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Article

## Short-Term Effect of Feedstock and Pyrolysis Temperature on Biochar Characteristics, Soil and Crop Response in Temperate Soils

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**Abstract:** At present, there is limited understanding of how biochar application to soil could be beneficial to crop growth in temperate regions and which biochar types are most suitable. Biochar's (two feedstocks: willow, pine; three pyrolysis temperatures: 450 °C, 550 °C, 650 °C) effect on nitrogen (N) availability, N use efficiency and crop yield was studied in northwestern European soils using a combined approach of process-based and agronomic experiments. Biochar labile carbon (C) fractions were determined and a phytotoxicity test, sorption experiment, N incubation experiment and two pot trials were conducted. Generally, biochar caused decreased soil NO<sub>3</sub><sup>-</sup> availability and N use efficiency, and reduced biomass yields compared to a control soil. Soil NO<sub>3</sub><sup>-</sup> concentrations were more reduced in the willow compared to the pine biochar treatments

and the reduction increased with increasing pyrolysis temperatures, which was also reflected in the biomass yields. Woody biochar types can cause short-term reductions in biomass production due to reduced N availability. This effect is biochar feedstock and pyrolysis temperature dependent. Reduced mineral N availability was not caused by labile biochar C nor electrostatic  $\text{NH}_4^+/\text{NO}_3^-$  sorption. Hence, the addition of fresh biochar might in some cases require increased fertilizer N application to avoid short-term crop growth retardation.

**Keywords:** biochar; nitrogen; crop growth; labile carbon; soil fertility; adsorption

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## 1. Introduction

Biochar application to soils has gained attention as a climate change mitigation strategy, as it could act as a long-term carbon (C) sink [1]. If in addition biochar could increase crop yields and improve soil quality, this would distinguish it from costly geo-engineering measures to mitigate climate change [2]. A meta-analysis by Jeffery *et al.* [3] revealed a small (*ca.* 10%), but statistically significant, average increase in crop productivity with biochar application in tropical and subtropical regions. Only one study from a temperate region (New Zealand) was included in their meta-analysis, showing the need for more research in temperate regions. Three years later, biochar research is emerging throughout these regions, including lab, pot and field studies, (e.g., [4–7]). This is also reflected in the meta-analysis from Biederman and Harpole [8], which included several studies from temperate regions. Their study confirmed the overall positive effect of biochar application on aboveground plant production and yield, but the authors simultaneously stressed the importance of feedstock source and pyrolysis settings on the effect size of biochar treatments.

At present, there is limited understanding of which biochar types are most suited for enhancing soil properties and processes in function of crop growth in temperate regions. Consequently, more insight is needed into biochar's effect on soil properties and processes, and crop growth. Results from incubation experiments often show net nitrogen (N) immobilization after applying biochar to soil [9–13]. Mostly, hypotheses for this observation include microbial immobilization (e.g., [10]) or ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) sorption (e.g., [11]) However, these studies are not linked to pot or field trials using the same soil and biochar types and hence cannot confirm if the observed N immobilization would reduce crop growth in the short term. Pot trials and field experiments with other biochar types did mostly not support the N immobilization results from incubation experiments, as equal or higher crop yields were often observed with biochar addition [5–7,14,15]. It is thus questionable whether incubation tests are of any value for the indication of short-term effects on crop growth. Therefore, there is a need for combining laboratory, pot and field experiments in order to get a better mechanistic insight into biochar soil-plant effects. Moreover, biochar properties depend on both feedstock and production conditions [16], which complicates identifying biochar types that are best suited to apply in temperate regions.

The objectives of this study were to investigate the effect of the application of biochar produced from different woody feedstocks (willow and pine) and pyrolysis temperatures (450 °C, 550 °C and

650 °C) on soil properties and processes, as well as crop growth through conducting a series of experiments, being (1) a mineral N adsorption experiment, (2) a phytotoxicity test, (3) an incubation experiment in which biochar's effect on soil mineral N availability was tested, and (4) pot experiments using two temperate soil types to assess biomass production and N use efficiency. The biochars were characterized and an incubation study was conducted to determine the labile carbon fractions. We hypothesized that biochar addition to soil reduces soil mineral N availability, which would be reflected in reduced crop yield. We further hypothesized that reduced mineral N availability would be due to N adsorption to biochar because of its high CEC or due to biotic N immobilization because of biochar's labile C fraction.

## 2. Materials and Methods

The six biochars were characterized and a phytotoxicity test, N incubation experiment and a pot trial were conducted at the Institute for Agricultural and Fisheries Research (ILVO) (Belgium). The pot trial was repeated at the Department of Chemical and Biochemical Engineering, Technical university of Denmark (DTU), Campus Risø. In the pot trials, a set of parameters was measured in common (soil pH, soil mineral N and dry matter yield); at ILVO, extra crop analyses, while at DTU, extra soil analyses were conducted.

### 2.1. Soil

The soil used in the phytotoxicity test, N incubation experiment and pot trial conducted at ILVO was a sandy loam (USDA) soil containing 8.8% clay (<2 µm), 24.6% silt (2–50 µm) and 66.6% sand (50–2000 µm). It was collected in August 2009 from the 0–20 cm layer of an agricultural field located at ILVO in Melle, Belgium (50°59' N, 3°47' E). It was air-dried until a water content below 50% WFPS (water-filled pore space) was reached after which it was sieved to obtain the <1 cm fraction. Soil pH was measured in a 1 M KCl solution (1:5 v:v) (ISO 10390). Total organic carbon (TOC) content was measured on oven dried (70 °C) soil samples (ISO 10694) by dry combustion using a TOC-analyzer (Primacs<sup>SLC</sup>, Skalar, the Netherlands). Total N content was also determined by dry combustion (Dumas principle, ISO 13878; Flash 4000, Thermo Scientific, Waltham, MA, USA).

The soil used in the pot trial conducted at DTU was a sandy loam (IUSS) soil containing 11% clay (<2 µm), 14% silt (2–20 µm), 49% fine sand (20–200 µm), and 25% coarse sand (200–2000 µm). The soil was collected in March 2009 from the 0–25 cm layer of an agricultural field located at Campus Risø in Roskilde, Denmark (55°41' N, 12°05' E). It was air-dried and sieved to obtain the <1 cm fraction. Soil pH was measured in a 1 M KCl solution (1:5 v:v) (ISO 10390), while TC and TN were measured using an elemental analyzer (EA-1110 CHN, CE Instruments, Wigan, UK).

### 2.2. Biochar Characterization

The six biochar types used were produced in a batch pyrolysis unit at the UK Biochar Research Centre (University of Edinburgh, Edinburgh, UK) from willow (*Salix viminalis* L.) and Scots pine (*Pinus sylvestris* L.) at three different treatment temperatures (450 °C, 550 °C and 650 °C). Each of the production operations ran from ambient up to the desired treatment temperature. Once the desired

treatment temperature was obtained, this was sustained until the flammable and visible gas flow from the non-condensable fractions had ceased. The residence time was recorded for the total duration of each operation and for the length of time held at each desired treatment temperature (Table 1). In between the changeover of feedstock, a steam clean of the entire equipment took place to minimize contamination.

Moisture content (mass of water:mass of dry biochar) was determined by oven-drying (24 h at 105 °C). Total CHN contents were determined using an elemental analyzer (FLASH 2000, Thermo Scientific, Waltham, MA, USA). Proximate analyses were performed according to ASTM D1762-84 [17]. Biochar pH was measured in a 1 M KCl solution (1:5 v:v) (ISO 10390). Mineral N content was extracted (1:5 w:v) in a 1 M KCl solution (ISO 14256-2) and measured using a continuous flow analyzer (FIAstar 5000, Foss, Denmark). The cation exchange capacities (CEC) were measured according to Chapman [18] by measuring the extracted  $\text{NH}_4^+$  using 1 M KCl, after cation exchange capacity of the biochars had been saturated using 1 M  $\text{NH}_4$ -acetate (pH 7, biochar-solution ratio of 1:50 (w:v) instead of the proposed ratio of 1:5). Total metal concentrations were measured using ICP-OES after ashing at 750 °C followed by extraction with aqua regia (nitro-hydrochloric acid). Biochar's labile C fraction was determined by assessing the microbial C mineralization of the biochar over time, as described by Nelissen *et al.* [19]. In short, a microbial inoculum and nutrient solution were added to 20 g sulfuric acid-washed sand mixed with 1 g of biochar (and a control containing only sand) in a 200 mL glass penicillin bottle. Moisture content was adjusted to 70% of field capacity (FC) using distilled water. In order to measure the microbial respiration,  $\text{CO}_2$  concentrations of the headspace were measured at days 0, 1, 2, 3, 6, 9, 13, 16, 20, 27, 34, 45, 64, 87, 111, 140, 199, 254 and 357 after closing the bottles using a gas chromatograph (Finnigan Trace GC Ultra, Thermo Scientific, Waltham, MA, USA) equipped with a thermal conductivity detector (TCD). The C mineralized from the biochar was calculated by subtracting the  $\text{CO}_2$ -C concentrations measured in the control treatment from the  $\text{CO}_2$ -C concentrations measured in the biochar treatment. The experiment was stopped after 357 days, as then the amount of  $\text{CO}_2$ -C produced was similar in the biochar as in the control treatments, indicating that all labile biochar C had been mineralized. The cumulative mineralized biochar C curves were fitted using a first order growth model:

$$C_{(t)} = C_{\max} (1 - e^{-kt}) \quad (1)$$

where  $C_{(t)}$  is the amount of cumulative mineralized biochar C as measured at time (t),  $C_{\max}$  is the maximum of the growth function, which corresponds to the labile C fraction, and k is the mineralization rate constant.

