The effect of wood ash application on growth, leaf morphological and physiological traits of trees planted in a cutaway peatland

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SUMMARY

Management of cutaway peatlands is a key issue in many countries. Whilst afforestation has been considered as a suitable after use option for cutaway peatlands, growing conditions after peat harvest are often adverse. To increase soil fertility and neutralise soil acidity, wood ash, an energy production by-product, can be used. In this study, we examined whether wood ash fertiliser affects growth (survival, height, root collar diameter), leaf morphological (mass, specific leaf area, leaf water content) and physiological traits (chlorophyll concentration, fluorescence parameters, photosynthetic and transpiration rate) of planted deciduous trees in a cutaway peatland in the central part of Latvia. At our study site in Latvia, we examined *Alnus glutinosa*, *Betula pendula* and *Populus* v. *Vesten* tree species and tested 0, 5, 10 and 15 t ha^{-1} wood ash doses in three replications. Tree species used in the study exhibited different reactions to the wood ash fertilisation. *A. glutinosa* traits were the least affected, whereas for *Populus* v. *Vesten* and *B. pendula* wood ash fertilisation was beneficial. In most cases we detected significant differences between unfertilised and fertilised plots, but rarely saw an effect of increased wood ash dose. Due to the lack of significant benefits and for environmental concerns the lowest wood ash dose is recommended. Overall, the fertilisation improved the success of afforestation and, by increasing the amount of leaf litter, is likely to indirectly alter understorey conditions.

KEY WORDS: afforestation, fertilisation, photosynthesis

INTRODUCTION

Due to land-use changes, especially in Europe, the peatland area has considerably decreased (Rochefort & Lode 2006). Of the Baltic states, Latvia has both the largest current peat extraction area and existing post-extraction and abandoned peatlands, whilst less than 20 % of these areas have been used after peat extraction (Karofeld *et al.* 2016). Peat extraction results in greenhouse gas emissions, loss of biodiversity and soil degradation (Wilson *et al*. 2016, Wüst-Galley *et al*. 2016, Leifeld & Menichetti 2018). Afforestation may increase nutrient pools and improve microclimatic conditions (cool the air, lower soil surface temperature and reduce temperature fluctuations) of peat extracted bogs (Paradis & Rochefort 2017). However, often due to factors such as extreme weather conditions or unfavourable soil conditions cutaway peatland afforestation trials fail (Renou-Wilson *et al*. 2006).

Prior to peat extraction the peatland area is drained. For horticulture, typically peat is extracted from the upper layers of lowland bogs, while for fuel purposes peat from blanket peatlands is also extracted from lower levels (Cruickshank *et al*. 1995). For horticultural peat, extraction is typically carried out using large vacuums pulled by tractors (Graf *et al.* 2012). The most suitable peat layer for horticulture is partly decomposed *Sphagnum* peat (Graf *et al.* 2012). After peat extraction, when the top layer of the soil has been removed, the remaining soil is typically acidic, with high nitrogen concentrations (mainly incorporated in organic matter), lacks phosphorus and potassium and the depth of the residual peat layer varies (Triisberg *et al*. 2013). Growing conditions can be improved by fertilising cutaway peatland with a by-product from energy production - wood ash. Wood ash is a liming material which provides nutrients that typically limit growth in cutaway peatland (Demeyer *et al*. 2001, Kikamägi *et al*. 2013).

Acclimatisation of plants to the specific growing conditions determines their survival in the environment. At species level stress may not induce instant phenotypical changes (Yordanov *et al*. 2000). Being able to follow tree vitality before observing visual stress signs is crucial, especially under current climate change conditions (Pontius & Hallet 2014). Some traits such as height are still currently used to evaluate species suitability despite the response time lag (Johnstone *et al*. 2013) whilst other physiological parameters, like chlorophyll fluorescence, have proved to be very sensitive to a number of factors (Renou-Wilson *et al*. 2008, Gorbe & Calatayud 2012, Kalaji *et al*. 2016). Ecophysiological traits describe not only species performance at the individual level (Violle *et al*. 2007), but also functioning of the ecosystem (nutrient cycling, decomposition, surface refractivity) (Myers-Smith *et al*. 2019) and provided ecosystem services after restoration (Lavorel 2013).

The main goal of the study was to investigate if growing conditions in a cutaway peatland can be improved by using waste material – wood ash. This was done by evaluating the ecophysiological traits (plant height, root collar diameter, leaf mass, specific leaf area, leaf water content, chlorophyll concentration, fluorescence parameters, photosynthetic and transpiration rate) of multiple tree species.

