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Effect of biochar amendment on the properties of growing media and growth of containerized Norway spruce, Scots pine, and silver birch seedlings

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Abstract

Common practices and several studies have demonstrated the positive effect of biochar amendment to climate change mitigation, soil properties and plant growth. We performed a greenhouse experiment to assess the potential of wood biochar to improve the properties of the growing media and the growth of seedlings in boreal tree species. We added willow biochar (0%, 5%, 10%, and 20%) to raw peat and measured the growth of Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and silver birch (*Betula pendula*) seedlings. In addition, the co-effect of biochar amendment with 0%, 50%, and 100% fertilization was estimated.

We found that using up to 10% of biochar did not reduce the water retention capacity of the growing media significantly. Moreover, biochar amendment increased significantly carbon, nitrogen, potassium and phosphorus concentrations and had significant liming effect on the growing media. The biochar amendment increased the aboveground growth of spruce seedlings, and root biomass as well as the root collar diameter of birch seedlings. Biochar amendment did not affect the quality of seedlings, estimated by the Dickson's quality index, for spruce and pine, while the quality of birch increased. Based on our results biochar has potential in forest seedling production.

Keywords: seedling growth; greenhouse experiment; biochar; growing media; fertilization

34 Introduction

35 Planting is the most common afforestation and reforestation practice in the Northern European
36 countries, including Finland. Special nurseries grow and provide seedlings, and since the 1960s
37 mainly containerized planting stock has been used (Räsänen 1982, Rikala 2002, 2012). Container
38 plants are preferred due to their increased resistance to planting stress and higher post-planting
39 survival (Griswold 1981, Thiffault et al. 2012). Although forest planting is an environmentally
40 friendly activity, there are some issues that are left unnoticed due to the common positive image
41 of forestation.

42 Peat is the most common growing medium used in tree seedling production throughout Northern
43 Europe, and there are presently no other good options on the market (Rikala 2012). However, the
44 Finnish Ministry of the Environment has listed peat extraction as one of the main stressors on the
45 quality of headwaters in Finland (Kauppila et al. 2016). Peat extraction for horticultural and
46 environmental purposes composes about 10% of overall peat production in Finland (Statistical
47 Yearbook of Finland 2017). Still, issues such as suspended solids in the runoff waters of milled
48 peat production (Selin 1996), reduced biodiversity, and increased carbon emissions (Alexander et
49 al. 2008, Caron and Rochefort 2013) could be decreased by reducing the share of peat used in
50 seedling production. A successful replacement of peat has to fulfill several requirements to gain
51 any interest among the nurseries. Grain size, porosity, water-holding capacity, pH, and cation
52 exchange capacity are the most important factors in terms of the plants (Heiskanen 1993a, Rikala
53 2012), while a low price of the substitute is significant for the producers. There are several options
54 for improving the quality of growing media and/or for reducing the use of peat. Significant amount
55 of published data can be found on the effects of growing media containing artificial mixtures of
56 peat and organic materials, such as rice hulls, kenaf core, coconut fiber, sand, sawdust etc.
57 (Worrall, 1981; Rose and Haase, 2000; Tsakalidimi, 2006; Tsakalidimi and Ganastas, 2016).
58 However, the costs of making these mixtures often exceed the received benefits (Heiskanen and

Rikala 1998, Rikala 2012). Perlite and vermiculite are the most commonly used additives to improve the aeration or soil water regulation of the growing media based on peat, respectively (Rikala 2012). The addition of compost to the peat-based growing media has also been suggested, but as the variation in the chemical, physical, and hygienic properties of compost-amended growing media is rather high, media-specific adjustments in growing conditions should be made (Heiskanen 2013). Experiments with Sphagnum mosses as a peat substitute in growing media have been rather successful (Tahvonen 2014, Kumar 2017), but the commercial use of mosses is still not common.

To achieve better growing conditions for the seedlings, the raw peat is limed and fertilized with synthetically produced inorganic salts (Rikala 2012), but the production and use of these components affect several environmental issues such as eutrophication, acidification, climate change and depletion of the ozone layer (Schultz and Waggoner 1999, Jacobs and Timmer 2005, Skowrońska and Filipek 2014). The use of fertilizers in forestry is insignificant compared with their use in agriculture, but still more than 10 million euros are spent on them in forestry in Finland every year (Statistical Yearbook of Finland 2017). Fertilizing cannot be totally avoided to promote the growth of seedlings (Jacobs and Timmer 2005), but decreased dosages could be beneficial for both nature and producers.

The use of biochar as an additive to or substitute for peat as a growing medium is not common and the idea is just starting to gain interest among both scientists and producers. Biochar is produced by burning biomass in conditions of limited oxygen and high temperatures, and it has been used as soil amendment to improve the fertility and physical properties of soil (Lehmann and Joseph 2015). Biochar addition to the soil could also be an efficient way to store carbon in the soil for a long period, thus helping to mitigate climate change (Lehmann and Joseph 2015). Although biochar as a growing medium component is rather expensive, the costs could be at least partly compensated for by the reduced need for fertilizers and liming components as dolomitic lime and

ground limestone. Biofuel–biochar coproduction, technological development, and policies promoting renewable fuels such as economic incentives and carbon credits will likely lower the price of biochar and promote the large-scale use of biochar as a growing medium and soil amendment in the future (Galinato et al. 2011, Campbell et al. 2018)

The type of feedstock used for biochar production is found to be important (Rajkovich et al. 2012, Lehmann and Joseph 2015). A study by Rajkovich et al. (2012) has shown that the type of feedstock used caused eight times more variation in maize growth compared with the variation caused by the pyrolysis temperature. Biochars produced from poultry litter increased the plant yield (Chan et al. 2008, Rajkovich et al. 2012) while biochar produced from some other organic waste feedstocks such as crab shells, and food waste have been shown to decrease the plant biomass (Rajkovich et al. 2012). Positive effect on both lettuce growth and soil microbial biomass was achieved by replacing up to 50% of peat by sewage sludge biochars (Méndez et al. 2017, Gaskó et al. 2018). Also, wood biochars have mostly been found to have positive effects on plant growth (Steiner and Harttung 2014, Nieto et al. 2016, Cho et al. 2017) due to their low contents of ash and other minerals (Gaskin et al. 2008).

Steiner and Harttung (2014) compared biochar with other growing media, such as perlite, clay granules, peat, and peat mixed with biochar, and they observed that after optimization (particle size and feedstock) biochar would be a perfect additive to reduce the amount of peat used in horticulture. Wood (pruning waste) biochar has also been tested as replacement of the peat by Nieto et al. (2016), who found that replacing brown peat with up to 75% of biochar increased significantly the plant biomass. Some studies of biochar as an additive to the growing media in forestry have been recently published (Robertson et al. 2012, Cho et al. 2017, Razaq et al. 2017, Dumroese et al. 2018). The results are rather promising as the growth of plants did not decrease with biochar treatment (Dumroese et al. 2018, Cho et al. 2017), and biochar amendment had a

significant positive impact on the root growth of all studied tree species (Robertson et al. 2012, Razaq et al. 2017).

However, the growth responses of three main nursery produced species in Northern Europe (Norway spruce (*Picea abies* (L.) H. Karst.), Scots pine (*Pinus sylvestris* L.), and silver birch (*Betula pendula* Roth)) to biochar amendment have not been studied. Containerized planting stock is highly preferred due to its better post-planting performance compared to bare root seedlings (Jäärats et al., 2016), but due to increasing environmental awareness, novel growing media mixtures are searched. The addition of biochar to the growing media of tree seedlings could be a novel solution to reduce the negative effects of tree seedling production on the environment, and it could also provide some other benefits both to the environment and to the producers. Still, optimal amounts of biochar to improve growth of seedlings and to reduce the need for fertilization and liming should be tested to provide important information for the producers of forest seedlings.

The main purpose of this study was to develop and test novel substrates in which to grow forest tree seedlings in nurseries. We aimed to study the impact of added wood biochar on the properties of the growing media and on the growth of Scots pine, Norway spruce, and silver birch seedlings. Specifically we aimed to test and to estimate the optimal amount of biochar to be added to the growing media to obtain the maximal growth of tree seedlings. Additionally, sub-treatments of fertilization were done, to test the interaction of biochar amendment and fertilization. Tested growing media included different amounts of biochar and fertilizers added to the raw peat. We hypothesized that: i) the use of liming components in growing media can be replaced by the use of biochar, ii) biochar amendment increases the growth of tree seedlings, iii) the optimal amount of biochar varies for different tree species, as species differ in their nutrient and water demand, and iv) the use of biochar allows a reduction in the amount of mineral fertilizers used in the production process of tree seedlings.

