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## Permanent grassland hay-derived biochar increases plant N, P and K uptake on an acidic soil

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### Abstract

The importance of permanent grasslands in the feed production has decreased in Estonia in the last decades, because of the low feeding value of the biomass. Therefore, there is a need for new solutions for utilization of this biomass. One way of giving value to this resource would be its application in the production of biochar (BC) and its subsequent use for increasing the carbon (C) and nutrient concentration of previously low-fertility soils. This study examined (i) the properties of biochar produced from permanent grassland hay at three (300°C, 550°C and 850°C) pyrolysis temperatures and (ii) the impact of biochar on the uptake of N, P, K, Ca and Mg and the biomass yield of perennial ryegrass (*Lolium perenne* L.) on a strongly acidic (pH 4.2) soil.

It was found that the permanent grassland hay dominated by reed canary grass (*Phalaris arundinacea* L.) is a suitable raw material for biochar production. This biochar is a potassium (K) rich fast-acting liming agent, which also contains a remarkable amount of N. The addition of this biochar into acidic soil reduces soil acidity and significantly increases plant uptake of N, P and K, which has a short term positive impact on biomass yield. The increase of pyrolysis temperature changes biochar properties like neutralization capacity, acidity (pH), nutrient concentration and the release of nutrients from biochar, but these changes do not have a significant impact on the effect the biochar has on plant nutrition and yield. The only exception was phosphorus (P) uptake, which was the highest when the biochar was produced at 550°C temperature.

Key words: biochar properties, biomass yield, nutrient release from biochar, nutrient uptake, pyrolysis temperature.

### Introduction

In Estonia, there are many high-yielding permanent types of grassland, which are cut once a year to avoid secondary forest growth. Today the harvested biomass is mostly left to decay at the edge of the grassland because of its low feeding value. It has been found that it could be used as a fuel in heating plants, but because of high concentration of ash and alkali metals and low energy density it is not economically viable (Heinsoo et al., 2010; Kukk et al., 2010). One way of giving value to this resource would be its application in the production of biochar, which could be used for improving the soil fertility.

Biochar is a solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment during pyrolysis and gasification process (Lehmann, Joseph, 2015). Both these processes are exothermic and are used for producing energy from biomass. When incorporated into the soil, biochar impacts soil properties including the soil C concentration (Sohi, 2012), specific surface area and porosity (Baiaomonte et al., 2015; Nelissen et al., 2015), field water-holding

capacity (Basso et al., 2013; Ma et al., 2016), cation-exchange capacity and acidity (Laghari et al., 2016), which could have a positive effect on plant growth (Ding et al., 2016). Due to the high concentration of stable carbon (C) compounds, the biochar decomposes very slowly in the soil that enables the long-term removal of C from the nutrient cycles, thereby being beneficial for climate change mitigation (Sohi, 2012). Also it has been found that biochar inhibits the release of greenhouse gases from the soil, but it has not been confirmed by all studies (Cayuela et al., 2014; Feng, Zhu, 2017; Buchkina et al., 2019; Escuer-Gatius et al., 2020).

Biochar can also be applied to act as an organic fertilizer that enriches the soil on a short-term basis with essential plant nutrients (Gaskin et al., 2010; Kloss et al., 2014). Nutrient amounts and their availability for plants in the biochar depends on its raw material (Enders et al., 2012; Rajkovich et al., 2012) and pyrolysis temperature (Gaskin et al., 2010; Kloss et al., 2012; Ippolito et al., 2015).

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Although the properties of biochar produced from various raw materials have been studied intensively, the application of graminaceous plants in temperate climate regions as a possible raw material for biochar production has received less attention. The majority of studies have been focused on the properties and impacts of biochar produced from graminaceous plants such as *Miscanthus* spp. and switchgrass (*Panicum virgatum* L.) growing in warm climate regions (Mimmo et al., 2014; Ding et al., 2016). Properties and impact of biochar produced from the biomass of temperate climate plants may be different, as was shown by Van de Voorde et al. (2014), who concluded that the species of the raw material plants has a significant impact on the properties of biochar and the effect it has on the crops.

To date, it remains unclear whether biochar effect on plant nutrition depends on the pyrolysis temperature, at which it was produced. There have been few experiments studying the effect of biochars produced from the same raw material at different temperatures on nutrient uptake and soil (Rajkovich et al., 2012; Zheng et al., 2013; Nelissen et al., 2014). In most cases, the effect of pyrolysis temperature on nutrient release from biochar has been evaluated analytically (Enders et al., 2012; Wang et al., 2012; Mukherjee, Zimmerman, 2013).

The purpose of this research was to examine (i) the properties of biochar produced from permanent grassland hay at three (300°C, 550°C and 850°C) pyrolysis temperatures and (ii) the impact of biochar on the uptake of N, P, K, Ca and Mg and perennial ryegrass (*Lolium perenne* L.) yield on a strongly acidic (pH 4.2) soil.

We hypothesized that permanent grassland hay is a suitable raw material for biochar production, whose fertilizing properties are significantly influenced by the pyrolysis temperature.