METHODS

Preparation of the experimental site

The experimental site was established on an 8 ha large cutaway peatland in the central part of Latvia (56° 43' 41.35" N 23° 34' 39.61" E, Figure 1). After the peat extraction for horticulture, the residual layer of peat was thicker than 0.5 m and consisted of acidic, moderately decomposed raised bog peat. Prior to soil preparation and tree planting, all the vegetation on the site and the build-up of material in the ditches was removed. Afterwards, wood ash was mixed with water, to reduce wind-driven wood ash dispersal, and mechanically applied to the top layer of soil. Four treatments of wood ash were tested (0, 5, 10 and 15 t ha^{-1} dry mass of wood ash). Wood ash was obtained from a nearby energy plant SIA "Fortum". On dry mass basis the wood ash consisted of K 24.7, Mg 18.2, Ca 120.4, P 6.6 g kg^{-1}. With the addition of wood ash, the pH of the soil changed from 3.5 in control plots with no wood ash to 5.9 in plots with the maximum wood ash dose. For a more detailed description of soil properties and the vegetation in the study site see Neimane *et al.* (2019). Each wood ash treatment was separated by a ditch and replicated three times, resulting in twelve 20 m wide and 236 m long columns. In each column in a 20 × 45 m large plot, 95 seedlings were planted in five rows. *Alnus glutinosa* containerised seedlings (obtained from tree nursery "Zābaki", certification no. EK:LV/616015), *Betula pendula* containerised seedlings (tree nursery "Zābaki", certification no. EK:LV/616011) and 1.8 m Poplar clone *Vesten* cuttings (Biopoplar s.r.l., Cavallermaggiore, Italy) were planted in the spring of 2017.

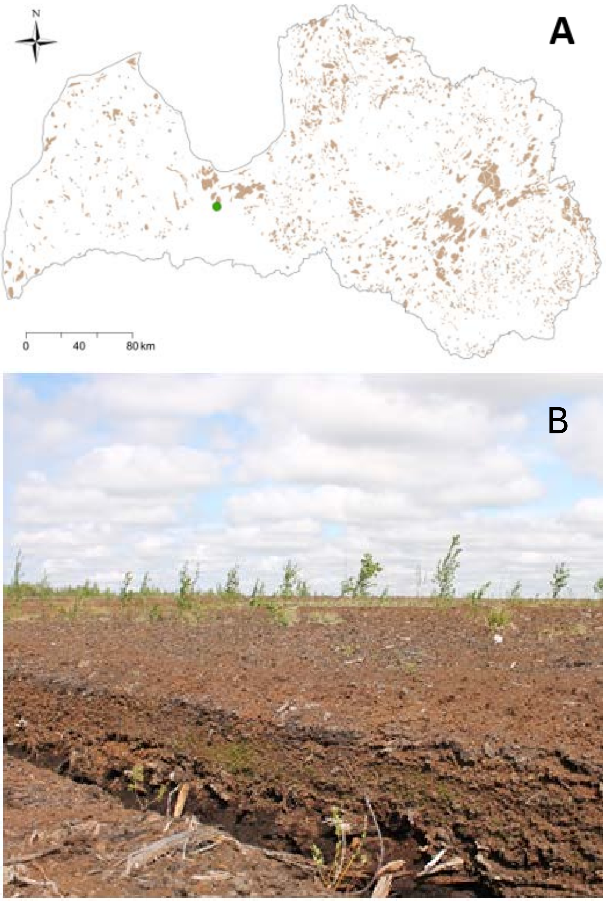

Figure 1. The experimental site "Kaigu purvs", Latvia. (A) Location of the experimental site (marked with a green dot) with a background map of border line of Latvia (grey lines), peat deposits or peat industrial sediments (coloured in brown). Map prepared by authors using the map database GIS Latvija 10.2 (SIA Envirotech). (B) Landscape of the study site with poplar hybrids growing in the background.

Data collection

Sampling

Leaf morphological traits (fresh mass, dry mass, leaf area, specific leaf area (SLA)), chlorophyll concentration and fluorescence traits were measured in the second growing season of 2018 at the beginning of August during the European heatwave. During the next growing season in August 2019 the photosynthetic and transpiration rates were measured, the number of leaves was counted and the survival rate was determined. In December 2019 the height and diameter at root collar was measured. In both years leaves from the same trees were measured. Detailed meteorological data are available from Latvian Environment, Geology and Meteorology Centre station Dobele, 20 km from the study site, available at www.meteo.lv.

To determine average survival rate, height and root collar diameter of the 6th to 12th tree in three rows in each sample plot were examined (in total 21 trees per sample plot). In case of die-back, the height and the diameter at root collar of all trees in the sample plot was measured (at least eight trees per sample plot). To determine leaf traits, one fully expanded undamaged sun leaf from six to nine trees in the centre of each sample plot was measured and sampled. Leaves were sampled according to the standardised methodology of Pérez-Harguindeguy *et al*. (2013).