**Table 1.** Production conditions and physico-chemical properties (mean  $\pm$  1 standard deviation) of biochars used based on oven-dry weight (105 °C) except for pH-KCl ( $n = 2$ ; for CEC,  $n = 1$ ).

Biochar type	Time at treatment temperature (min)	Total time in reactor (min)	Moisture (%)	C (%)	H (%)	N (%)	C:N	Volatile matter (%)	Ash (%)	pH-KCl	CEC (cmol <sub>c</sub> kg <sup>-1</sup> )
Willow-450 °C	29	89	3.2 $\pm$ 0.4	78.4 $\pm$ 0.7	2.03 $\pm$ 0.04	0.82 $\pm$ 0.02	96	11.2 $\pm$ 0.6	4.3 $\pm$ 0.1	7.3 $\pm$ 0.1	33.4
Willow-550 °C	23	100	3.2 $\pm$ 0.6	86.3 $\pm$ 0.6	1.95 $\pm$ 0.10	0.85 $\pm$ 0.14	102	6.7 $\pm$ 0.7	3.2 $\pm$ 0.0	7.5 $\pm$ 0.0	42.8
Willow-650 °C	28	118	5.3 $\pm$ 0.7	84.8 $\pm$ 0.7	1.14 $\pm$ 0.02	1.00 $\pm$ 0.02	85	6.0 $\pm$ 0.4	4.9 $\pm$ 0.1	8.1 $\pm$ 0.3	59.1
Pine-450 °C	88	151	2.6 $\pm$ 0.1	86.8 $\pm$ 1.0	2.80 $\pm$ 0.09	0.19 $\pm$ 0.05	457	12.1 $\pm$ 1.6	0.9 $\pm$ 0.2	6.7 $\pm$ 0.0	38.6
Pine-550 °C	60	159	3.3 $\pm$ 0.6	91.6 $\pm$ 1.6	2.13 $\pm$ 0.01	0.19 $\pm$ 0.04	482	7.3 $\pm$ 1.7	1.0 $\pm$ 0.3	6.8 $\pm$ 0.0	52.1
Pine-650 °C	33	148	3.4 $\pm$ 0.5	92.6 $\pm$ 0.2	1.68 $\pm$ 0.01	0.15 $\pm$ 0.00	617	6.0 $\pm$ 1.7	1.1 $\pm$ 0.2	7.7 $\pm$ 0.2	68.8

C denotes Carbon, H denotes hydrogen, N denotes Nitrogen, CEC denotes Cation exchange capacity.

To test how much  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N could be adsorbed to biochar, first a preliminary sorption experiment was conducted with the willow-450 °C, willow-650 °C, pine-450 °C and pine-650 °C biochars. Twenty-five ml of  $\text{NH}_4\text{Cl}$  or  $\text{KNO}_3$  solutions with different N concentrations (10, 25, 50, 100 mg N  $\text{L}^{-1}$  for  $\text{NH}_4\text{Cl}$ ; 1, 5, 10 mg N  $\text{L}^{-1}$  for  $\text{KNO}_3$ ) was added to 0.5 g (oven-dry basis) of each biochar type (1 replicate). In addition, a control treatment without biochar (so only solution) was included. These mixtures were shaken for 1 hour and then filtered (MN 640 w filters), after which  $\text{NH}_4^+$  or  $\text{NO}_3^-$  concentrations were measured in the filtrates using a continuous flow analyzer (FIAstar 5000, Foss, Denmark). It has to be noted that we did not test when equilibrium was reached. Sorption of N was calculated as the difference between the amount of added N, as measured in the control, and the amount of N in the extract after shaking. For both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , sorption did not increase when adding higher N concentrations [20]. After this preliminary study, the experiment was repeated with 3 replicates per treatment and with a concentration of 10 mg N  $\text{L}^{-1}$  for both  $\text{NH}_4\text{Cl}$  and  $\text{KNO}_3$ .

### 2.3. Phytotoxicity Test

Phytotoxicity was tested in soil mixed with biochar, as well as in sulfuric acid-washed sand mixed with biochar. For the former, the soil was sieved (2 mm), and water was added to obtain a moisture content of 50% water filled pore space (WFPS). The six biochar types (sieved to <0.5 cm) were mixed with soil at a dose of 10 g fresh biochar  $\text{kg}^{-1}$  dry soil each. Petri dishes were packed with moist soil (equivalent to 60 g oven-dried soil) until a bulk density of 1.3 g  $\text{cm}^{-3}$  was obtained. For the second toxicity test, which was based on a standard phytotoxicity test for compost (CMA/2/IV/12, in Dutch [21]), the sand was mixed with each of the six biochars in a 50:50 volume ratio. These mixtures were brought to 50% FC (determined as described previously) and spread in the petri dishes. Control treatments contained only sand or soil. For both toxicity tests, 50 *Lepidium sativum* L. seeds were spread on top of every mixture and all petri dishes were covered and artificially lighted (12/12 hrs, 750 to 1250 lux, 20 °C  $\pm$  2 °C) for 10 days. The experimental design used was a randomized block design with four replicates. Germinated seeds were counted, investigated for normal root and shoot growth, and removed after 4 and 10 days. Phytotoxicity was then calculated using the equation (CMA/2/IV/12, [21]):

$$\text{Phytotoxicity (\%)} = (\text{Kr} - \text{Ks})/\text{Kr} \times 100 \quad (2)$$

where Kr is the germinative capacity (sum of the normally developed seeds from both counts) of *L. sativum* in the control treatments (sand or soil) and Ks the germinative capacity of *L. sativum* in sand- or soil-biochar mixtures.

### 2.4. Nitrogen Incubation Experiment

Biochar's effect on soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations was tested in a four-week incubation experiment. Sieved biochar (<0.5 cm) was mixed with moist soil (equivalent to 263 g oven-dried soil; adjusted with distilled water to 50% WFPS) at a dose of 10 g fresh biochar  $\text{kg}^{-1}$  dry soil. PVC tubes (h = 12 cm, r = 2.3 cm) were filled with these mixtures in order to reach a bulk density of 1.3 g  $\text{cm}^{-3}$ . To each biochar treatment and a control treatment without biochar, N fertilizer was added as  $\text{NH}_4\text{NO}_3$  solution in 3 different dosages: 0, 12.8 and 38.5 mg N  $\text{kg}^{-1}$ , corresponding to field application rates of

0, 50, and 150 kg N ha<sup>-1</sup> (assuming a soil depth of 0.30 m and soil bulk density equal to 1.3 g cm<sup>-3</sup>), resulting in 21 treatments in total. The tubes were covered with a single layer of gas permeable parafilm to avoid water evaporation and subsequently incubated at 15 °C and 70% relative humidity. The experimental design used was a completely randomized design with three replicates. Twelve tubes per treatment were set up in order to be able to analyze soil mineral N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) destructively at 7, 14, 21 and 28 days after the start of the incubation. For that purpose, soil from three replicates per treatment was extracted using 1 M KCl (1:5 w:v) (ISO 14256-2) after which the mineral N concentration of the extracts was measured using a continuous flow analyzer (FIAstar 5000, Foss, Denmark). One extra soil or soil-biochar sample per treatment was prepared and immediately extracted in order to analyze mineral N at the start of the experiment.

