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Hydrochar-Amended Substrates for Production of Containerized Pine Tree Seedlings under Different Fertilization Regimes

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Abstract: There is a growing body of research that recognizes the potentials of biochar application in agricultural production systems. However, little is known about the effects of biochar, especially hydrochar, on production of containerized seedlings under nursery conditions. This study aimed to test the effects of hydrochar application on growth, quality, nutrient and heavy metal contents, and mycorrhizal association of containerized pine seedlings. The hydrochar used in this study was produced through hydrothermal carbonization of paper mill biosludge at 200 °C. Two forms of hydrochar (powder and pellet) were mixed with peat at ratios of 10% and 20% (v/v) under three levels of applied commercial fertilizer (nil, half and full rates). Application of hydrochar had positive or neutral effects on shoot biomass and stem diameter compared with control seedlings (without hydrochar) under tested fertilizer levels. Analysis of the natural logarithmic response ratios (LnRR) of quality index and nutrient and heavy metal uptake revealed that application of 20% (v/v) hydrochar powder or pellet with 50% fertilizer resulted in same quality pine seedlings with similar heavy metal (Cu, Ni, Pb, Zn and Cr) and nutrient (P, K, Ca and Mg) contents as untreated seedlings supplied with 100% fertilizer. Colonization percentage by ectomycorrhizae significantly increased when either forms of hydrochar were applied at a rate of 20% under unfertilized condition. The results of this study implied that application of proper rates of hydrochar from biosludge with adjusted levels of liquid fertilizer may reduce fertilizer requirements in pine nurseries.

Keywords: containerized production systems; heavy metals; paper mill sludge; biochar-ash pellet; quality index

1. Introduction

Sweden is the world's second largest exporter of pulp, paper and wood products; this amounted to ≅ 125 billion Swedish krona (SEK)in 2017 [1]. There is about 28 million hectares of forest land in Sweden and pine trees constitute 40% of the total standing volume [2]. Although approximately 80 million m^3 of Swedish forest stands are harvested annually, the total standing volume has considerably increased in the last century [3]. In fact, a total of 400 million containerized tree seedlings are produced by Swedish forest nurseries to restock forests each year [3]. However, intensive annual forest harvests remove essential soil nutrients, which may cause problems for forest productivity.

In Sweden, container-grown seedlings are dominantly produced in peat and peat-based growth media [4]. Peat-based substrates have many advantages such as long-term drainage ability, good aeration for tree seedling roots, good fertilizer absorbance and release capability. However, peat-based media are considered non-sustainable as their extraction have adverse environmental impacts [5,6]. Hence, researchers tend to evaluate various growing substrate alternatives that could fully or partially replace peat [7]. Particularly, nutrient-rich growing media have become a special area of interest for research, as the growing global demand for wood harvest will cause further depletion of soil nutrients. Therefore, sustainable approaches towards forest production and plantation management are urgently needed.

Biochar has been introduced as an environmentally sustainable option to replace peat in nursery conditions [8–11]. Biochar is the carbonaceous residue of rapid biomass combustion in the absence or partial supply of oxygen (pyrolysis) [12]. Biochars as soil amendment not only contribute to storing carbon in the soils but also act as fertilizers [13,14], which subsequently reduces the environmental impact [15,16] and economic cost of plant production [17]. Moreover, biochar can increase substrate pH, improve water-holding capacity and enhance phyto-available nutrients by decreasing nutrient leaching and increasing cation exchange capacity (CEC) and consequently, enhance plant growth and productivity [18–21]. Biochar from paper sludge has also been characterized with high specific surface area [22] and has shown promising effect on simulating growth of plants such as *Lolium perenne* [23]. Biochar from pulp and paper mill sludge has been reported to have higher environmental performance relative to conventional disposal methods for sludge such as landfilling or incineration [24,25]. In many studies, the term 'biochar' is generally understood to mean 'pyrochar', which is a coproduct of fast pyrolysis or gasification. Another type of biochar is hydrochar, which is produced through hydrothermal carbonization (HTC). Therefore, it may have different physical structure and chemical composition, and consequently cause different effects on nutrient availability and plant growth compared to pyrochar [26,27]. Hydrochar is characterized by a low pH, high carbon content, high nutrient levels, good heavy metal absorption capacity and a carbon supply for microorganisms in the soil [18,28,29]. Generally, the effects of biochar on plant growth and physicochemical properties of the growing media vary depending on biochar feedstock, particle size and production process, e.g., temperature and heating duration [27,30]. Potentials of pyrochars and hydrochars as substrate components for production of tree seedlings in nurseries have not been well documented. Pyrochar has been evaluated in forms of either pellet or powder and shown promising results as nursery substrate for pine tree seedlings as well as sequestering carbon as part of normal reforestation [10,31,32]. Few empirical studies investigated the effects of hydrochar on tree seedling and soil nutrient cycling. Some studies reported adverse impacts of hydrochar derived from beet root chips on plant productivity and seed germination even when applied as low as 20–25% of volume of the growth mixture [33,34]. However, other studies offered contradictory findings about the effects of hydrochar on a fast-growing tree species: Baronti et al. [35] and George et al. [27] showed that biomass productivity and nitrogen use efficiency increased in poplar tree seedlings treated with hydrohar derived from maize (*Zea mays* L.) silage feedstock. These contradictory results concerning the effects of hydrochar on plant productivity call for careful choice of feedstock, application rate and the target species to ensure optimum growth benefits.

As hydrochar is usually friable and dusty, pelletization decreases dust formation, unifies its shape and size and facilitate transportation and distribution. Other benefits of reducing the loss of nutrients (e.g., nitrate and phosphate) and water, reducing bulk density and providing a beneficial environment for microbes as well as improved total porosity and aeration porosity in containers have been also reported by Di Lonardo et al. [20] and Dumroese et al. [10]. Thus far, however, there has been no discussion about the use of hydrochar pellets in nursery substrate constituent. Moreover, despite the potential significance of hydrochar, the effects of this byproduct on plant and microorganism interactions, e.g., mycorrhizal associations, is poorly understood. Ectomycorrhizal (ECM) associations play a key role in nutrient uptake in many woody plants, e.g., pine trees, such that growth and survival of these plants in forest ecosystems considerably rely on ECM fungi [36]. The existing body of research on biochar suggests positive effects of pyrochar on mycorrhizal symbiosis of the host plants, however, some substantial differences between hydrochar and pyrochar require assessment of hydrochar for any potential negative effects [33].

Therefore, the aim of this paper is to study the effects of hydrochar, derived from paper mill biosludge, on growth, quality, mycorrhizal associations and nutrient/heavy metal uptake of pine tree seedlings. We analyzed whether effects varied significantly between hydrochar forms (powder or pellets) or hydrochar proportions mixed with peat (10% or 20% hydrochar v/v). The effects of hydrochar addition on pine tree seedling was evaluated under three fertilization regimes (no fertilizer, 50% fertilizer and 100% fertilizer). We hypothesized that the growth, quality and mycorrhizal colonization of pine tree seedlings grown in substrate mixed with hydrochar would improve. We also expected pine tree seedlings grown with hydrochar to require less fertilizer to achieve similar or higher growth, mycorrhizal colonization and associated nutrient uptake relative to seedlings grown without hydrochar but with optimum rates of fertilizer (100% fertilizer). To the best of our knowledge, this current study is the first paper to explore the potentials of hydrochar powder and pellets for being used as a growing media component in production of containerized pine tree seedlings.

2. Materials and Methods

2.1. Hydrochar Preparation and Properties: Powder and Pellets

The hydrochar used in this study was produced through hydrothermal carbonization (HTC) of biosludge from a market kraft pulp mill. The HTC process started with combusting biosludge in Innventia's Parr-reactor (Relzow, Germany) for 2 h at 200 °C [37]. The attained solid and liquid were filtered and the resultant solid was then reslurried in deionized water at 70 °C and was filtered for a second time. We used the second filtered char as the experimental hydrochar. We enriched the experimental hydrochar by dry blending it with 5% wood ash (w/w) in a rotating mixer for 1 h. The main reason of adding wood ash to hydrochar was to improve the nutrient supply and to counteract the low pH of the raw hydrochar (pH 4.9). Moreover, having ash is in compliance with the current common practice of spreading ash into Swedish forests. Samples of hydrochar and ash were dried at 105 °C overnight and then ground to < 2 mm; the dry materials were then acid digested with ultra-wave digestion technique (Milestone© with unique Single Reaction Chamber (SRC)). Element contents were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The pH of hydrochar and ash was determined according to the methods outlined by Chen et al. [38]. Carbon and nitrogen content was quantified using Elementar Vario EL with TCD detector. Properties of ash and hydrochar are summarized in Table 1. Hydrochar pellets were produced at the discipline of Environmental and Energy Systems, Karlstad University (Karlstad, Sweden) by feeding the finely ground ash-enriched hydrochar powder into a single pellet press unit. The measured technical parameters for densification process of hydrochar included temperature (100 °C), compressive force (4 kN), holding time (30 s) and the speed of girder moving (5 mm/min). The length (18.32 mm) and diameter (8.85 mm) of hydrochar pellets were also measured.