Measurement of chlorophyll a fluorescence, chlorophyll and leaf mass

Chlorophyll *a* fluorescence was measured (at a light intensity of 3500 µmol m^{-2} s^{-1}) and indices calculated using Handy PEA (Hansatech, Kings Lynn, United Kingdom) fluorimeter and software. Chlorophyll fluorescence was measured using the Kautsky fluorescence induction which provides that, if the photosynthetic part of the plant is adapted in the dark, the Photosystem II acceptor pool is re-oxidised until all reaction centres of Photosystem II are able to engage in photochemical reactions (Kautsky & Hirsch 1931). During this process, three measurements of fluorescence were taken: F_o, which refers to the fluorescence origin and shows the emissions by excited chlorophyll *a* molecule in Photosystem II; F_m, which refers to the fluorescence maximum and is attributable to the time when electron acceptor is fully reduced; and F_v, or variable fluorescence value, obtained by subtracting F_o from the F_m (Handy Pea 2006). The F_m/F_v ratio is used to determine the maximum effectiveness and photosynthesis performance of Photosystem II, as it is a sensitive parameter to environmental factors (Ibaraki & Murakami 2007). The performance index was calculated automatically in the Handy PEA software by using the concentration of active reaction centres, light reactions and dark reactions (F_v, F_m, F_o) in the Nernst equation, which describes the force of redox reactions and Gibbs's energy movements in biochemical systems (Handy Pea 2006). Prior to measurement leaves were dark-adapted by using Handy PEA clips for 30 minutes. After each fluorescence measurement the leaf petiole was removed and the leaf blade was put in a plastic bag with a zipper, the bag was breathed into to minimise transpiration water loss and the bag was closed. All plastic bags with leaves were put in a heat-insulated bag with a cooling element. After all the samples were collected, they were weighed (0.001 g precision) to obtain fresh mass. Leaves were later put on a flat surface and covered with transparent plastic cover, to avoid overlapping, and photographed, with a levelled camera, to calculate leaf area. Leaf area was calculated using ImageJ (Rasband 1997–2018, Schneider *et al*. 2012) software following the protocol of Wang (2017). The leaf chlorophyll concentration was then measured both with the SPAD-502 (Konica-Minolt, Japan) and the CCM-300 (Opti-Sciences, Hudson, USA) in roughly the same spot in the middle of the leaf blade, avoiding midribs and large veins. Later, leaves were put in paper envelopes and dried at a temperature of 37 °C until reaching constant dry mass and then weighed again. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass. Leaf water content was calculated following this formula: (fresh mass – dry mass) / dry mass.

Measurement of photosynthetic performance

The photosynthetic performance reflects the functionality of both photosystem I and II and provides quantitative information about carbon fixation (Živčák *et al*. 2008). Photosynthetic and transpiration rate was determined by using LCpro-SD (ADC BioScientific Limited, Hertfordshire, United Kingdom) portable infrared gas analyser system on the same tree and type of leaves as in the previous season. Measurements were taken over three consecutive days from early morning until midday. Each leaf was kept in the leaf chamber for at least five minutes to acclimate before taking the measurement. Broad Leaf Chamber was used and set at 25 °C temperature, the LED mixed red/blue light unit to PAR of 1500 µmol m^{-2} s^{-1} and to the ambient CO_2 concentration. The mean external CO_2 concentration was 400 ppm.

Data analysis

R version 3.6.2 (2019) was used for data analysis and visualisation. Statistical differences in tree survival were analysed with a generalised linear model for binary data with survival as response binary variable with "logit" link function, wood ash fertiliser dose as categorical explanatory variable and plot number as a random factor. For post hoc analysis Tukey tests were used under the glht function in the multcomp package.

For other tree traits normality assumptions were checked with Shapiro–Wilk and Levene tests. Statistical tests showed significant deviations from normality even after data transformation, therefore for further data analysis non-parametric methods were used. To determine if wood ash fertiliser affected tree traits the Kruskal-Wallis test was used for each species separately. Afterwards Dunnett's test with Bonferroni correction was used to see if the effect of wood ash was dose-specific.

RESULTS

Whole plant traits

After three growing seasons regardless of the studied tree species and wood ash fertilisation dose, the mean survival rate was higher than 77.8 % in all cases (Table 1).

The lowest survival rate was detected for *A. glutinosa* species in fertilised plots. Wood ash fertilisation improved growing conditions for *B. pendula* as survival significantly increased by more than 10 % in the plots fertilised with only 5 t ha^{-1} of wood ash in comparison with the control where no wood ash was applied. An increase, though not statistically significant, was also observed for *P.* v. *Vesten*. Moreover, there was no recorded dieback for *P.* v. *Vesten* under the maximum wood ash fertiliser dose (15 t ha^{-1}).

Dose-specific response to wood ash fertiliser was observed in the case of tree height (Figure 2a). The highest total growth after the first three growing seasons was recorded for *Populus* v. *Vesten* in both plots fertilised with 10 t ha^{-1} (mean height 3.21 m ± standard error 0.05 m; root collar diameter 54.3 mm ± standard error 0.9 mm) and 15 t ha^{-1} (mean height 3.21 m ± standard error 0.06 m; root collar diameter 54.0 mm ± standard error 0.9 mm) wood ash (Figure 2a, 2b).

Leaf traits

In fertilised plots all species had larger leaf fresh and dry mass and increased the total number of leaves (Figure 3a, 3b, 3f). Of the studied tree species *B. pendula* had both the smallest and the lightest leaves, while the SLA index was similar, though highly variable, for all species (Figure 3b, 3c, 3d).

Table 1. Mean survival of multiple tree species in cutaway peatland fertilised with different doses of wood ash. Letters denote significant ($p_{adj.}$ < 0.05) difference between fertiliser dose for each species.