### 2.5. ILVO Pot Trial

A pot trial was conducted in a greenhouse with a light regime of 16 h day/8h night using two crops (radish (*Raphanus sativus* L.) and spring barley (*Hordeum vulgare* L.)), 2 fertilizer doses (0 and 12.8 mg N kg<sup>-1</sup>, equating to field application rates of 0 and 50 kg N ha<sup>-1</sup>, assuming a soil depth of 0.30 m and soil bulk density equal to 1.3 g cm<sup>-3</sup>) and 2 biochar doses (0 and 10 g fresh biochar kg<sup>-1</sup> dry soil). Tap water and fertilizer solution (NH<sub>4</sub>NO<sub>3</sub>) were added to the soil in order to reach a water content of 50% WFPS, after which sieved biochar (<0.5 cm) was added and mixed thoroughly with the soil. For growing radish and spring barley, respectively, two sizes of plastic pots were used: 2 L (h = 13.2 cm; d = 16.7 cm; 2 kg soil on a dry weight basis) and 5 L pots (h = 18.1 cm; d = 22.5cm; 5 kg soil on a dry weight basis). The soil bulk density was 1.3 g cm<sup>-3</sup>. Ten seeds of radish or 14 seeds of spring barley were sown. The experimental design used was a randomized block design with four replicates. One extra soil or soil-biochar sample per treatment was prepared at the start of the experiment in order to determine soil mineral N content and pH-KCl (analyzed as described previously). After emergence, seedlings were thinned to 5 for radish and 8 for spring barley. During the experiment, the soil moisture content was kept at 50% WFPS.

The experiment was terminated 5 weeks after sowing and pH-KCl and soil mineral N content were determined as described previously. Since no tuber growth was observed in radish plants (probably due to light deficiency in the initial growth stadium), only aboveground biomass (per pot) was harvested in both radish and spring barley. Subsequently, the plant material was dried at 70 °C to constant weight and dry matter yield (per pot) was determined. N content was determined in ground plant material by the block digestion/steam distillation method (Kjeldahl method, ISO 5983-2). Aboveground biomass N uptake (per pot) was calculated by multiplying the aboveground dry matter production per pot by the aboveground biomass N concentration. N use efficiency (NUE) was calculated as follows:

$$\text{NUE (\%)} = [(N_f) - (N_{nf})]/R \quad (3)$$

where N<sub>f</sub> is the aboveground biomass N uptake in the fertilized treatment (mg N kg<sup>-1</sup> dry soil), N<sub>nf</sub> the aboveground biomass N uptake in the unfertilized control treatment (mg N kg<sup>-1</sup> dry soil) and R the fertilizer dose applied (12.8 mg N kg<sup>-1</sup> dry soil).

## 2.6. DTU Pot Trial

A pot trial with identical treatments as for the ILVO pot trial was conducted in a growth chamber with a light regime of 16 h day/8 h night and a temperature regime of 19 °C day/12 °C night. One extra soil sample for each treatment (analyzed in triplicate) was prepared at the start of the experiment in order to determine initial mineral N content, pH-KCl, total N (TN), total dissolved N (TDN), total carbon (TC) and dissolved organic carbon (DOC). Soil was extracted in a 0.01 M CaCl<sub>2</sub> solution (1:5 w:v), after which mineral N and TDN was measured in the extracts using a continuous flow analyzer (AA3, Bran and Luebbe, Germany). DOC was analyzed in the same extracts on a TOC-VCPH (Shimadzu Corp., Kyoto, Japan). Soil pH was measured in a 1 M KCl solution (1:5 v:v) (ISO 10390). TC and TN were measured using an elemental analyzer (EA-1110 CHN, CE Instruments, Wigan, UK). At the end of the experiment, six weeks after sowing, mineral N content, pH-KCl, TDN and DOC were determined as described above. For radish, belowground (roots) and aboveground (leaves) fresh biomass were harvested separately. Spring barley was harvested by cutting it just above soil level. The plant material was dried at 70 °C to constant weight in order to determine dry matter yield (per pot).

## 2.7. Statistical Analyses

Phytotoxicity data were analyzed using a one-tailed *t*-test, in order to verify whether phytotoxicity was larger than zero.

For the N incubation experiment, the effect of biochar addition on NO<sub>3</sub><sup>-</sup> concentrations after four incubation weeks was investigated using a three step approach. First, we determined if the presence of biochar or not (factor 1; levels: yes or no) and fertilizer dose (factor 2) had an effect on NO<sub>3</sub><sup>-</sup> concentrations using a two-way analysis of variance (ANOVA). Second, we tested which biochar treatments were different from the control regarding NO<sub>3</sub><sup>-</sup> concentrations at the end of the incubation period for every biochar treatment individually, separately for each fertilizer dose, using independent *t*-tests. Last, we investigated the effect of biochar feedstock, pyrolysis temperature and fertilizer dose using a three-way ANOVA, with the relative difference (in %) in NO<sub>3</sub><sup>-</sup> concentrations between the biochar and control treatments as dependent variable. There were no significant interactions between the factors. A post-hoc Scheffé test was used to compare the effect of the individual levels of factor pyrolysis temperature on the NO<sub>3</sub><sup>-</sup> concentration. The effect of biochar addition on NH<sub>4</sub><sup>+</sup> concentrations after four weeks of incubation was investigated using a two-way ANOVA including the factors presence of biochar and fertilizer dose. As the factor presence of biochar was not significant, no further analyses were undertaken.

The data from the pot trials, except for NUE, were analyzed using two-way ANOVA, including the factors biochar type (including the control) and fertilizer dose. In case the interaction term was significant ( $P < 0.05$ ), a one-way ANOVA including the factor biochar type was run for each fertilizer dose separately. A post-hoc Scheffé-test was used to compare the effect of the individual levels of the factor biochar type. For the NUE data, a one-way ANOVA was conducted, and treatment means were compared using a post-hoc Scheffé-test. For all statistical analyses, SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used.



### 3. Results

#### 3.1. Soil and Biochar Characterization

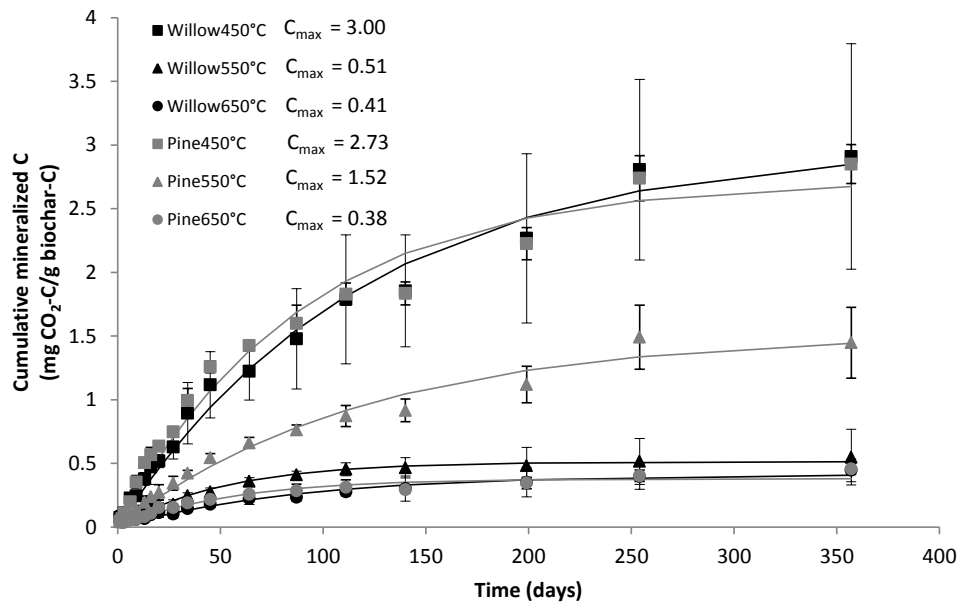
The soil from ILVO was rather low in OC content (TOC = 1.00%) and pH-KCl (5.5), while DTU soil had a slightly higher C content (TC = 1.47%) and neutral pH-KCl (7.1). Total N of the ILVO and DTU soil were respectively 0.11% and 0.16%, resulting in C:N ratios of 9.1 and 9.2. Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were respectively 1.1 and 12.9 mg N  $\text{kg}^{-1}$  at ILVO and 1.5 and 21.6 mg N  $\text{kg}^{-1}$  at DTU. The biochars had C contents between 78.4%–92.6% and pH-KCl ranging from 6.7 to 8.1 (Table 1). Pine derived biochar was generally more acidic than the willow chars and the pH-KCl increased with pyrolysis temperatures. The pine chars showed N contents lower than 0.2% resulting in a high C:N ratio (>456). Biochar  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were low, <1.1 and <0.4 mg N  $\text{kg}^{-1}$  biochar, respectively. Ash content in the pine chars was lower compared to the willow chars. The biochar CEC increased with increasing pyrolysis temperature (Table 1). From the adsorption experiment, it was observed that the willow-450 °C, willow-650 °C, pine-450 °C and pine-650 °C biochars can absorb  $4.9 \pm 2.8$ ,  $5.9 \pm 2.3$ ,  $10.0 \pm 2.7$  and  $15.3 \pm 5.3$  mg  $\text{NH}_4^+$ -N  $\text{kg}^{-1}$  biochar, and  $3.7 \pm 1.4$ ,  $19.0 \pm 1.6$ ,  $12.2 \pm 5.9$  and  $12.3 \pm 2.3$  mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  biochar, respectively (mean  $\pm$  standard deviation). Consequently, when biochar is applied to soil at a dose of 10 g biochar (dry base)  $\text{kg}^{-1}$  soil (dry base), a maximum of 0.15 mg  $\text{NH}_4^+$ -N  $\text{kg}^{-1}$  soil and 0.19 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  soil can be adsorbed to biochar.