132

133 Materials and methods

134 Study design for greenhouse experiment

135 A greenhouse experiment was performed in June–November 2017 to study the effect of biochar
136 in growing media on the growth of tree seedlings. We tested the three most common tree species
137 grown in nurseries in Northern Europe: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea*
138 *abies* (L.) H. Karst.), and silver birch (*Betula pendula* Roth) (n = 588 seedlings in total per species).
139 Raw Sphagnum peat (Kekkilä, Brown 015W, pH 4.7, Finland; natural peat without fertilizers and
140 liming component), was used as the growing medium base and it was fertilized with a nitrogen /
141 phosphorus / potassium / magnesium oxide (NPK/MgO) starter fertilizer blend with micronutrients
142 (12-14-24+2) (Haifa North West Europe, Belgium) according to the product information provided
143 by the producer. To study the effect of biochar on the growing media and the growth of tree
144 seedlings, we used biochar produced from willow (Carbons Oy, Finland). The temperature of
145 pyrolysis was 450°C, and the particle size of the biochar was > 5 mm. Original pH of used biochar
146 was 6.4, and concentrations of main nutrients were: C = 3815.6 g m⁻³, N = 115.4 g m⁻³, P = 6.62 g
147 m⁻³ and K = 20.72 g m⁻³. Treatments with biochar included 0% (control), 5%, 10%, and 20%
148 (volume) of biochar (referred in text as B0%, B5%, B10% and B20%, respectively) added to the
149 growing medium (n = 147 seedlings per biochar treatment for each species). Plantek 49 F
150 containers with size 49 × 49 × 100 mm and cell volume 155 cm³ were used for growing the
151 seedlings (1 container of 49 plants for each combination of biochar and fertilization treatment).
152 These containers are typically used for growing medium sized spruce and pine seedlings (plant
153 height up to 26 cm for spruce and 16 cm for pine) (Rikala 2012).

154 Seeds of all tested species were provided by a certified provider and had tested germination rates
155 of 97%, 98%, and 66% for pine, spruce, and birch, respectively (Tapio Silva Oy, Finland). Three

pine seeds, three spruce seeds, and 10 birch seeds were sown per container in June, 2017. Two weeks after sowing, only one seedling was left to grow per cell.

Growing conditions were based on literature sources (Rowan 1987, Räsänen 1982, Zhigunov et al. 2011, Rikala 2012) as no commercial nursery was willing to share their exact practices. The seedlings were grown in a greenhouse under controlled conditions (temperature $> 20^{\circ}\text{C}$ (night) and $> 25^{\circ}\text{C}$ (day), air humidity 50%), and shade was provided when the solar radiation was $> 450 \text{ W m}^{-2}$. In August, extra light was used to extend the period of daylight. We decreased both night and daytime temperatures in September and October by 5°C per month to prepare the plants for winter. The containers were irrigated every second day with approximately 2.5 L of water per container, and the position of the trays was changed every two weeks to reduce the variation associated with environmental conditions such as unequal water spraying and light.

To investigate the interaction of biochar and fertilization, the study design included three sub-treatments: 0% (control), 50%, and 100% fertilization compared with common nursery practice (referred in the text as F0%, F50% and F100%, respectively) (Rowan 1987, Zhigunov et al. 2011). We used NPK (17-4-25) (Kekkilä kastelu, Kekkilä Oy, Finland) during July and August and PK (16-26) (Kekkilä syys, Kekkilä Oy, Finland) in September. Fertilizers were dissolved in water and added to the containers while watering the seedlings. In total, the 100% fertilized seedlings received $8.5 \text{ g m}^{-2} \text{ N}$, $11.6 \text{ g m}^{-2} \text{ P}$, and $28.1 \text{ g m}^{-2} \text{ K}$ during the growing period from July to October. For the 50% fertilization sub-treatment, the amount of each fertilizer was reduced by half.

Measurements

The water content of the growing medium was measured periodically during the whole growing period (June–November, 2017) (number of measurements: $n = 93$ per biochar treatment). Before every watering, we measured the soil water content in the containers, which had undergone different treatments and contained different species. For that, a soil moisture sensor (ThetaProbe

ML3, Delta-T Devices Ltd, Cambridge, UK) connected to a data reader (HH2 moisture meter, Delta-T Devices Ltd, Cambridge, UK) was used.

A ceramic pressure plate extraction method was used to measure the soil water retention capacity for different growing media mixtures and pure biochar (Klute 1986). The system was set up for pressure heads (water columns) of 10 cm, 30 cm, 60 cm, 100 cm, and 1000 cm (100 kPa). The change in the weight of the growing media was used to calculate the soil water potential.

Seedling growth was estimated by measuring their morphological traits at the end of the greenhouse experiment. A randomly selected 21 seedlings per treatment from every species were removed from the containers. The characteristics measured were the dry weight of the above- and belowground biomass, height of the plant, root length, root collar diameter, and the number of buds (both terminal and lateral buds counted). In addition, the root:shoot mass ratio was calculated. To characterize the quality of analyzed seedlings, we used Dickson's quality index (*DQI*) as an estimate of seedling quality (Dickson et al. 1960):

$$DQI = \frac{DW}{H} \frac{1}{\frac{RCD}{S:R} + 1}$$

, where *DW* = plant biomass dry weight (g), *H* = plant height (cm), *RCD* = root collar diameter (mm), and *S:R* = shoot:root ratio. Higher values of *DQI* potentially imply a higher quality of seedlings. Calculation and comparison of the Dickson's quality index is rather reliable and common practice to estimate the quality of seedlings (Dickson et al. 1960, Haase 2008, Tsakalimi et al. 2013, Currey et al. 2013, Lin et al. 2018). This quality index was originally designed for *Picea abies* (L.) H. Karst. and *Pinus strobus* L. (Dickson et al. 1960), and is proven to provide an objective tool for seedling quality estimation (Currey et al. 2013, Lin et al. 2018).

At the end of the experiment, physical and chemical traits of the growing medium were analyzed from six randomly selected samples per biochar×fertilization treatment. The pH and concentration

of carbon (C), nitrogen (N), potassium (K), and phosphorus (P) were analyzed from the growing media for all treatments at the end of the experiment (6 soil samples per treatment, in total $n = 72$). The pH of the growing medium was analyzed from a soil extract with a glass electrode (Standard pH meter, Radiometer Analytical, Lyon, France) in 35 mL soil suspensions consisting of 10 mL of soil sample and 25 mL of ultrapure Milli-Q water (left to stand overnight after mixing). The elemental concentrations of the growing media were analyzed from dried (at 105°C until constant weight) and homogenized (ground with a mortar grinder: Retsch, type RMO, Bioblock Scientific, Haan, Germany) samples. C and N concentrations were measured with an elemental analyzer (Vario Max CN elemental analyzer, Elementar Analysensysteme GmbH, Germany) operated in C/N mode. Concentrations of potassium (K) and phosphorus (P) were determined from $\text{HNO}_3\text{-H}_2\text{O}_2$ digestion (Koplik et al. 1998) by an inductively coupled plasma atomic emission spectrometer (ARL 3580 OES, Fison Instruments, Valencia, USA).

Data analysis

Data was balanced, with variances in the treatment and sub-treatment levels being similar. Normality check for the data and for the residuals of the linear mixed effect model was done with the Shapiro–Wilk test ($p > 0.05$), and logarithm transformations were applied for plant dry weight, shoot dry weight, root dry weight and plant height. The homogeneity of variances was tested using Levene's test.

A linear mixed effect model (PROC MIXED) was used to assess the effect of biochar, fertilization and their interaction on soil properties (pH, C, N, C:N ratio, P and K), and seedling growth parameters (plant dry weight, plant height, shoot dry weight, root dry weight, root length, root:shoot ratio, number of buds, root collar diameter and *DQI*) for each species separately. As *DQI* calculations are taking into account most of the measured variables (plant biomass dry weight, plant height, root collar diameter and shoot:root ratio), the linear mixed effect model was used to

227 explain how experimental factors affected the *DQI* of different tree species seedlings. In all models
228 container was a random factor, and seedlings within container were random and nested factors.
229 Biochar, fertilization and their interaction (biochar×fertilization) were fixed factors.
230 Tukey's HSD post hoc test (for all pairwise tests) and/or Dunnett's T3 test (comparing one level
231 of treatment pairwise with all the other treatment levels) were employed to compare differences
232 within treatments.
233 All the statistical analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC,
234 USA). All probabilities were tested at a significance level of $p < 0.05$.

235

236 Results

237 Impact of biochar amendment and fertilization on the growing media

238 The water retention capacity of the peat was much higher compared with that of the biochar, so
239 adding biochar decreased the water retention of the growing media. However, the water retention
240 capacity of the growing media with 10% added biochar was rather similar to that of the pure peat
241 (Fig. 1a). The amount of added biochar decreased significantly ($p < 0.001$) soil moisture content
242 with soil moisture content being the lowest for the 20% biochar treatment (Fig. 1b).

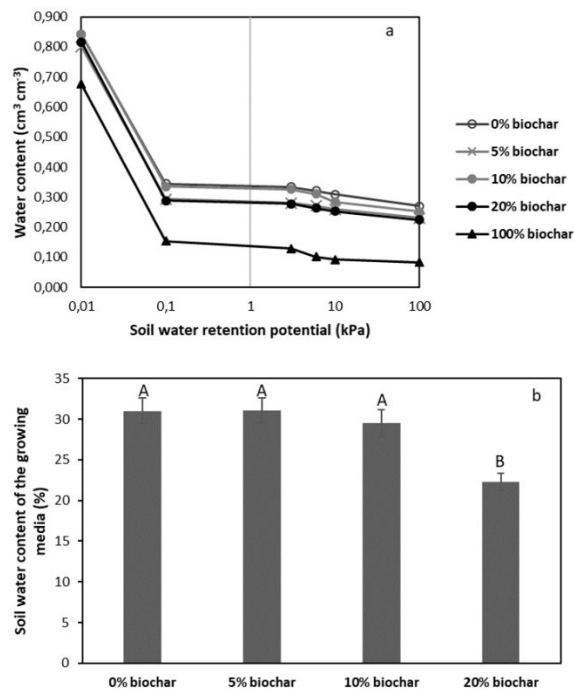


Figure 1. Impact of biochar amendment on the hydrological conditions of the growing media. a) Water retention curves of control (pure peat), three biochar treatments and pure biochar. Water retention capacity measured at pressure heads of 10, 30, 60, 100, and 1000 cm (100 kPa). b) Average soil water content of the growing media during the first growing season. Soil water content was measured before every watering ($n = 93$ measurements per treatment). Error bars represent the standard error. Letters above the bars indicate the statistically significant difference ($p < 0.05$).