Table 1. The pH, nutrient and heavy metal concentrations of the peat, hydrochar and wood ash used in this study.

Parameters	Peat	Hydrochar	Wood Ash
pH	5.17 ± 0.17	5.59 ± 0.12	11.95 ± 0.00
Total Carbon (%)	48.82 ± 0.04	49.20 ± 1.51	3.16 ± 0.88
Total N (%)	1.04 ± 0.01	2.26 ± 0.02	0.05 ± 0.01
P (g/kg)	0.28 ± 0.00	2.77 ± 0.66	8.68 ± 0.08
Ca (g/kg)	7.92 ± 0.08	2.72 ± 0.04	216.66 ± 4.26
K (g/kg)	0.39 ± 0.00	1.34 ± 0.02	19.33 ± 0.12
Mg (g/kg)	2.39 ± 0.01	1.34 ± 0.01	9.55 ± 0.13
Na (g/kg)	0.23 ± 0.00	0.81 ± 0.01	6.93 ± 0.09
Zn (g/kg)	0.01 ± 0.00	0.43 ± 0.00	1.19 ± 0.00
Cu (g/kg)		0.07 ± 0.00	0.06 ± 0.00
Ni (g/kg)		0.02 ± 0.00	0.01 ± 0.00
Pb (g/kg)		0.02 ± 0.00	0.02 ± 0.00
Cd (g/kg)		0.01 ± 0.00	
Cr (g/kg)		0.04 ± 0.00	0.06 ± 0.00

2.2. Experimental Design and Growth Condition

The experiment consisted of 12 unique treatment combinations: two factor levels of the factor "hydrochar type" (powder—PW; pellets—PL), two factor levels of the factor "hydrochar proportion" (10% and 20% v/v) and three factor levels of the factor "liquid fertilizer" (none, half and full rate). Square plastic containers (60 mL) were hand filled with a mixture of peat and either hydrochar powder or pellets at rates of 10% or 20% on a volume basis. Ten and 20 percent volume rates corresponded to mass-based proportions of 23% and 35% for the hydrochar powder and 35% and 49% for the hydrochar pellet treatment, respectively. Element contents of peat summarized in Table 1 was determined through the same approaches as for hydrochar and ash. The pH of peat was measured in a 1:5 solid water suspension (Table 1). Fertilizer regimes were determined and adjusted to our pot volume according to recommended Nitrogen (N) rates by Jackson et al. [39] for pine seedlings. Three pine seeds (*Pinus sylvestris*) were sown in each pot. Three weeks after sowing, seedlings were thinned to one per pot and were then fertilized once a week with a commercial liquid fertilizer (N:P:K, 100:13:65 + 4 Mg w/v; Wallco, Sweden) for 20 weeks. This resulted in a full rate of 2.4 mg N per seedling per week (100% fertilizer treatment) and a half rate of 1.2 mg N per seedling per week (50% fertilizer treatment). Treatments assigned to be unfertilized received only deionized water at fertilization times. There were 10 replicate pots in each treatment combination, including a control with neither forms of hydrochar applied. Irrigation frequency was determined using weight loss method [40] (Table A1). Therefore, the mass of each empty pot and its oven-dried growing medium (60 °C for 72 h) was recorded. Pots filled with assigned growing medium were watered with deionised water to their capacity (until saturated) and then left to freely drain for 60 min. The water content of each pot at container capacity was calculated by subtracting the weight of empty pot and oven-dry medium from the weight of the pot at container capacity (after 60 min of drainage). Pots were weighed daily and manually irrigated to container capacity when the average mass of the water in three pot replicates reached 75% of the water content at container capacity. The required amount of fertilizer was dissolved in the calculated irrigating water in designated fertilized treatments once a week. In order to adjust for substrate shrinkage and seedling biomass, recalculation of container capacity mass was done every two months.

The experiment was conducted at a fully controlled plant growth room at Karlstad University, Karlstad (59°24′12.59″ N and 13°34′32.39″ E). Pots were placed randomly in the growth room with constant temperature maintained at 23 °C throughout the first three weeks after sowing (germination stage). Temperature were then controlled between 23 °C and 16 °C for day and night, respectively. Relative humidity and photoperiod of the room was set at 60% and 18 h (~40,000 lux light intensity), respectively. Pine tree seedlings were grown in the aforementioned conditions for a period of six months and were rearranged monthly to minimize the effects of bench location.

2.3. Seedling Measurements

At 6 months after sowing, seedlings were harvested and their height and stem diameter at the root collar were measured. Seedlings were then dissected into root and shoot sections and the fresh mass of each section determined. Afterwards, they were dried in a fan-forced oven at 60 °C for 72 h and their dry mass were recorded. Determination of the nutrient/heavy metal composition of triplicate shoot samples (dried and ground to <2 mm) was done with ICP-OES following digestion using an ultra-wave digestion technique (Milestone© with unique Single Reaction Chamber (SRC)). Nitrogen content of plant samples was measured using Elementar Vario EL with TCD detector.

To compare the robustness and biomass distribution equilibrium in the pine tree seedlings, the quality index of Dickson (QID) was calculated using a formula first described by Dickson et al. [41] (Equation (1)):

$$QID = \frac{Total\ dry\ biomass\ (g)}{\left(\frac{Height\ (cm)}{Stem\ diameter\ (mm)} + \frac{Shoot\ dry\ weight\ (g)}{Root\ dry\ weight\ (g)}\right)} \quad (1)$$

2.4. Mycorrhizal Colonization Test

A second experiment was designed to examine potential effects of hydrochar on mycorrhizal symbiosis of pine tree seedlings. We inoculated hydrochar-amended substrates (mixed with 20% powder or pellet by volume) with a specific commercial ectomycorrhizal inoculant for coniferous trees (ectovit®-Mycorrhiza for Trees). These treatments were either fully fertilized to the recommended rate by Jackson et al. [39] or remained unfertilized (receiving only deionized water at fertilization times). A non-inoculated control treatment was included for each hydrochar type (PW and PL) in both fertilized and unfertilized regimes. We used 60 mL pots and 10 replicates in the same growth condition as in the first experiment. Seedlings were irrigated according to the weight loss method [40].

At six months after sowing, the root systems of the three replicates in each treatment were carefully washed free of soil with deionized water. Roots were cut into 2 cm segments and stored in 50% ethanol at 4 °C for further analysis. In order to quantify mycorrhizal root colonization, the gridline intersect method for ectomycorrhizal associations was used with a stereo microscope [42].

2.5. Data Analysis

The statistical analysis of the experiments was designed based on the hypotheses elaborated earlier in this paper. Firstly, in order to investigate the potential positive/negative effects of hydrochar type and application rates on growth of pine tree seedlings under the tested fertilizer levels, we used the natural logarithmic response ratios (LnRR), which is frequently used in meta-analysis (e.g., Brose et al. [43]) but also in laboratory experiments (e.g., Loydi et al. [44]). Each dependent variable, e.g., shoot dry mass and stem diameter, was standardized by the mean of the associated control treatment (without hydrochar addition) at each fertilization scenario. LnRR was calculated according to Goldberg and Scheiner [45] (Equation (2)):

$$LnRR = Ln\,\frac{(PT)}{(PC)} \tag{2}$$

where PT is the value of the treated sample and PC is the mean value of the control treatment. The effects of hydrochar type and hydrochar proportion were considered significantly different from the control at each fertilization regime, when the 95% confidence interval (CI) did not overlap with zero. This analysis allowed to directly compare pine seedling growth responses to 10 and 20% hydrochar powder and pellet addition in growing media under the tested nutritional regimes. To examine the significance of differences among treatments, the LnRR was then subjected to a General Linear Model (GLM) using IBM SPSS Statistics for Windows, Version 25.0.0.2 (Released 2017. Armonk, NY: IBM Corp).