## Materials and methods

**The biochar (BC) and soil.** The BC was produced from permanent grassland hay dominated by reed canary grass (*Phalaris arundinacea* L.), which is very common high yielding species on wet permanent grasslands in Estonia. The grass was cut at the seed-ripening phase. The ash concentration of harvested hay biomass was 9.12%, and the total (tot) concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (hereafter  $N_{tot}$ ,  $P_{tot}$ ,  $K_{tot}$ ,  $Ca_{tot}$  and  $Mg_{tot}$ , respectively) was 2.3, 0.25, 2.22, 0.92 and 0.46 %, respectively. The hay was pressed into pellets at Ecopellet Ltd. (Estonia) and torrefied at a temperature of 300°C in the Fraunhofer Institute (Germany). From there, the torrefied biochar (hereafter  $BC_{300}$ ) pellets were taken to the Lithuanian Energy Institute, where they were pyrolysed at 550°C (hereafter  $BC_{550}$ ) or 850°C (hereafter  $BC_{850}$ ) temperatures. The average length of biochar pellets was 10–20 mm, the diameter 7 mm.

The plant growth substrate was the upper 20 cm of eroded *Dystric Endostagnic Glossic Retisol (Colluvic)* (WRB, 2014) excavated from permanent grassland. The soil texture was sandy loam (73% sand, 22% silt and 5% clay). Before the experiment, soil was dried and sieved through a 1-cm sieve for removal of stones and plant roots. The soil  $N_{tot}$ ,  $C_{tot}$ ,  $P_{tot}$ ,  $K_{tot}$ ,  $Ca_{tot}$  and  $Mg_{tot}$  concentrations were 0.07, 12.4, 0.214, 0.715, 0.538 and 1.48 g kg<sup>-1</sup>, respectively. Plant-available P, K, Ca and Mg concentrations of the soil determined by ammonium lactate (AL) method (Egner et al., 1960) (hereafter  $P_{AL}$ ,  $K_{AL}$ ,  $Ca_{AL}$  and  $Mg_{AL}$ , respectively) were 0.02, 0.07, 0.475 and 0.155 g kg<sup>-1</sup>, respectively.

**Experimental design.** The pot experiment was conducted under controlled growing chamber conditions over 60 days at the Estonian University of Life Sciences in 2017. The experimental factors were (i) the presence or absence of biochar and (ii) biochar pyrolysis temperatures (300°C, 550°C and 850°C). The experiment also included a control treatment, in which unamended soil was used. The total number of treatments in the experiment was 4, all with four replications.

PVC tubes (Wavin, The Netherlands) with a length of 30 cm, diameter of 10.5 cm and surface area of 0.0095 m<sup>2</sup> were used as growing pots in the experiment. The tubes were sealed at the bottom with a 3-cm-thick styrofoam cap with a hole in the centre for free water drainage. The total soil volume in one pot was 2.4 L and the soil density in the pot 1.3 g cm<sup>-3</sup>. The pots were filled with soil in two parts, the lower 10–27 cm layer and then the upper 0–10 cm layer. Prior to the addition of the upper soil layer, biochar pellets (8.7 g per pot, 915.8 g m<sup>-2</sup>) were mixed into the added soil.

After filling the pots, tap water was added to the soil until field capacity was reached (volumes of applied and drained water were equal) and then perennial ryegrass (*Lolium perenne* L.) cultivar 'Jubilee EG' 100 seeds were sown into each pot. During the experiment, growth room air temperature was maintained at 17°C, and the relative air humidity at 60%. The light mode during the entire experiment was 13/11 h light/darkness. During the first 30 days of the growing period, the plants were irrigated manually three times a week at a rate of 125 ml per pot. This was increased to 150 ml per pot in the second growing period. In both growing periods, the irrigation rate was increased by 25 ml one week before harvest. The water amount was adjusted according to plant biomass size to avoid leaching.

**Data analysis.** Soil agrochemical parameters were determined at the beginning and at the end of the experiment. At the beginning of the experiment, a single average soil sample was taken before the soil was put into the pots. At the end of the experiment, a sample was taken from the upper (0–10 cm) and lower (10–27 cm) soil layers of all pots. The following parameters were determined in the soil sample taken at the beginning and the end of the experiment from the 0–10 cm soil layer: soil acidity with a pH meter SevenCompact (Mettler Toledo Inc., Canada), soil ratio to KCl solution 1:2.5 and the  $N_{tot}$ ,  $C_{tot}$ ,  $P_{tot}$ ,  $Ca_{tot}$ ,  $Mg_{tot}$ ,  $P_{AL}$ ,  $K_{AL}$ ,  $Ca_{AL}$  and  $Mg_{AL}$  concentrations. To clarify the extent to which N mobility was affected by the applied biochar, the  $N_{tot}$  concentration was also determined in the 10–27 cm soil layer.

For the determination of  $P_{tot}$ ,  $K_{tot}$ ,  $Ca_{tot}$  and  $Mg_{tot}$ , the soil sample was at first dried, then ground and mineralized with 2.5 ml HNO<sub>3</sub> and 7.5 ml HCl in a microwave oven (microwave digestion method, <https://www.berghof-instruments.com/en/product/speedwave-entry/>).