Wood ash dose, t ha^{-1}	**Survival (%)**		
	A. glutinosa	*B. pendula*	*P.* v. *Vesten*
0	90.1	84.0 *b*	93.8
5	82.7	97.5 *a*	98.8
10	77.8	95.1 *ab*	98.8
15	86.4	96.3 *ab*	100.0

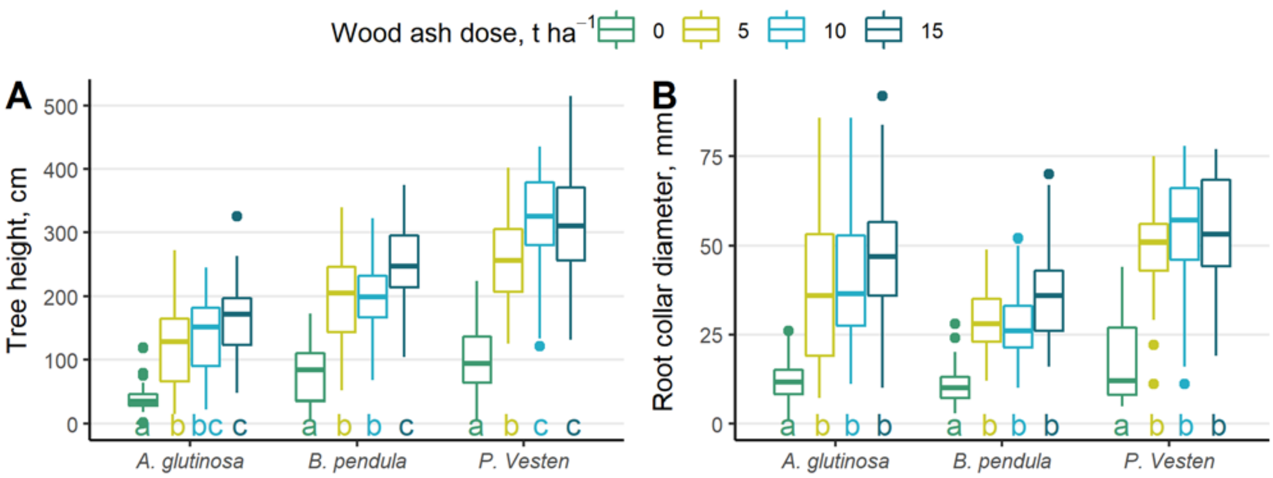

Figure 2. Studied tree species traits. (A) tree height; (B) root collar diameter. Letters denote significant ($p_{adj.}$ < 0.05) differences between fertiliser dose for each species. In box-plot graphs vertical lines show either the minimum or maximum value in 1.5*interquartile range (upper) or minus 1.5*interquartile range (lower), lower hinge - 25% quantile, upper hinge - 75% quantile, horizontal line in the middle represents the median. Dots show outlier values.

A. glutinosa species SLA ratio significantly decreased and average leaf area increased with the addition of wood ash to the soil. Only *A. glutinosa* leaves had greater leaf area and SLA with increased wood ash dose (Figure 3c, 3d). *A. glutinosa* leaves in unfertilised plots were the smallest (with an average leaf area of less than 30 cm^2) and in the plots with the maximum dose of wood ash 15 t ha^{-1} the average leaf size was 52 cm^2. Leaf water content was similar for all species and did not significantly differ between