Secondly, in order to test our second hypothesis about lower fertilizer requirements of hydrochar-treated seedlings compared to full-fertilized untreated ones (common nursery practice), LnRR of QID and nutrient/heavy metal uptake were standardized with the mean of the untreated seedlings grown with 100% fertilizer and were then subjected to a GLM using IBM SPSS Statistics for Windows, Version 25.0.0.2 (Released 2017. Armonk, NY: IBM Corp). This analysis allowed for examining whether addition of hydrochar may produce the same quality of seedlings but using less fertilizer relative to the control seedlings. We also examined the LnRR of mycorrhizal colonization percentage standardized with the mean of the full-fertilized control treatment. The discrepancy between untreated and hydrochar treated seedling were considered significantly different when the 95% confidence interval did not overlap with zero.

3. Results

3.1. Effects of Hydrochar on Growth Parameters

3.1.1. Shoot Dry Mass

Hydrochar application had either positive ($P < 0.05$) or neutral effects on shoot dry mass of pine seedlings in tested under different nutritional scenarios (Figure 1a). In the unfertilized treatment, shoot dry mass in pine seedlings receiving hydrochar powder was ~3 times higher than in their counterparts treated with hydrochar pellets (fertilizer × hydrochar type $P < 0.05$; mean ± standard error (SE): 0.225 ± 0.025 versus 0.080 ± 0.006) (Figure 1a). Higher rates of hydrochar application (20%) improved seedling shoot biomass in unfertilized and half fertilized conditions (fertilizer × hydrochar rate $P < 0.05$). The interaction among hydrochar type, application rate and fertilizer level was not significant ($P > 0.05$) (Figure 1a).

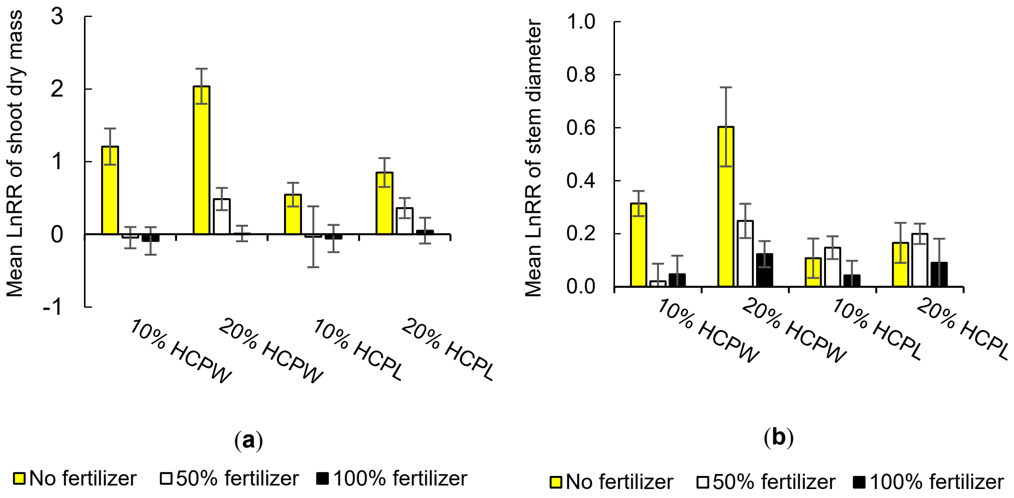

Figure 1. Mean (±95 CI, n = 10) natural logarithmic response ratios (standardized with the untreated controls at the same fertilizer level) of shoot dry mass (**a**) and stem diameter (**b**) of pine tree seedlings grown in peat mixed with 10% and 20% (v/v) hydrochar powder (HCPW) and hydrochar pellet (HCPL) in unfertilized (yellow bars), half (open bars) and full fertilized (black bars) conditions. Effects of hydrochar type and application rate were considered significant when 95% CI did not overlap with zero.

3.1.2. Stem Diameter

The results showed that replacing 20% of the growing media volume with hydrochar powder significantly increased the stem diameter compared to their untreated counterparts in all experimental fertilization scenarios ($P < 0.05$) (Figure 1b). Stem diameter increased when the 20% of hydrochar was mixed with the substrate under unfertilized and 50% fertilizer rate condition (fertilizer rate × hydrochar rate $P = 0.052$) (Figure 1b). Hydrochar powder increased stem diameter by 40% relative to hydrochar pellet under unfertilized condition (Fertilizer rate × hydrochar type $P < 0.05$; mean ± SE: 1.21 ± 0.02 versus 0.86 ± 0.06). However, there was no significant interaction between hydrochar type, rate and fertilizer addition ($P = 0.08$) (Figure 1b).

3.1.3. Quality Index of Dickson (QID)

To test whether hydrochar-treated seedlings needed less fertilizer than untreated ones, natural logarithmic response ratio of QID was standardized with the mean of untreated full-fertilized control. The results suggested that seedlings treated with 20% hydrochar powder or pellets, which received a 50% fertilizer dose, had similar QID than pine seedlings without any hydrochar addition that received 100% fertilizer (Figure 2, 95% CI overlapping with zero). When seedling were fertilized with 100% fertilizer, those grown with 20% hydrochar powder had 75% higher QID values than their untreated counterparts (mean ± SE: 0.07 ± 0.012 versus 0.04 ± 0.004, respectively) (Figure 2). There was no significant interaction among hydrochar type, hydrochar proportion and fertilizer addition ($P > 0.05$) (Figure 2).

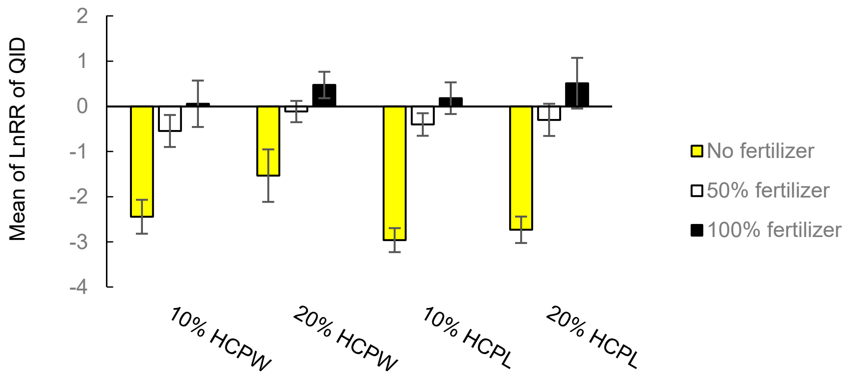

Figure 2. Mean (±95 CI, n = 4) natural logarithmic response ratios (standardized with the untreated full-fertilized control) of Quality Index of Dickson (QID) of pine tree seedlings grown in peat mixed with 10% and 20% (v/v) hydrochar powder (HCPW) and hydrochar pellet (HCPL) in unfertilized (yellow bars), half (open bars) and full fertilized (black bars) conditions. Effects of hydrochar type and application rate were considered significant, relative to controls receiving 100% fertilizer, when 95% CI did not overlap with zero in each nutritional regime.

3.2. Effects of Hydrochar on Above-Ground Nutrient and Heavy Metal Uptake

Shoot N, P, K, Ca and Mg uptake of pine tree seedlings grown with hydrochar under unfertilized condition was significantly lower than uptake of seedlings receiving 100% fertilizer ($P < 0.05$) (Figure 3). Nitrogen uptake reflected QID responses (Figure 3a). Using 20% hydrochar powder or pellet with 50% fertilizer resulted in pine seedling with similar N content to untreated seedlings grown under 100% fertilizer. Adding hydrochar under 100% fertilizer had mostly neutral impact on N uptake (Figure 3a). Shoot P uptake of seedlings treated with hydrochar powder and half or full rates of liquid fertilizer was similar to full-fertilized control ones (Figure 3b). Application of hydrochar pellets decreased shoot P acquisition in 50% fertilizer regime compared to untreated ones with 100% fertilizer rate ($P < 0.05$) (Figure 3b). Comparison of K uptake of hydrochar treated seedlings under 50% and 100% fertilizer with untreated seedlings receiving 100% fertilizer showed mostly positive or neutral impacts of hydrochar addition (Figure 3c). Addition of hydrochar powder and pellet with half and full rates of chemical fertilizer did mostly not significantly affect shoot Ca and Mg uptake. Only seedlings grown under fully fertilized conditions in peat amended with 20% (v/v) hydrochar had 63% higher Ca uptake than untreated seedlings (mean ± SE: 6.14 ± 0.14 versus 3.77 ± 0.60, respectively). There was no significant interaction between hydrochar type, rate and fertilizer level for shoot N, P, K, Ca and Mg uptake ($P > 0.05$).

