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Intensive initial care of silver fir using improving compounds: A way to support diverse forests?

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Abstract: In some cases, it is difficult to promote climax tree species in the forest stand composition. In the Czech Republic, silver fir (*Abies alba* Mill.) is a typical example. This study offers an evaluation of the use of two improving compounds for tree plantations in the initial stage of growth in Central Bohemia. In the experiment, we measured the initial growth performance of a young fir plantation treated with a brassinolide compound (concentration 1 : 100 and 1 : 200) and Bio-Algeen® prior to planting and compared it with control treatment: we assessed height, root collar diameter, vitality and mortality rate during the period 2014–2019. Cumulative mortality rate of the plantation reached 25% at the end of the monitoring period, without any significant differences between variants. Height increment of the variant treated with Bio-Algeen® was significantly ($P < 0.05$) lower than the growth of brassinolide-treated variants, all treated variants were comparable with the control variant. The plantation underwent a drought-stress period in 2014 and 2015, which resulted in worsened vitality and colour across variants. As a result of the simple economic analysis, the most expensive inputs are planting stock and labour, therefore the price and application of additional substances should not affect decision-making.

Keywords: *Abies alba*; brassinolide; Bio-Algeen®; forest regeneration; silviculture

Forests are an essential part of the terrestrial environment. In some parts of the world, previous woodland areas are devastated or already nonexistent due to human activity and it has a drastic impact on the well-being of humans (Hobley 2005; Vacek et al. 2021b). Where diverse forests are present, also the support of biodiversity is very high in comparison with non-forested areas like agricultural land (Grainger 2013).

In Central Europe, some forests have been drastically changed in the past in terms of structure and tree species composition (Holubík et al. 2014; Cukor et al. 2020). Silver fir (*Abies alba* Mill.) was one of the most impacted climax species, which was an abundant species on the area of today's Czech Republic, as it expanded naturally across Central Europe in response to climatic change after the last glacial period, before the beginning of extensive

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Neolithic human activities (Tinner, Lotter 2006). But through history, other species, particularly Norway spruce (*Picea abies* L. Karst.), were preferred at the expense of silver fir (Vacek et al. 2015; Podrázský et al. 2018).

Silver fir is the main canopy tree species especially in the 4th–6th forest vegetation zone (Žárník, Holuša 2005; Poleno et al. 2009). The most favourable conditions for silver fir are northern slopes with high air humidity (Dinca et al. 2022). In these conditions it forms mixed forests together with European beech (*Fagus sylvatica* L.) and Norway spruce (the so-called Hercynian mixture) (Musil 2003). The uneven-aged mixed stands with a complicated internal structure are typical (Hofmeister et al. 2008; Slanař et al. 2017; Štefančík et al. 2021; Dinca et al. 2022). In the other forest vegetation zones, fir usually occurs only as a mixed or interspersed tree species and it usually has reduced vitality. It maintains its share there mainly thanks to its ability to survive in the shade for a long time, possibly thanks to its ability to grow on alternately moist and waterlogged soils, where it has an irreplaceable function in terms of the stability of forest stand production (Korpeľ 1989; Dobrowolska et al. 2017; Mikulenka et al. 2020). Silver fir is also an important tree species in terms of wood production (Štefančík 2019; Mikulenka et al. 2020; Prokůpková et al. 2021). Stands of silver fir showed lower sensitivity to different climatic factors than Norway spruce stands in recent studies (van der Maaten-Theunissen et al. 2013; Dobrowolska et al. 2017; Vitali et al. 2017). In provenance trials, Czech populations showed better growth in comparison with foreign, particularly Italian provenances (Fulín et al. 2023).

Since the mid-19th century, the decline and even mortality of silver fir have been recorded. The dieback reached the peak in the second half of the 20th century. The air pollution is considered as the main but not the only reason for fir dieback. Nowadays, a substantial improvement in the health status is observed across Europe (Elling et al. 2009). Unsuitable clear-cutting management, air pollution and outbreaks of *Dreyfusia* caused a rapid decline of this species (Mrkva 1994; Boettger et al. 2014; Dobrowolska 2017; Novák et al. 2019; Mikulenka et al. 2020). In recent years, the decline of silver fir has also been attributed to climate change (Hanewinkel et al. 2013; Boettger et al. 2014; Konôpková et al. 2018). In particular, the repeated summer heatwaves and long-term drought periods had a significant impact on the health status of silver fir (Büntgen et al. 2014; Gazol et al. 2015; Konôpková et al. 2018). Silver fir is often preferred by ungulates for browsing that is a substantial obstacle to natural or artificial regeneration (Häsler, Senn 2012).

Ministry of Agriculture of the Czech Republic (Ministry of Agriculture 2020) estimates the original proportion of silver fir in the forest cover before the intensive influence of humans to 19.8%, but nowadays its representation is only around 1.2% in the Czech Republic. Meanwhile, the outlined recommended representation by Ministry of Agriculture (2020) in the future is 4.4%. Generally, silver fir is considered as an ameliorative (or also "site improving") and stand stabilising species on many sites, similarly like European beech (Podrázský, Remeš 2010; Gallo et al. 2018). According to the Czech legislation (Regulation No. 298/2018), silver fir is included among the tree species with site improving and stand stabilizing functions. In the above-mentioned regulation the minimal share of tree species is demanded through forest restoration. Its ameliorating effects were confirmed by recent studies (Podrázský et al. 2018). It is also important that the roots of silver fir reach much deeper and are able to gain sufficient nutrients from the whole soil profile (Novotný 2023). Other studies showed, however, only a minor ameliorating effect of silver fir in comparison with Norway spruce. Higher content of total N, P, Ca and slightly accelerated litter decomposition were recorded, pedochemical characteristics were comparable (Třeštík, Podrázský 2017).

Silver fir is often preferred for forest reforestation, in the efforts for diversification and increasing the stability of forests (Pach, Podlaski 2015). Fir is considered as a shade-tolerant climax tree species which can survive the under the crown canopy for decades, but insolation is needed to reach a substantial height increment (Polách, Špulák 2022). On the other hand, the environmental conditions on large-scale salvage-logged clear-cuts are adverse (Poleno et al. 2009; Vaněk, Mauer 2014). The planting of fir in the conditions of full insolation usually does not show a negative influence on the height growth, however, the conditions of the clear-cut area initially contribute to increased mortality and frost injury, and worsened vitality of the young plants (Vaněk, Mauer 2014). Therefore, silver fir is a desirable tree species for use in stand reconstructions as underplanting under dying Norway spruce stands (Simon et al. 2006; Vencurik et al. 2015). The appropriate way may be to plant silver fir in the mixture with rowan tree (*Sorbus aucuparia*) which offers the desired protective shelter but does not substantially inhibit the growth of fir (Kacálek et al. 2023).

A possible solution is the two-phase forest regeneration, which, however, takes a longer period of time, in the order of decades to reach the climax state (Šafránek et al. 2018; Polách, Špulák 2022). Is it possible to support climax tree species in a way so that at least in some parts of large areas it is possible to count on their existence and production already in the first stages of forest regeneration?

The aim of this study was to find differences between the plantation treated with a brassinolide compound and Bio-Algeen® and the control variant to decide whether these substances could be useful for the initial intensive care of a target tree species – silver fir. The specific objectives were to determine differences between variants with regard to (*i*) mortality rate, (*ii*) height and root collar diameter, (*iii*) height and diameter increment, (*iv*) vitality and (*v*) economic costs. It was hypothesized that the brassinolide compound (BRs)- and Bio-Algeen®-treated part of the plantation would show increased growth and vitality and decreased mortality.

MATERIAL AND METHODS

Study site

The experimental plot was situated at the Truba Research Station close to Kostelec nad Černými lesy, Czech Republic (GPS: 50°0.36'N, 14°50.25'E, altitude 365 m a.s.l.). The surface is flat, the soil is sandy loam, and the area is exposed to direct sunlight for most of the day. The stand conditions represent afforestation of abandoned agricultural land. The area was protected by a deer-exclosure fence.