Total Cu, Zn, Na, Ca, Mg, K, Sr, and B concentrations were higher in the willow biochars compared to the pine biochars, while for Fe and Ti the opposite was the case (Supplementary data). The cumulative biochar-C mineralization rate (Figure 1) was highest during the first days of incubation, after which the rate decreased as a function of time to reach a steady state at end of incubation. The best fit for this curve could be achieved using a first order growth model (Equation 1). As expected, the labile C fraction ( $C_{\text{max}}$ ) increased with decreasing pyrolysis temperature. It was highest for willow-450 °C (3 mg C  $\text{g}^{-1}$  biochar-C) and lowest for pine-650 °C (0.38 mg C  $\text{g}^{-1}$  biochar-C), but generally, labile C fractions were low (max. 0.3% of biochar-C).

#### 3.2. Phytotoxicity Test

When a biochar dose of 10 g  $\text{kg}^{-1}$  was added to soil, the 6 biochars were not phytotoxic ( $P \geq 0.14$ ; Table 2). At a very high biochar dose (50:50 v:v, biochar:sand), three of the six biochars turned out to be phytotoxic ( $P \leq 0.05$ ; Table 2). This was the case for the two chars produced at 650 °C and the willow char produced at 550 °C. In these three treatments, it was visually observed that root and shoot growth were suppressed, although 97% ( $\pm 2.7$ ) of the seeds had germinated.

**Figure 1.** Cumulative carbon mineralization originating from biochar measured during 357 days for 6 biochar types (symbols) and predicted by the equation  $C_{(t)} = C_{\max}(1 - e^{-kt})$  (lines); error bars indicate  $\pm 1$  standard deviation ( $n = 3$ ).



**Table 2.** Mean phytotoxicity of 6 biochars ( $\pm 1$  standard deviation;  $n = 4$ ) at a biochar application rate of  $10 \text{ g kg}^{-1}$  soil and in a biochar-sand mixture (50:50 v:v).

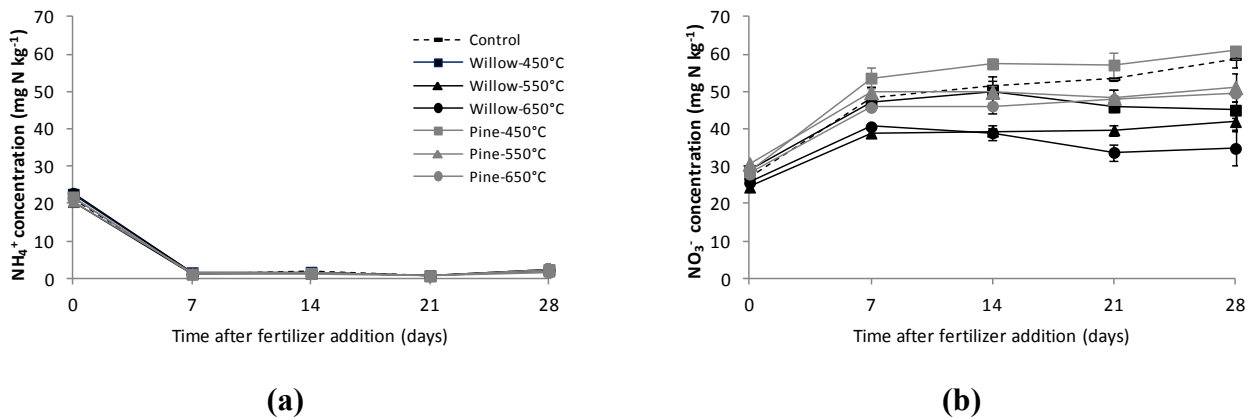
	Phytotoxicity (%)	
	$10 \text{ g kg}^{-1}$	50:50 v:v
Willow-450 °C	$0.5 \pm 1.0$	$1.0 \pm 2.9$
Willow-550 °C	$1.0 \pm 1.6$	$54.0 \pm 4.2$
Willow-650 °C	$2.0 \pm 2.0$	$86.4 \pm 14.1$
Pine-450 °C	$2.5 \pm 2.5$	$2.5 \pm 1.9$
Pine-550 °C	$0.0 \pm 2.0$	$2.5 \pm 1.9$
Pine-650 °C	$0.0 \pm 1.2$	$72.7 \pm 33.0$

### 3.3. Nitrogen Incubation Experiment

Biochar addition to soil did not significantly affect  $\text{NH}_4^+$  concentrations when compared to the control treatment four weeks after fertilizer application (Figure 2a for  $150 \text{ kg N ha}^{-1}$ ; data for 0 and  $50 \text{ kg N ha}^{-1}$  are similar and not shown). In the fertilized treatments, added  $\text{NH}_4^+$  was nitrified within one week (Figure 2b). After four weeks of incubation, biochar addition to soil reduced  $\text{NO}_3^-$  concentrations significantly compared to the control (two-way ANOVA,  $P < 0.001$ ). However,  $t$ -tests show that  $\text{NO}_3^-$  concentrations were not significantly decreased in all biochar treatments (Table 3). The relative difference (in %) in  $\text{NO}_3^-$  concentrations between the biochar and control treatments was significantly higher in the willow compared to the pine biochar treatments ( $P < 0.001$ ), and this relative difference was significantly higher at  $650 \text{ °C}$  than at  $450 \text{ °C}$  and  $550 \text{ °C}$  ( $P < 0.001$ ) (three-way ANOVA). This means that generally, (i) willow biochar addition reduced  $\text{NO}_3^-$  concentrations more than pine biochar addition compared to the control and (ii) this  $\text{NO}_3^-$  reduction increased with increasing pyrolysis temperature. Therefore, applying willow- $650 \text{ °C}$  to the soil reduced

$\text{NO}_3^-$  concentrations to the largest extent compared to the control, *i.e.* by  $24.0 \text{ mg N kg}^{-1}$  soil at the highest fertilizer dose (Figure 2b, Table 3). This corresponds to  $93.6 \text{ kg N ha}^{-1}$  when assuming a soil depth of  $0.30 \text{ m}$  and a soil bulk density equal to  $1.3 \text{ g cm}^{-3}$ .

**Figure 2.** (a)  $\text{NH}_4^+$  and (b)  $\text{NO}_3^-$  concentrations in the control soil and biochar-amended treatments at a fertilizer dose of  $150 \text{ kg N ha}^{-1}$  ( $38.5 \text{ mg N kg}^{-1}$ ); error bars indicate  $\pm 1$  standard deviation ( $n = 3$ ).



**Table 3.**  $\text{NO}_3^-$  concentrations ( $\text{mg N kg}^{-1}$ ) in the control and biochar treatments (mean  $\pm 1$  standard deviation;  $n = 3$ ) after four incubation weeks for three fertilizer doses ( $0, 50$  and  $150 \text{ kg N ha}^{-1}$ ).