Concerning heavy metals, our results suggested that the acquisition of Cu, Ni, Pb, Zn and Cr in seedling grown with 20% hydrochar in 50% fertilizer was not significantly different from what was observed in untreated seedlings (Figure 4). However, under the 100% fertilizer regime, application of both hydrochar powder and pellets resulted in significantly higher shoot Cu and Zn content in pine seedlings in comparison with untreated seedlings (Figure 4). Cadmium uptake also increased by 2–5 times when 20% of pot volume was mixed with hydrochar pellet or powder under 50% fertilizer application, respectively ($P < 0.05$) (Figure 4e). However, application of hydrochar pellet resulted in less Cd uptake by seedling compared to hydrochar powder in all tested nutritional regimes (fertilizer rate × hydrochar type $P = 0.004$) (Figure 4e). The shoot heavy metal uptake was not significantly affected by the interaction between hydrochar type, rate and fertilizer ($P > 0.05$).

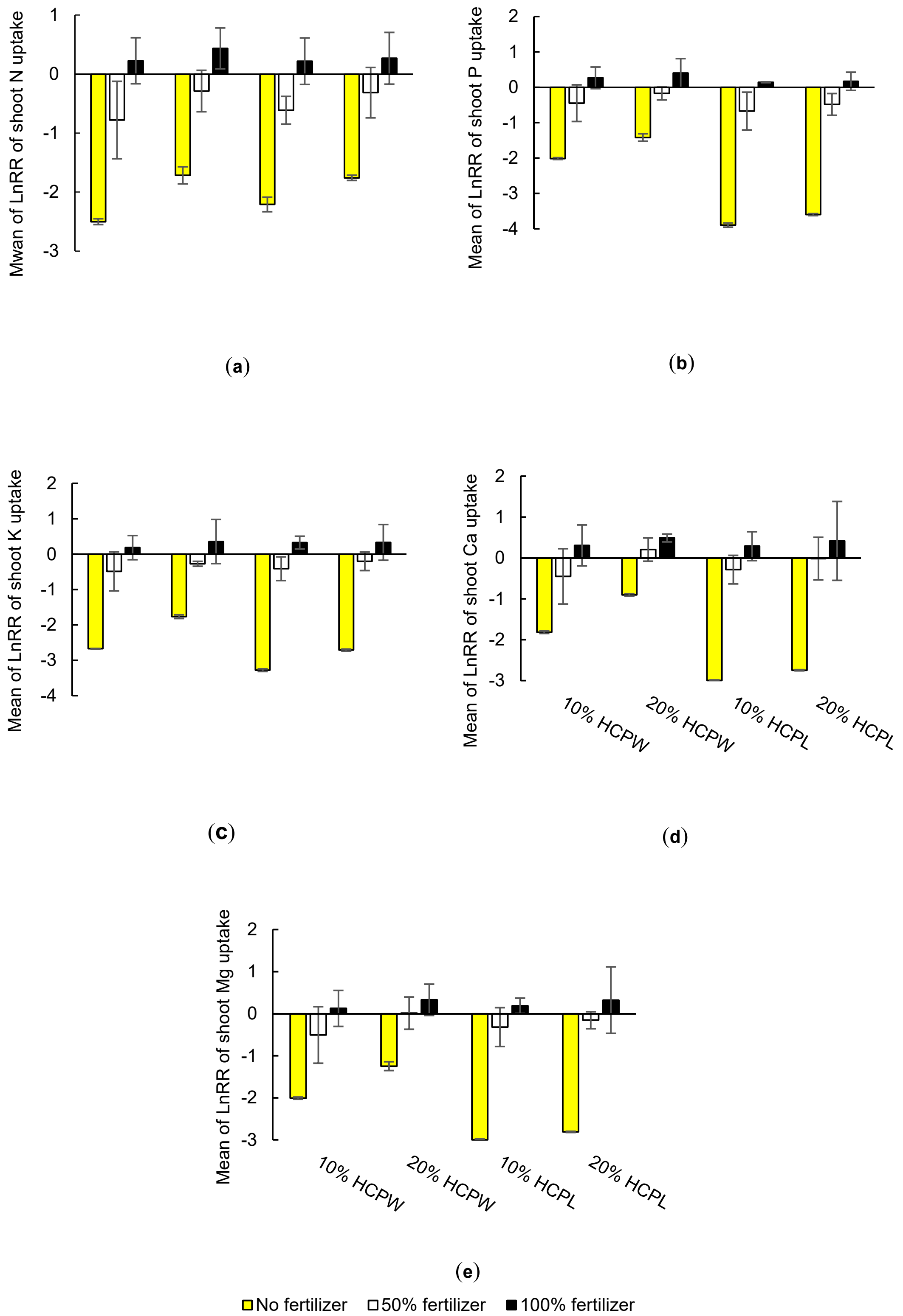

Figure 3. Mean (±95 CI, n = 3) natural logarithmic response ratios (standardized with the untreated fully-fertilized control) of shoot N (**a**), P (**b**), K (**c**), Ca (**d**) and Mg (**e**) uptake of pine tree seedlings grown in peat mixed with 10% and 20% (v/v) hydrochar powder (HCPW) and hydrochar pellet (HCPL) in unfertilized (yellow bars), half (open bars) and full fertilized (black bars) conditions. Effects of hydrochar type and application rate were considered significant, relative to controls receiving 100% fertilizer, when 95% CI did not overlap with zero in each nutritional regime.