Considering that the positive effect of both used improving substances should manifest more clearly in the adverse conditions, we intentionally chose the locality for establishing an experimental plantation with such stand conditions that do not represent the optimal stand demands of silver fir.

An automatic meteorological station is monitoring weather conditions within the experimental plantation (data logger type: LEC 3010, Libor Daneš Company, Czech Republic; GPS: 50°0.382'N, 14°50.236'E; elevation: 365 m a.s.l., 3rd forest vegetation zone). Long-term average annual temperature is 7.9 °C and annual rainfall around 670 mm (1961–2021; Ondřejov meteorological station, 490 m a.s.l.). The comparison with basic climatic conditions measured on the research plot in 2014–2019 is shown in Table 1. Precipitation throughout the year was measured by a standard non-heated tipping bucket rain gauge with the circular surface of 500 cm^2, i.e. diameter ≈ 25.3 cm. Precipitation in the vegetation period was calculated as the sum of daily precipitation in the period from April to September. Analysis of soils on this research site was done in detail in a previous study (Gallo et al. 2020).

Planting and planting stock

Five-year-old bare-rooted planting stock of silver fir was used. Planting stock was grown in the Burda Forest Nursery, Milevsko, Southern Bohemia, according to the 2 + 3 cultivation formula, i.e. seedlings were grown on a seedbed for two years, then they were transplanted to a nursery bed and grown there for another three years. The experimental plantation was established at 1 m × 1 m spacing in April 2014 and the rows of the control and treated seedlings alternated. The unusually dense spacing was used in order to restrict the used area to achieve as homogeneous stand conditions as possible.

In total, 880 transplants were planted: 352 plants treated with Bio-Algeen®, 176 BRs-treated (concentration 1 : 100), 176 BRs-treated (concentration 1 : 200) and 176 plants in the control variant. Application of Bio-Algeen and BRs was done twice, on May 20, 2014 and on June 9, 2015. Control trees were without any treatment. Weed control was

Table 1. Climatic conditions on the experimental plot measured by automatic station

Year	Average annual temperature	Temperature in vegetation period	Sum of annual precipitation	Precipitation in vegetation period
	(°C)		(mm)	
2014	10.4	15.5	563	379
2015	10.6	16.2	451	211
2016	9.3	15.4	525	301
2017	9.6	15.5	533	340
2018	10.9	18.2	389	219
2019	10.2	15.9	528	315
Mean	10.2	16.1	498	294

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done by trampling during annual measurements and engine-trimmer was used, as required, at least once a year. The monocotyledonous grass (e.g. the genera *Calamagrostis*, *Festuca*, *Arrhenatherum*, *Alopecurus*, *Holcus*) prevailed, there were also dicotyledonous genera (e.g. *Hypericum*, *Acetosella*, *Urtica*). Initial measurements of height and root collar diameter were performed in spring 2014 (initial values). Periodic measurements of height and root collar as well as records of mortality rates were taken annually in autumn in 2014–2019 (after the vegetation period). Mortality rates were calculated as the percentage of dead transplants related to the initial numbers of plants. Vitality and colour of trees were assessed visually according to a vitality scale (Kuneš et al. 2011; Gallo et al. 2017).

Treatment

Two improving substances were used to enhance initial vitality of the plantation – a brassinolide compound (BRs) and Bio-Algeen®. The brassinolide compound is a part of brassinosteroids group (Nováková et al. 2014, 2015; Gallo et al. 2017) and it was patented in 2003 (Kohout et al. 2003). Bio-Algeen® is a preparation based on the hydrolyzate of the brown alga *Ascophyllum nodosum* produced in the form of granules, spray or wet root coating material (Hanzal et al. 2015).

Brassinolide. The norm was 20 mL of the solution per tree. In the original preparation, the concentration of brassinolide was 0.4 $mg\cdot L^{-1}$. This was diluted at 1 : 100 or 1 : 200, i.e. in 1 : 100 dilution there was 0.004 $mg\cdot L^{-1}$ and in 1 : 200 dilution there was 0.002 $mg\cdot L^{-1}$. Divided by 50, the dose in mg of brassinolide per one seedling comes out.

***Bio-Algeen*®.** Regarding the application, it was sprinkled with a dropper, 1 L per seedling, which was 352 litres per plantation. It was done immediately after planting.

Economic calculation

One of the important aspects that is monitored when assessing the effectiveness of new methods is the financial performance and evaluation of economic parameters. The table below shows the financial values of the primary inputs for growing transplants that were used for the research. Basic costs comprise transport costs, planting stock, outplanting labour, fencing and subsequent weeding and protection. Extra costs comprise particularly the treatments with different substances. An overview of costs entering the simple economic analysis is in Table 2.

Table 2. Overview of costs entering simple economic analysis

Item / Activity	$EUR\cdot unit^{-1}$	per 1 ha	$EUR\cdot ha^{-1}$
Basic costs			
1 plant (transplant)	0.5	4 444 (spacing 1.5 × 1.5)	2 222
Labour costs planting 1 tree	0.5	–	2 222
Transport	0.4 ($EUR\cdot km^{-1}$)	2 × 100 km	80
Weeding	300	–	300
Fence	–	–	400
Extra costs			
BRs 1 : 100	50	–	50
BRs 1 : 200	50	–	25
Bioalgeen®	70	–	70
BRs preparation and app.	10	–	10
Bioalgeen® preparation and app.	20	–	20

BR – brassinolide

The costs related to the purchase of planting stock, outplanting labour, transport, weeding and protection were compared. The common spacing (rather sparser in comparison with the experimental plantation) was used in the model forest stand for calculation. The transport costs are, of course, strongly dependent on the distance. The price of planting stock was derived from the current prices in the Czech Republic (Burda 2019), the exchange rate of EUR to CZK was 1 : 26. For the purposes of the analysis, we considered the labour costs of outplanting of one transplant to be EUR 0.5. The fencing costs were estimated to be 4 000 $EUR\cdot km^{-1}$, based on our previous experience. In this case, 500 m to fence 1 ha (400 m of a perfect square plus extra 100 m for irregularities in the real shape) was considered.

Transport costs are calculated as the costs of vehicle with trailer per km, which were approximated to 0.4 $EUR\cdot km^{-1}$. In our model case, the transport from Milevsko nursery to Truba Research Station was calculated (100 km). In the case of purchasing a larger number of trees, transport is usually free.

Application of BRs was done to 352 individuals, spreading duration and movement from tree to tree were approximately 10 s per tree. 352 plants therefore mean 3 520 s + considering the dilution and preparatory work around, resulting in app. 2 h. The price could not be established as it is an experimental product, but considering the amount, it can be almost negligible.

Application time of Bio-Algeen® was also 2 h – delivery and sprinkling of the seedling are a matter of 2 s, but everything around (10 s) the total time is 2 h. The 10-litre canister cost was ca 100 EUR, 1 seedling was diluted = 1 L containing 2.5 mL of Bio-Algeen®. Total cost of Bio-Algeen® can be estimated to 10 EUR.

Control. The control variant had an obvious advantage because neither cost of additive chemicals nor additional labour of the application of those chemicals was required.

Data analysis

The analysed data included the trees which had survived until autumn 2019. Data relating to the trees that died by autumn 2015 were retrospectively excluded. Some severely damaged living trees (especially those injured by a brush cutter during weed cutting) were also excluded.

Data were digitized and adjusted in MS Excel (Version 365, 2022). Statistical analyses were done in R software (Version 4.0.2, 2020). Annual mortality rate was assessed by test for quantities corresponding to binomial distribution using a predefined script [Linda (2020) according to Agresti et al. (2008)]. In height and root collar diameter increments, the Shapiro-Wilk test was used to test normality of data. Subsequently (as the data showed other than normal distribution), the Kruskal-Wallis test with multiple comparisons was used to find possible significant differences between the variants. Then, the Kruskal-Wallis multiple comparison test from the package ’pgirmess’ was used to test particular differences. The principal component analysis (PCA) was performed in Canoco 5 program (Šmilauer, Lepš 2014) to evaluate the relationship between seedling parameters, climate factors and treatment variants. Data were log-transformed and standardized before analysis. The results of the multidimensional PCA analysis were visualized in the form of ordination diagram.