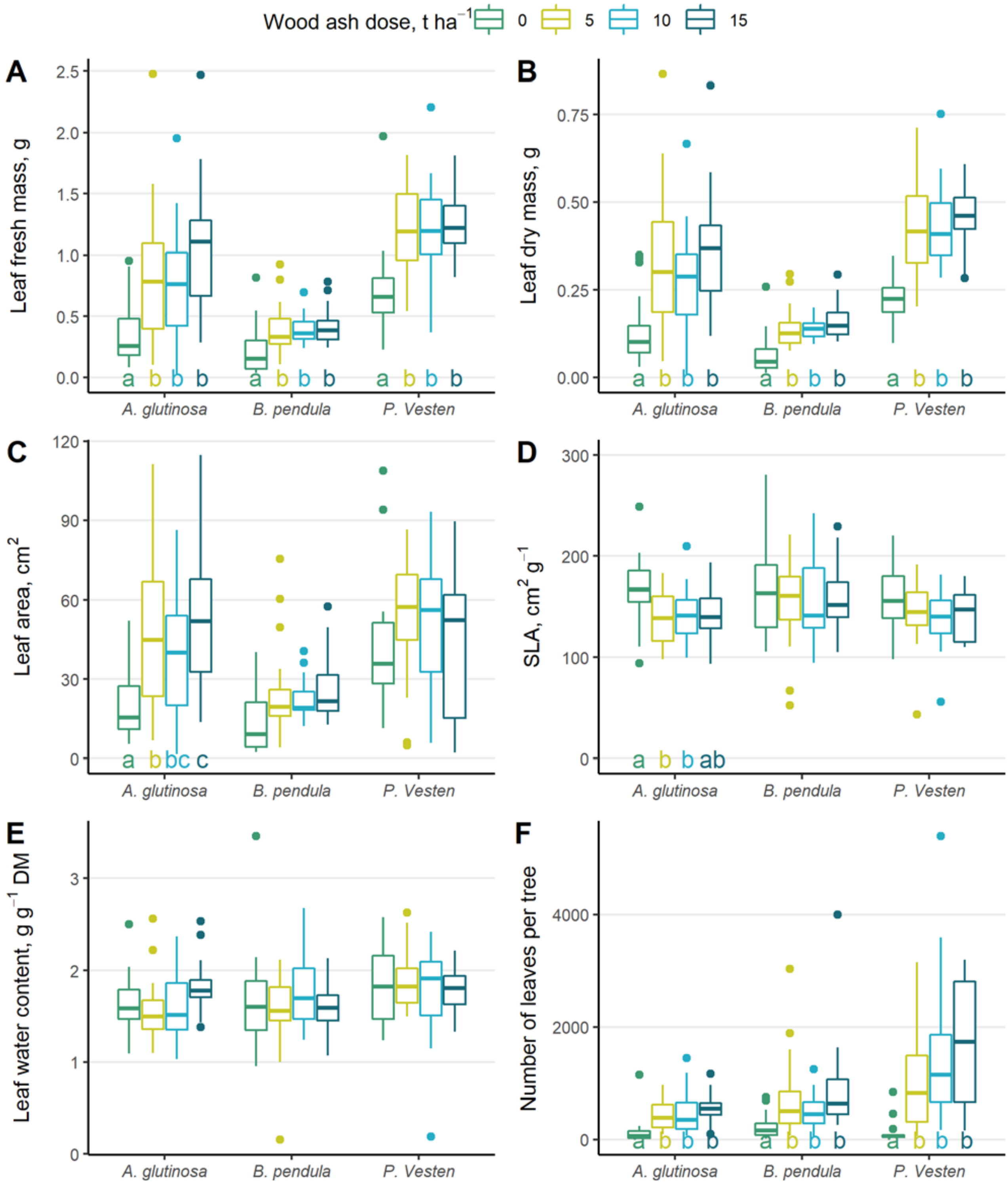

Figure 3. Studied tree species traits. (A) leaf fresh mass (B) leaf dry mass (C) leaf area (D) SLA (specific leaf area) (E) leaf water content per dry mass (F) number of leaves per tree. Letters denote significant ($p_{adj.} < 0.05$) differences between fertiliser dose for each species.

control plots and plots with added wood ash fertiliser (Figure 3e). Neither SPAD-502 nor CCM-300 chlorophyll measurement device detected significant differences in the chlorophyll content in the leaves for all studied tree species (Figure 4a, 4b).

The average F_v/F_m ratio was below 0.783 for all species in control plots and any of the fertilisation treatments (Figure 5e). Only *P.* v. *Vesten* species had reduced Photosystem II inhibition under wood ash fertilisation. A significant increase relative to the control plot in the Performance Index (PI) was recorded only in tree leaves under the highest (15 t ha^{-1}) wood ash dose for *P.* v. *Vesten* and second highest (10 t ha^{-1}) for *B. pendula* (Figure 5d). Significantly decreased initial fluorescence (F_o), the fluorescence in the absence of photosynthetic light, was observed for *A. glutinosa* species (Figure 5a). *B. pendula* had significantly higher maximal fluorescence (F_m) and variable fluorescence (F_v) in the fertilised plots (Figure 5b, 5c).

Fertilisation with wood ash did not significantly affect the rate of transpiration for any of the studied tree species (Figure 6b). An increased rate of photosynthesis was observed for *B. pendula* and *P.* v. *Vesten* species with the addition of even the lowest dose (5 t ha^{-1}) of wood ash fertiliser (Figure 6a). The physiological performance (chlorophyll content, photosynthetic and transpiration rate, and fluorescence parameters, except for F_o) of *A. glutinosa* leaves was not affected by soil fertilisation with wood ash.

DISCUSSION

This study shows that it is possible to successfully afforest industrially cutaway peatland with deciduous tree species. Afforestation as a re-cultivation method would offer carbon storage in woody biomass and thus biomass production possibilities. It also affords a level of soil protection from direct sunlight and wind erosion in a way that improves microclimatic conditions on the site, while fertilisation with wood ash promotes natural colonisation by vegetation (Silvan & Hytönen 2016). The ecophysiological mechanism that ensures survival and growth under such unfavourable conditions as in cutaway peatlands is not clear.

In this study, 18 traits of three deciduous tree species were examined. Chlorophyll fluorescence traits indicate plant condition in the current moment and can change rather rapidly, whereas leaf morphological traits indicate plant condition over a longer period of time, and whole tree growth accumulates during the whole growing period. Therefore, the information time lag on adverse growth conditions or stressor presence, as well as duration, is different for each trait. By assessing a combination of these traits it is possible to provide a comprehensive understanding about short term stressor presence and long term adverse growing conditions. The studied tree species, *B. pendula*, *A. glutinosa*, *P.* v. *Vesten*, differ in their reproduction and growth strategies as well as site requirements for