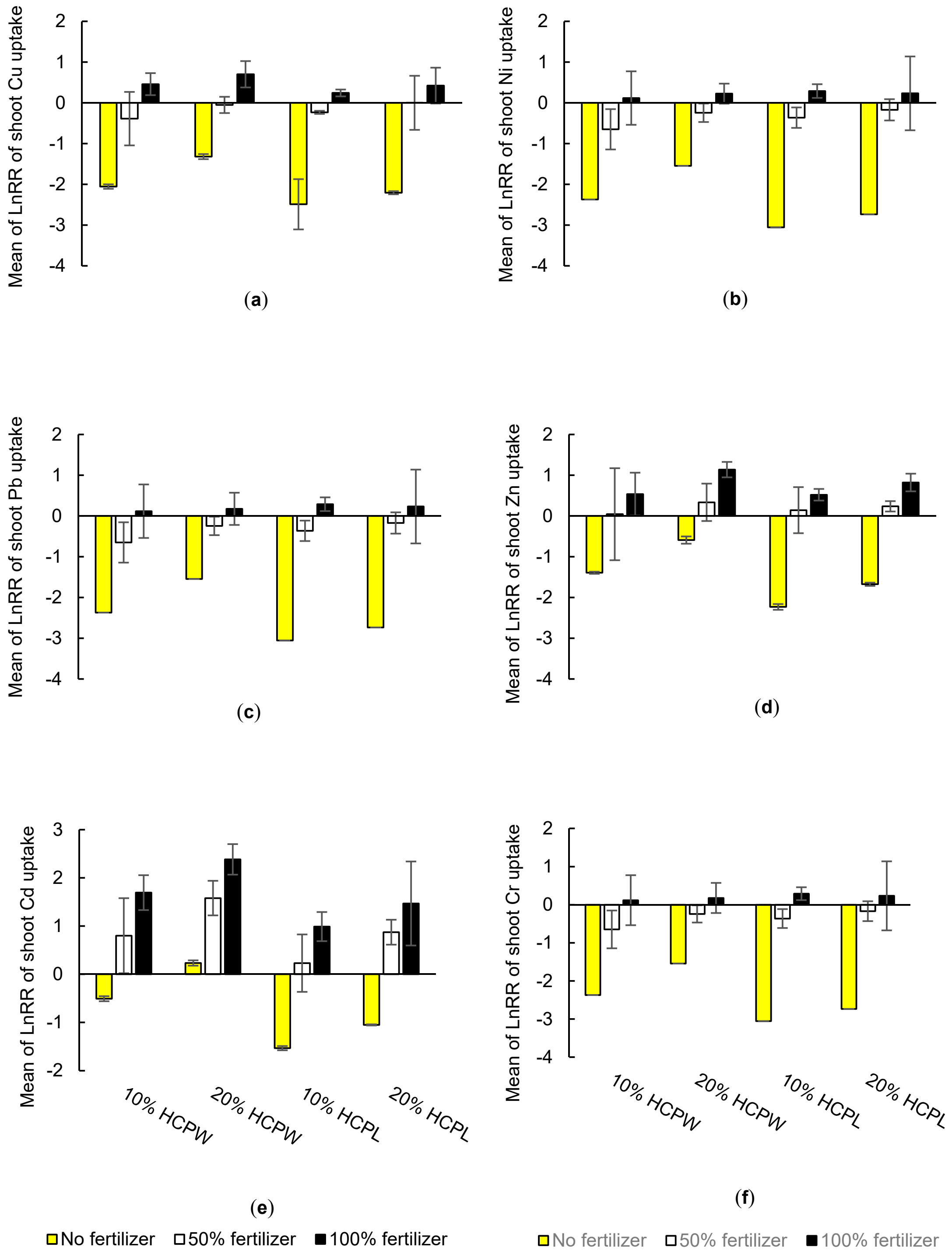

Figure 4. Mean (±95 CI, n = 3) natural logarithmic response ratios (standardized with the untreated fully-fertilized control) of shoot Cu (**a**), Ni (**b**), Pb (**c**), Zn (**d**), Cd (**e**) and Cr (**f**) uptake of pine tree seedlings grown in peat mixed with 10% and 20% (v/v) hydrochar powder (HCPW) and hydrochar pellet (HCPL) in unfertilized (yellow bars), half (open bars) and full fertilized (black bars) conditions. Effects of hydrochar type and application rate were considered significant, relative to controls receiving 100% fertilizer, when 95% CI did not overlap with zero in each nutritional regime.

3.3. Mycorrhizal Colonization Response to Hydrochar

The results of the second experiment showed that percentage of root length colonized by ectomycorrhizal fungi in seedlings grown with hydrochar amendment under unfertilized condition was 18% higher than in those grown without hydrochar but with 100% fertilizer ($P < 0.05$; mean ± SE: 39.6 ± 2.62 versus 21.5 ± 2.1) (Figure 5). However, neither powder nor pellet of hydrochar affected the colonization percentage of pine seedling roots under full-fertilized condition ($P > 0.05$) (Figure 5). The two-way interaction between fertilizer rate and hydrochar type was not significant ($P > 0.05$).

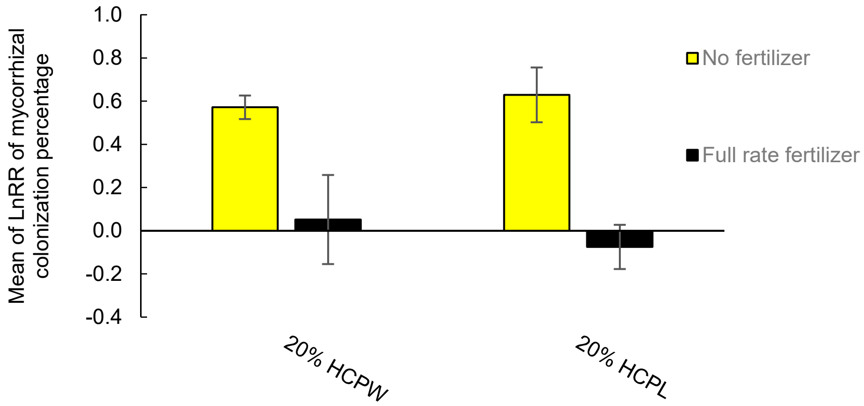

Figure 5. Mean (±95 CI, n = 3) natural logarithmic response ratios (standardized with the untreated fully-fertilized control) of mycorrhizal colonization percentage of pine tree seedlings grown in peat mixed with 20% (v/v) hydrochar powder (HCPW) and hydrochar pellet (HCPL) in unfertilized (yellow bars) and full fertilized (black bars) regimes. Effects of hydrochar type and application rate were considered significant, relative to controls receiving 100% fertilizer, when 95% CI did not overlap with zero in each nutritional regime.

4. Discussion

4.1. Shoot Dry Mass and Stem Diameter

This study is the first report of the effects of ash-enriched hydrochar powder/pellet applied as substrate constituents on growth and quality of pine tree seedlings. Amending growing media with hydrochar had positive or neutral effects on shoot biomass of seedlings compared to those grown in non-amended substrates under all experimental fertilization regimes (0%, 50% and 100% fertilizer), which supports the first hypothesis of this study (Figure 1). Although there is no published study on the potentials of hydrochar for production of containerized pine tree seedlings, our results are in accordance with those of George et al. [27] who reported 82% increase in above-ground biomass of hydrochar treated poplar seedlings in the first year and neutral effect in the second year of their study. We suggest that seedlings treated with hydrochar enriched with 5% ash in this study benefitted from improved nutrient availability in the rhizosphere that consequently enhanced shoot biomass. George et al. [27] showed that >24% of the total N in hydrochar treated poplar tree seedlings came from the char proving that hydrochar may behave as a direct source of nutrients for poplar seedlings. Our results contradict those of Belda et al. [30] who found that hydrochar from forest waste decreased stem dry weight of myrtle (*Myrtus communis* L.) and mastic (*Pistacia lentiscus*) seedlings by up to 75% and 49%, respectively. While limited studies are available on the use of hydrochar for container grown seedlings, the effects of pyrochar on pine seedlings have recently been investigated [10], showing similar growth of seedlings grown with ≤50% pyrochar (v/v) and in those grown in untreated peat. Under higher rates of nitrogen fertilizer, morphological traits were similar between pine seedlings grown with either 25% pyrochar powder or pyrolyzed wood pellets and in those grown in raw peat without biochar addition [10]. This is consistent with the results of the present study (Figure 1). Therefore, the growth response of plants may be idiosyncratic, depending on the biochar feedstock, biochar production processes and plant species. Toufiq et al. [28] showed that phytotoxic effects of hydrochar depended on feedstock and production conditions and do not necessarily occur in every hydrochar application.

Lower effects on seedling shoot biomass grown in peat amended with hydrochar pellets compared to those grown with hydrochar power under unfertilized condition, might be related to the fact that pellets release growth-affecting nutrients much more slowly. This may have important implications for potential long-term benefits of hydrochar pellets in forest soils after transplantation.

We found that amending peat with 20% hydrochar powder (v/v) increased the stem diameter compared to untreated control under all fertilizer regimes. Stem diameter is important for the survival of containerized pine and spruce tree seedlings after transplantation [46,47]. Approximately 20% of pine tree seedlings die during the first couple of growing seasons [48]. There is a strong positive relationship between initial stem-base diameter of spruce seedlings and their survival rate [47] so that containerized spruce with ~8 mm stem diameter showed low mortality rate through Pine Weevils (*Hylobius* spp.) and may be an alternative for insecticide-treated seedlings. Our finding, while preliminary, suggests that hydrochar application, especially hydrochar powder, in containerized pine tree seedlings may also enhance the survival rates after transplantation.

4.2. Quality of The Pine Tree Seedlings

Another aim of the present study was to estimate the potential of ash-enriched hydrochar for compensating fertilizer requirements of containerized pine tree seedlings. This was done using QID of different hydrochar application under the various fertilization scenarios. QID is one of the most comprehensive indices evaluating seedling quality [49]. We found that application of 20% (v/v) hydrochar as either powder or pellet and a 50% fertilizer dose resulted in the same quality of pine seedlings as seedlings growing in non-amended substrate but receiving full rate of fertilizer (optimum growth condition) (Figure 2). This result can be explained by boosted N, P, Ca and Mg availability in hydrochar-amended substrates (Figure 3).