For vitality assessment, a scale for rapid visual assessment of the health status was used (Gallo et al. 2017): A – excellent; B – good; C – somewhat worsened; D – significantly worsened; E – dry. Dead trees were also excluded from the analysis of vitality and colour. For evaluation of needle colour, a three-degree scale was used (1 – green, 2 – green-yellow, 3 – yellow). Vitality and colour assessment was done only in the years 2014 and 2015. In the next years these parameters were not recorded because the majority of trees overcame the postplanting shock (or died) and no extensive colour and vitality differences were observed.

RESULTS

Mortality rate. No statistical differences in annual mortality between variants were observed. Notably increased annual mortality was registered in 2015 for all variants. Otherwise, values of annual mortality were generally low, in BRs 1 : 100 and control variant they were zero in the last three monitored seasons (Table 3). Although the climatic conditions in 2018 were similar to those in 2015, almost no annual mortality was registered. In the fifth season after planting (2018), the root systems of the trees were well developed. Therefore, the survival rate in the extreme drought period was very high, contrarily to the second season (2015).

Cumulative mortality rate of the plantation reached 25% at the end of the monitoring period in 2019. Mortality according to each treatment is shown in Figure 1. The lowest cumulative mortality was registered in control treatment (20.5%), while the highest was in BRs 1 : 100 treatment (29.5%). *P*-values were not statistically significant.

Height and root collar diameter. Mean height development of fir individuals differentiated according to variants is shown in Figure 2. It was similar in all treatments with Bio-Algeen® show-

Table 3. Annual mortality rate (%) of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

Treatment	Year					
	2014	2015	2016	2017	2018	2019
Bio-Algeen®	2.27^a	21.22^a	1.11^a	0.37^a	0.00^a	0.37^a
BRs 1 : 100	0.00^a	26.70^a	3.10^a	0.00^a	0.00^a	0.00^a
BRs 1 : 200	0.57^a	24.00^a	1.50^a	0.00^a	0.76^a	0.00^a
Control	0.57^a	18.86^a	1.41^a	0.00^a	0.00^a	0.00^a

Different letters indicate significant differences; BRs – brassinolide

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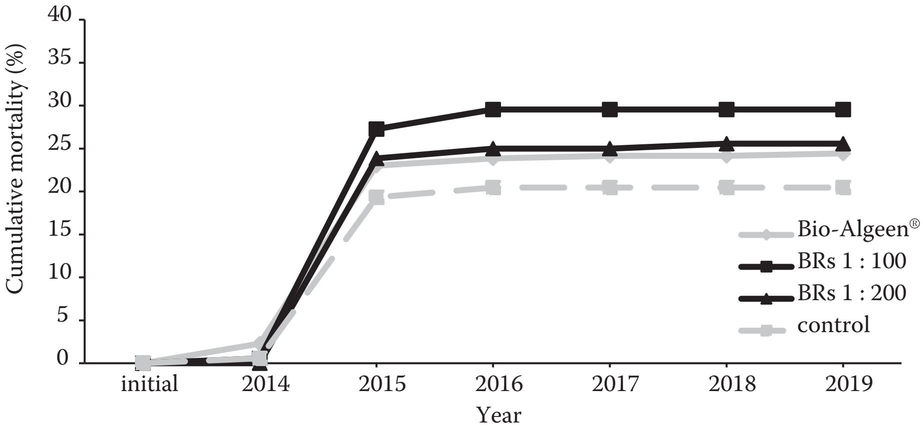

Figure 1. Cumulative mortality (%) of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

BRs – brassinolide

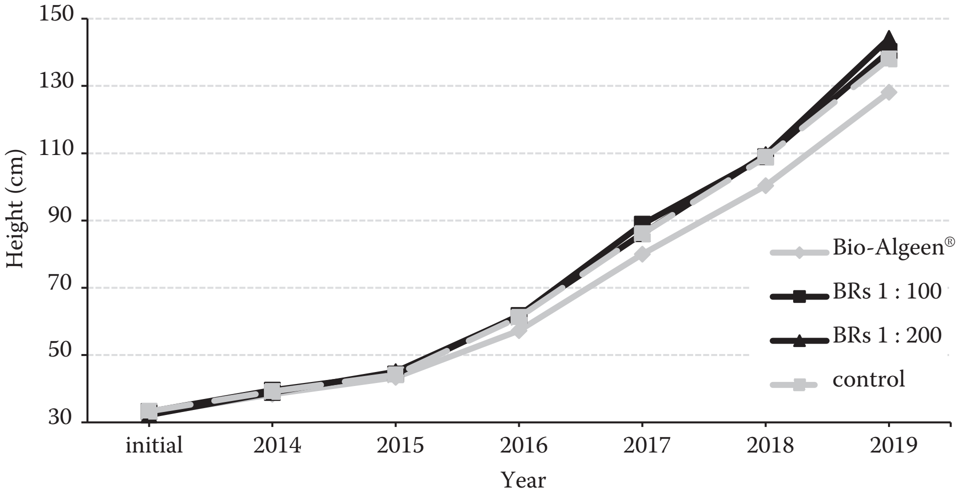

Figure 2. Mean height (cm) development of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

BRs – brassinolide

ing the lowest values (128.1 cm) at the end of the monitoring period. The highest values were shown by BRs 1 : 200 treatment (144.1 cm). BRs 1 : 100 and control treatment showed 140.3 cm and 138.1 cm, respectively.

Mean root collar diameter development is shown in Figure 3. The yearly values were similar in all treatments. In absolute numbers, control treatment showed the lowest root collar diameter (28.7 mm) at the end of the monitoring period. BRs

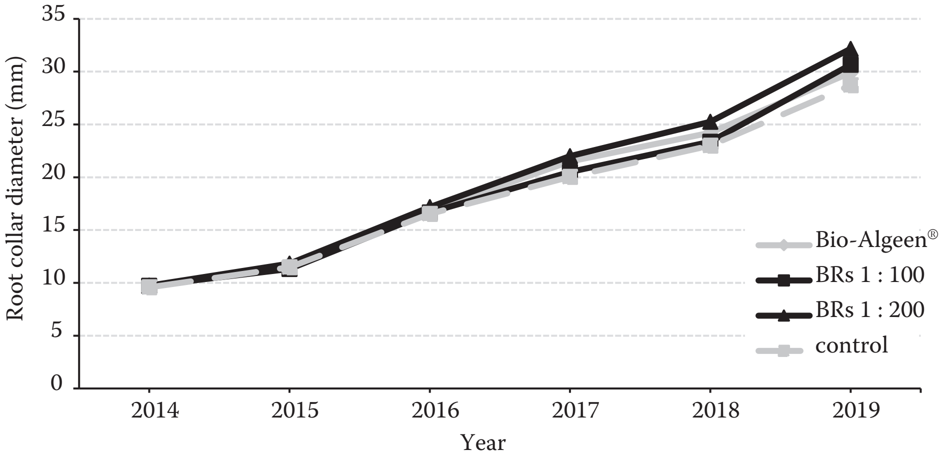

Figure 3. Mean root collar diameter (cm) development of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

BRs – brassinolide

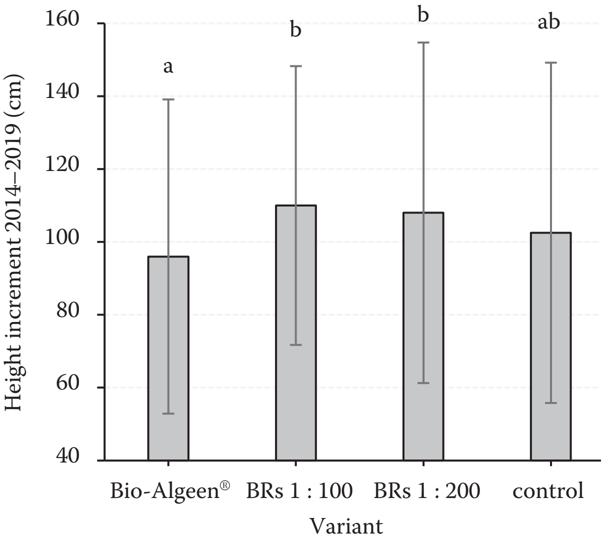

Figure 4. Median height increment (cm) of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

Different letters indicate significant differences according to Kruskal-Wallis multiple comparisons test; error bars – standard deviation; BRs – brassinolide

1 : 200 showed the highest value (32.2 mm), while BRs 1 : 100 and Bio-Algeen® reached 30.7 mm and 29.9 mm, respectively.