Pure biochar had $\text{pH} = 6.40$, which was much more alkaline compared with the used raw peat (measured value of $\text{pH} = 4.40$). Biochar amendment had a significant liming effect ($p < 0.0001$) (Table 2a) reflected in significantly higher growing media pH, ranging between 4.28 – 4.63. Within different biochar treatments, fertilization decreased pH (Table 1 and Table 2a). However, we found that biochar \times fertilization interaction had significant effect on pH, thus, the growing media mix with 20% added biochar and 100% fertilized sub-treatment (F100% B20%) had significantly smaller pH ($p < 0.0001$) compared to 0% and 50% fertilized and 20% biochar amended sub-treatments (Table 1). Similar trend was also observed for 10% biochar treatment, where 50% and

100% fertilization sub-treatments had significantly lower pH compared to 0% fertilization sub-treatment. However, in case of 5% biochar treatment, there was no significant difference between the sub-treatments; the addition of fertilizers did not change the soil pH of 5% biochar treatment significantly.

The effects of added biochar on nutrient concentrations in the growing media were measured at the end of the growing season. Biochar amendment affected significantly C ($365.8 - 450.5 \text{ g m}^{-3}$), N ($11.9 - 12.6 \text{ g m}^{-3}$), P ($1.86 - 3.23 \text{ g m}^{-3}$) and K ($7.04 - 10.31 \text{ g m}^{-3}$) concentration of the growing media (Table 2a). C concentration of the growing media amended with 10 and 20% of biochar was significantly ($p < 0.0001$) higher compared to 0 and 5% biochar treatments (Table 1). Fertilization had no effect on the C concentration of the growing media (Table 2a). C:N ratio of the growing media increased with the amount of added biochar ($30.7 - 35.7$) (Table 1), and it was significantly affected by the amount of added biochar and fertilization to the growing media, and by biochar×fertilization interaction (Table 2a). The N concentration of the growing media was lowest in 0% biochar treatment, followed by the 5 and 20% biochar, and the highest N concentration was in the 10% biochar treatment (Table 1). Fertilization itself had no effect on the N concentration (Table 2a), but while studying the biochar×fertilization interactions we found that concurrently highest N concentrations were in the growing media with 10% added biochar and 100 and 50% fertilized sub-treatments (F100% B10% and F50% B10%) (Table 1). The lowest N concentration ($p < 0.0001$) was detected for the sub-treatment with no added biochar and 50% fertilized (F50% B0%) growing media (Table 1, 2a). Addition of biochar and fertilization affected significantly the concentration of P and K in the growing media (Table 2a). The highest concentration of both nutrients was found in the 20% biochar treatment and for the 100% fertilized sub-treatment (Table 1), respectively, P and K concentration was significantly affected by biochar×fertilization interaction (Table 2a). The highest P concentration was observed in the 100% fertilized and no biochar added growing media (F100% B0%), followed by F100% B20%, F100% B10% and

F100% B5%, respectively (Table 1). For K concentration the highest values were measured in the growing media fertilized by 100% and 5% biochar amended sub-treatment (F100% B5%) (Table 1).

Impact of biochar amendment on the growth of Norway spruce seedlings

Biochar amendment had a significant effect on the shoot dry weight and the height of spruce seedlings (Table 2b). Overall, fertilization increased seedling dry weight, shoot dry weight, height and root collar diameter, while the interaction of biochar and fertilization had no effect on the growth of spruce seedlings (Table 2b). For the shoot dry weight of the spruce seedlings the best growing media mixture had 10% of added biochar and it was fertilized as in common nursery practices (F100% B10%), as spruce seedlings grown with this mixture had significantly higher shoot dry weight compared to others (Table 3a). The same growing media mixture (F100% B10%) also resulted in the largest height growth of spruce seedlings, although the difference with the other combinations was not significant (Table 3a). The seedling quality of spruce, in terms of *DQI* was not affected by any factor (Table 2b, Fig. 2a).

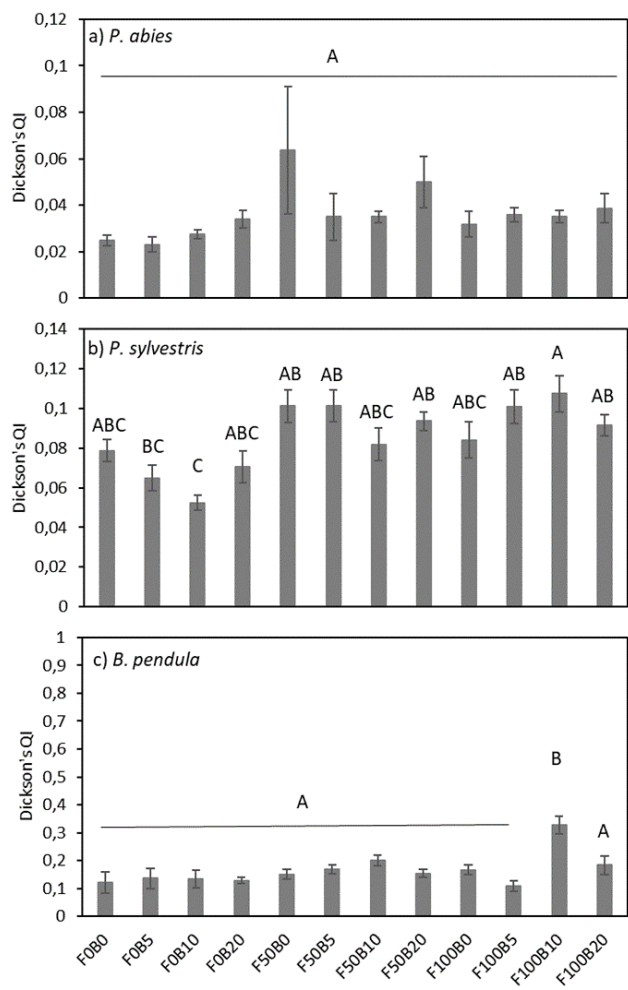


Figure 2. Dickson's quality index (*DQI*) of Norway spruce (*P. abies*) (a), Scots pine (*P. sylvestris*) (b), and silver birch (*B. pendula*) (c) seedlings for different biochar treatments (B) and fertilization sub-treatments (F). Numbers following F and B on the x-axis indicate the amount of fertilizer (% of nursery practice) and biochar (% volume of growing media) used, respectively. Letters above the bars indicate the statistically significant difference between treatments ($p < 0.05$).

Impact of biochar amendment on the growth of Scots pine seedlings

Biochar amendment decreased significantly the shoot dry weight and height of pine seedlings (Table 2c), as the highest values for both of these variables were observed for pine seedlings grown with 0% biochar (Table 3b). Fertilization increased almost all measured and calculated variables,

excluding root length (Table 2c). Biochar×fertilization interaction affected shoot dry weight, root length, root collar diameter and *DQI* of pine seedlings (Table 2c). Fertilization had the biggest effect on the shoot dry weight of pine seedlings, as the significantly highest values were observed in seedlings grown with 100% fertilization and with 0, 10 and 20% of biochar amendment (F100% B0%, F100% B10% and F100% B20%) (Table 3b). In case of root collar diameter the significantly highest values for pine seedlings were observed in case of F100% B10% and F50% B0% (Table 2c, 3b). However, fertilization increased the growth of root collar diameter of pine seedlings (Table 3b). Seedling *DQI* was not affected by biochar amendment ($p = 0.5383$), while the effect of fertilization ($p < 0.0001$) and biochar×fertilization interaction ($p = 0.0498$) were both significant (Table 2c, Fig. 2b). The lowest *DQI* values were observed for seedlings grown without fertilization (F0%), and the significantly highest value was observed for seedlings amended with 10% of biochar and fertilized as in common nursery practices (F100% B10%) (Fig. 2b).

Impact of biochar amendment on the growth of birch seedlings

Biochar amendment affected significantly root dry weight, root collar diameter and *DQI* of birch seedlings (Table 2d). Fertilization increased significantly all measured and calculated variables (Table 2d). Biochar×fertilization interaction affected significantly the number of buds, root dry weight, root length, root collar diameter and *DQI* of birch seedlings (Table 2d). Fertilization had bigger effect on the growth of birch seedlings compared to biochar amendment, as the lowest values for almost all measured variables were observed 0% fertilized birch seedlings (Table 3c). Seedlings amended with 10% biochar and 100% fertilizers (F100% B10%) had the highest shoot and root dry weight, number of buds and root collar diameter, which also resulted in highest *DQI* values i.e. the quality of seedlings (Table 3c, Fig. 2c).

Discussion

The main aim of this study was to determine the effect of wood biochar amendment on the properties of the growing media and on the growth of spruce, pine and birch seedlings. Our results suggest that willow biochar could be an option to reduce the use of peat in forest nurseries. A species-specific optimal amount of biochar used in the tree seedling nurseries would have a positive effect on the growth of spruce (20% of biochar) and pine seedlings (10-20% of biochar), and on the quality of birch seedlings (10% of biochar). In addition, it would have a significant environmental effect through a reduced need for peat, liming, and fertilization during the seedling production process.