These results suggest the possibility of decreasing chemical fertilizer application in pine tree nurseries by partially mixing the growing media with hydrochar powder or pellet. This probability had been previously brought to attention by Steinbeiss et al. [50], who believed that hydrochar, from yeast and glucose, could be added into soil as a fertilizer while also acting as a decadal carbon pool. The fertilizer effects of hydrochar might be attributed to stimulation of soil microorganisms that participate in nutrient recycling, improved water retention and CEC of the growing media due to large surface area of hydrochar [50]. However, our results should be interpreted with caution since we found only a relatively small reduction in irrigation frequency requirement (3–10%) (Table A1) and we did not analyze microbial activity or composition of the experimental growing media used in this study.

Under the full fertilizer regime, we found 75% increment in the quality index of the pine seedlings when grown with 20% hydrochar powder (v/v) in comparison with untreated seedlings. This is corroborated by Rezende et al. [51] and Aung et al. [49], who also found that biochar treatment significantly increased the quality index in *Tectona grandis*, *Quercus serrata* and *Prunus sargentii*. These researchers explained the observed increase in QID with improved water retention in growing substrates amended with biochar in a containerized production system. However, this might not be the case here as no significant difference was recorded in irrigation frequency demand of seedling grown with 100% fertilizer and 20% hydrochar powder and those with only full rate of fertilizer (Table A1). Indeed, it may be that these seedlings benefitted from better nutrient availability through either improved nutrient recycling by simulated beneficial microbes or direct supplement from hydrochar powder.

4.3. Nutrient and Heavy Metal Contents

The results of the current study showed that nutrient uptake of seedlings grown with either forms of ash-enriched hydrochar without addition of chemical fertilizer was significantly lower than in those grown in untreated peat with 100% fertilizer. Similarly, Sarauer and Coleman [21] found that N concentration in Douglas-fir seedlings was significantly lower in biochar treated seedlings fertilized with $\frac{1}{4}$ rate fertilizer relative to untreated ones with full rate of fertilizer. Therefore, it can be concluded that application of 10–20% hydrochar as powder or pellet without added fertilizer will not provide the seedlings with sufficient nutrients to achieve optimal growth and nutrient acquisition. Substrates amended with 20% hydrochar and 50% fertilizer produced seedling with similar N, P, K, Ca and Mg contents as non-amended but fully fertilized ones, which may result in similar seedling quality in both groups. Therefore, mixing 20% volume of the growing media with ash-enriched hydrochar from biosludge may provide sufficient plant-available nutrients to compensate for halved levels of fertilizer. Prior studies have noted that amending peat and perlite media with biochar can improve nutrient availability in these substrates [52]. Similarly, enhanced phytonutrient availability and phyto-stimulant ability of hydrochar-peat mixtures have been shown by Álvarez, et al. [53]. Furthermore, wood ash application to peat media can also boost foliar P and K concentrations [54].

Sludge of pulp and paper mill effluent contains significant amounts of heavy metals [55]. Due to environmental issues, considering data about heavy metal content is crucial before introducing hydrochar to either forestry or agriculture sectors. Hydrochar treated seedlings with 50% fertilizer that showed the same quality index as full-fertilized control ones also had similar heavy metal contents except for Cd. This may provide some support for the conceptual premise that the bioavailability of heavy metals existing in biosludge reduce when the sludge are hydrothermally carbonized. Devi and Saroha [55] believed that the bioavailability and eco-toxicity of the heavy metals in biochar derived from pulp and paper mill sludge declined because bioavailable and mobile heavy metals became relatively stable when pyrolyzed. Similarly, Liu et al. [56] found that exchangeable and reducible fraction of heavy metals in sewage sludge decreased by hydrothermal carbonization. The immobilized heavy metals in hydrochar considerably reduce the terrestrial ecotoxicity [24] and aquatic ecotoxicity impacts [25] of soil application of biochar.

Accumulation of heavy metals were previously suggested to cause negative effects of hydrochar on plants [28]. However, our results, while preliminary, proposes that hydrochar from biosludge, in combination with proper levels of fertilizer may safely be used in forestry and agriculture. However, the potential of using hydrochar in agricultural systems warrants further research.

However, Cd uptake in hydrochar-treated seedling receiving 50% fertilizer was significantly higher than in untreated seedlings receiving 100% fertilizer (Figure 4e). Likewise, unfertilized seedlings in peat amended with 20% hydrochar powder had greater Cd values than those grown fully fertilized without hydrochar. Considering the heavy metal contents of the peat, hydrochar and ash summarized in Table 1, accessible Cd for pine tree seedling was from the hydrochar source. The reason for this is not clear but it may be related to relatively high leaching potential of Cd from the hydrochar at low pH conditions of the experimental growing media (pH ~ 5). According to Devi and Saroha [55], Cd in biochar from paper mill sludge is mainly associated with the oxidizable fraction, which represents potentially bioavailable metals. They also found that potential ecological risk index for Cd in biochar derived from paper mill sludge pyrolyzed at 200 °C was significantly higher than for other heavy metals, i.e., Cr, Cu, Ni, Zn, Pb.

Our results showed increased accumulation of heavy metals by using hydrochar powder/pellet combined with 100% fertilizer. This result must be interpreted with caution because the interaction between root system and hydrochar-peat mixture is very complex, and therefore, it may partly be explained by either increased leaching of heavy metals from hydrochar in response to acidic exudates released by the developing root system and microbial community or direct uptake from hydrochar sources. Further work is required to test these relationships.

4.4. Colonization with Ectomycorrhizal Fungi

This is the first study describing ectomycorrizal responses of pine tree seedlings to hydrochar addition. Our results confirmed that application of either hydrochar powder or pellets, at a rate of 20% (v/v), boosted the percentage of root length colonized by ectomycorrhizal fungi of pine tree seedlings grown with no added fertilizer. Rillig et al. [33] reported higher root colonization percentage, spore germination and arbuscule formation by arbuscular mycorrhizae when *Taraxacum* was grown on soil mixed with 20% (v/v) hydrochar. This might be due to changes in physicochemical properties of the soil, e.g., nutrient status and pH, and signaling interaction in the root zone [33,57]. On the other hand, George et al. [58] found reduced mycorrhizal colonization and arbuscule formation when hydrochar was applied at rates of 5–10%. Indeed, different hydrothermal carbonization conditions and feedstock used to produce hydrochar might be responsible for contradictory results on mycorrhizal associations [28]. Unchanged mycorrhizal colonization percentage in hydrochar amended seedlings grown with 100% fertilizer relative to their untreated counterparts was not surprising, since pine tree seedlings might have reduced carbon transfer to the fungus due to high nutrient availability in the rhizosphere.

4.5. Environmental and Economic Implications of Using Hydrochar

Based on the results of the current study, peat substrate can successfully be replaced with hydrochar up to ratio of 20% (v/v). This substitution of peat, and even fertilizer, with hydrochar is important from environmental and economic point of views, particularly for nitrogen (N) fertilizer, the production of which is a greenhouse gas and energy intensive process. Moreover, peat use reduction due to replacement with hydrochar will result in less peat extraction and, therefore, will decrease the associated environmental impacts. Results of our previous studies showed that pyrochar and hydrochar production from paper mill sludge can significantly increase the environmental performance of sludge management and contribute to sustainable forest ecosystems [24,25]. Hydrochar-amended substrates can contribute to climate change mitigation with carbon sequestration as part of the normal reforestation. Potential contribution to climate change mitigation have been supported with studies of the life cycle carbon footprint of biochar systems [16,59,60] Moreover, potentials of using hydrochar pellets in containerized production systems add to the economic viability of HTC and densification processes. To date, no studies assessing the environmental and economic impacts of using hydrochar powder/pellets in nursery production or forest landscape have been published. Therefore, further study is recommended for scientific evidence of the sustainability and productivity of hydrochar use to assess the economic market and to identify and manage the associated risks of broad application in forestry sector.