The chemical properties of the ground biochar were analysed twice, at the beginning and at the end of the experiment. In the first analysis, pH,  $N_{tot}$ , NO<sub>3</sub>-N, NH<sub>4</sub>-N,  $C_{tot}$ ,  $P_{tot}$ ,  $K_{tot}$ ,  $Ca_{tot}$ ,  $Mg_{tot}$ ,  $P_{AL}$ ,  $K_{AL}$ ,  $Ca_{AL}$ ,  $Mg_{AL}$ , water-extractable N, ash and biochar acid-neutralising capacity were determined. The pH of the biochar and total nutrient concentrations were determined with similar methods to those for soil.

The water-extractable N was analysed separately in the whole and ground pellets granules by shaking them in water for 1 h. The  $N_{tot}$  concentration

was determined by Dumas dry combustion method with the CNS elemental analyser (Elementar). The nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) were analysed by using flow injection analyses by Tecator ASN 65-32/84 (FOSS, Sweden) (Ruzicka, Hansen, 1988). The biochar acid-neutralising capacity was analysed by using 1 M HCl solution according to standard EVS-EN 12945:2014+A1:2016 (Liming materials - Determination of neutralizing value - Titrimetric methods) and ash concentration by heating biochar samples for 6 h at 750°C temperature in a muffle furnace.

At the end of the experiment, biochar pellets were sieved out from the soil and analysed for  $\text{N}_{\text{tot}}$ ,  $\text{C}_{\text{tot}}$ ,  $\text{P}_{\text{tot}}$ ,  $\text{K}_{\text{tot}}$ ,  $\text{Ca}_{\text{tot}}$  and  $\text{Mg}_{\text{tot}}$  concentration similarly to the samples at the beginning of the experiment to determine the loss of those nutrients from biochar.

The biochar cumulative pore volume was determined according to Density Functional Theory (DFT) method (Lastoskie et al., 1993) and the specific area by Brunauer-Emmett-Teller (BET) method (Brunauer et al., 1938).

The aboveground biomass yield was determined twice during the experiment, both times after a 30-day growing period. Plants were cut 2 cm above the soil. Root yield was determined at the end of the experiment. Roots were removed from the soil by sieving with a 2-cm sieve. The raw material was weighed, air-dried at 105°C temperature and weighed again. Based on the weight difference, the dry matter (DM) concentration (%) in biomass was calculated. The biomass DM yield (DMY) was calculated based on DM concentration and fresh biomass yield. From dry biomass that was first ground and then ashed in sulphuric acid (AOAC, 1990), the P, K, Ca and Mg were determined with a micro-plasma atomic emission spectrometer (Agilent). The  $\text{N}_{\text{tot}}$  concentration was determined by the Dumas dry combustion method with the CNS elemental analyser (Elementar). The nutrient concentration (%) in hay (raw material for the biochar) was analysed similarly to aboveground biomass harvested from pots.

*Calculations.* Nutrient uptake (NUP) ( $\text{g m}^{-2}$ ) was determined as follows:

$$\text{NUP} = \text{DMY} \times \text{N}_c / 100,$$

where DMY is biomass dry matter yield ( $\text{g m}^{-2}$ ),  $\text{N}_c$  – nutrient concentration in dry biomass (%).

Total nutrient uptake ( $\text{NUP}_{\text{tot}}$ ) ( $\text{g m}^{-2}$ ) per whole experimental period was calculated as follows:

$$\text{NUP}_{\text{total}} = \text{NUP}_{\text{AGB1}} + \text{NUP}_{\text{AGB2}} + \text{NUP}_{\text{R}}$$

where  $\text{NUP}_{\text{AGB}}$  is nutrient uptake by aboveground biomass ( $\text{g m}^{-2}$ ),  $\text{NUP}_{\text{R}}$  – nutrient uptake by roots ( $\text{g m}^{-2}$ ); indexes 1 and 2 indicate the 1<sup>st</sup> and 2<sup>nd</sup> cuts, respectively.

*Statistical analysis.* One-way analysis of variance (ANOVA) was used for the assessment of the influence of biochar incorporation on soil pH, plant-available and total nutrient concentrations and on cumulative nutrient uptake by plants. The significance of differences between treatment means (control,  $\text{BC}_{300}$ ,  $\text{BC}_{550}$  and  $\text{BC}_{850}$ ) was assessed by Tukey's post hoc test at level  $p < 0.05$ . The relationships between nutrient uptake from soil with soil agrochemical parameters were tested with Pearson correlation analysis. Statistical analysis was conducted by the software *Statistica*, version 13 (StatSoft Inc., USA).

## Results and discussion

*Properties of hay biochar (BC).* Biochar properties were significantly ( $p < 0.01$ ) influenced by the pyrolysis temperature. The rise in pyrolysis temperature increased biochar ash concentration, acid-neutralization capacity and acidity (Table 1) supporting findings of previous studies for biochar produced from wood, maize, manure, paper waste and hazelnut (Enders et al., 2012; Rajkovich et al., 2012; Zeng et al., 2013).

In  $\text{BC}_{300}$ ,  $\text{P}_{\text{tot}}$ ,  $\text{K}_{\text{tot}}$ ,  $\text{Ca}_{\text{tot}}$  and  $\text{Mg}_{\text{tot}}$  concentrations were similar to those in the raw material, but in  $\text{BC}_{550}$  and  $\text{BC}_{850}$ , they were more than twice as high as those in  $\text{BC}_{300}$  (Table 2). This was due to greater loss of volatile compounds at the higher pyrolysis temperatures (Novak et al., 2009; Zheng et al., 2013).