We observed that addition of biochar up to 10% had no significant impact on the water-holding capacity of the growing media (Fig. 1a). Results of earlier studies suggest that biochar amendment has a strong positive effect on the water-holding capacity of the growing media (Novak et al. 2012, Basso et al. 2013, Mulcahy et al. 2013, Githinji 2014, Rasa et al. 2018, Dumroese et al. 2018), but in these cases sandy or clay substrates were used as bases for the growing media. The water-holding capacity of the raw peat is rather high (Kitir et al. 2018, Dumroese et al. 2018, Markoska et al. 2018), even up to 90% (Heiskanen 1993b, Rikala 2012). It is significantly higher compared with that of the willow biochar used in our study, so adding biochar decreased the water retention of the studied peat growing media. So based on our results it is important to consider the amount of biochar in the peat-based growing media due to biochars' lower water holding capacity compared to raw peat (Fig. 1a).

Due to its alkaline character, biochar could easily replace the liming components of the growing media. At the moment, limestone or dolomite dust is incorporated into peat-based substrates to neutralize acidity (Fisher et al. 2006, Rikala 2012). Extraction of these materials has a considerable environmental effect, which could be reduced by lower utilization. Steiner and Harttung (2014)

studied the liming effect of rather high concentrations of biochar added to peat and found that biochar has a significant liming effect. We observed that biochar amendment had significant effect on the pH values of growing media (Table 2a), and even small amounts of added biochar significantly increased the pH values of the growing media (Table 1). Wood biochars are found to have lower pH values than manure biochars (Rajkovich et al. 2012, Lehmann and Joseph 2015), so their liming effect might be less efficient. However, even if the original pH of the willow biochar used in our treatments was not highly alkaline, it still increased significantly the pH of the growing media (Table 2a), and therefore it would be a suitable replacement for the current liming components.

Depending on the feedstock and pyrolysis time and temperature, biochar contains a significant amount of nutrients, varying in quantity and quality (Lehmann and Joseph 2015), and therefore can significantly affect the elemental concentrations of the growing media. As biochar used in our experiment contained much more N compared with the peat used, we expected that the biochar amendment would have a significant effect on the N concentration of the growing media. This result was well pronounced, as both biochar and biochar×fertilization interaction had significant effect on the N concentration of the growing media (Table 2a). Biochar as a C-rich substance (Lehmann and Joseph 2015) significantly increased the C concentration of the growing media (Table 2a), resulting in higher C:N ratios (Table 1). It has been suggested that biochar with a C:N ratio lower than 30 could be used as a soil conditioner (Lehmann and Joseph 2015). The C:N ratio of biochar used in our treatments was slightly higher (33.1) compared with that of pure peat (30.7) (Table 1). Besides the quality/availability of C and N, the soil C:N ratio is known to be a major factor determining the microbial community and its activity in the soil (Rousk et al. 2011), and through that it is a significant promoter of plant growth (Jacoby et al., 2017). However, final conclusions on that matter cannot be made at this point, as additional analysis of the microbial community is needed.

The biochar used had higher concentrations of K and P compared with those of the pure peat, thus adding biochar to the growing media significantly increased the concentrations of both of these nutrients (Table 2a). The importance of these nutrients increases at the end of the growing season, as K and P are needed for the winter preparation of seedlings (Zhigunov et al. 2011). Although some sources claim that the significance of K during autumn fertilization is not proven (Rowan 1987, Rikala 2012), we used protocols provided by Zhigunov et al. (2011) to calculate the amount of fertilizers for the sub-treatments. Fertilization had a significant impact on the P and K concentrations of the growing media, while N was not affected (Table 1, 2a). However, we analyzed the nutrient concentrations at the end of the growing season, so at that time most of the available N was used by the seedlings, while additional P and K were provided for the fertilization sub-treatments during the last month before the analysis.

Different studies in the field of agriculture have proven that biochar has a significant effect on plant growth (especially on the growth of roots) and on the drought resistance of seedlings (Rajkovich et al. 2012, Mulcahy et al. 2013, Prendergast-Miller et al. 2013). We observed that biochar amendment had a species-specific effect on the growth of tree seedlings, but the effects were strongly influenced by fertilization. Our results on the root growth of birch seedlings are similar to Mulcahy et al. (2013) and Razaq et al. (2017), who also observed that biochar had positive effect on the root growth. While for spruce and pine seedlings biochar amendment had no effect on the growth of root biomass and root length (Table 3). Possible reasons for the promoted root growth could be decreased bulk density of the growing media caused by biochar addition (Lehmann and Joseph 2015) and/or a decreased amount of available inorganic N for seedlings. Biochar particles can effectively retain soluble plant-available inorganic N in the soil (Gruffman et al. 2012, Prendergast-Miller et al. 2013). Increased root growth and higher root:shoot ratios of seedlings are generally associated with improved post-planting establishment and better field performance (Grossnickle 2005, Gruffman et al. 2012). Biochar amendment did not affect the

root:shoot ratio of analyzed species as we expected, as there was no statistically significant effect for any analyzed species (Table 2b, 2c, 2d). The main effect on the root:shoot ratio of pine and birch seedlings came from fertilization. Increased root:shoot ratio was measured for pine seedlings in case if no fertilizers were used (for F0% sub-treatments) (Table 3b), while for the birch seedlings these sub-treatments resulted in the lowest root:shoot ratios (Table 3c). Based on our results, we cannot definitely conclude that adding biochar to the growing media might improve the post-planting survival of these species. In addition, significant negative effect of biochar on shoot growth of pine (Table 3b) can also have significant negative effect on the vitality of these seedlings.

The most used seedling quality standards are seedling height and root collar diameter (Haase 2008, Ivetić et al. 2016). Adding willow biochar to the growing media increased significantly the height and shoot dry weight of the spruce seedling, and root collar diameter of the birch seedlings (Table 2), which confirms that biochar amendment has some positive effect on the quality of these two studied species. The tallest seedlings and largest root collar diameters were achieved for all studied species, if fertilizers were used as in common nursery practices (100% fertilization) (Table 3). These results are similar to Robertson et al. (2012), who also found that biochar+fertilizer treatment increased the shoot growth and biomass of lodgepole pine and sitka alder. While their treatments included biochar amendment up to 10% biochar (dry mass basis), during our experiment the highest values of seedling height and root collar diameter were achieved with 10-20%, 0-5% and 10-20% of added biochar for spruce, pine and birch, respectively (Table 3).

Increased soil fertility is commonly associated with a decreased root:shoot ratio, as fertilization (as any other factor that improves the growing conditions) decreases the need for larger root biomass to absorb more nutrients (Harris 1992). We found that fertilization had no significant effect on the root:shoot ratio of spruce seedlings (Table 2b). However, we observed smaller values of the root:shoot ratio for fertilized (both for F50% and F100%) pine and birch seedlings (Table

3). Dickson's quality index takes into account several variables and therefore could provide significant information about the overall quality of the seedlings (Tsakalimi et al. 2013, Currey et al. 2013, Lin et al. 2018). As expected, fertilization significantly increased the growth of all analyzed species, resulting in increased values of *DQI* (Fig. 2). This result allows us to assume that the overall seedling quality benefited from fertilization. For spruce, the highest *DQI* values were achieved for three sub-treatments: 50% fertilized and 0% biochar amended seedlings (F50% B0%), 50% fertilized and 20% biochar amended (F50% B20%), and for 100% fertilized and 20% biochar amended seedlings (F100% B20%) (Fig. 2a). However, the difference between the treatments was not statistically significant due to high variation within the treatments. According to these results, the quality of Norway spruce seedlings was not affected by the amount of added biochar (Table 2b, Fig 2a). Based on the possible future restrictions on the peat extraction (Kauppila et al. 2016), and the missing negative effect of biochar amendment on the growth of spruce seedlings, suggested optimal amount of biochar in the growing media for Norway spruce could be at least up to 20% of the volume. Biochar amendment did not affect the quality of pine seedlings (Fig. 2b). However, the highest quality of seedlings for pine was achieved by sub-treatment with 100% fertilizing and 10% added biochar (F100% B10%) (Fig. 2b). Based on our results (trends visible on Fig. 2) we can assume that up to 10% of peat could be replaced with biochar for pine seedlings. We found that the quality of birch seedling was significantly affected by biochar amendment (Table 2d), and the highest *DQI* values were achieved with the growing media amended with 100% fertilization and 10% biochar (Fig. 2c). Therefore, based on received results, 10% of peat could be replaced by biochar in the growing media for birch. Birch is considered to be a pioneer tree species for fire disturbed areas (Whelan 1995), so we expected biochar to have a positive effect on the growth of its seedlings. Also, several studies have proved that biochar amendment has a positive effect on the growth of other broad-leaved species like *Acer mono* (Razaq et al. 2017), *Zelkova serrata* (Cho et al. 2017), *Quercus serrata*, and *Prunus sargentii*

(Aung et al. 2018). Our conclusions about the optimal amount of biochar to be added to the growing media, are similar to those obtained by Dumroese et al. (2018), who found that replacing 25% of the peat-based growing media with biochar results in acceptable growth of *Pinus ponderosa* seedlings. For all studied species there was no statistically significant variation in *DQI* values for fertilized (50% and 100%) sub-treatments (except for pine and birch 100% fertilized and 10% biochar amended sub-treatment) (Fig. 2). The reduced need for fertilization could have been caused by the original nutrients available in the biochar used and/or by the effect of biochar on the specific nutrient transformations, microbial communities or physical and chemical properties of the growing media (Lehmann and Joseph 2015). The exact pathways are not studied here, but based on the results obtained in this study the use of mineral fertilizers could be decreased. However, final conclusions should be made after further studies on the aforementioned mechanism.