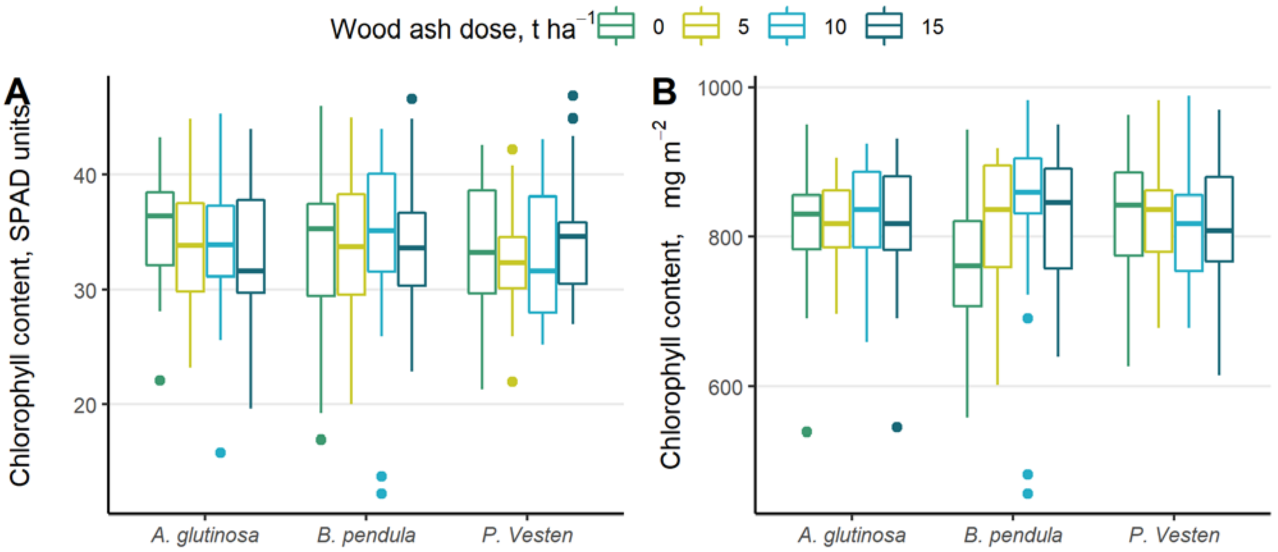

Figure 4. Studied tree species traits. (A) chlorophyll content I (SPAD) (B) chlorophyll content (CCM-300). Letters denote significant ($p_{adj.} < 0.05$) differences between fertiliser dose for each species.

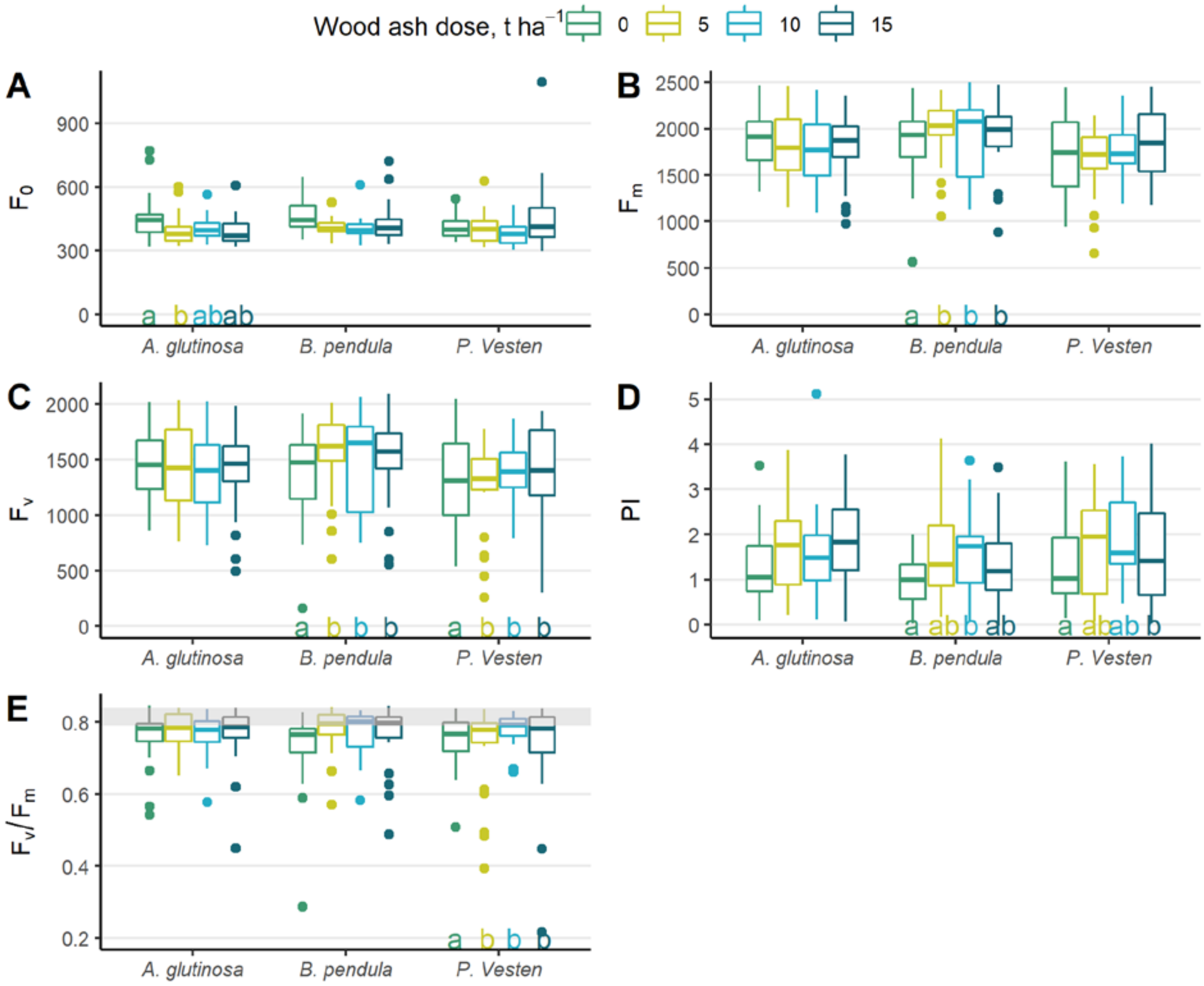

Figure 5. Studied tree species traits. (A) initial fluorescence, F_0 (B) maximal fluorescence, F_m (C) variable fluorescence, F_v (D) Performance index (PI) (E) F_v and F_m ratio (optimal region for this parameter is coloured in grey). Letters denote significant ($p_{adj.} < 0.05$) differences between fertiliser dose for each species.