Height and root collar diameter increment. Total number of evaluated trees was 626. In height increment over the monitoring period, the Kruskal-Wallis test showed significant differences between variants (Kruskal-Wallis chi-squared = 12.371, *df* = 3, *P*-value = 0.006213). Subsequent Kruskal-Wallis multiple comparison test revealed that height increment of the variant treated with Bio-Algeen® was significantly lower than the growth of BRs-treated variants. All treated variants were comparable to control variant (Figure 4).

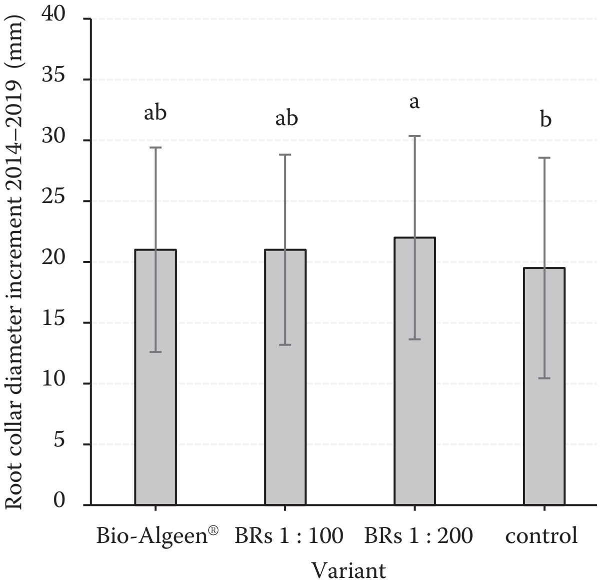

Figure 5. Median root collar diameter increment (mm) of different treatments of silver fir plantation in the period 2014–2019 on the research plot Truba

Different letters indicate significant differences according to Kruskal-Wallis multiple comparisons test; error bars – standard deviation; BRs – brassinolide

In evaluating the root collar diameter increment over the monitoring period, BRs 1 : 200 exhibited significantly higher increment in comparison with control treatment. However, it was not significantly different from BRs 1 : 100 or Bio-Algeen®, which were not significantly different from the control (Figure 5).

Vitality. Visually assessed vitality and colour are in Tables 4 and 5, respectively. In case of Bio-Algeen®, most trees were in vitality category B in 2014, but they shifted to C in 2015. In contrast, B remained the most abundant category in 2014 and in 2015 for BRs 1 : 100, even though the qual-

Table 4. Relative frequency (%) of silver fir individuals in different vitality classes according to different variants in 2014 and 2015 on the research plot Truba

Year	2014					2015				
Variant	A	B	C	D	E	A	B	C	D	E
Bio-Algeen®	34.66	40.91	17.61	4.55	2.27	7.10	23.30	39.49	7.10	23.01
BRs 1 : 100	38.07	53.98	6.25	1.14	0.57	8.52	28.41	25.57	10.23	27.27
BRs 1 : 200	46.29	40.57	12.00	1.14	0.00	20.00	24.57	25.71	5.71	24.00
Control	43.18	46.02	9.66	0.57	0.57	12.50	25.00	28.98	14.20	19.32

Vitality classes: A – excellent; B – good; C – somewhat worsened; D – significantly worsened; E – dry; BRs – brassinolide

Table 5. Relative frequency (%) of *Abies alba* individuals in different colour classes according to different variants in 2014 and 2015 on the research plot Truba

Year	2014			2015		
Variant	1	2	3	1	2	3
Bio-Algeen®	34.6	60.2	5.2	66.1	27.7	6.3
BRs 1 : 100	37.7	58.9	3.4	58.6	31.3	10.2
BRs 1 : 200	41.7	56.0	2.3	70.9	23.1	6.0
Control	38.3	53.7	8.0	58.5	27.5	14.1

Colour classes: 1 – green, 2 – green-yellow, 3 – yellow; BRs – brassinolide

ity also heavily shifted to other categories. For BRs 1 : 200 in 2014, the most abundant category was A, but then C and B in the next year. For the control, a similar pattern like in other treatments was registered, i.e., shift from better to worsened vitality.

Regarding the visually assessed parameter − colour of previous year needles, the development was contrary to overall vitality – it mostly shifted from yellow-green in 2014 to green in 2015 in all variants (Table 3).

Economic analysis. Estimated results of the simple economic analysis are shown in Table 6. The input costs are the same (planting stock, outplanting, transport, weed control and fence), the differences are in the extra care of individual treatments. The most expensive was Bio-Algeen® treatment (total cost 5 254 EUR·ha^{-1}) compared to the cheapest control variant (5 224 EUR·ha^{-1}). The extra care of BRs variants was 10 EUR·ha^{-1} lower.

Relationship between increment, mortality, climate and treatments. Results of the principal component analysis expressing the relationship between seedling parameters, climate factors and fir treatments are presented in the form of ordination diagram in Figure 6. The first ordination axis represents

Table 6. Results of simple economic analysis comparing three different variants: Bioalgeen®, BRs and control at the experimental plantation of *Abies alba* at Truba research plot

Item / activity	Bio-Algeen®	BRs	Control
	(EUR·ha^{-1})		
Planting stock	2 222	2 222	2 222
Outplanting	2 222	2 222	2 222
Transport	80	80	80
Weed control	300	300	300
Fence	400	400	400
Extra care	30	20	0
Total	5 254	5 244	5 224

BRs – brassinolide

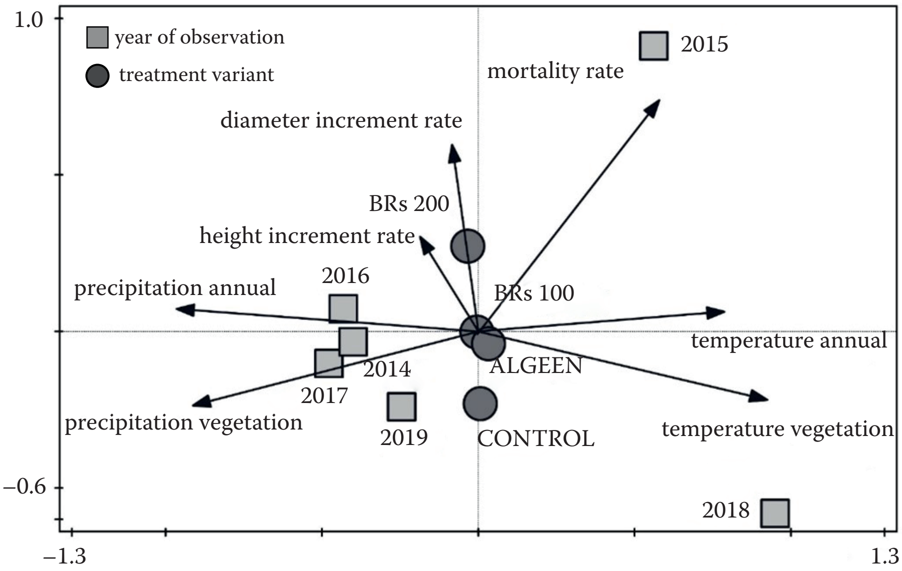

Figure 6. Ordination diagram showing results of principal components analysis of relationship between seedling parameters (root collar diameter increment rate, height increment rate, annual mortality rate), climate factors (annual temperature, temperature in vegetation period, sum of annual precipitation, sum of precipitation in vegetation period) and treatments (control, BRs 100, BRs 200 and Bio-Algeen®)