**Table 1.** Selected physical and chemical properties of the hay biochar (BC) produced at different pyrolysis temperatures

Treatment	Acidity (pH)	Ash %	Acid neutralization capacity, $\text{CaCO}_3$ %	Surface area $\text{m}^2 \text{g}^{-1}$ (BET)	Cumulative pore volume $\text{cm}^3 \text{g}^{-1}$ (DFT)	Concentration of volatile compounds %
$\text{BC}_{300}$	6.8	10.4	4.35	0.99	0.0015	62.9
$\text{BC}_{550}$	10.1	20.3	8.15	3.91	0.018	16.1
$\text{BC}_{850}$	11.8	23.0	8.11	6.17	0.008	9.5

*Note.* The index indicates the temperature (300°C, 550°C or 850°C), at which the biochar was produced; BET – Brunauer-Emmett-Teller method; DFT – Density Functional Theory method.

**Table 2.** Nutrient concentration (% in dry matter) in biochar (BC)

Treatment	$\text{N}_{\text{tot}}$	$\text{C}_{\text{tot}}$	$\text{P}_{\text{tot}}$	$\text{K}_{\text{tot}}$	$\text{Ca}_{\text{tot}}$	$\text{Mg}_{\text{tot}}$
At the beginning of the experiment						
$\text{BC}_{300}$	2.82 ± 0.01	53.5 ± 0.03	0.24 ± 0.01	2.28 ± 0.03	0.93 ± 0.03	0.47 ± 0.04
$\text{BC}_{550}$	2.92 ± 0.01	65.9 ± 0.01	0.54 ± 0.01	4.62 ± 0.11	2.11 ± 0.01	0.93 ± 0.04
$\text{BC}_{850}$	2.56 ± 0.01	68.1 ± 0.32	0.60 ± 0.02	5.10 ± 0.24	2.44 ± 0.04	1.03 ± 0.04
At the end of the experiment						
$\text{BC}_{300}$	1.76 ± 0.08	35.9 ± 1.07	0.09 ± 0.01	0.06 ± 0.01	0.74 ± 0.01	0.23 ± 0.01
$\text{BC}_{550}$	1.32 ± 0.04	33.3 ± 0.71	0.46 ± 0.02	0.46 ± 0.01	1.41 ± 0.11	0.79 ± 0.03
$\text{BC}_{850}$	0.79 ± 0.04	28.9 ± 1.07	0.48 ± 0.01	0.93 ± 0.04	1.64 ± 0.08	0.80 ± 0.01

*Note.* The index indicates the temperature (300°C, 550°C or 850°C), at which the biochar was produced; the values are presented as mean ± standard error (n = 4).

$N_{\text{tot}}$  concentration was high in all three biochars. This high  $N_{\text{tot}}$  amount has been noted earlier for lucerne (*Medicago sativa* L.) (3.1%) and switchgrass (*Panicum virgatum* L.) (2.3%) biochar (Wang et al., 2015) and tomato (*Solanum lycopersicum* L.) (2.6%) aboveground biomass biochar (Smider, Singh, 2014); however, there are also many studies, which suggest that the  $N_{\text{tot}}$  concentration may be two times lower in herbaceous biomass biochars (Ippolito et al., 2015).

$N_{\text{tot}}$  concentration in biochar was affected by pyrolysis temperature, but its impact on  $N_{\text{tot}}$  amount was in the current study less than it was showed previously. Wang et al. (2015) found that an increase in pyrolysis temperature from 300°C to 600°C reduced  $N_{\text{tot}}$  concentration of biochar produced from lucerne and switchgrass by 28% and 52%, respectively. In the current study similarly to Wang et al. (2015), the lowest  $N_{\text{tot}}$  was in the  $BC_{850}$  that was produced at the highest temperature, but its difference from  $BC_{300}$  was only 10%. The reason for the N concentration being so high in  $BC_{850}$  was probably the short duration of pyrolysis process (1 h at 850°C).

#### Release of nutrients from biochar in soil.

During the two-month experiment, nutrient concentration in biochar were decreased significantly in the soil (Table 2). The most decreased elements were K and N, and less changed concentration was of P, Ca and Mg. The greatest  $N_{\text{tot}}$  reduction occurred in  $BC_{550}$  and  $BC_{850}$ , which was unexpected, as most of the studies to date have shown that the amount of extractable N in biochar decreases with increasing pyrolysis temperature (Wang et al., 2012; Mukherjee, Zimmerman, 2013; Naem et al., 2014).

Analysis of water-extractable N of whole and ground biochar pellets showed that slightly more N was extracted from ground  $BC_{300}$  – 0.89 and 1.33 mg g<sup>-1</sup>, respectively (n = 4). However, the water-extractable N for whole and ground biochar was similar in  $BC_{850}$  – 0.11 and 0.08 mg g<sup>-1</sup>, respectively (n = 4) and  $BC_{550}$  – 0.04 and 0.05 mg g<sup>-1</sup>, respectively (n = 4). This result suggests that in  $BC_{300}$ , N compounds were dispersed throughout the granule, whereas in  $BC_{550}$  and  $BC_{850}$  they were more often located in the surface layer.

