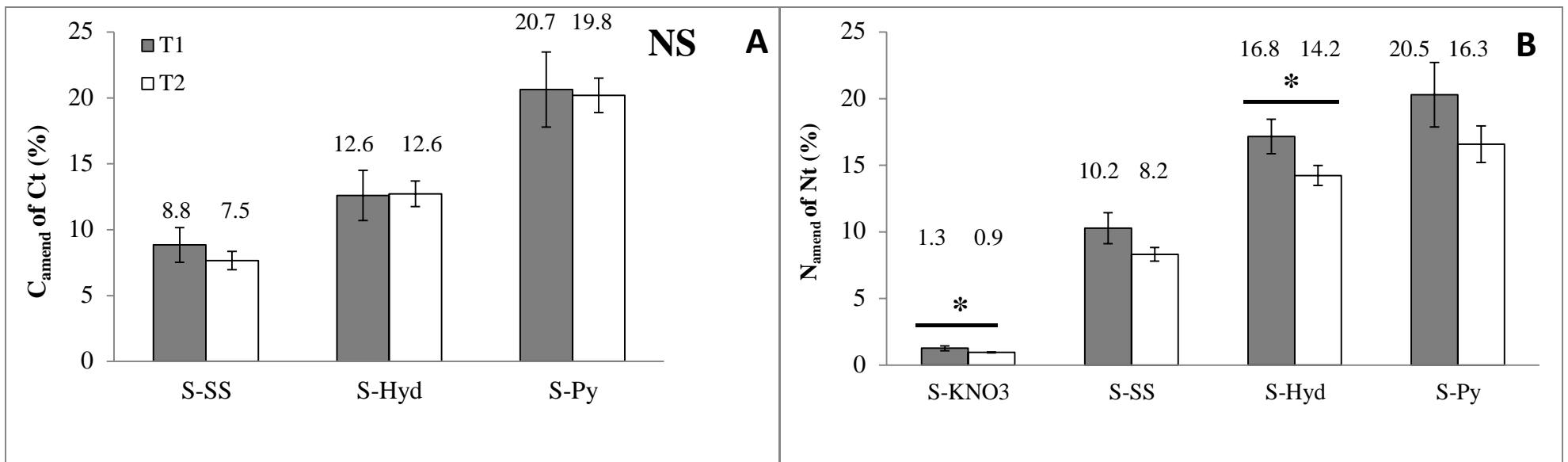
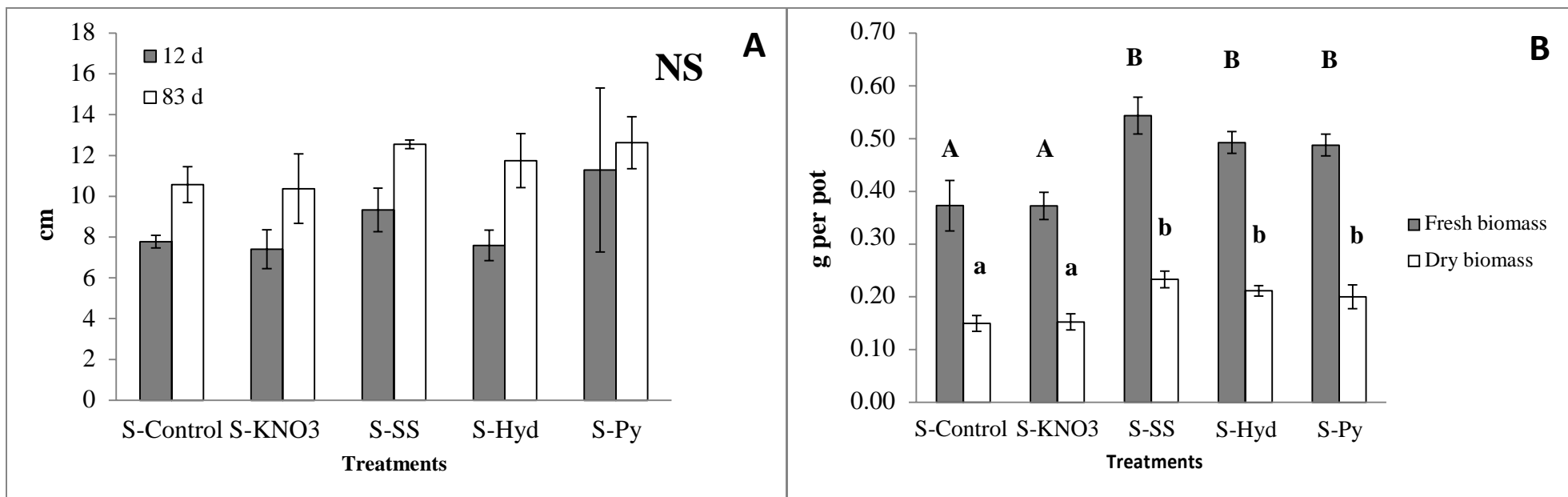


Figure 3



**Figure 4**