The importance of peatlands as a valuable habitat and a significant store of C cannot be underestimated. However, the need for peat as a growing medium increases with the growth of the population. In the European Union (EU), the reported amount of peat used as a growing medium in forestry is more than 6 million m³ per year (Kitir et al. 2018). The use of alternatives has been strongly encouraged by the governments of EU countries, but the high usage of peat as a growing medium still continues (Kitir et al. 2018). The use of biochar in forest tree seedling nurseries would have a significant environmental effect due to the reduced need for peat, fertilization, and liming, and it would also have a positive effect on the growth of seedlings at the early growth stage. Still, continued research is needed to estimate the effect of biochar amendment on the soil processes and post-planting survival of seedlings.

Conclusions

482 To our knowledge, this is the first study to examine the effect of biochar amendment on the growth
483 of Norway spruce, Scots pine and silver birch seedlings. We found that biochar amendment
484 slightly reduced the soil water retention of the growing media due to the high water holding
485 capacity of raw peat. Still, the reduction in water holding capacity became statistically significant
486 if the amount of added biochar was 20% of the total volume. Due to its alkaline character, biochar
487 had a significant liming effect and could replace other liming components in the growing media.
488 In addition, biochar amendment had significant effect on the nutrient concentration of the growing
489 media, as it increased significantly the concentrations of all analyzed nutrients (C, N, P and K).
490 Biochar affected the aboveground growth of spruce and pine seedlings, and increased the root dry
491 weight and root collar diameter of birch seedlings. Fertilization significantly reduced the
492 root:shoot ratio of all species but improved the quality of seedlings as measured by *DQI*. We also
493 observed no variation in *DQI* values for 50% and 100% fertilized seedlings, which suggests that
494 the use of fertilizers could be reduced with biochar amendment. Adding up to 20% biochar for
495 spruce, pine and birch did not reduce overall seedling performance when fertilization was used.
496 We can conclude that biochar could be used as a peat additive in the nursery production of tree
497 seedlings. The results suggest that the growing media could be amended with 20% of biochar for
498 spruce, and 10% of biochar for pine and for birch seedlings. Increased root growth and higher
499 root:shoot ratios of seedlings observed in our current study were found to improve the post-
500 planting establishment and field performance. In addition, the transition to biochar–peat mixtures
501 in nurseries would decrease the environmental impact of the extraction of peat, limestone, and
502 dolomite due to the reduced need for liming and fertilization.

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References

- Alexander, P.D., Bragg, N.C., Meade, R., Padelopoulos, G. and Watts, O. 2008. Peat in horticulture and conservation: the UK response to a changing world. *Mires Peat*. **3**: 1–10.
- Aung, A., Han, S.H., Youn, W.B., Meng, L., Cho, M.S. and Park, B.B. 2018. Biochar effects on the seedling quality of *Quercus serrata* and *Prunus sargentii* in a containerized production system. *Forest Sci Tech*. **14**(3): 112–118. doi 10.1080/21580103.2018.1471011.
- Basso, A.S., Miguez, F.E., Laird, D.A., Horton, R. and Westgate, M. 2013. Assessing potential of biochar for increasing water-holding capacity of sandy soils. *GCB Bioenergy*. **5**: 132–143.
- Campbell, R.M., Anderson, N.M., Daugaard, D.E. and Naughton, H.T. 2018. Financial viability of biofuel and biochar production from forest biomass in the face of market price volatility and uncertainty. *Applied Energy*. **230**: 330–343.
- Caron, J. and Rochefort, L. 2013. Use of peat in growing media: State of the art on industrial and scientific efforts envisioning sustainability. *Acta Hortic*. **982**: 15–22.
- Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A. and Joseph, S. 2008. Using poultry litter biochars as soil amendments. *Aust J Soil Res*. **46**: 437–444.

- 528 Cho, M.S., Meng, L., Song, J.-H., Han, S.H., Bae, K. and Park, B.B. 2017. The effects of biochars
529 on the growth of *Zelkova serrata* seedlings in a containerized seedling production system. Forest
530 Sci Tech. **13**(1): 25–30. doi: 10.1080/21580103.2017.1287778.
- 531 Currey, C.J., Torres, A.P., Lopez, R.G. and Jacobs, D.F. 2013. The quality index – A new tool for
532 integration quantitative measurements to assess quality of young floriculture plants. In Proc. VIIth
533 IS on New Floricultural Crops. Facciuto, G., Sánchez, M.I. (eds.). Acta Hort. **1000**: ISHS. 385–
534 392 pp.
- 535 Dickson, A., Leaf, A.L. and Hosner, J.F. 1960. Quality appraisal of white spruce and white pine
536 seedling stock in nurseries. The Forestry Chronicle. **36**(1): 10–13. doi: 10.5558/tfc36010-1.
- 537 Dumroese, R.K., Pinto, J.R., Heiskanen, J., Tervahauta, A., McBurney, G., Page-Dumroese, D.S.
538 and Englund, K. 2018. Biochar can be a suitable replacement for sphagnum peat in nursery
539 production of *Pinus ponderosa* seedlings. Forests. **9**: 232. doi: 10.3390/f9050232.
- 540 Fisher, P.R., Huang, J. and Argo, W.R. 2006. Modeling lime reaction in peat-based substrates.
541 Acta Horticulturae. **718**: 461–468.
- 542 Galinato, S.P., Yoder, J.K. and Granatstein, D. 2011. The economic value of biochar in crop
543 production and carbon sequestration. Energy Policy. **39**: 6344–6350.
- 544 Gaskin, J.W., Steiner, C., Harris, K., Das, K.C. and Bibens, B. 2008. Effect of low-temperature
545 pyrolysis conditions on biochar for agricultural use. Transactions of the American Society and
546 Agricultural and Biological Engineers. **51**: 2061–2069.
- 547 Gascó, G., Álvarez, M.L., Paz-Ferreiro, J., San Miguel, G. and Méndez, A. 2018. Valorization of
548 biochars from pinewood gasification and municipal solid waste torrefaction as peat substitutes.
549 Environ Sci Pollut R. **25**: 26461–26469. doi: 10.1007/s11356-018-2703-x.

- 550 Githinji, L. 2014. Effect of biochar application rate on soil physical and hydraulic properties of
551 sandy loam. *Arch Agr Soil Sci.* **60**(4): 457–470.
- 552 Griswold, H.C. 1981. Barerooted versus containerized loblolly pine seedlings – six-year
553 performance. *Res Note.* 88 pp.
- 554 Grossnickle, S.C. 2005. Importance of root growth in overcoming planting stress. *New Forests.*
555 **30**: 273–294. doi: 10.1007/s11056-004-8303-2.
- 556 Gruffman, L., Ishida, T., Nordin, A. and Näsholm, T. 2012. Cultivation of Norway spruce and
557 Scots pine on organic nitrogen improves seedling morphology and field performance. *Forest Ecol*
558 *Manag.* **276**: 118–124. doi: 10.1016/j.foreco.2012.03.030.
- 559 Haase, D.L. 2008. Understanding forest seedling quality: measurements and interpretation. *Tree*
560 *Planters' Notes.* **52**(2): 24–30.
- 561 Harris, R.W. 1992. Root-shoot ratios. *J Arboricult.* **18**(1): 39–42.
- 562 Heiskanen, J. Rikala, R. 1998. Influence of different nursery container media on rooting of Scots
563 pine and silver birch seedlings after transplanting. *New Forests.* **16**: 27–42.
- 564 Heiskanen, J. 1993a. Favourable water and aeration conditions for growth media used in
565 containerized tree seedling production: A review. *Scand J Forest Res.* **8**(1-4): 337–358. doi:
566 10.1080/02827589309382782.
- 567 Heiskanen, J. 1993b. Variation in water retention characteristics of peat growth media used in tree
568 nurseries. *Silva Fenn.* **27**(2): 77–97. doi: 10.14214/sf.a15664.
- 569 Heiskanen, J. 2013. Effects of compost additive in sphagnum peat growing medium on Norway
570 spruce container seedlings. *New Forests.* **44**: 101–118. doi: 10.1007/s11056-011-9304-6.

- 571 Ivetić, V., Devetaković, J. and Maksimović, Z. 2016. Initial height and diameter are equally related
572 to survival and growth of hardwood seedlings in first year after field plantin. *Reforesta*. **2**: 6–21.
573 doi: 10.21750/REFOR.2.02.17.
- 574 Jacobs, D.F. and Timmer, V.R. 2005. Fertilizer-induced changes in rhizosphere electrical
575 conductivity: relation to forest tree seedling root system growth and function. *New Forests*. **30**:
576 147–166. doi: 10.1007/s11056-005-6572-z.
- 577 Jacoby, R., Peukert, M., Succorro, A., Koprivova, A. and Kopriva, S. 2017. The role of soil
578 microorganisms in plant mineral nutrition – current knowledge and future directions. *Front Plant*
579 *Sci.* **8**: 1617. doi: 10.3389/fpls.2017.01617.
- 580 James, G., Witten, D., Hastie, T. and Tibshirani, R. 2000. An introduction to statistical learning.
581 *Curr. Med. Chem.* **7**. doi: 10.1007/978-1-4614-7138-7.
- 582 Jäärats, A., Tullus, A. and Seemen, H. 2016. Growth and survival of bareroot and container palts
583 of *Pinus sylvestris* and *Picea abies* during eight years in hemiboreal Estonia. *Balt For.* **22**(2): 365–
584 374.
- 585 Kauppila, T., Ahokas, T., Nikolajev-Wikström, L., Mäkinen, J., Tammelin, M.H. and Meriläinen,
586 J.J. 2016. Aquatic effects of peat extraction and peatland forest drainage: a comparative sediment
587 study of two adjacent lakes in Central Finland. *Environ Earth Sci.* **75**: 1473. doi: 10.1007/s12665-
588 016-6278-x.
- 589 Kitir, K., Yildirim, E., Şahin, Ü., Turan, M., Ekinci, M., Ors, S., Kul, R., Ünlü, H. and Ünlü, H.
590 2018. Peat Use in Horticulture. In *Peat*. Edited by Topcuoğlu B., Turan, M. IntechOpen, doi:
591 10.5772/intechopen.79171.
- 592 Klute, A. 1986. Water retention: Laboratory methods. In *Methods of Soil Analysis. Part 1, Physical*
593 *and Mineralogical Methods*. Edited by Klute, A. ASA and SSSA, Madison, 635–662.