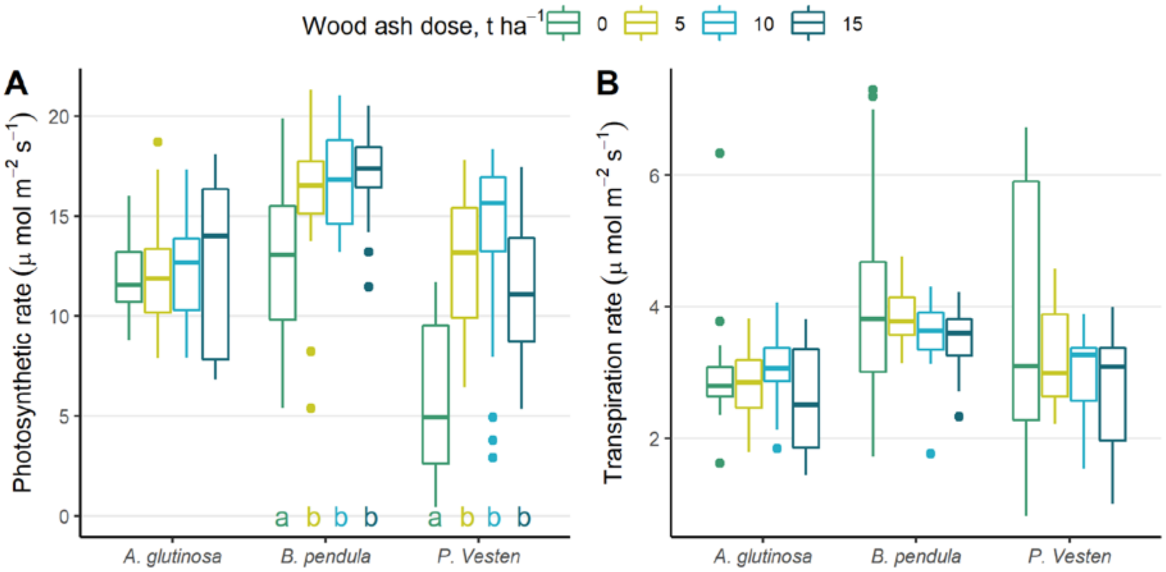

Figure 6. Studied tree species traits. (A) photosynthetic rate (B) transpiration rate. Letters denote significant ($p_{adj.} < 0.05$) value difference between fertiliser dose for one species.

optimal growth. *B. pendula* and *A. glutinosa* can survive in broader pH range compared to *Populus* spp. (Griffiths & McCormick 1984, Lutter *et al*. 2015, Hjelm & Rytter 2018). *Populus* spp. uses water more conservatively than *B. pendula* and *A. glutinosa* and responds to drought conditions more efficiently by osmotic adjustments and limiting leaf expansion, while *A. glutinosa* in extreme conditions reduces transpiration by shedding leaves (Perala & Alm 1990, Herbst *et al*. 1998, Baird *et al*. 2017, Tschaplinski *et al*. 2019). Therefore, the observed trait response to fertilisation was also species-specific in most cases.

Tree survival and growth

The survival rate of all the studied tree species (*A. glutinosa*, *B. pendula*, *P*. v. *Vesten*) was higher than 78 % both in the control and the fertilised plots. In early years the survival rate of *A. glutinosa* and *P*. v. *Vesten* was not significantly affected by the application of wood ash to the soil. No distinct trend in the survival of *A. glutinosa*, with respect to fertilisation, was observed in this study or others (Renou-Wilson *et al*. 2009, Finnegan *et al*. 2012, Lazdiņa *et al*. 2017). This suggests that other factors such as moisture level could be of greater importance. For example, Rodríguez-González *et al.* (2014) have shown that hydrological conditions affect *A. glutinosa* growth. As for *P*. v. *Vesten*, *Populus* spp. survival rate on peat soil without fertilisation is generally low: ranging from less than 20 % (Bussières *et al.* 2008) to 60 % (Kikamägi *et al.* 2013) in the first three to four years respectively. *Populus* spp. is sensitive to soil pH as acidity can inhibit root development and dieback occurs when internal nutrient reserves of the plant are used up (Hjelm & Rytter 2016, 2018). Thus, it is expected that in the following few seasons *P*. v. *Vesten* in control plots, where pH value is 3.5, could die out even though the early survival rates are high and similar across all treatments. Only *B. pendula* in this study showed a significantly better survival rate under fertiliser. This coincides with a similar study by Kikamägi *et al.* (2013) where in control plots 98 % and 88 % *B. pendula* survival rate was observed. Just as in this study, wood ash fertilisation improved the rate of survival to 100 % at 5 t ha^{-1} dose and to 92 %–100 % at 10 t ha^{-1} dose (Kikamägi *et al.* 2013).

At a plant level, plant growth is limited under stress (Yordanov *et al.* 2000). Wood ash incorporation into the soil improved growth (both tree height and root collar diameter) of all studied tree species. Even though fertilisation significantly increased total tree growth of *A. glutinosa*, the plants still grew less than when grown in agricultural soil (Liepiņš & Liepiņš 2010). The observed total growth of *B. pendula* in fertilised plots was similar to growth performance observed in a pot experiment on nutrient-poor forest soil (Kula *et al*. 2012).