BRs – brassinolide

48.24%, the first two axes 66.21% and the four axes together account for 95.20% of data variability. The x-axis represents the root collar diameter increment rate and the y-axis represents climate factors (air temperatures and sums of precipitation). Mortality rate of seedlings was increasing with decreasing sum of precipitation and increasing temperature. Height increment was positively correlated with root collar diameter increment. The lowest explanatory variable in the ordination diagram was height increment ratio. The differences between treatment variants were significant, compared to small differences between years in the period 2014–2019. Generally, BRs 1 : 200 treatment was the most suitable for the growth of silver fir compared to the control.

DISCUSSION

The existence of forests in many areas is threatened. An ideal way of the ecosystem preservation is natural dynamics and natural regeneration (Vacek et al. 2019; Brichta et al. 2020; Prokůpková et al. 2020; Fuchs et al. 2021; Hammond et al. 2021). These processes are not functional on many areas for different reasons. One of possible ways to save forests is artificial regeneration (reforestation) and afforestation on new non-forest areas and areas where forests were previously removed (Vacek et al. 2018, 2021a; Cavalli et al. 2022; Cukor et al. 2022; Zeidler et al. 2022). In such cases, intensive care is efficient to decrease the initial mortality of trees (Gallo et al. 2020). Moreover, afforestation plays a significant role in the mitigation of climatic changes due to the global carbon cycle (Cukor et al. 2017a).

In many cases, planting trees became a controversial topic (Chytrý et al. 2001). Phrases like "more harm than good" or "controversial strategy" are used regarding the effects of plantations on climate change and fires (Elbein 2019; Yanes 2020). Their effect on plant communities is also significant (Andrés, Ojeda 2002). It is important to understand historical context of deforestation, scale of original forest cover and all benefits of forests and trees.

A number of field experiments done as well as practical experience showed that the cultivation of climax species under the conditions of large-scale clear-cuts is difficult and much more demanding on post-planting care in comparison with the use of pioneer species which grow spontaneously on many sites (not on all however) (Eisenhauer et al. 2001; Martiník et al. 2018). It is up to the owners to decide whether they want to invest in more demanding measures. Silver fir requires fencing against game damage and often also weeding or at least weed control by trampling (Vacek et al. 2014; Vacek 2017; Gallo et al. 2020). Regeneration of fir is strongly influenced by vegetation cover. For example, Prokůpková et al. (2021) from Poland showed that the tallest fir regeneration individuals were observed in the *Rubus* cover, while the highest density of fir natural regeneration was observed in the moss cover. A number of papers also reported that a warm summer and repeated long-term drought with lower precipitation total had a significant negative impact on the vitality and health of silver fir (Thomas et al. 2002; Camarero et al. 2011; Linares 2011; Linares, Camarero 2012; Büntgen et al. 2014; Gazol et al. 2015; Vejpustková et al. 2023). Similarly, a high mortality rate was observed in our study in 2015 (second year after planting) due to the synergism of extremely high air temperature in vegetation and droughts.

BRs 1 : 200 treatment performed the best in relation to mean root collar diameter, as well as vitality and colour changes under drought stress. It is in accordance with previous research, where a particular concentration of BRs performed better than the control, but different concentration performed worse (Nováková et al. 2015). However, the results of different studies are inconsistent. BRs also showed a positive effect on overcoming drought stress in the germination of Norway spruce, Scots pine (*Pinus sylvestris* L.) and Douglas fir (Kuneš et al. 2016). BRs had negative effects on growth and mortality of Scots pine in another study (Nováková et al. 2014).

Bio-Algeen® treatment showed lower height increment in comparison with BRs treatments, but comparable with the control variant while showing root collar diameter increment comparable with all other variants. Benefits of Bio-Algeen® were therefore not proven. Study by Lorenc et al. (2016) recommended Bio-Algeen® for application in forest nurseries, as its application boosted growth. Similarly, another study showed positive economic and production results regarding the application of Bio-Algeen® to seedlings in both nursery and plantation site (Hanzal et al. 2015). Another way of applying algae-related material is the application of alginite rocks (Tužinský et al. 2015; Cukor et al. 2017b, c).

The simple economic analysis showed moderate differences in costs over the variants, as the additional substances are not crucial in the inputs, especially in comparison with the costs of planting stock and labour. On the other hand, from the ecological point of view, the ameliorative effects of silver fir are not clearly determined, in most factors it has better results than Norway spruce, but not very convincingly and its sensitivity to environmental conditions is problematic (Třeštík, Podrázský 2017; Podrázský et al. 2018).

There are still many questions regarding the research of BRs and Bio-Algeen®. What about the effects of the combination of those substances? What about combining BRs and Bio-Algeen® with different fertilizers or alginite rocks? There are many possible combinations of different substances to enhance growth and vitality of young forest plantations. Such activities of initial care are aimed at creating more diverse and stable ecosystems in future, ensuring their functionality in later stages using mostly silvicultural measures (Vacek et al. 2020).

CONCLUSION

Silvicultural measures to support biodiversity are crucial for nature protection under the current conditions of intensive human impact on forest ecosystems and ongoing climate change. In this experiment, the plantation of target tree species *Abies alba* was treated with a brassinolide compound and with Bio-Algeen® alongside with the control variant to find the best possible treatment for optimized growth and vitality of this infrequent species. The plantation showed stable growth and vitality and minimal rates of mortality, except the year 2015 with hot and dry summer. None of the treated variants showed significantly better values of growth and mortality in comparison with the control. Therefore, we cannot confirm the hypothesis that brassinolide compound or Bio-Algeen application increased growth and vitality and decreased mortality of silver fir plantation. Regarding the economic aspect, control variant is the cheapest, missing all additive operations, but there can be some benefits brought by additional chemicals like improved radial growth, vitality and colour under stressful conditions. With the combined knowledge and practice of natural regeneration support, as well as intensive care of the plantation of valuable target species, it is possible to preserve and even extend the areas of fully functional forest ecosystems. Depending on the level of previous transformation of forest stands, different methods of afforestation and management intensity might be used. On suitable sites, it is possible to cultivate rare and demanding tree species directly without the preparatory stage of forest. Silviculture is the only tool to reintroduce rare tree species to places where they have been missing for a long time due to previous human activity and impact.

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REFERENCES

Agresti A., Bini M., Bertaccini B., Ryu E. (2008): Simultaneous confidence intervals for comparing binomial parameters. Biometrics, 64: 1270–1275.

Andrés C., Ojeda F. (2002): Effects of afforestation with pines on woody plant diversity of Mediterranean heathlands in southern Spain. Biodiversity & Conservation, 11: 1511–1520.

Boettger T., Haupt M. Friedrich M., Waterhouse J.S. (2014): Reduced climate sensitivity of carbon, oxygen and hydrogen stable isotope ratios in tree-ring celulose of silver fir (*Abies alba* Mill.) influenced by background SO_2 in Franconia (Germany, Central Europe). Environmental Pollution, 185: 281–294.

Brichta J., Bílek L., Linda R., Vítámvás J. (2020): Does shelterwood regeneration on natural Scots pine sites under changing environmental conditions represent a viable alternative to traditional clear-cut management? Central European Forestry Journal, 66: 104–115.