Nitrogen emissions from biochar intensify when pyrolysis temperature rises above 400°C (Zheng et al., 2013). It is probable that evaporated N that did not escape the granule during the pyrolysis condensed in the top layer of the  $BC_{550}$  and  $BC_{850}$  pellets. The biochar pellets retained their shape in soil; therefore, the release of N was possible only from the pellets surface layer. This was probably the reason, why greater reduction of  $N_{\text{tot}}$  occurred in  $BC_{550}$  and  $BC_{850}$ .

On the other hand, it is notable that the water-extractable N concentration in the surface layer of  $BC_{300}$  was 8–20 times higher than that of  $BC_{550}$  and  $BC_{850}$  but the amount of  $N_{\text{tot}}$  released from  $BC_{300}$  in soil was lower (10.6 mg g<sup>-1</sup>  $N_{\text{tot}}$ ) than from  $BC_{550}$  (16.0 mg g<sup>-1</sup> N) and  $BC_{850}$  (17.7 mg g<sup>-1</sup> N). This result indicates that N release from  $BC_{300}$  should be (i) lower in the soil than in water extract or (ii)  $BC_{300}$  not only released N into the soil, but it also adsorbed it from there. Experiment in aqueous environment by Gai et al. (2014) showed that biochar produced at 400...500°C temperature adsorbed  $NH_4$ -N and released  $NO_3$ -N, but it is not clear if similar process takes place also in the soil. The capability of biochar to adsorb  $NH_4$ -N depends on the amount of negatively charged functional groups that is reduced with higher pyrolysis temperatures (Li et al., 2013; Gai et al., 2014). Among the biochars used in the current experiment, the amount of negatively charged functional groups was the greatest on the surface of the biochar produced at the lowest temperature (Escuer-Gatius et al., 2020) indicating that  $BC_{300}$  has prerequisite for  $NH_4$ -N adsorption. Release and adsorption of N in the soil by hay biochar produced at low temperature needs precise study in the future.

**Biochar impact on N, P, K, Ca and Mg uptake by perennial ryegrass.** The biochar increased plant N, P and K uptake ( $NUP_{\text{tot}}$ ) and had no effect on Ca and Mg uptake (Table 3). The relative importance of biochar (indicated by % of  $R^2$ ) was the highest for K uptake (Table 4).

The increased nutrient uptake was related to the increased aboveground biomass yield ( $p < 0.01$ ) and higher concentration of P ( $p < 0.01$ ) and K ( $p < 0.01$ )

**Table 3.** Cumulative nutrient uptake by perennial ryegrass (above + below ground biomass) (g m<sup>-2</sup>)

Treatment	Uptake				
	N	P	K	Ca	Mg
Control	9.60 B ± 0.24	1.30 C ± 0.02	15.30 B ± 0.18	4.40 AB ± 0.04	2.56 A ± 0.09
$BC_{300}$	11.63 A ± 0.60	1.53 BC ± 0.01	20.49 A ± 0.41	4.89 A ± 0.09	2.76 A ± 0.05
$BC_{550}$	11.45 A ± 0.17	1.75 A ± 0.06	20.40 A ± 0.20	4.13 AB ± 0.32	2.83 A ± 0.16
$BC_{850}$	12.06 A ± 0.29	1.66 AB ± 0.07	20.9 A ± 0.56	3.93 B ± 0.25	2.64 A ± 0.11

Note. The index indicates the temperature, at which the biochar (BC) was produced (300°C, 550°C or 850°C); the values are presented as mean ± standard error (n = 4); results marked with different capital letters are statistically different among treatments.

**Table 4.** Results of one-way ANOVA testing the effects of amendment of biochar on nutrient uptake

Biochar	N		P		K		Ca		Mg	
	% of $R^2$	P	% of $R^2$	P	% of $R^2$	P	% of $R^2$	P	% of $R^2$	P
		65.5	0.01	58.8	0.01	92.0	0.01	0.7	0.75	13.5
	$R^2 = 0.63$	0.01	$R^2 = 0.56$	0.01	$R^2 = 0.91$	0.01	$R^2 = 0.06$	0.75	$R^2 = 0.07$	0.16

Note. Relative importance of factor is indicated by % of  $R^2$  (n = 16).

in shoots and roots and N only in shoots ( $p < 0.01$ ). The methodology used in the experiment did not allow us to determine precisely how much of the nutrients assimilated by plants originated from biochar. It was only possible to estimate indirectly based on the nutrient

amount released from biochar, the nutrient uptake and the change of the nutrient amount in the soil during the experiment. The hay biochar used in the current study was rich in K, and during the experiment K was released to the soil in much higher quantities (32.2 g m<sup>-2</sup>) than it

was assimilated by plants ( $20.6 \text{ g m}^{-2}$ ). At the end of the experiment, soil  $K_{\text{tot}}$  and  $K_{\text{AL}}$  in the biochar treatments were higher ( $p < 0.01$ ) than in the control (Tables 5 and 6) and also when compared with the concentration at the beginning experiment. This result suggests that increase of K uptake was mainly due to K, which was released from the biochar. The exception was  $BC_{300}$  treatment, where  $K_{\text{tot}}$  concentration in the soil at the end

of the experiment did not differ significantly from that of the control. A positive effect of biochar on soil and plant biomass K uptake has been noted in many studies (Gaskin et al., 2010; Kloss et al., 2014; Zemanová et al., 2017), but it is significant only when the concentration of K in biochar is high (Gaskin et al., 2010), as was the case with hay biochar in this experiment.