Leaf characteristics

For all studied tree species, the response to wood ash fertiliser presented as an increased fresh and dry leaf mass and total number of leaves. An increased photosynthetic area is a typical response to increased nutrient availability (Maier *et al*. 2008, Aguraijuja *et al*. 2015). In combination with growth parameters, these traits show that fertilisation with wood ash did increase above ground biomass accumulation for all three species. Similar values for *A. glutinosa* traits (leaf area, specific leaf area and dry mass) as in unfertilised plots in this experiment have been observed also on abandoned agricultural land by Uri *et al.* (2007). In other studies, *B. pendula* has been shown to maintain normal foliar processes under unfavourable conditions, whilst *P. tremula* reduces leaf size and invests more resources in perennial parts of the plant (Possen *et al*. 2011). However, in this study both *B. pendula* and *P*. v. *Vesten*, as well as *A. glutinosa* in plots without fertiliser, had smaller leaves. No change was detected in leaf water content between treatments for all species. Leaf water content is a measure that indirectly describes leaf tissue density similarly to SLA (Meziane & Shipley 1999). SLA did not differ between treatments for *B. pendula* and *P*. v. *Vesten.* SLA index is species-dependent and describes the relation of leaf dry biomass and area, indicating the carbon uptake relative to water loss and thus the strategy between resource uptake and preservation (Poorter *et al*. 2009, Liu *et al*. 2017). Lack of SLA and leaf water content value differences between treatments may indicate that the main limiting factor in this case was not nutrient availability or soil pH (which were improved by fertilisation with wood ash), but rather drought. These traits have shown to be responsive to limited water accessibility and climatic conditions (Niinemets 2001, Milla *et al.* 2008). In addition, SLA and leaf water content are typically lower in high irradiance conditions. In case of high irradiance, nutrient availability has minimal effect on SLA, yet in low irradiance conditions, nutrient availability is an important factor determining SLA (Meziane & Shipley 1999). However, in case of *A. glutinosa*, higher SLA was observed in control plots, indicating frugal investments in leaf construction, pointing to either high nutrient stress or more rapid resource allocation response compared to *B. pendula* and *P*. v. *Vesten* (Reich *et al.* 1992).

Chlorophyll fluorescence parameters are considered to be one of the most sensitive traits to environmental stressors and there are many examples in the literature (e.g. Damour *et al*. 2009, Gorbe & Calatayud 2012, Johnstone *et al*. 2013, Kalaji *et al*. 2016). These parameters have been shown to vary amongst species, individuals and even their development state (Santiago 2015). Fluorescence parameters are not only depended on photosynthetic processes but will also be modified by alterations in the metabolic pools needed for photosynthesis and possibly even metabolic inhibitors that are not directly related to photosynthetic metabolism (Baker & Rosenqvist 2004). Addition of wood ash to the soil had no significant effect on the physiological traits (apart from increased F_o) of *A. glutinosa* leaves. The F_v/F_m ratio indicated functional impairments to Photosystem II in almost all measured leaves even in plots with added wood ash fertiliser. This points to the presence of environmental stressors in both control plots and treated plots. However, fertilisation with wood ash had significant positive effect on *P*. v. *Vesten*, even though F_v/F_m was still below optimal levels (Figure 5e). This indicates that, even under unfavourable environmental conditions, fertilisation can increase the vitality of *P*. v. *Vesten.*

In this study, no significant pair-wise differences in leaf chlorophyll content could be found between fertilised and control plots even though chlorophyll content typically depends on nutrient availability, stress physiology, heavy metal contamination and other abiotic factors (Richardson *et al*. 2002, Ling *et al*. 2011, Pérez-Jiménez *et al*. 2019). Presumably since wood ash as fertiliser lacks nitrogen, a major component of chlorophyll, fertilisation did not affect nitrogen availability across all treatments.

In conclusion, *B. pendula* and *P*. v. *Vesten* tree species growing conditions in cutaway peatland can be improved by incorporating wood ash into the soil. However, increase of wood ash dose rarely resulted in statistically improved performance. Therefore, due to the risk of nutrient leaching into groundwater and toxicity risks (Pitman 2006), one of the lowest doses would be recommended.

ACKNOWLEDGEMENTS

The study was a part of the project MAGIC – “Marginal Lands for Growing Industrial Crops: Turning a burden into an opportunity” (Horizon2020, Grant agreement ID: 727698) and Research program on improvement of forest growth conditions, 2016–2021 (JSC "Latvia's State Forests"). The experimental plot was established as a part of the LIFE Restore project (LIFE14 CCM/LV/001103). We thank Zaiga Landorfa-Svalbe for helping with the acquisition of the chlorophyll measurement devices, Toms A. Štāls and K. Dūmiņš for help measuring tree height and survival.

AUTHOR CONTRIBUTIONS

DL set up the experimental site, SN, SC and DL designed the study, SN, SC and AZ collected the data, SN analysed the data and wrote the paper, SC, SN, AZ, DL and GI revised the manuscript.

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Submitted 27 Nov 2020, revision 16 Jun 2021
Editor: Gareth Clay

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