5. Conclusions

The present study is the first study assessing the potentials of ash-enriched hydrochar application in containerized pine seedling production. We examined the effects of hydrochar addition in two forms (powder and pellets) and two mixing rates (10% and 20% v/v) under different nutritional regimes (no fertilizer, 50% fertilizer and 100% fertilizer). The most important finding was that hydrochar derived from biosludge did not adversely affect the growth of containerized pine tree seedlings. Moreover, we could show that pine tree seedlings grown in peat mixed with 20% hydrochar and 50% fertilizer rate had similar quality index values and nutrient and heavy metal uptake (except for Cd) to those that did not receive hydrochar but supplied with 100% fertilizer. Percentage of root length colonized with ectomycorrhizae also showed positive responses to hydrochar application. The results of our study imply that application of proper rates of hydrochar from biosludge with adjusted levels of liquid fertilizer may reduce fertilizer requirements in pine nurseries through recycling nutrients from forest waste materials. Furthermore, these findings have significant implications for the understanding of how forest industry can approach a circular bioeconomy. However, more information about the effects of hydrochar on chemistry of growing media and microbial activity would help to establish a greater degree of accuracy on this matter.

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Appendix A

Table A1. Irrigation frequency for 6 months-old pine tree seedlings grown with 10% and 20% hydrochar powder and pellet standardized with values of control pines (without hydrochar) with 100% fertilizer.

	Percentage of Peat Replaced (v/v)						
	0		10			20	
Fertilizer rate	100%	0%	50%	100%	0%	50%	100%
Peat	100						
Hydrochar Powder		66	94	96	72	97	100
Hydrochar Pellet		61	92	92	61	90	93

References

1. The Swedish Forest Industries' Statistics. Economic Importance. Available online: http://www.forestindustries.se/forest-industry/statistics/economic-importance/ (accessed on 4 April 2019).
2. Nilsson, P. Forest Land. Available online: https://www.slu.se/en/Collaborative-Centres-and-Projects/the-swedish-national-forest-inventory/forest-statistics/forest-statistics/skogsmark (accessed on 21 August 2018).
3. Mattsson, A. Reforestation challenges in Scandinavia. *Reforesta* **2016**, 67–85. [CrossRef]
4. Bohlin, C.; Holmberg, P. Peat: Dominating growing medium in Swedish horticulture. In Proceedings of the International Symposium on Growing Media and Hydroponics, Alnarp, Sweden, 8–14 September 2001; pp. 177–181.
5. Cleary, J.; Roulet, N.T.; Moore, T.R. Greenhouse Gas Emissions from Canadian Peat Extraction, 1990–2000: A Life-cycle Analysis. *Ambio J. Hum. Environ.* **2005**, *34*, 456–461. [CrossRef]
6. Alexander, P.; Bragg, N.; Meade, R.; Padelopoulos, G.; Watts, O. Peat in horticulture and conservation: The UK response to a changing world. *Mires Peat* **2008**, *3*, 8.
7. Dombrowsky, M.; Dixon, M. Sustainable growing substrates for potted greenhouse gerbera production. In Proceedings of the International Symposium on Responsible Peatland Management and Growing Media Production, Quebec City, Canada, 13–17 June 2011; pp. 61–68.
8. Tian, Y.; Sun, X.; Li, S.; Wang, H.; Wang, L.; Cao, J.; Zhang, L. Biochar made from green waste as peat substitute in growth media for *Calathea rotundifola* cv. *Fasciata*. *Sci. Hortic.* **2012**, *143*, 15–18. [CrossRef]
9. Vaughn, S.F.; Eller, F.J.; Evangelista, R.L.; Moser, B.R.; Lee, E.; Wagner, R.E.; Peterson, S.C. Evaluation of biochar-anaerobic potato digestate mixtures as renewable components of horticultural potting media. *Ind. Crop. Prod.* **2015**, *65*, 467–471. [CrossRef]
10. Dumroese, K.R.; Pinto, J.R.; Heiskanen, J.; Tervahauta, A.; McBurney, K.G.; Page-Dumroese, D.S.; Englund, K. Biochar Can Be a Suitable Replacement for Sphagnum Peat in Nursery Production of Pinus ponderosa Seedlings. *Forests* **2018**, *9*, 232. [CrossRef]
11. Matt, C.P.; Keyes, C.R.; Dumroese, R.K. Biochar effects on the nursery propagation of 4 northern Rocky Mountain native plant species. *Nativ. Plants J.* **2018**, *19*, 14–26. [CrossRef]
12. Huber, G.W.; Iborra, S.; Corma, A. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem. Rev.* **2006**, *106*, 4044–4098. [CrossRef]
13. Glaser, B.; Haumaier, L.; Guggenberger, G.; Zech, W. The 'Terra Preta' phenomenon: A model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **2001**, *88*, 37–41. [CrossRef]
14. Marris, E. Black is the new green. *Nature* **2006**, *442*, 624–626. [CrossRef]

15. Mohammadi, A.; Cowie, A.; Anh Mai, T.L.; de la Rosa, R.A.; Kristiansen, P.; Brandão, M.; Joseph, S. Biochar use for climate-change mitigation in rice cropping systems. *J. Clean. Prod.* **2016**, *116*, 61–70. [CrossRef]
16. Mohammadi, A.; Cowie, A.L.; Anh Mai, T.L.; Brandão, M.; Anaya de la Rosa, R.; Kristiansen, P.; Joseph, S. Climate-change and health effects of using rice husk for biochar-compost: Comparing three pyrolysis systems. *J. Clean. Prod.* **2017**, *162*, 260–272. [CrossRef]
17. Mohammadi, A.; Cowie, A.L.; Cacho, O.; Kristiansen, P.; Anh Mai, T.L.; Joseph, S. Biochar addition in rice farming systems: Economic and energy benefits. *Energy* **2017**, *140*, 415–425. [CrossRef]
18. Hu, B.; Wang, K.; Wu, L.; Yu, S.-H.; Antonietti, M.; Titirici, M.-M. Engineering Carbon Materials from the Hydrothermal Carbonization Process of Biomass. *Adv. Mater.* **2010**, *22*, 813–828. [CrossRef] [PubMed]
19. Laird, D.; Fleming, P.; Wang, B.; Horton, R.; Karlen, D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma* **2010**, *158*, 436–442. [CrossRef]
20. Di Lonardo, S.; Baronti, S.; Vaccari, F.P.; Albanese, L.; Battista, P.; Miglietta, F.; Bacci, L. Biochar-based nursery substrates: The effect of peat substitution on reduced salinity. *Urban For. Urban Green.* **2017**, *23*, 27–34. [CrossRef]
21. Sarauer, J.L.; Coleman, M.D. Biochar as a growing media component for containerized production of Douglas-fir. *Can. J. For. Res.* **2018**, *48*, 581–588. [CrossRef]
22. Paneque, M.; De la Rosa, J.M.; Franco-Navarro, J.D.; Colmenero-Flores, J.M.; Knicker, H. Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. *Catena* **2016**, *147*, 280–287. [CrossRef]
23. De la Rosa, J.M.; Paneque, M.; Miller, A.Z.; Knicker, H. Relating physical and chemical properties of four different biochars and their application rate to biomass production of *Lolium perenne* on a Calcic Cambisol during a pot experiment of 79days. *Sci. Total Environ.* **2014**, *499*, 175–184. [CrossRef]
24. Mohammadi, A.; Sandberg, M.; Venkatesh, G.; Eskandari, S.; Dalgaard, T.; Joseph, S.; Granström, K. Environmental analysis of producing biochar and energy recovery from pulp and paper mill biosludge. *J. Ind. Ecol.* **2019**. [CrossRef]
25. Mohammadi, A.; Sandberg, M.; Venkatesh, G.; Eskandari, S.; Dalgaard, T.; Joseph, S.; Granström, K. Environmental performance of end-of-life handling alternatives for paper-and-pulp-mill sludge: Using digestate as a source of energy or for biochar production. *Energy* **2019**, *182*, 594–605. [CrossRef]
26. Kambo, H.S.; Dutta, A. A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renew. Sustain. Energy Rev.* **2015**, *45*, 359–378. [CrossRef]
27. George, E.; Ventura, M.; Panzacchi, P.; Scandellari, F.; Tonon, G. Can hydrochar and pyrochar affect nitrogen uptake and biomass allocation in poplars? *J. Plant Nutr. Soil Sci.* **2017**, *180*, 178–186. [CrossRef]
28. Toufiq, M.R.; Andert, J.; Wirth, B.; Busch, D.; Pielert, J.; Lynam, J.G.; Mumme, J. Hydrothermal carbonization of biomass for energy and crop production. *Appl. Bioenergy* **2014**, *1*, 11–29. [CrossRef]
29. Fornes, F.; Belda, R.M. Biochar versus hydrochar as growth media constituents for ornamental plant cultivation. *Sci. Agric.* **2018**, *75*, 304–312. [CrossRef]
30. Belda, R.M.; Lidón, A.; Fornes, F. Biochars and hydrochars as substrate constituents for soilless growth of myrtle and mastic. *Ind. Crop. Prod.* **2016**, *94*, 132–142. [CrossRef]
31. Dumroese, R.K.; Heiskanen, J.; Englund, K.; Tervahauta, A. Pelleted biochar: Chemical and physical properties show potential use as a substrate in container nurseries. *Biomass Bioenergy* **2011**, *35*, 2018–2027. [CrossRef]
32. Robertson, S.J.; Rutherford, P.M.; Lopez-Gutierrez, J.C.; Massicotte, H.B. Biochar enhances seedling growth and alters root symbioses and properties of sub-boreal forest soils. *Can. J. Soil Sci.* **2012**, *92*, 329–340. [CrossRef]
33. Rillig, M.C.; Wagner, M.; Salem, M.; Antunes, P.M.; George, C.; Ramke, H.-G.; Titirici, M.-M.; Antonietti, M. Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. *Appl. Soil. Ecol.* **2010**, *45*, 238–242. [CrossRef]
34. Busch, D.; Kammann, C.; Grunhage, L.; Muller, C. Simple biotoxicity tests for evaluation of carbonaceous soil additives: Establishment and reproducibility of four test procedures. *J. Environ. Qual.* **2012**, *41*, 1023–1032. [CrossRef]
35. Baronti, S.; Alberti, G.; Camin, F.; Criscuoli, I.; Genesio, L.; Mass, R.; Vaccari, F.P.; Ziller, L.; Miglietta, F. Hydrochar enhances growth of poplar for bioenergy while marginally contributing to direct soil carbon sequestration. *GCB Bioenergy* **2017**, *9*, 1618–1626. [CrossRef]