**Table 5.** Soil acidity (pH), total (tot) nutrient concentration ( $\text{g kg}^{-1}$  DM) in the 0–10 cm soil layer at the end of the experiment

Biochar (BC)	pH	$N_{\text{tot}}$	$P_{\text{tot}}$	$K_{\text{tot}}$	$Ca_{\text{tot}}$	$Mg_{\text{tot}}$
Control	5.1 A $\pm$ 0.09	0.641 A $\pm$ 0.01	0.23 A $\pm$ 0.02	0.71 A $\pm$ 0.02	0.77 A $\pm$ 0.08	1.64 A $\pm$ 0.04
$BC_{300}$	5.4 B $\pm$ 0.07	0.65 A $\pm$ 0.01	0.13 B $\pm$ 0.01	0.85 A $\pm$ 0.05	0.80 A $\pm$ 0.04	1.72 A $\pm$ 0.05
$BC_{550}$	5.8 C $\pm$ 0.09	0.73 A $\pm$ 0.03	0.13 B $\pm$ 0.03	1.18 B $\pm$ 0.03	0.88 A $\pm$ 0.05	1.72 A $\pm$ 0.03
$BC_{850}$	5.8 C $\pm$ 0.03	0.67 A $\pm$ 0.03	0.14 B $\pm$ 0.02	1.24 B $\pm$ 0.06	0.78 A $\pm$ 0.03	1.65 A $\pm$ 0.04

*Note.* The index indicates the temperature ( $300^\circ\text{C}$ ,  $550^\circ\text{C}$  or  $850^\circ\text{C}$ ), at which the biochar was produced; the values are presented as mean  $\pm$  standard error ( $n = 4$ ); the results marked with different capital letters are statistically different at the 0.05 level; prior to the analysis, the biochar granules were removed from soil by sieving.

**Table 6.** Soil plant-available nutrient concentration ( $\text{mg kg}^{-1}$  DM) in the 0–10 cm soil layer at the end of the experiment

Biochar (BC)	$P_{\text{AL}}$	$K_{\text{AL}}$	$Ca_{\text{AL}}$	$Mg_{\text{AL}}$
Control	12.3 A $\pm$ 1.75	3.5 D $\pm$ 2.3	565.3 A $\pm$ 27.5	116.2 A $\pm$ 4.5
$BC_{300}$	8.9 A $\pm$ 1.63	125.9 C $\pm$ 5.7	551.6 A $\pm$ 9.3	133.5 A $\pm$ 3.7
$BC_{550}$	14.2 A $\pm$ 1.45	277.1 A $\pm$ 9.4	729.7 A $\pm$ 41.0	137.9 A $\pm$ 5.5
$BC_{850}$	16.8 A $\pm$ 2.62	243.6 B $\pm$ 8.4	742.6 A $\pm$ 113.1	128.8 A $\pm$ 8.8

*Note.* The index indicates the temperature ( $300^\circ\text{C}$ ,  $550^\circ\text{C}$  or  $850^\circ\text{C}$ ), at which the biochar was produced; the values are presented as mean  $\pm$  standard error ( $n = 4$ ); the results marked with different capital letters are statistically different at the 0.05 level; prior to the analysis, the biochar pellets granules were removed from soil by sieving.

The P uptake ( $1.7 \text{ g m}^{-2}$  P) was approximately 1.5 times higher compared with the amount of P ( $1.1 \text{ g m}^{-2}$  P) released into the soil from biochar. The concentration of  $P_{\text{tot}}$  in the soil at the end of the experiment was significantly lower compared with that at the beginning of the experiment, suggesting that major part of P assimilated by plants during experiment originated from soil. The impact of biochar on P uptake was associated with the reduction of soil acidity, which increased P mobility in the soil. This was indicated by the positive relationship between P uptake and soil acidity (Table 7).

**Table 7.** Pearson correlation coefficients of the linear relationship between nutrient uptake and soil acidity (pH) and total nutrient and plant-available nutrient concentration at the end of the experiment

Characteristic	Uptake				
	N	P	K	Ca	Mg
pH	<b>0.74</b>	<b>0.78</b>	<b>0.76</b>	<b>-0.50</b>	0.21
$N_{\text{tot}}$	0.04	0.30	0.21	<b>-0.52</b>	0.04
$P_{\text{tot}}$	<b>-0.72</b>	<b>-0.52</b>	<b>-0.72</b>	0.27	-0.00
$K_{\text{tot}}$	<b>0.63</b>	<b>0.80</b>	<b>0.68</b>	-0.43	0.28
$Ca_{\text{tot}}$	0.12	0.30	0.22	-0.11	0.29
$Mg_{\text{tot}}$	0.04	0.15	0.10	0.25	0.52
$P_{\text{AL}}$	0.10	0.32	-0.06	0.07	0.14
$K_{\text{AL}}$	<b>0.68</b>	<b>0.85</b>	0.77	-0.50	0.25
$Ca_{\text{AL}}$	0.47	<b>0.65</b>	0.40	-0.28	0.19
$Mg_{\text{AL}}$	<b>0.65</b>	<b>0.61</b>	<b>0.60</b>	-0.02	0.37
$Ca_{\text{tot}}:K_{\text{tot}}$	<b>-0.81</b>	<b>-0.79</b>	<b>-0.94</b>	0.19	-0.33
$Mg_{\text{tot}}:K_{\text{tot}}$	0.18	0.42	0.12	-0.37	-0.03

*Note.* Correlations marked in bold are significant at the 0.05 level ( $n = 3$ ).