- 594 Kumar, S. 2017. Sphagnum moss as a growing media constituent: some effects of harvesting,
595 processing and storage. *Mires and Peat*. **20**: 1–11. doi: 10.19189/MaP.2016.OMB.232.
- 596 Koplik, R., Curdova, E. and Suchanek, M. 1998. Trace element analysis in CRM of plant origin
597 by inductively coupled plasma mass spectrometry. *Fresenius Journal of Analytical Chemistry*.
598 **360**: 449–451.
- 599 Lehmann, J. and Joseph, S. 2015. *Biochar for Environmental Management – Science, Technology*
600 *and Implementation*. TJ International Ltd., Padstow, Cornwall, Great Britain.
- 601 Lin, K.-H., Wu, C.-W. and Chang, Y.-S. 2018. Applying Dickson quality index, chlorophyll
602 fluorescence, and leaf area index for assessing plant quality of *Pentas lanceolata*. *Notulae*
603 *Botanicae Horti Agrobotanici Cluj-Napoca*. **47**(1): 169–176. doi: 10.15835/nbha47111312.
- 604 Markoska, V., Spalevic, V., Lisichkov, K., Atkovska, K. and Gulaboski, R. 2018. Determination
605 of water retention characteristics of perlite and peat. *Agr For*. **64**(3): 113–126. doi:
606 10.17707/AgricultForest.64.3.10.
- 607 Mendéz, A., Cárdenas-Aguilar, E., Paz-Ferreiro, J., Plaza, C. and Gascó, G. 2018. The effect of
608 sewage sludge biochar on peat-based growing media. *Biol Agric Hortic*. **33**(1): 40–51. doi:
609 10.1080/01448765.2016.1185645.
- 610 Mulcahy, D.N., Mulcahy, D.L. and Dietz, D. 2013. Biochar soil amendment increases tomato
611 seedling resistance to drought in sandy soils. *J Arid Environ*. **88**: 222–225. doi:
612 10.1016/j.jaridenv.2012.07.012.
- 613 Nieto, A., Gascó, G., Paz-Ferreiro, J., Fernández, J.M., Plaza, C. and Méndez, A. 2016. The effect
614 of pruning waste and biochar addition on brown peat based growing media properties. *Sci Hortic*.
615 **1999**: 142–148. doi: 10.1016/j.scienta.2015.12.012.

- 616 Novak, J.M., Busscher, W.J., Watts, D.W., Amonette, J.E., Ippolito, J.A., Lima, I.M., Gaskin, J.,
617 Das, K.C., Steiner, C., Achmedna, M., Rehrah, D. and Schomberg, H. 2012. Biochars impact on
618 soil-moisture storage in an ultisol and two aridisols. *Soil Sci.* **177**(5): 310–320. doi:
619 10.1097/SS.0b013e31824e5593.
- 620 Prendergast-Miller, M.T., Duvall, M. and Sohi, S.P. 2013. Biochar-root interactions are mediated
621 by biochar nutrient content and impacts on soil nutrient availability. *European Journal of Soil Sci.*
622 **65**: 173–185. doi: 10.1111/ejss.12079.
- 623 Rajkovich, S., Enders, A., Hanley, K., Hyland, C., Zimmerman, A.R. and Lehmann, J. 2012. Corn
624 growth and nitrogen nutrition after additions of biochar with varying properties to a temperate soil.
625 *Biol Fert Soils.* **48**: 271–284. doi: 10.1007/s00374-011-0624-7.
- 626 Rasa, K., Heikkinen, J., Hannula, M., Arstila, K., Kulju, S. and Hyväluoma, J. 2018. How and why
627 does willow biochar increase a clay soil water retention capacity? *Biomass Bioenerg.* **119**: 346–
628 353.
- 629 Räsänen, P.K. 1982. Containerized forest tree seedling production and development prospects in
630 Finland and Scandinavia. In *Proceedings, Canadian Containerized Tree Seedling Symposium*;
631 1981 September 14–16. Edited by Scarratt, J.B., Glerum, C. Plexman, C.A. Toronto, ON.
632 COJFRC Symp. Proc. O-P-10. Sault Ste. Marie, ON: Canadian Forestry Service, Great Lakes
633 Forest Research Centre: 9–17.
- 634 Rikala, R. 2002. *Metsätaimiopas – taimien valinta ja käsittely tarhalta uudistusalalle.*
635 *Metsätutkimuslaitoksen tiedonantoja.* **881**: 107 p. (In Finnish).
- 636 Rikala, R. 2012. *Metsäpuiden paakkutaimien kasvatusopas.* Vammalan Kirjapaino. 247 p. (In
637 Finnish).

- Robertson, S.J., Rutherford, M., López-Gutiérrez, J.C. and Massicotte, H.B. 2012. Biochar enhances seedling growth and alters root symbioses and properties of sub-boreal forest soils. *Can J Soil Sci.* **92**: 329–340. doi:10.4141/CJSS2011-066.
- Rose, R. and Haase, D.L. 2000. The use of coir as a containerized growing medium for Douglas-fir seedlings. *Native Plants Journal.* **1**: 107-111. doi: 10.3368/npj.1.2.107.
- Rousk. J., Brookes, P.C. and Bååth, E. 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. *FEMS Microbiol Ecol.* **76**: 89–99.
- Rowan, S.J. 1987. Effects of potassium fertilization in the nursery on survival and growth of pine seedlings in the plantation. *Georgia Forest Research Paper.* **68**: 1–8.
- Razaq, M., Salahuddin, Shen, H., Sher, H. and Zhang, P. 2017. Influence of biochar and nitrogen on fine root morphology, physiology and chemistry of *Acer mono*. *Sci Rep.* **7**: 5367. doi: 10.1038/s41598-017-05721-2.
- Schultz, J.J. and Waggoner, D.R. 1999. Current Environmental Issues of Fertilizer Production. *Proceedings of an International Workshop, June 7-9, 1999. Prague, Czech Republic.* pp. 249.
- Selin, P. 1996. Releases to watercourses from peat harvesting and their control. In *Peatlands in Finland*. Edited by H. Vasander. Helsinki, Finnish Peat Land Society, pp 150–154.
- Skowrońska, M. and Filipek, T. 2014. Life cycle assessment of fertilizers: a review. *Int Agrophys.* **28**: 101–110. doi. 10.2478/intag-2013-0032.
- Steiner, C. and Harttung, T. 2014. Biochar as a growing media additive and peat substitute. *Solid Earth.* **5**: 995–999.
- Tahvonen, R. 2014. Sammalesta kasvualusta ja kitusuot sammalen tuotantoon (Sphagnum as a substrate and nutrient-poor peatlands in moss production). *Suo* **65**(1): 23–26. (In Finnish).

- 661 Thiffault, N., Lafleur, B., Roy, V. and DeBlois, J. 2012. Large planting stock type and mechanical
662 release effects on the establishment success of *Picea glauca* plantations in Quebec, Canada. Int J
663 For Res. Article id 617392: 12 pp. doi: 10.1155/2012/617392.
- 664 Tsakalimi, M. 2006. Kenaf (*Hibiscus cannabinus* L.) core and rice hulls as components of
665 container media for growing *Pinus halepensis* M. seedlings. Bioresour Technol. **97**(14): 1631-9.
666 doi: 10.1016/j.biortech.2005.07.027.
- 667 Tsakalimi, M., Ganatsas, P. and Jacobs, D.F. 2013. Prediction of planted seedling survival of five
668 Mediterranean species based on initial seedling morphology. New Forests. **44**: 327–339. doi:
669 10.1007/s11056-012-9339-3.
- 670 Tsakalimi, M. and Ganatsas, P. 2016. A synthesis of results on wastes as potting media substitutes
671 for the production of native plant species. Reforesta. **1**: 147–163. doi: 10.21750/REFOR.1.08.8.
- 672 Whelan, R.J. 1995. The Ecology of Fire. Cambridge University Press, UK, pp. 349.
- 673 Worrall, R.J. 1981. Comparison of composted hardwood and peat-based media for the production
674 of seedlings, foliage and flowering plants. Scientia Hortic. **15**: 311–319. doi: 10.1016/0304-
675 4238(81)90085-6.
- 676 Zhigunov, A., Saksa, T., Sved, J. and Nerg, J. 2011. Fundamentals of container tree seedling
677 production. St. Petersburg Forestry Research Institute and METLA. St. Petersburg. 28 p.
- 678 Statistical Yearbook of Finland, 2017:
679 https://www.stat.fi/tup/julkaisut/tiedostot/julkaisuluettelo/yyti_stv_201700_2017_17863_net.pdf
680 (14.08.2019)

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682 Table 1. Average values and standard errors (\pm) of measured soil pH, carbon (C), nitrogen (N), C:N ratio, phosphorus (P) and potassium (K) for
 683 tested growing media mixes. Treatments include growing media samples (n=18 per treatment) with 0%, 50% and 100% fertilization (F) and 0%,
 684 5%, 10% and 20% of added biochar (B). Statistical significance ($p < 0.05$) of variables is marked with letters.