	Fertilizer dose ( $\text{kg N ha}^{-1}$ )		
	0	50	150
Control	$16.77 \pm 0.39$	$28.18 \pm 2.65$	$58.79 \pm 2.27$
Willow-450 °C	$10.71 \pm 0.36^{**}$	$21.96 \pm 1.53^*$	$45.02 \pm 2.16^*$
Willow-550 °C	$11.82 \pm 3.77^{ns}$	$18.55 \pm 1.87^*$	$41.95 \pm 1.99^*$
Willow-650 °C	$6.73 \pm 0.53^{**}$	$15.13 \pm 1.96^*$	$34.79 \pm 4.63^*$
Pine-450 °C	$14.58 \pm 0.31^*$	$26.28 \pm 1.67^{ns}$	$60.86 \pm 0.46^{ns}$
Pine-550 °C	$12.12 \pm 1.36^*$	$26.27 \pm 1.27^{ns}$	$51.11 \pm 3.9^*$
Pine-650 °C	$8.997 \pm 0.37^*$	$19.17 \pm 2.17^*$	$49.58 \pm 0.87^*$

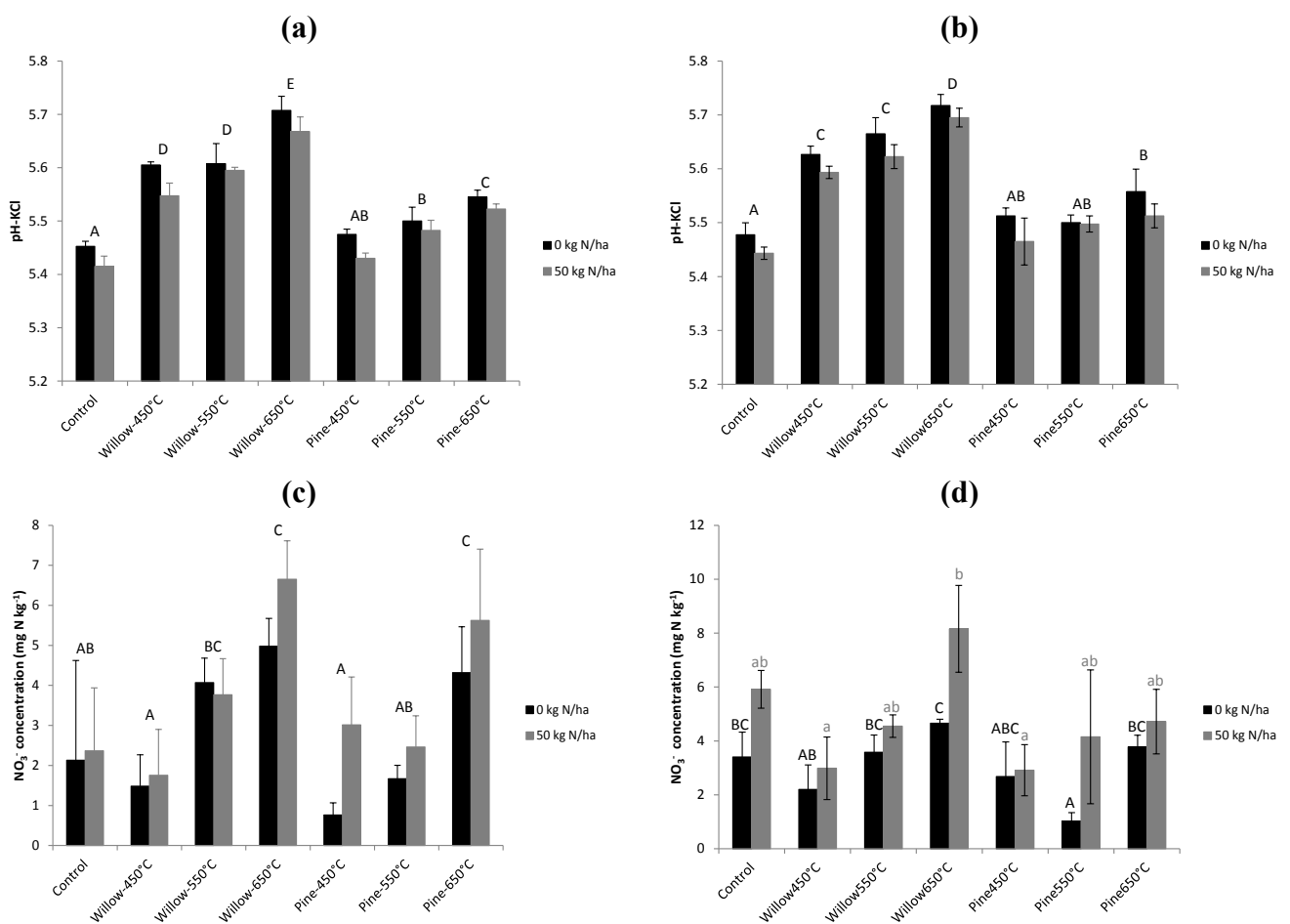
Treatments with \* or \*\* are significantly different from the control at  $P < 0.05$  (\*) or at  $P < 0.001$  (\*\*), *ns* denotes not significant.

### 3.4. ILVO Pot Trial

At radish harvest, soil pH was significantly higher in all biochar treatments ( $P < 0.05$ ) compared to the control, except for the pine-450 °C treatment (Figure 3a). The pH results at spring barley harvest confirm this observation (Figure 3b). Similar to the biochar pHs, soil pH results demonstrated two trends, (i) a higher pH increase with willow biochar addition compared to pine biochar addition and (ii) a higher pH increase with increasing pyrolysis temperatures (Figure 3a,b). At radish and spring barley harvest, there were no significant differences between the treatments for soil  $\text{NH}_4^+$  concentrations, which were very low in all treatments ( $< 1.5 \text{ mg N kg}^{-1}$ ). Soil  $\text{NO}_3^-$  concentrations were generally increased with increasing pyrolysis temperature. For radish,  $\text{NO}_3^-$  concentrations in the willow-650 °C and pine-650 °C treatments were significantly higher than in the control soil (Figure 3c). For spring

barley, no significant differences were found except for lower  $\text{NO}_3^-$  concentrations in the unfertilized pine-550 °C treatment than in the control (Figure 3d).

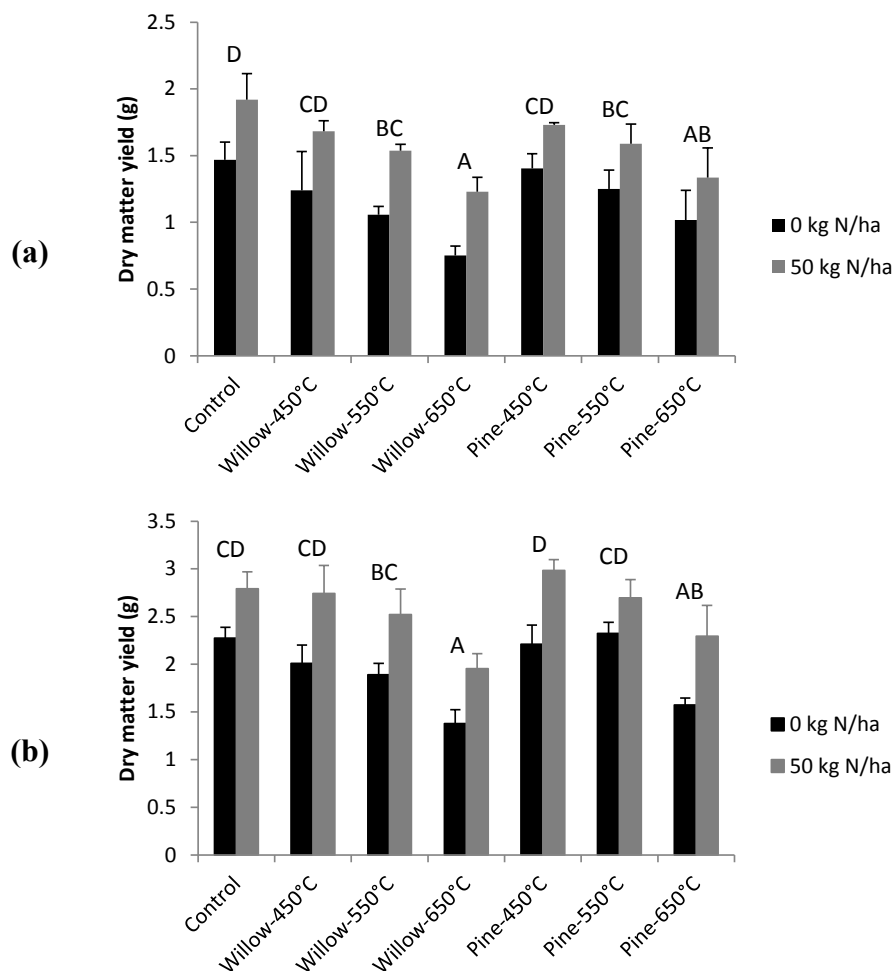
**Figure 3.** (a,b) pH-KCl and (c,d)  $\text{NO}_3^-$  concentrations in the control and biochar-amended treatments at two fertilizer doses (0 and 50 kg N ha<sup>-1</sup>) at the end of the ILVO pot trial with radish (a,c) and spring barley (b,d); error bars indicate  $\pm 1$  standard deviation ( $n = 4$ ); treatments with different letters differ significantly ( $P < 0.05$ ) according to a Scheffé-test. In case of no interaction between the factors biochar type and fertilizer dose, there is only one letter per biochar treatment (a,b,c); in case of interaction, there is one letter on top of each bar (d) (capital and lowercase letters for unfertilized treatments and fertilized treatments, respectively).