Büntgen U., Tegel W., Kaplan J.O., Schaub M., Hagedorn F., Bürgi M. Brázdil R., Helle G., Carrer M., Heussner K.U., Hofmann J., Kontic R., Kyncl T., Kyncl J., Camarero J.J., Tinner W., Esper J., Liebhold A. (2014): Placing unprecedented recent fir growth in a European-wide and Holocene-long context. Frontiers in Ecology and the Environment, 12: 100–106.

Burda P. (2019): Burda lesní školky – Ceník. Available at: http://www.pavelburda.cz/cenik/ (in Czech).

Camarero J.J., Bigler C., Linares J.C., Gil-Pelegrín E. (2011): Synergistic effects of past historical logging and drought on the decline of Pyrenean silver fir forests. Forest Ecology and Management, 262: 759–769.

Cavalli A., Francini S., Cecili G., Cocozza C., Congedo L., Falanga V., Spadoni G.L., Maesano M., Munafò M., Chirici G., Mugnozza G.S. (2022): Afforestation monitoring through automatic analysis of 36-years Landsat Best Available Composites. iForest – Biogeosciences and Forestry, 15: 220–228.

Chytrý M., Kučera T., Kočí M., Grulich V., Lustyk P. (2001): Katalog biotopů České republiky. Praha, Agentura ochrany přírody a krajiny ČR: 304. (in Czech)

Cukor J., Vacek Z., Linda R., Bílek L. (2017a): Carbon sequestration in soil following afforestation of former agricultural land in the Czech Republic. Central European Journal of Forest Science, 63: 97–104.

Cukor J., Vacek Z., Linda R., Remeš J., Bílek L., Sharma R.P., Baláš M., Kupka I. (2017b): Effect of mineral eco-fertilizer on growth and mortality of young afforestations. Austrian Journal of Forest Science, 134: 367–385.

Cukor J., Linhart L., Vacek Z., Baláš M., Linda R. (2017c): The effects of Alginite fertilization on selected tree species seedlings performance on afforested agricultural lands. Central European Forestry Journal, 63: 48–56.

Cukor J., Zeidler A., Vacek Z., Vacek S., Šimůnek V., Gallo J. (2020): Comparison of growth and wood quality of Norway spruce and European larch: Effect of previous land use. European Journal of Forest Research, 139: 459–472.

Cukor J., Vacek Z., Vacek S., Linda R., Podrázský V. (2022): Biomass productivity, forest stability, carbon balance, and soil transformation of agricultural land afforestation: A case study of suitability of native tree species in the submontane zone in Czechia. Catena, 210: 105893.

Dinca L., Marin M., Radu V., Murariu G., Drasovean R., Cretu R., Georgescu L., Timis-Gânsac V. (2022): Which are the best site and stand conditions for silver fir (*Abies alba* Mill.) located in the Carpathian mountains? Diversity, 14: 547.

Dobrowolska D., Bončina A., Klumpp R. (2017): Ecology and silviculture of silver fir (*Abies alba* Mill.): A review. Journal of Forest Research, 22: 326–335.

Eisenhauer D.R., Baeucker B., Helbig M. (2001): The importance of stand succession for elasticity and conversion of damaged spruce forest and birch pioneer woods. Journal of Forest Science, 47: 117–127.

Elbein S.G. (2019): Tree-planting programs can do more harm than good. Available at: https://pulitzercenter.org/stories/tree-planting-programs-can-do-more-harm-good (accessed Oct 3, 2022).

Elling W., Dittmar C., Pfaffelmoser K., Rötzer T. (2009): Dendroecological assessment of the complex causes of decline and recovery of the growth of silver fir (*Abies alba* Mill.) in Southern Germany. Forest Ecology and Management, 257: 1175–1187.

Fuchs Z., Vacek Z., Vacek S., Gallo J. (2021): Effect of game browsing on natural regeneration of European beech (*Fagus sylvatica* L.) forests in the Krušné hory Mts. (Czech Republic and Germany). Central European Forestry Journal, 67: 166–180.

Fulín M., Dostál J., Čáp J., Novotný P. (2023): Evaluation of silver fir provenances at 51 years of age in provenance trials in the Předhoří Hrubý Jeseník and Nízký Jeseník Mts. regions, Czech Republic. Journal of Forest Science, 69: 44–59.

Gallo J., Baláš M., Linda R., Kuneš I. (2017): Growth performance and resistance to near-ground late frosts of *Fagus sylvatica* L. plantation treated by a brassinosteroid compound. Journal of Forest Science, 63: 117–125.

Gallo J., Baláš M., Linda R., Cukor J., Kuneš I. (2018): Initial evaluation of experimental plantation with new generation semi-saplings of European beech on nutrient-rich site with a tendency to dry-out in Vintířov-Sedlec. In: Baláš M., Podrázský V., Gallo J. (eds): Proceedings of Central European Silviculture. Volume 8: Pěstování lesů ve střední Evropě. Doksy, Sept 4–5, 2018: 39–46.

Gallo J., Baláš M., Linda R., Kuneš I. (2020): The effects of planting stock size and weeding on survival and growth of small-leaved lime under drought-heat stress in the Czech Republic. Austrian Journal of Forest Science, 137: 43–66.

Gazol A., Camarero J.J., Gutierrez E., Popa I., Andreu-Hayles L., Motta R., Nola P., Ribas M., Sangüesa-Barreda G., Urbinati C., Carrer M. (2015): Distinct effects of climate warming on populations of silver fir (*Abies alba*) across Europe. Journal of Biogeography, 42: 1150–1162.

Grainger A. (2013): Controlling Tropical Deforestation. London, Routledge: 314.

Hammond M.E., Pokorný R., Dobrovolný L. (2021): Gap regeneration and dynamics: The case study of mixed forests at Křtiny in the Czech Republic. Central European Forestry Journal, 67: 135–147.

Hanewinkel M., Cullmann D.A., Schelhaas M.J., Nabuurs G.J., Zimmermann N.E. (2013): Climate change may cause severe loss in the economic value of European forest land. Nature Climate Change, 3: 203–207.

Hanzal V., Janiszewski P., Kubeček J., Bergman J., Gjurov V., Tužinský M., Baláš M. (2015): Support of the growth and prosperity of the Norway spruce (*Picea abies*) seedlings in forest nursery by the Bio-Algeen system – preliminary results. Polish Journal of Natural Sciences, 30: 217–224.

Häsler H., Senn J. (2012): Ungulate browsing on European silver fir *Abies alba*: The role of occasions, food shortage and diet preferences. Wildlife Biology, 18: 67–74.

Hobley M. (2005): The impacts of degradation and forest loss on human well-being and its social and political relevance for restoration. In: Mansourian S., Vallauri D., Dudley N. (eds): Forest Restoration in Landscapes. New York, Springer: 22–30.

Hofmeister Š., Svoboda M., Souček J., Vacek S. (2008): Spatial pattern of Norway spruce and silver fir natural regeneration in uneven-aged mixed forests of northeastern Bohemia. Journal of Forest Science, 54: 92–101.

Holubík O., Podrázský V., Vopravil J., Khel T., Remeš J. (2014): Effect of agricultural lands afforestation and tree species composition on the soil reaction, total organic carbon and nitrogen content in the upper-most mineral soil profile. Soil and Water Research, 9: 192–200.

Kacálek D., Bartoš J., Špulák O. (2023): Positive effect of fir-rowan intimate mixture on new forest floor and topsoil following afforestation. Journal of Forest Science, 69: 80–92.

Kohout L., Kasal A., Chodounská H., Slavíková B., Hniličková J. (2003): The use of 2α, 3α, 17SS-trihydroxy-5α-androstan-6-one as active ingredient for plant development regulation. Patent International Publication No. WO 03/003834 A1, Application Number PCT/CZ2002/000041. Geneva, World Intellectual Property Organisation, International Bureau: 17.

Konôpková A., Kurjak D., Kmeť J., Klumpp R., Longauer R., Ditmarová L., Gömöry D. (2018): Differences in photochemistry and response to heat stress between silver fir (*Abies alba* Mill.) provenances. Trees, 32: 73–86.