36. Nara, K. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytol.* **2006**, *169*, 169–178. [CrossRef] [PubMed]
37. Ahlroth, M.; Bialik, M.; Jensen, A. Hydrothermal Carbonization of Pulp-and Paper Mill Effluent Sludge. *Bioresource Technol.* **2016**, *200*, 15–489.
38. Chen, H.; Zhou, Y.; Zhao, H.; Li, Q. A comparative study on behavior of heavy metals in pyrochar and hydrochar from sewage sludge. *Energy Sources Part A Recovery Util. Environ. Eff.* **2018**, *40*, 565–571. [CrossRef]
39. Jackson, D.P.; Dumroese, K.R.; Barnett, J.P. Nursery response of container *Pinus palustris* seedlings to nitrogen supply and subsequent effects on outplanting performance. *For. Ecol. Manag.* **2012**, *265*, 1–12. [CrossRef]
40. Dumroese, R.K.; Pinto, J.R.; Montville, M.E. Using container weights to determine irrigation needs: A simple method. *Nativ. Plants J.* **2015**, *16*, 67–71. [CrossRef]
41. Dickson, A.; Leaf, A.L.; Hosner, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. *For. Chron.* **1960**, *36*, 10–13. [CrossRef]
42. Brundrett, M.; Bougher, N.; Dell, B.; Grove, T.; Malajczuk, N. *Working with Mycorrhizas in Forestry and Agriculture*; Australian Centre for International Agricultural Research Canberra: Canberra, Australia, 3 January 1996.
43. Brose, P.H.; Dey, D.C.; Phillips, R.J.; Waldrop, T.A. A Meta-Analysis of the Fire-Oak Hypothesis: Does Prescribed Burning Promote Oak Reproduction in Eastern North America? *For. Sci.* **2012**, *59*, 322–334. [CrossRef]
44. Loydi, A.; Donath, T.W.; Eckstein, R.L.; Otte, A. Non-native species litter reduces germination and growth of resident forbs and grasses: Allelopathic, osmotic or mechanical effects? *Biol. Invasions* **2015**, *17*, 581–595. [CrossRef]
45. Goldberg, D.E.; Scheiner, S.M. ANOVA and ANCOVA: Field competition experiments. In *Design and Analysis of Ecological Experiments*, 2nd ed.; Scheiner, S.M., Gurevitch, J., Eds.; Oxford University Press Inc: Oxford, UK, 2001; pp. 77–98.
46. Selander, J.; Immonen, A.; Raukko, P. Resistance of naturally regenerated and nursery-raised Scots pine seedlings to the large pine weevil. *Folia For.* **1990**, *766*, 19.
47. Thorsen, Å.A.; Mattsson, S.; Weslien, J. Influence of Stem Diameter on the Survival and Growth of Containerized Norway Spruce Seedlings Attacked by Pine Weevils (*Hylobius* spp.). *Scand. J. For. Res.* **2001**, *16*, 54–66. [CrossRef]
48. Köster, E.; Pumpanen, J.; Köster, K. Biochar as a possible new tool for afforestation practices. In Proceedings of the EGU General Assembly Conference Abstracts, Vienna, Austria, 8–13 April 2018; p. 6082.
49. Aung, A.; Han, S.H.; Youn, W.B.; Meng, L.; Cho, M.S.; Park, B.B. Biochar effects on the seedling quality of Quercus serrata and Prunus sargentii in a containerized production system. *For. Sci. Technol.* **2018**, *14*, 112–118. [CrossRef]
50. Steinbeiss, S.; Gleixner, G.; Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol. Biochem.* **2009**, *41*, 1301–1310. [CrossRef]
51. Rezende, F.A.; Santos, V.A.H.F.D.; Maia, C.M.B.D.F.; Morales, M.M. Biochar in substrate composition for production of teak seedlings. *Pesqui. Agropec. Bras.* **2016**, *51*, 1449–1456. [CrossRef]
52. Locke, J.C.; Altland, J.E.; Ford, C.W. Gasified Rice Hull Biochar Affects Nutrition and Growth of Horticultural Crops in Container Substrates. *J. Environ. Hortic.* **2013**, *31*, 195–202. [CrossRef]
53. Álvarez, M.L.; Gascó, G.; Plaza, C.; Paz-Ferreiro, J.; Méndez, A. Hydrochars from Biosolids and Urban Wastes as Substitute Materials for Peat. *Land Degrad. Dev.* **2017**, *28*, 2268–2276. [CrossRef]
54. Hytönen, J. Wood ash fertilisation increases biomass production and improves nutrient concentrations in birches and willows on two cutaway peats. *Balt* **2016**, *22*, 98–106.
55. Devi, P.; Saroha, A.K. Risk analysis of pyrolyzed biochar made from paper mill effluent treatment plant sludge for bioavailability and eco-toxicity of heavy metals. *Bioresour. Technol.* **2014**, *162*, 308–315. [CrossRef]
56. Liu, T.; Liu, Z.; Zheng, Q.; Lang, Q.; Xia, Y.; Peng, N.; Gai, C. Effect of hydrothermal carbonization on migration and environmental risk of heavy metals in sewage sludge during pyrolysis. *Bioresour. Technol.* **2018**, *247*, 282–290. [CrossRef]
57. Warnock, D.D.; Lehmann, J.; Kuyper, T.W.; Rillig, M.C. Mycorrhizal responses to biochar in soil—Concepts and mechanisms. *Plant Soil* **2007**, *300*, 9–20. [CrossRef]
58. George, C.; Wagner, M.; Kücke, M.; Rillig, M.C. Divergent consequences of hydrochar in the plant—Soil system: Arbuscular mycorrhiza, nodulation, plant growth and soil aggregation effects. *Appl. Soil Ecol.* **2012**, *59*, 68–72. [CrossRef]

59. Mohammadi, A.; Cowie, A.; Mai, T.L.A.; de la Rosa, R.A.; Brandão, M.; Kristiansen, P.; Joseph, S. Quantifying the Greenhouse Gas Reduction Benefits of Utilising Straw Biochar and Enriched Biochar. *Energy Procedia* **2016**, *97*, 254–261. [CrossRef]
60. Cowie, A.; Woolf, D.; Gaunt, J.; Brandão, M.; Anaya de la Rosa, R.; Cowie, A. Biochar, carbon accounting and climate change. In *Biochar for Environmental Management: Science, Technology, and Implementation*; Lehmann, J., Joseph, S., Eds.; Routledge: Milton Park, UK, 2015; pp. 763–794.