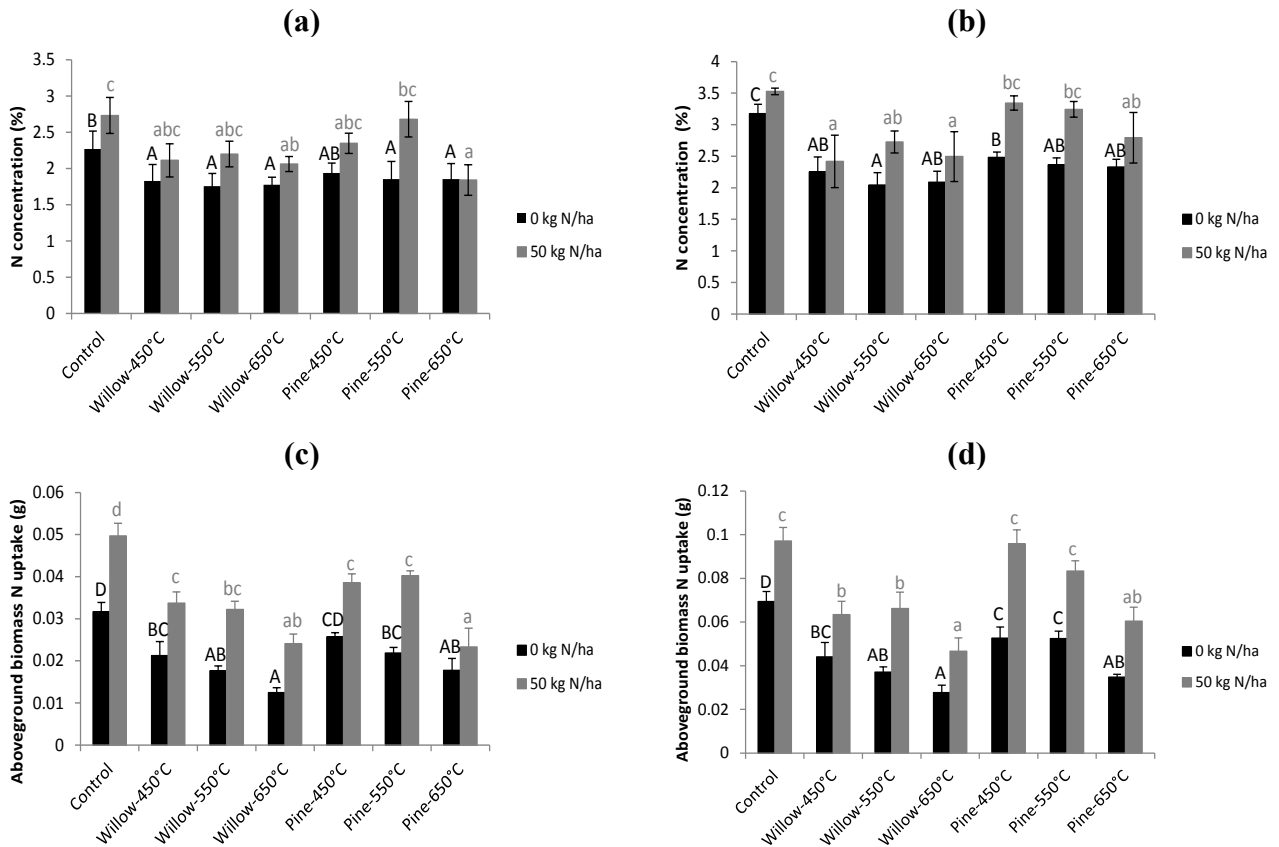
For both fertilizer treatments, radish dry matter yields were generally decreased with biochar addition, and this decrease became more pronounced with increasing pyrolysis temperatures (Figure 4a). The yield reduction was significant for all biochars produced at 550 °C and 650 °C. For spring barley, this was only the case for the 650 °C-treatments (Figure 4b). For both crops and when not fertilized, crop N concentrations were significantly lower ( $P < 0.05$ ) in the biochar treatments (except for radish in pine-450 °C treatment) compared to the control. In the fertilized radish

treatments, N concentrations were significantly reduced in both the 650 °C treatments, while for spring barley, this was also the case in the willow-450 °C and willow-550 °C treatments (Figure 5a,b). Aboveground biomass N uptake was significantly lower in the biochar treatments (both unfertilized and fertilized) compared to the control, except for the unfertilized radish control *versus* pine-450 °C and the fertilized spring barley control *versus* pine-450 °C and pine-550 °C treatments (Figure 5c,d). The N use efficiency results demonstrate lower NUE-values in the biochar treatments compared to the control soil (Figure 6). In some cases, NUE was even negative, meaning that N uptake in these fertilized biochar treatments was lower than in the unfertilized control.

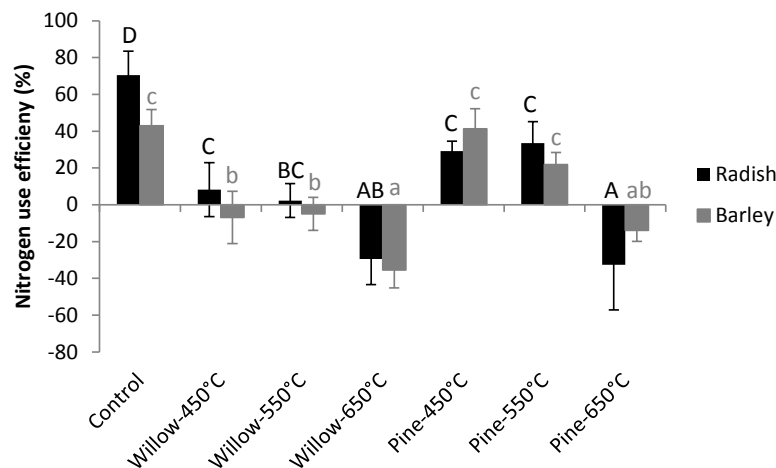
**Figure 4.** (a) Radish and (b) spring barley dry matter yield (per pot) in the control and biochar-amended treatments at two fertilizer doses (0 and 50 kg N ha<sup>-1</sup>) in the ILVO pot trial; error bars indicate  $\pm 1$  standard deviation ( $n = 4$ ); treatments with different letters differ significantly ( $P < 0.05$ ) according to a Scheffé-test (one letter per biochar treatment due to no interaction between factor biochar type and fertilizer dose).



**Figure 5.** (a,b) N concentration and (c,d) aboveground biomass N uptake for radish (a,c) and spring barley (b,d) in the control and biochar-amended treatments at two fertilizer doses (0 and 50 kg N ha<sup>-1</sup>) in the ILVO pot trial; error bars indicate ± 1 standard deviation (*n* = 4); treatments with different letters differ significantly (*P* < 0.05) according to a Scheffé-test (capital and lowercase letters for unfertilized treatments and fertilized treatments, respectively).



**Figure 6.** Nitrogen use efficiency in the control and biochar-amended treatments in the ILVO pot trials; error bars indicate ± 1 standard deviation (*n* = 4); treatments with different letters differ significantly (*P* < 0.05) according to a Scheffé-test (capital and lowercase letters for radish and spring barley, respectively).