Korpeľ Š. (1989): Pralesy Slovenska. Bratislava, Veda: 328. (in Slovak)

Kuneš I., Baláš M., Millerová K., Balcar V. (2011): Vnášení listnaté příměsi a jedle do jehličnatých porostů Jizerských hor. Lesnický průvodce 9/2011. Jíloviště-Strnady, VULHM: 36. (in Czech)

Kuneš I., Baláš M., Linda R., Gallo J., Nováková O. (2016): Effects of brassinosteroid application on seed germination of Norway spruce, Scots pine, Douglas fir and English oak. iForest – Biogeosciences and Forestry, 10: 121.

Linares J.C. (2011): Biogeography and evolution of *Abies* (*Pinaceae*) in the Mediterranean Basin: The roles of long-term climatic change and glacial refugia. Journal of Biogeography, 38: 619–630.

Linares J.C., Camarero J.J. (2012): From pattern to process: Linking intrinsic water-use efficiency to drought-induced forest decline. Global Change Biology, 18: 1000–1015.

Linda R. (2020): Binomial test for R. Available at: https://github.com/Lindros9203/mcbintest

Lorenc F., Pešková V., Modlinger R., Podrázský V., Baláš M., Kleinová, D. (2016): Effect of Bio-Algeen® preparation on growth and mycorrhizal characteristics of Norway spruce seedlings. Journal of Forest Science, 62: 285–291.

Martiník A., Sendecký M., Urban J. (2018): Survival and early growth of silver fir and pioneer species on two sites in nurse crop regeneration systems in the Czech Republic. Dendrobiology, 80: 81–90.

Mikulenka P., Prokůpková A., Vacek Z., Vacek S., Bulušek D., Simon J., Šimůnek V., Hájek V. (2020): Effect of climate and air pollution on radial growth of mixed forests: *Abies alba* Mill. vs. *Picea abies* (L.) Karst. Central European Forestry Journal, 66: 23–36.

Ministry of Agriculture (2020): Zelená zpráva o stavu lesa a lesního hospodářství České republiky v roce 2019. Available at: https://eagri.cz/public/web/mze/lesy/lesnictvi/zprava-o-stavu-lesa-a-lesniho/zprava-o-stavu-lesa-a-lesniho-2019.html (in Czech)

Mrkva R. (1994): Korovnice kavkazská (*Dreyfusia nordmannianae* Eckstein), obrana proti ní a její podíl na ústupu jedle. Lesnictví – Forestry, 40: 361–370. (in Czech)

Musil I. (2003): Lesnická dendrologie I. Praha, Academia, Praha: 177. (in Czech)

Novák J., Dušek D., Kacálek D., Slodičák M. (2019): Adequate silviculture management of silver fir stands in the Czech Republic. In: Kohnle U., Klädtke J. (eds.): Berichte zur Jahrestagung 2019. Deutscher Verband Forstlicher Versuchsanstalten Sektion Ertragskunde. Freiburg i. Br., Germany, June 3-5, 2019: 112–114.

Nováková O., Kuneš I., Gallo J., Baláš M. (2014): Effects of brassinosteroids on prosperity of Scots pine seedlings. Journal of Forest Science, 60: 388–393.

Nováková O., Gallo J., Baláš M., Špulák O., Kuneš I. (2015): Influence of brassinosteroids on height increment, chlorophyll fluorescence and mortality of seedlings of Norway spruce and Scots pine in forest nursery conditions. Zprávy lesnického výzkumu, 60: 122–129. (in Czech)

Novotný R. (2023): Nutrition of silver fir (*Abies alba* Mill.) and its comparison with Norway spruce (*Picea abies* L. H. Karst) from the same forest sites in the Czech Republic. Journal of Forest Science, 69: 60–66.

Pach M., Podlaski R. (2015): Tree diameter structural diversity in Central European forests with *Abies alba* and *Fagus sylvatica*: Managed versus unmanaged forest stands. Ecological Research, 30: 367–384.

Podrázský V., Remeš J. (2010): Effects of the species composition change on the humus form state in the forest stands on the territory of the University Training Forest at Kostelec nad Černými lesy. Zprávy Lesnického Výzkumu, 55: 71–77. (in Czech)

Podrázský V., Vacek Z., Kupka I., Vacek S., Třeštík M., Cukor J. (2018): Effects of silver fir (*Abies alba* Mill.) on the humus forms in Norway spruce (*Picea abies* (L.) H. Karst.) stands. Journal of Forest Science, 64: 245–250.

Polách R., Špulák O. (2022): Prosperity of silver fir planted under preparatory stands of pioneer broadleaves of different stocking and age. Zprávy lesnického výzkumu, 67: 269–277. (in Czech)

Poleno Z., Vacek S., Podrázský V., Remeš J., Štefančík I., Mikeska M., Kobliha J., Kupka I., Malík V., Turčáni M., Dvořák J., Zatloukal V., Bílek L., Baláš M., Simon J. (2009): Pěstování lesů III. Praktické postupy pěstování lesů. Kostelec nad Černými lesy, Lesnická práce: 952. (in Czech)

Prokůpková A., Vacek Z., Vacek S., Blažejová J., Schwarz O., Bulušek D. (2020): Dynamics of natural regeneration of mountain forests after wind disturbance: Model study for the Krkonoše Mts. (Czech Republic). Zprávy lesnického výzkumu, 65: 72–81. (in Czech)

Prokůpková A., Brichta J., Vacek Z., Bielak K., Andrzejczyk T., Vacek S., Štefančík I., Bílek L., Fuchs Z. (2021): Effect of vegetation on natural regeneration of mixed silver fir forests in lowlands: a case study from the Rogów region in Poland. Sylwan, 165: 779–795.

Šafránek Z., Martiník A., Vala V. (2018): Model economic comparison of forest regeneration treatments after calamity events of allochthonous spruce standa: Conventional artificial regeneration vs. preparatory-birch stands. Zprávy lesnického výzkumu, 63: 92–101. (in Czech)

Simon J., Vacek S., Schwarz O., Minx T., Hofmeister Š. (2006): Simulace vývoje autochtonního smrkobukového porostu v Bažinkách s virtuální podsadbou jedle bělokoré (*Abies alba*). In: Neuhöferová P. (ed.): Zvýšení podílu přírodě blízké porostní složky lesů se zvláštním statutem ochrany. [Increase of Close-to-Nature Stand Component of Forests with Special Protection Status]. Kostelec nad Černými lesy, Ústav hospodářské úpravy lesů LDF MZLU v Brně, Katedra pěstování lesů FLE ČZU v Praze: 1–16. (in Czech)

Slanař J., Vacek Z., Vacek S., Bulušek D., Cukor J., Štefančík I., Bílek L., Král J. (2017): Long-term transformation of submontane spruce-beech forests in the Jizerské hory Mts.: Dynamics of natural regeneration. Central European Forestry Journal, 63: 213–225.

Šmilauer P., Lepš J. (2014): Multivariate Analysis of Ecological Data using CANOCO 5. Cambridge, Cambridge University Press: 361.

Štefančík I. (2019): Comparison of growth of silver fir (*Abies alba* Mill.) in pure and mixed spruce, fir and beech stands. Zprávy lesnického výzkumu, 64: 94–101. (in Slovak)

Štefančík I., Petráš R., Mecko J., Novák J. (2021): Qualitative and value production of tree species in mixed spruce-fir-beech stands under the conditions of the Western Carpathians. Central European Forestry Journal, 67: 155–165.

Thomas A.L., Gegout J.C., Landmann G., Dambrine E., King D. (2002): Relation between ecological conditions and fir decline in a sandstone region of the Vosges mountains (northeastern France). Annals of Forest Science, 59: 265–273.