Treatment	pH	g C m ²	g N m ²	C:N ratio	g P m ³	g K m ³
F0%B0%	4.06(\pm 0.06) ^D	365.77(\pm 7.47) ^{EF}	11.93(\pm 0.22) ^{CD}	30.65(\pm 0.18) ^G	1.86(\pm 0.08) ^F	7.44(\pm 1.06) ^D
F0%B5%	4.19(\pm 0.02) ^C	407.91(\pm 6.65) ^{CD}	12.59(\pm 0.18) ^{BC}	32.39(\pm 0.22) ^{DE}	2.14(\pm 0.02) ^{EF}	7.05(\pm 0.26) ^D
F0%B10%	4.39(\pm 0.01) ^B	416.18(\pm 6.93) ^{BCD}	12.24(\pm 0.25) ^{CD}	34.06(\pm 0.29) ^{BC}	2.66(\pm 0.09) ^{EF}	7.23(\pm 0.24) ^D
F0%B20%	4.55(\pm 0.03) ^A	450.53(\pm 5.69) ^{AB}	12.62(\pm 0.16) ^{BC}	35.71(\pm 0.17) ^A	3.23(\pm 0.10) ^E	10.32(\pm 0.36) ^D
F50%B0%	3.90(\pm 0.02) ^E	325.22(\pm 10.71) ^G	10.66(\pm 0.35) ^E	30.51(\pm 0.30) ^G	5.97(\pm 0.35) ^D	15.07(\pm 0.65) ^C
F50%B5%	4.18(\pm 0.01) ^{CD}	391.25(\pm 8.64) ^{DE}	12.23(\pm 0.31) ^{CD}	32.05(\pm 0.18) ^E	6.10(\pm 0.19) ^D	16.44(\pm 0.46) ^C
F50%B10%	4.21(\pm 0.01) ^C	454.35(\pm 8.40) ^A	13.67(\pm 0.24) ^{AB}	33.26(\pm 0.26) ^{CD}	6.27(\pm 0.18) ^D	16.77(\pm 0.42) ^C
F50%B20%	4.56(\pm 0.02) ^A	459.03(\pm 7.55) ^A	12.89(\pm 0.21) ^{BC}	35.62(\pm 0.13) ^A	7.10(\pm 0.17) ^{CD}	21.14(\pm 1.55) ^B
F100%B0%	3.81(\pm 0.01) ^E	349.82(\pm 4.26) ^{FG}	11.29(\pm 0.13) ^{ED}	30.99(\pm 0.11) ^{FG}	10.07(\pm 0.59) ^A	23.98(\pm 1.09) ^{AB}
F100%B5%	4.22(\pm 0.03) ^C	387.86(\pm 8.10) ^{DE}	12.16(\pm 0.34) ^{CD}	32.01(\pm 0.32) ^{EF}	8.09(\pm 0.21) ^{BC}	26.23(\pm 1.60) ^A
F100%B10%	4.26(\pm 0.03) ^C	454.77(\pm 7.06) ^A	14.07(\pm 0.21) ^A	32.34(\pm 0.24) ^{DE}	9.11(\pm 0.34) ^{AB}	23.11(\pm 0.43) ^{AB}
F100%B20%	4.42(\pm 0.02) ^B	438.41(\pm 9.61) ^{ABC}	12.72(\pm 0.25) ^{BC}	34.46(\pm 0.20) ^B	10.01(\pm 0.44) ^A	24.87(\pm 0.93) ^{AB}

Table 2. Results of linear mixed effects model (type 3 test results) to test the effects of biochar amendment, fertilization and biochar×fertilization interaction on soil properties (a), and seedling growth for Norway spruce (b), Scots pine (c) and silver birch (d) seedlings. C = carbon; N = nitrogen; C:N = C:N ratio; P = phosphorus; K = potassium; DW = dry weight; L = length; R:S ratio = root:shoot ratio; RCD = root collar diameter; DQI = Dickson's quality index.

a) Soil properties

	Biochar		Fertilization		Biochar×Fertilization	
	F	p-value	F	p-value	F	p-value
pH	272.08	< 0.0001	23.92	< 0.0001	8.38	< 0.0001
C	111.57	< 0.0001	0.14	0.87	6.20	< 0.0001
N	35.90	< 0.0001	0.93	0.40	7.28	< 0.0001
C:N	212.99	< 0.0001	11.11	< 0.0001	4.90	0.0001
P	11.61	< 0.0001	602.25	< 0.0001	3.68	0.0017
K	8.67	< 0.0001	352.82	< 0.0001	2.78	0.0127

b) Norway spruce seedlings

	Biochar		Fertilization		Biochar-Fertilization	
	F	p-value	F	p-value	F	p-value
Plant DW	2.20	0.0977	7.19	0.0016	1.28	0.2820
Height	2.80	0.0473	3.56	0.0347	1.12	0.3598
Shoot DW	2.78	0.0488	15.06	< 0.0001	1.36	0.2448
Root DW	1.73	0.1714	1.41	0.2520	1.37	0.2395
Root L	2.48	0.0701	0.75	0.4770	2.02	0.0762
R:S ratio	0.99	0.4028	1.61	0.2098	0.78	0.5881
No of buds	0.36	0.7902	1.03	0.3641	0.38	0.8912
RCD	0.17	0.9144	9.27	0.0003	1.88	0.0982
DQI	0.60	0.6151	2.79	0.0692	0.60	0.7315

c) Scots pine seedlings

	Biochar		Fertilization		Biochar-Fertilization	
	F	p-value	F	p-value	F	p-value
Plant DW	1.48	0.2289	47.76	< 0.0001	2.01	0.0785
Height	5.44	0.0022	28.00	< 0.0001	1.51	0.1913
Shoot DW	2.96	0.0393	93.03	< 0.0001	3.57	0.0043
Root DW	0.52	0.6700	6.11	0.0008	1.52	0.1862
Root L	1.56	0.2112	1.81	0.1729	2.41	0.0377

720	R:S ratio	1.60	0.9930	60.50	< 0.0001	1.75	0.1258
721	No of buds	1.38	0.2571	13.82	< 0.0001	1.63	0.1554
722	RCD	1.39	0.2555	38.72	< 0.0001	2.35	0.0422
723	DQI	0.73	0.5383	18.28	< 0.0001	2.26	0.0498

724 d) silver birch seedlings

725	Biochar			Fertilization		Biochar-Fertilization	
726		F	p-value	F	p-value	F	p-value
727	Plant DW	2.01	0.1228	64.98	< 0.0001	1.52	0.1879
728	Height	0.14	0.9379	181.12	< 0.0001	0.86	0.5264
729	Shoot DW	1.52	0.2186	92.58	< 0.0001	1.19	0.3241
730	Root DW	3.02	0.0367	13.41	< 0.0001	2.36	0.0409
731	Root L	0.66	0.5818	5.36	0.0072	3.21	0.0084
732	R:S ratio	0.68	0.5660	122.42	< 0.0001	1.09	0.3774
733	No of buds	2.45	0.0709	82.05	< 0.0001	2.48	0.0332
734	RCD	3.83	0.0141	56.89	< 0.0001	2.44	0.0356
735	DQI	6.29	0.0009	6.45	0.0029	3.55	0.0045

736 Table 3. Average values and standard errors (\pm) of measured variables for Norway spruce (a), Scots pine (b) and silver birch (c). DW = dry weight;
737 RC = root collar. Treatments include seedlings grown in growing media mixes with 0%, 50% and 100% fertilization (F) and 0%, 5%, 10% and
738 20% of added biochar (B). Statistical significance ($p < 0.05$) of measured and calculated variables is marked with letter.