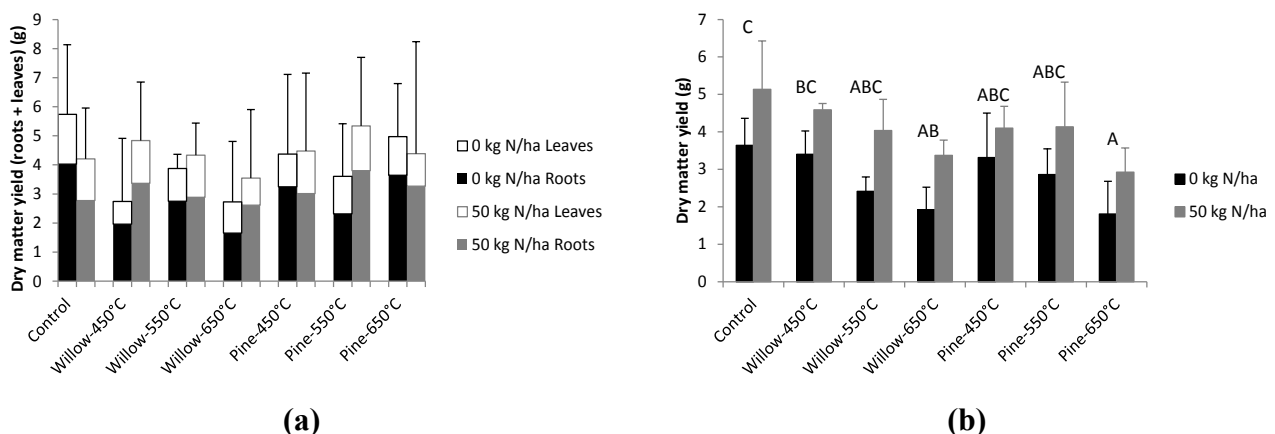


### 3.5. DTU Pot Trial

Soil pH was not influenced by biochar addition at the start of the pot trial [22]. Also TN, TDN and DOC concentrations were not significantly affected by biochar addition, while TC was, as expected, significantly increased in the biochar treatments compared to the control [22].

In contrast to the ILVO radish trial, no significant differences were found in pH-KCl between the control and biochar treatments at harvest [22], probably because the initial soil pH was already rather high and in the range of the biochar pH. Both  $\text{NH}_4^+$  ( $1.3 \pm 0.9 \text{ mg N kg}^{-1}$ ) and  $\text{NO}_3^-$  ( $0.5 \pm 0.1 \text{ mg N kg}^{-1}$ ) concentrations were not significantly influenced by biochar at harvest, and had decreased compared to the initial values ( $1.7 \pm 0.7 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ;  $21.3 \pm 2.2$  and  $31.6 \pm 2.5 \text{ mg NO}_3^-\text{-N kg}^{-1}$  for the unfertilized and fertilized treatments, respectively). DOC and TDN concentrations were not influenced by biochar addition, but were lower ( $26.1 \pm 6.0 \text{ mg DOC kg}^{-1}$ ;  $15.7 \pm 3.1$  and  $16.9 \pm 2.9 \text{ mg TDN kg}^{-1}$  for unfertilized and fertilized radish treatments, respectively) compared to the initial values ( $31.7 \pm 8.2 \text{ mg DOC kg}^{-1}$ ;  $37.7 \pm 3.2$  and  $47.8 \pm 4.0 \text{ mg TDN kg}^{-1}$  for unfertilized and fertilized treatments, respectively). For spring barley, the soil results at harvest were similar to those for radish [22]. Radish dry matter yield was decreased with biochar addition in the unfertilized treatments, although not statistically significant, while in the fertilized treatments no trend was observed (Figure 7a). The barley dry matter yield at harvest was significantly lower in the 650 °C -biochar treatments compared to the control soil (Figure 7b).

**Figure 7.** (a) Radish and (b) spring barley dry matter yield (per pot) in the control and biochar-amended treatments at two fertilizer doses (0 and 50 kg N ha<sup>-1</sup>) in the DTU pot trial; error bars indicate  $\pm 1$  standard deviation ( $n = 4$ ); for (a) no significant differences were found; for (b) treatments with different letters differ significantly ( $P < 0.05$ ) according to a Scheffé-test (one letter per biochar treatment due to no interaction between factors biochar type and fertilizer dose).



## 4. Discussion

### 4.1. Biochar and Toxicity

Our phytotoxicity results (Table 2) at a biochar dose of  $10 \text{ g kg}^{-1}$  (corresponding to  $40 \text{ ton ha}^{-1}$ ) are comparable to results from Van Zwieten *et al.* [23]. Their germination test with various biochar

(10 ton ha<sup>-1</sup>), crop and soil types did not reveal negative effects of biochar on plant germination. In contrast, our results at a 50:50 (v:v) dose, which is an unrealistically high application rate in agriculture, demonstrate that three out of six biochar types suppressed root and shoot growth (Table 2). Rogovska *et al.* [24] performed germination tests using aqueous biochar extracts and attributed differences in shoot lengths to the inhibiting effect of PAHs and/or other organic compounds present in water extracts of biochars. The biochars' heavy metal content (Supplementary data) cannot be related to phytotoxicity, but the presence and inhibiting effect of unidentified organic compounds cannot be excluded. In contrast to the purpose of our study, Oleszczuk *et al.* [25] investigated the effect of biochar on phytotoxicity when mixed with sewage sludge. Reduced phytotoxicity of the contaminated sludge after biochar application was observed. This was explained by surface sorption of contaminants or a change in matrix properties (e.g., pH), through which the mobility of the contaminants was reduced.

#### 4.2. Mechanisms for Reduced N Availability after Biochar Application

Generally, biochar treatments reduced NO<sub>3</sub><sup>-</sup> availability compared to the control (up to 93.6 kg N ha<sup>-1</sup> after applying 150 kg N ha<sup>-1</sup>), implying a risk for retarded crop growth and lower crop yield in the short term, especially at the high temperature biochars (Figure 2 and Table 3). The reduced N availability was also feedstock dependent, as it was larger for willow than for pine biochar. There are five possible explanations for these observations: biochar addition caused (i) biotic N immobilization, (ii) reduced soil organic matter (SOM) mineralization, (iii) suppressed nitrification, (iv) increased gaseous losses or (v) abiotic NH<sub>4</sub><sup>+</sup> and/or NO<sub>3</sub><sup>-</sup> immobilization.

The labile C fractions of the chars were too low (maximum 3 mg C g<sup>-1</sup> biochar-C (Figure 1), which corresponds in our study to 30 mg C kg<sup>-1</sup> soil) to cause microbial immobilization (hypothesis i). Assuming an average microbial biomass C:N ratio of 8 and a microbial efficiency of 0.4 [26], microorganisms could immobilize a maximum of 1.5 mg N kg<sup>-1</sup> soil, while e.g., in the willow-650 °C treatment 24 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> soil was immobilized. Moreover, biochar labile fractions are increased with decreasing pyrolysis temperature while the opposite is true for the observed amounts of available NO<sub>3</sub><sup>-</sup>. However, other micro-organism stimulating processes induced by biochar, e.g., bacterial adhesion or a change in soil pH, could possibly increase the total microbial abundance or activity [27], thereby consuming more N and thus immobilizing N biotically. Also Bruun *et al.* [9] observed short-term N immobilization with biochar addition, and explained this by the high C:N ratio and labile C fraction of the fast-pyrolysis biochar produced at 525 °C. They hypothesize that a biochar produced at high temperatures by slow pyrolysis would probably have less impact on short-term N dynamics, but our results show the opposite.

Reduced soil organic matter mineralization in the presence of biochar (hypothesis ii), as hypothesized e.g., by Knowles *et al.* [11], seems unlikely, as the reduced NO<sub>3</sub><sup>-</sup> availability in the biochar treatments occurred already after one week. At that time, a significant mineralization was not yet observed in the control treatment, as the increase in NO<sub>3</sub><sup>-</sup> measured after the first week was equal to the decrease in (nitrified) NH<sub>4</sub><sup>+</sup>.

If suppression of autotrophic nitrification (hypothesis iii) had occurred, a higher NH<sub>4</sub><sup>+</sup> content would have been measured in the biochar treatments compared to the control, which was not the case.



Moreover, biochar application is supposed to enhance nitrification due to adsorption of certain organic compounds like phenolics [28] or due to a soil pH increase with biochar addition [12].

A water-filled pore space of 50% was probably too low for high N<sub>2</sub>O and N<sub>2</sub> emissions, but some N<sub>2</sub>O and N<sub>2</sub> could have occurred in water-logged, anaerobic microsites. Biochar could have stimulated NH<sub>3</sub> volatilization (hypothesis iv) in high pH micro-sites close to biochar particles. Moreover, the biochar types with higher pH tended to cause higher NO<sub>3</sub><sup>-</sup> reduction, indicating that biochar pH could have contributed to NH<sub>3</sub> volatilization. The produced NH<sub>3</sub> itself can be absorbed on or within biochar in multiple ways (physical or chemical) [29]. Taghizadeh-Toosi *et al.* [30] also showed that biochar can adsorb NH<sub>3</sub>, whereby it was reasonable to assume that this was a more dominant adsorption mechanism compared to NH<sub>4</sub><sup>+</sup>-N adsorption, and that the adsorbed NH<sub>3</sub>-N remained bioavailable.