This result is in accordance with the previous studies (Kloss et al., 2014; Jeffery et al., 2017), which have also shown that biochar incorporation into acidic soil reduces its acidity resulting in increased P availability.

Nitrogen uptake in biochar treatments was significantly higher than in the control. The biggest difference was seen during the 1<sup>st</sup> cut when plants in biochar treatments assimilated on average 42% more N ( $p < 0.001$ ) than from the control. During the 2<sup>nd</sup> cut the difference was 8% ( $p < 0.05$ ). The N concentration of biomass harvested from biochar and control treatment differed only during the 2<sup>nd</sup> cut ( $p < 0.001$ ). The amount of N released from biochar in every treatment was different (Table 8), but there was no significant relationship between released N amount and N uptake, which shows that direct effect of biochar as N source on the plants was low.

Nitrogen uptake significantly correlated with  $P_{\text{tot}}$  and  $K_{\text{AL}}$  concentration in the soil at the end of experiment suggesting that plants assimilated more N in biochar treatments mainly due to the higher amount of  $P_{\text{AL}}$  and  $K_{\text{AL}}$  in the soil (Table 7). Also was found that biochar increased substantially the N loss from soil (Table 8), which was partly due to increased ( $p < 0.01$ ) nitrous oxide ( $N_2O$ ) emission from soil (Escuer-Gatius et al., 2020). This result suggests that part of N released from biochar could have emitted from the soil during experiment, and this could be one of the reasons why the impact of N, released from biochar, on plants N uptake was low.

So far the research has shown that biochar increases Ca and Mg uptake (Laghari et al., 2016), but it can also reduce Ca uptake if it is adsorbed onto Fe and Al oxides (Smider, Singht, 2014). The reason for the neutral impact of biochar on Ca and Mg uptake in the present research could be due to its very high K concentration,

**Table 8.** The amount of nitrogen (N) in the soil at the beginning and at the end of the experiment, amount of N released from biochar (BC) during experiment, N uptake and N loss (g pot<sup>-1</sup>)

Treatment	N amount in soil at the beginning of experiment	N amount released from biochar during experiment	N <sub>tot</sub> amount in soil at the end of experiment	N uptake	N loss
Control	2.24	–	2.01 ± 0.05	0.09 ± 0.002	0.14 ± 0.05
BC <sub>300</sub>	2.24	0.09 ± 0.06	1.94 ± 0.14	0.12 ± 0.006	0.27 ± 0.13
BC <sub>550</sub>	2.24	0.14 ± 0.04	1.96 ± 0.04	0.11 ± 0.0002	0.31 ± 0.04
BC <sub>850</sub>	2.24	0.15 ± 0.03	2.08 ± 0.05	0.12 ± 0.003	0.19 ± 0.05

which could have caused the unbalanced ratio of K and Ca as well as K and Mg in the soil. At the beginning of the experiment, the soil Ca<sub>AL</sub>:K<sub>AL</sub> was 7:1, but by the end of experiment it was decreased to 3:1, because by biochar application 2–3 times more K relative to Ca was taken into the soil. At the start of the experiment, the ratio of Mg<sub>AL</sub>:K<sub>AL</sub> in the soil was 2:1, but it decreased to 1:1.4 by the end of the experiment. It has been previously shown also by Zemanová et al. (2017) that the application of biochar into the soil changes the ratio of Ca:K and

Mg:K, due to which the plant availability of Ca and Mg decreased because of antagonistic interaction mechanism between Ca, Mg and K. This shows that one should be careful when using biochar with high K concentration in the soil with low concentration of Ca and Mg, where it could decrease the uptake of these elements by the plants.

Biochar impact on nutrient uptake did not depend significantly on temperature, at which it was produced (Table 9).

**Table 9.** Results of analysis of one-way ANOVA testing the effects of pyrolysis temperature of biochar on nutrient uptake by perennial ryegrass

Uptake	N		P		K		Ca		Mg	
	% of R <sup>2</sup>	P	% of R <sup>2</sup>	P	% of R <sup>2</sup>	P	% of R <sup>2</sup>	P	% of R <sup>2</sup>	P
Pyrolysis temperature	12.4	0.55	49.7	0.045	8.76	0.66	48.71	0.051	14.1	0.51
	R <sup>2</sup> = 0.12	0.55	R <sup>2</sup> = 0.50	0.045	R <sup>2</sup> = 0.09	0.66	R <sup>2</sup> = 0.49	0.049	R <sup>2</sup> = 0.14	0.51

Note. The relative importance of factor is indicated by % of R<sup>2</sup> (n = 12).