739	a) Norway spruce	Plant height	Shoot DW	Root DW	Root length	Root:Shoot	No of buds	RC diameter
740		(cm)	(g)	(g)	(cm)			(mm)
741	F0% B0%	4,82 \pm 0,28	0,09 \pm 0,00 ^{BC}	0,05 \pm 0,01	14,02 \pm 1,09 ^A	0,61 \pm 0,05	4,17 \pm 0,70	1,20 \pm 0,05 ^{AB}
742	F0% B5%	4,47 \pm 0,47	0,07 \pm 0,01 ^C	0,05 \pm 0,01	14,27 \pm 0,63 ^A	0,80 \pm 0,10	3,67 \pm 0,56	1,05 \pm 0,03 ^B
743	F0% B10%	4,35 \pm 0,25	0,08 \pm 0,00 ^C	0,06 \pm 0,01	15,23 \pm 0,77 ^A	0,83 \pm 0,12	3,00 \pm 0,52	1,18 \pm 0,04 ^{AB}
744	F0% B20%	4,97 \pm 0,46	0,09 \pm 0,01 ^{ABC}	0,09 \pm 0,01	17,57 \pm 1,18 ^{AB}	0,94 \pm 0,04	3,67 \pm 0,76	1,19 \pm 0,04 ^{AB}
745	F50% B0%	6,50 \pm 0,65	0,17 \pm 0,02 ^{AB}	0,21 \pm 0,12	18,42 \pm 1,57 ^B	1,08 \pm 0,56	4,67 \pm 0,71	1,33 \pm 0,09 ^{AB}
746	F50% B5%	4,32 \pm 0,79	0,11 \pm 0,03 ^{AB}	0,06 \pm 0,02	13,83 \pm 0,51 ^A	0,55 \pm 0,02	4,17 \pm 0,87	1,33 \pm 0,07 ^{AB}
747	F50% B10%	6,15 \pm 0,90	0,17 \pm 0,02 ^{AB}	0,07 \pm 0,01	15,60 \pm 0,79 ^A	0,46 \pm 0,02	3,67 \pm 0,88	1,29 \pm 0,10 ^{AB}
748	F50% B20%	4,90 \pm 0,57	0,11 \pm 0,02 ^{ABC}	0,14 \pm 0,08	15,82 \pm 1,31 ^{AB}	1,56 \pm 0,99	3,83 \pm 0,60	1,10 \pm 0,05 ^B
749	F100% B0%	5,75 \pm 0,69	0,14 \pm 0,02 ^{ABC}	0,06 \pm 0,01	14,67 \pm 0,86 ^A	0,47 \pm 0,02	3,83 \pm 0,54	1,35 \pm 0,06 ^{AB}
750	F100% B5%	4,97 \pm 0,52	0,13 \pm 0,01 ^{ABC}	0,07 \pm 0,01	14,48 \pm 0,74 ^A	0,53 \pm 0,04	4,50 \pm 0,34	1,39 \pm 0,08 ^{AB}
751	F100% B10%	6,53 \pm 0,66	0,18 \pm 0,02 ^A	0,08 \pm 0,01	15,48 \pm 0,79 ^A	0,48 \pm 0,05	4,33 \pm 0,71	1,30 \pm 0,13 ^{AB}
752	F100% B20%	6,08 \pm 0,69	0,15 \pm 0,02 ^{ABC}	0,08 \pm 0,01	15,73 \pm 1,24 ^{AB}	0,53 \pm 0,03	4,67 \pm 1,02	1,47 \pm 0,08 ^A
753	b) Scots pine	Plant height	Shoot DW	Root DW	Root length	Root:Shoot	No of buds	RC diameter
754		(cm)	(g)	(g)	(cm)			(mm)
755	F0% B0%	5,55 \pm 0,36 ^{BCDE}	0,26 \pm 0,02 ^{AB}	0,16 \pm 0,01 ^{AB}	19,17 \pm 0,99 ^A	0,65 \pm 0,04 ^{BCD}	4,67 \pm 0,56 ^{ABC}	1,48 \pm 0,07 ^{BCD}

756	F0% B5%	5,20±0,21 ^{DE}	0,20±0,02 ^A	0,15±0,01 ^{AB}	23,22±1,51 ^B	0,73±0,02 ^{ABC}	3,83±0,48 ^{BC}	1,30±0,06 ^D
757	F0% B10%	5,28±0,54 ^{DE}	0,14±0,01 ^A	0,12±0,01 ^{AB}	18,75±1,08 ^A	0,85±0,04 ^A	3,17±0,40 ^C	1,30±0,10 ^D
758	F0% B20%	5,02±0,27 ^E	0,20±0,02 ^A	0,16±0,02 ^B	19,55±0,70 ^A	0,78±0,03 ^{AB}	3,50±0,43 ^{BC}	1,33±0,04 ^{CD}
759	F50% B0%	7,13±0,23 ^{ABC}	0,39±0,03 ^B	0,20±0,02 ^A	24,87±3,23 ^B	0,51±0,04 ^{DE}	4,33±0,42 ^{ABC}	1,87±0,06 ^A
760	F50% B5%	6,28±0,39 ^{ABCDE}	0,36±0,02 ^{AB}	0,20±0,02 ^A	21,07±0,95 ^{AB}	0,54±0,05 ^{CDE}	5,17±0,48 ^{ABC}	1,77±0,08 ^{AB}
761	F50% B10%	7,22±0,36 ^{AB}	0,37±0,03 ^{AB}	0,18±0,02 ^{AB}	23,62±1,96 ^{AB}	0,50±0,05 ^{DE}	5,00±0,77 ^{ABC}	1,59±0,08 ^{ABCD}
762	F50% B20%	6,55±0,24 ^{ABCDE}	0,38±0,03 ^{AB}	0,18±0,02 ^{AB}	18,50±1,24 ^A	0,47±0,05 ^{DE}	4,00±0,68 ^{BC}	1,75±0,07 ^{AB}
763	F100% B0%	7,78±0,44 ^A	0,41±0,05 ^B	0,18±0,02 ^{AB}	22,40±1,12 ^B	0,45±0,03 ^E	5,17±0,60 ^{ABC}	1,66±0,12 ^{ABC}
764	F100% B5%	5,53±0,27 ^{CDE}	0,35±0,03 ^{AB}	0,16±0,02 ^{AB}	19,65±1,55 ^{AB}	0,47±0,04 ^{DE}	6,00±0,26 ^{AB}	1,89±0,05 ^A
765	F100% B10%	7,07±0,44 ^{ABC}	0,43±0,03 ^B	0,20±0,01 ^A	25,35±1,39 ^B	0,47±0,03 ^{DE}	6,83±0,60 ^A	1,82±0,06 ^{AB}
766	F100% B20%	6,85±0,28 ^{ABCD}	0,39±0,02 ^B	0,18±0,01 ^{AB}	21,47±2,55 ^{AB}	0,47±0,03 ^{DE}	5,17±0,60 ^{ABC}	1,67±0,05 ^{ABC}
767	c) silver birch	Plant height	Shoot DW	Root DW	Root length	Root:Shoot	No of buds	RC diameter
768		(cm)	(g)	(g)	(cm)			(mm)
769	F0% B0%	7,00±1,43 ^A	0,27±0,10 ^A	0,23±0,07 ^A	15,03±0,61 ^{AB}	0,94±0,09 ^A	9,17±0,60 ^A	2,32±0,35 ^E
770	F0% B5%	8,22±1,63 ^A	0,34±0,11 ^A	0,25±0,06 ^A	15,15±1,09 ^{AB}	0,79±0,06 ^A	9,67±0,67 ^A	2,73±0,34 ^{CDE}
771	F0% B10%	6,85±1,42 ^A	0,33±0,10 ^A	0,22±0,05 ^A	15,35±0,61 ^{AB}	0,81±0,09 ^A	9,50±0,67 ^A	2,48±0,20 ^{ED}
772	F0% B20%	7,37±0,46 ^A	0,28±0,04 ^A	0,24±0,02 ^A	18,45±2,00 ^A	0,91±0,08 ^A	9,33±0,42 ^A	2,60±0,14 ^{ED}
773	F50% B0%	24,88±0,80 ^{BC}	1,04±0,05 ^B	0,33±0,03 ^A	17,30±1,10 ^{AB}	0,31±0,02 ^B	15,00±0,63 ^B	4,21±0,28 ^{AB}
774	F50% B5%	21,87±2,45 ^{BC}	0,99±0,14 ^B	0,35±0,04 ^A	14,97±0,96 ^{AB}	0,38±0,04 ^B	14,83±1,11 ^B	4,31±0,29 ^{AB}
775	F50% B10%	24,55±2,19 ^{BC}	1,16±0,09 ^B	0,43±0,04 ^A	18,27±0,90 ^A	0,38±0,03 ^B	14,83±0,70 ^B	4,68±0,28 ^{AB}
776	F50% B20%	21,68±1,61 ^B	0,86±0,10 ^{AB}	0,32±0,06 ^A	14,30±0,65 ^{AB}	0,38±0,06 ^B	15,17±0,95 ^B	4,24±0,35 ^{AB}

777	F100% B0%	26,78±1,22 ^{BC}	1,22±0,09 ^B	0,40±0,05 ^A	14,87±0,77 ^{AB}	0,33±0,02 ^B	15,00±0,68 ^B	4,02±0,14 ^{BC}
778	F100% B5%	25,48±2,98 ^{BC}	0,94±0,17 ^B	0,24±0,04 ^A	14,38±0,67 ^{AB}	0,26±0,02 ^B	14,50±1,26 ^B	3,75±0,31 ^{BCD}
779	F100% B10%	29,10±2,35 ^{BC}	1,86±0,16 ^C	0,70±0,07 ^B	13,88±0,73 ^{AB}	0,37±0,01 ^B	19,33±0,84 ^C	5,57±0,30 ^A
780	F100% B20%	30,82±2,52 ^C	1,40±0,22 ^{BC}	0,47±0,10 ^{AB}	13,60±0,45 ^B	0,32±0,04 ^B	17,33±0,99 ^{BC}	4,51±0,35 ^{AB}

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Figure Captions

Figure 1. Impact of biochar amendment on the hydrological conditions of the growing media. a) Water retention curves of control (pure peat), three biochar treatments and pure biochar. Water retention capacity measured is at pressure heads of 10, 30, 60, 100, and 1000 cm (100 kPa). b) Average soil water content of the growing media during the first growing season. Soil water content was measured before every watering ($n = 93$ measurements per treatment). Error bars represent the standard error. Letters above the bars indicate the statistically significant difference ($p < 0.05$).

Figure 2. Dickson's quality index (DQI) of Norway spruce (*P. abies*) (a), Scots pine (*P. sylvestris*) (b), and silver birch (*B. pendula*) (c) seedlings for different biochar treatments (B) and fertilization sub-treatments (F). Numbers following F and B on the x-axis indicate the amount of fertilizer (% of nursery practice) and biochar (% volume of growing media) used, respectively. Statistical significances of biochar treatment (p_B), fertilization sub-treatment (p_F), and the co-effect of biochar amendment and fertilization (p_{B*F}) are given. Letters above the bars indicate the statistically significant difference between treatments ($p < 0.05$).