Another possible explanation for the lower NO<sub>3</sub><sup>-</sup> levels is abiotic N immobilization upon biochar addition (hypothesis v). Due to the high CEC of the biochars, (mineralized) NH<sub>4</sub><sup>+</sup> might be adsorbed and thus become unavailable for nitrification, resulting in reduced NO<sub>3</sub><sup>-</sup> production. This could explain the lower NO<sub>3</sub><sup>-</sup> availability at higher pyrolysis temperatures as these biochars show an increased CEC. However, adsorption did not seem to have occurred immediately, as differences in NH<sub>4</sub><sup>+</sup> (and NO<sub>3</sub><sup>-</sup>) concentrations between the treatments at the start of the experiment were small. Moreover, as 1 M KCl was the extraction agent for both CEC and mineral N determination, it would also be expected that NH<sub>4</sub><sup>+</sup> adsorbed to biochar due to CEC was KCl-extractable. The sorption experiment indicates that the biochars could not adsorb much NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (maximum 0.15 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> soil and 0.19 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> soil compared to 24 mg N kg<sup>-1</sup> soil immobilized in the willow-650 °C treatment). As a consequence, our findings do not support abiotic electrostatic NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> immobilization as main immobilization mechanism. However, it has to be noted that (i) a slight increase in soil CEC after biochar addition can improve crop nutrition by nutrient retention [31], (ii) through aging, biochar CEC can increase [32], leading to greater nutrient retention by aged compared to fresh biochar through which nutrient leaching can be reduced and soil fertility improved, and (iii) biochar can adsorb dissolved organic carbon compounds [33], thereby changing surface charge characteristics. Furthermore, other papers show the capacity of biochar to adsorb NH<sub>4</sub><sup>+</sup>. Yao *et al.* [34] conducted a sorption experiment, in which most biochars had capacity for removing NH<sub>4</sub><sup>+</sup> from aqueous solutions, independent of the biochar production temperature. In contrast, most tested biochars showed no sorption ability for NO<sub>3</sub><sup>-</sup>, except for the ones produced at the highest pyrolysis temperature (600 °C). Jones *et al.* [6] observed a similar trend, as sorption isotherms of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> with a woody high pH biochar revealed NH<sub>4</sub><sup>+</sup> (maximum about 30 mg N kg<sup>-1</sup> biochar) but almost no NO<sub>3</sub><sup>-</sup> sorption. It can, however, not be excluded that non-electrostatic sorption of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> occurred. The latter could be explained by the high pore volume of biochar, which increases at higher pyrolysis temperatures [35]. Major *et al.* [36] mention that biochar porosity could contribute to nutrient adsorption by trapping nutrient-containing water, which possibly also occurred in our experiment. This does not correspond to the observations in the sorption experiment, but the behavior of N containing water was most probably different under soil circumstances compared to during the N sorption experiment. Also Knowles *et al.* [11], Nelissen *et al.* [12] and Novak *et al.* [13] observed net NO<sub>3</sub><sup>-</sup> immobilization with biochar addition, but the mechanism could not be elucidated.

### 4.3. Crop Growth Effects

The biomass yield data from both pot experiments corroborated the reduced N availability data obtained in the incubation study, as generally dry matter yield, crop N uptake and NUE were decreased with biochar addition, especially at the high pyrolysis temperatures (Figures 4, 5, 6 and 7). For the willow-650 °C treatment, aboveground biomass N uptake was less than 50% of the N uptake in the control treatment. In some fertilized biochar treatments, N uptake was even lower compared to the unfertilized control treatment, resulting in a negative NUE. Only the radish dry matter yield in the DTU experiment was not significantly affected by biochar application. However, radish plant growth showed a high variation and there was no clear positive growth response to N addition even in the untreated controls. Therefore, a reduced N availability can probably not be expected to show any distinct effects.

Deenik *et al.* [37] observed reduced crop growth and N uptake when charcoal with a high content of volatile matter was applied, which was explained by the presence of readily available C sources stimulating microbial activity and N immobilization. Our results show that also a biochar low in volatile matter can decrease crop growth. In contrast to our results, an increased dry matter yield and N fertilizer use efficiency were observed with biochar application in several pot and field experiments [15,23,38,39]. However, many of these experiments have been carried out at very low pH soils, as e.g., Van Zwieten *et al.* [23] observed an increased dry matter yield and N uptake efficiency in soil with pH-CaCl<sub>2</sub> 4.2, while in a higher pH soil (pH-CaCl<sub>2</sub> 7.7), the effects of biochar were inconsistent. This indicates that the positive response in the low pH soil could be partly explained by biochar's liming value. Soil pH was also significantly increased in the ILVO pot trial (by 0.02 to 0.25 units), but this was not translated into positive yield results, probably because the initial value of pH 5.5 was not limiting to plant growth and soil processes. Vaccari *et al.* [15] hypothesized that the improvement of physical soil factors such as lower bulk density and higher soil temperatures contributed to positive yield responses after biochar application in the field. However, such factors are probably not crucial for plant growth in pots and the reduced N availability outweighed potential positive effects.

In the ILVO trial, the higher soil NO<sub>3</sub><sup>-</sup> concentrations with biochar-650 °C addition compared to the control in the radish experiment seem unexpected. One possible explanation could be again the higher pore volume of the high temperature biochars. Some N could have been trapped into the micropores, unavailable for plant roots but still KCl-extractable. Biochar did not influence the DOC and TDN concentrations, nor at the start, nor at the end of the DTU experiment. This could be expected as both the labile biochar-C fractions and mineral N contents were very low, and is in line with results from Bruun *et al.* [4].

## 5. Conclusions

The results from our pot trials confirmed the expected reduced crop growth and NUE upon biochar application due to reduced soil NO<sub>3</sub><sup>-</sup> availability, as observed in an incubation experiment. This effect was biochar type dependent: the higher the pyrolysis temperature, the greater the reduction in NO<sub>3</sub><sup>-</sup> availability; adding willow biochar lowered NO<sub>3</sub><sup>-</sup> availability more than applying pine biochar. The

reduced  $\text{NO}_3^-$  availability was not caused by biochar's labile C fraction nor electrostatic  $\text{NH}_4^+/\text{NO}_3^-$  sorption to biochar. Hypotheses that deserve further investigation are non-electrostatic sorption of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  and stimulation of  $\text{NH}_3$  volatilization.

In conclusion, our research demonstrates that care has to be taken when applying freshly produced biochar to the field in temperate regions, as it could reduce short-term crop growth as a result of N immobilization. Hence, biochar addition might in some cases require increased fertilizer N application to avoid crop growth retardation. As a precautionary measure, it is therefore recommended to apply biochar some months before the main crop season starts to avoid negative effects of N immobilization on crop performance. More research is needed to (i) further clarify the exact mechanism causing reduced N availability and (ii) to study the fate of the immobilized N, as the observed N immobilization is possibly short-term. Furthermore, our results indicate that attention has to be paid when extrapolating results from one biochar-soil-crop combination. Our study also shows the need for combining process and agronomic experiments in order to get a better mechanistic insight into biochar soil-plant effects.

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## Supplementary

**Table S1.** Total metal concentrations of the biochars used ( $n = 1$ ).

	Al	Cu	Fe	Mn	Ni	Si	Ti	Zn
	mg kg <sup>-1</sup>							
Willow-450 °C	64.1	20.8	59.8	34.5	1.1	293	0.5	80.3
Willow-550 °C	208	20.1	121	46.3	3.2	494	2.8	95.5
Willow-650 °C	133	25.6	154	44.7	8.6	227	3.9	145
Pine-450 °C	191	5.4	173	44.5	2.0	321	5.0	1.0
Pine-550 °C	370	10.4	368	35.6	3.2	198	11.2	2.2
Pine-650 °C	205	16.9	135	40.7	1.9	153	6.1	5.1
	V	Ba	Na	Ca	Mg	K	Sr	B
	mg kg <sup>-1</sup>							
Willow-450 °C	2.7	11.2	627	9,755	1617	1858	81.6	15.1
Willow-550 °C	5.7	12.6	748	12,384	1863	3392	114	19.1
Willow-650 °C	<2.6	11.9	512	13,618	2093	4446	142	21.7
Pine-450 °C	<2.6	9.1	35.4	3,454	517	67.0	18.4	2.3
Pine-550 °C	<2.6	12.3	60.6	3,438	674	239	18.9	2.9
Pine-650 °C	<2.6	14.2	85.2	2,863	541	379	16.4	4.8

Concentrations of As, Be, Cd, Cr, Pb and Hg were below the detection limit.

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