The exception was P uptake, which was the highest in BC<sub>550</sub> and lowest in BC<sub>300</sub> treatment (Table 3). This result shows that the changes occurring in biochar with the increasing pyrolysis temperature such as increased nutrient concentration and increase in acid neutralization capacity do not have a major influence on the impact the biochar has on nutrient uptake. We noted at the end of the experiment that in BC<sub>300</sub> treatment the pellets granules were so strongly colonized by plant roots that they had to be removed with force. This suggests that in the BC<sub>300</sub> treatment the plants assimilated nutrients also directly from the surface of the biochar, which could have partly compensated for the lower effect of biochar on soil acidity and on P availability in the soil. In contrast, plant roots did not attach to the BC<sub>550</sub> and BC<sub>850</sub> pellets because of their very high acidity.

**Biomass yield of perennial ryegrass.** The biochar increased the 1<sup>st</sup> cut yield of perennial ryegrass on average by 40% ( $p < 0.01$ ) compared to the control treatment (Table 10), but it did not have a significant effect on the 2<sup>nd</sup> cut yield, which is consistent with the conclusion by Rajkovich et al. (2012), which suggests that graminaceous biochar improves the plant growth over a short period. The effect of biochar on the yield showed no significant relationship with the pyrolysis temperature.

**Table 10.** Perennial ryegrass aboveground biomass yield (g DM m<sup>-2</sup>)

Treatment	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
Control	194.7 A ± 9.1	189.5 A ± 1.0
BC <sub>300</sub>	271.1 B ± 9.0	189.5 A ± 7.4
BC <sub>550</sub>	268.4 B ± 13.9	184.2 A ± 6.8
BC <sub>850</sub>	276.3 B ± 13.8	197.4 A ± 9.0

Results of our experiment demonstrated that yield increase (40% on average) in biochar treatments was mainly due to the reduction of soil acidity, which increased P availability to plants. Moreover, the large amount of K that was released from biochar could have had a positive influence on the yield, as the K concentration of the soil itself was low. This result supports the findings of previous research, according to which biochar acts as a liming agent and short-term fertilizer in low-nutrient acidic soils (Kloss et al., 2014; Jeffrey et al., 2017).

## Conclusions

1. The permanent grassland hay dominated by reed canary grass is a suitable raw material for biochar (BC) production. This biochar is a fast-acting potassium (K) rich liming agent, which also contains remarkable amount of nitrogen (N). The addition of this biochar into acidic soil reduced the soil acidity and significantly increased plant N, P and K uptake, which had positive impact on the biomass yield of perennial ryegrass.

2. The increase of pyrolysis temperature changed biochar properties like neutralization capacity, acidity (pH), nutrient concentration and the release of nutrients from biochar, but these changes did not have significant impact on the effect the biochar had on plant nutrition and yield as the effect of all three biochars on them was similar. The only exception was phosphorus (P) uptake, which was the highest when the biochar was produced at 550°C temperature.

3. The results of the present research suggested that the biochar produced from permanent grassland hay at 300°C temperature could concurrently release and adsorb N in the soil. This hypothesis needs to be proved or disapproved in future studies.

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## Bioanglis, pagaminta iš daugiamečių pievų žolės, didina augalų N, P ir K įsisavinimą rūgščiame dirvožemyje

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### Santrauka

Estijoje yra daug derlingų ilgalaikių pievų, kuriose žolės pjaunamos kartą per metus, kad būtų išvengta antrinio miško medžių augimo, tačiau nupjauta biomasė dėl mažos pašarinės vertės dažniausiai paliekama pūti. Vienas būdų šiems ištekliams suteikti vertę būtų jų panaudojimas bioanglies gamybai, siekiant padidinti anglies ir maisto medžiagų kiekį mažo derlingumo dirvožemiuose. Buvo tirta: (i) bioanglis, pagaminta iš ilgalaikių pievų žolių, naudojant tris (300°C, 550°C ir 850°C) pirolizės temperatūras ir (ii) bioanglies įtaka daugiamečių svidrės (*Lolium perenne* L.) maisto medžiagų N, P, K, Ca bei Mg įsisavinimui ir biomasės derliui rūgščiame (pH 4,2) dirvožemyje. Nustatyta, kad ilgalaikės pievos, kuriose vyravo nendrinis dryžutis (*Phalaris arundinacea* (L.)), žolė yra tinkama žaliava bioanglies gamybai. Tokia bioanglis turi daug kalio (K), kaip greitai veikiančios kalkinės medžiagos, ir didelį kiekį azoto (N). Ją įterpus į rūgštų dirvožemį, sumažėja jo rūgštumas ir reikšmingai padidėja daugiamečių svidrių N, P ir K įsisavinimas, o tai turi trumpalaikę teigiamą įtaką biomasės derliui. Pirolizės temperatūrų didinimas keičia bioanglies savybes – neutralizacijos gebą, rūgštumą (pH), maisto medžiagų koncentraciją ir jų atpalaidavimą, tačiau šie pokyčiai neturi reikšmingos įtakos bioanglies poveikiui daugiamečių svidrių mitybai ir derliui. Išimtis buvo fosforo (P) įsisavinimas, kuris buvo didžiausias tręšiant bioanglimi, pagaminta 550°C temperatūroje.

Reikšminiai žodžiai: biomasės derlius, maisto medžiagų atpalaidavimas iš bioanglies, maisto medžiagų įsisavinimas, bioanglies savybės, pirolizės temperatūra.