Tinner W., Lotter A.F. (2006): Holocene expansions of *Fagus silvatica* and *Abies alba* in Central Europe: Where are we after eight decades of debate? Quaternary Science Reviews, 25: 526–549.

Třeštík M., Podrázský V. (2017): Soil improving role of the silver fir (*Abies alba* Mill.): A case study. Zprávy lesnického výzkumu, 62: 182–188. (in Czech)

Tužinský M., Kupka I., Podrázský V., Prknová H. (2015): Influence of the mineral rock alginite on survival rate and re-growth of selected tree species on agricultural land. Journal of Forest Science, 61: 399–405.

Vacek Z. (2017): Structure and dynamics of spruce-beech-fir forests in Nature Reserves of the Orlické hory Mts. in relation to ungulate game. Central European Forestry Journal, 63: 23–34.

Vacek Z., Vacek S., Bílek L., Král J., Remeš J., Bulušek D., Králíček I. (2014): Ungulate impact on natural regeneration in spruce-beech-fir stands in Černý důl Nature Reserve in the Orlické Hory Mountains, case study from Central Sudetes. Forests, 5: 2929–2946.

Vacek S., Vacek Z., Bulušek D., Bílek L., Schwarz O., Simon J., Štícha V. (2015): The role of shelterwood cutting and protection against game browsing for the regeneration of silver fir. Austrian Journal of Forest Science, 132: 81–102.

Vacek Z., Cukor J., Vacek S., Podrázský V., Linda R., Kovařík J. (2018): Forest biodiversity and production potential of post-mining landscape: Opting for afforestation or leaving it to spontaneous development? Central European Forestry Journal, 64: 116–126.

Vacek Z., Vacek S., Slanař J., Bílek L., Bulušek D., Štefančík I., Králíček I., Vančura K. (2019): Adaption of Norway spruce and European beech forests under climate change: From resistance to close-to-nature silviculture. Central European Forestry Journal, 65: 129–144.

Vacek Z., Prokůpková A., Vacek S., Cukor J., Bílek L., Gallo J., Bulušek D. (2020): Silviculture as a tool to support stability and diversity of forests under climate change: Study from Krkonoše Mountains. Central European Forestry Journal, 66: 116–129.

Vacek Z., Cukor J., Vacek S., Linda R., Prokůpková A., Podrázský V., Gallo J., Vacek O., Šimůnek V., Drábek O., Hájek V., Spasić M., Brichta J. (2021a): Production potential, biodiversity and soil properties of forest reclamations: Opportunities or risk of introduced coniferous tree species under climate change? European Journal of Forest Research, 140: 1243–1266.

Vacek Z., Linda R., Cukor J., Vacek S., Šimůnek V., Gallo J., Vančura K. (2021b): Scots pine (*Pinus sylvestris* L.), the suitable pioneer species for afforestation of reclamation sites? Forest Ecology and Management, 485: 118951.

Van der Maaten-Theunissen M., Kahle H.P., van der Maaten E. (2013): Drought sensitivity of Norway spruce is higher than that of silver fir along an altitudinal gradient in southwestern Germany. Annals of Forest Science, 70: 185–193.

Vaněk P., Mauer O. (2014): Regeneration of silver fir (*Abies alba* Mill.) on clear-cut areas. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 62: 267–277.

Vejpustková M., Čihák T., Fišer P. (2023): The increasing drought sensitivity of silver fir (*Abies a*lba Mill.) is evident in the last two decades. Journal of Forest Science, 69: 67–79.

Vencurik J., Kucbel S., Saniga M., Jaloviar P., Pittner J., Vajduliak T., Hunčaga M. (2015): The effect of light and competition on height growth and crown morphology of Norway spruce (*Picea abies* [L.] Karst.) and silver fir

(*Abies alba* Mill.) regeneration in spruce stand in conversion. Zprávy lesnického výzkumu, 60: 281–286. (in Slovak)

Vitali V., Büntgen U., Bauhus J. (2017): Silver fir and Douglas fir are more tolerant to extreme droughts than Norway spruce in south-western Germany. Global Change Biology, 23: 5108–5119.

Yanes A. (2020): Planting trees, a controversial strategy against climate change. Available at: https://www.bbvaopenmind.com/en/science/environment/planting-trees-a-controversial-strategy-against-climate-change/ (accessed Oct 3, 2022).

Žárník M., Holuša O. (2005): Jedle bělokorá (*Abies alba*) v lesnicko-typologických stupních Českého masivu, Západních a Východních Karpat. In: Jedle bělokorá – 2005, conference proceedings. Srní, Oct 31–Nov 1, 2005: 83–87. (in Czech)

Zeidler A., Vacek Z., Cukor J., Borůvka V., Vacek S., Prokůpková A., Linda R., Vacek O. (2022): Is European larch (*Larix decidua* Mill.) a suitable substitute for Norway spruce (*Picea abies* (L.) Karst.) for agricultural land afforestation? Forest Ecology and Management, 517: 120257.

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Effect of forest management on the genetic diversity of *Abies hidalgensis*, a threatened species with restricted distribution

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Abstract: *Abies hidalgensis* is an endemic species from the state of Hidalgo, Mexico, that has been registered only in nine fragmented relict populations that have a total of 1 000 individuals among them. Intensive forest management takes place in five of the populations under specific programs focused on *Pinus* spp. Still, it is necessary to know the impact of these activities on the genetic diversity of the threatened species, if restoration and conservation strategies are to be proposed. The aim of this work was to estimate the effect of forest management on the genetic structure of *A. hidalgensis* using seven nuclear molecular markers developed for *A. guatemalensis* (Ab07, Ab08, Ab09, Ab12, Ab15, Ab20, Ab23). The species was sampled growing under two different conditions; (*i*) areas under forest management and (*ii*) conserved areas. Two indexes of genetic diversity were evaluated, observed and expected heterozygosity. The genetic structure was determined by an analysis of molecular variance and a Bayesian assignment model. A bottleneck analysis was also carried out. The populations were found to have a common genetic base (differentiation coefficient F_{ST} = 0.056, number of migrants per generation Nm = 43), which suggests recent fragmentation of the distribution, which in turn increases the bottleneck effect in managed areas (Wilcoxon probability Wp = 0.007 and 0.016). This explains the apparently high heterozygous level (H_e = 0.69) and low inbreeding. Our results are important as they may be used to design strategies for management and conservation of *A. hidalgensis.*

Keywords: bottleneck; fir; genetic structure; genetic variation; population genetics

Diversity and genetic structure are critical population attributes for management and conservation of plant species classified under some level of risk due to their size, fragmentation, sensitivity, or restricted distribution (Gordon et al. 2012; Awad et al. 2014; Wu et al. 2015) because they fluctuate more quickly in response to spatial and temporal factors such as isolation, extent of the original distribution, deforestation, fragmentation, intrinsic demographic factors of the species, and associations such as competition, pollination or predation, life forms, harvesting, stochastic natural events, or anthropogenic dispersal, among others (Hedrick 2005; Conord et al. 2012; Vranckx et al. 2012; Zhang et al. 2012; Wyatt et al. 2021).

In particular, the fragmentation associated with forest management for timber and non-timber purposes has controversial effects on plants, with a varying intensity, depending on silvicultural practices, stand structure, and species characteristics present in the community before and during extraction (Gautam et al. 2021). For example, pioneer species benefit from management because it increases their population size while they share a common genetic base (post-colonisation expansion); whereas long-lived trees mostly suffer a reduction in fertility and consequently lose their genetic variability (Poelchau, Hamrick 2013). The negative effects for long-lived species may include a reduction in the number of alleles and genotypes, loss of heterozygosity, and reduction of gene flow and effective population size (Lowe et al. 2015). Nevertheless, for some widely distributed species, there are no differences in genetic diversity between managed and unmanaged stands (Paffetti et al. 2012; Aravanopoulos 2018; Rungis et al. 2019).

However, in principle, relict species with restricted distribution, endemics, and species with low population sizes should not be subjected to forest exploitation. Nevertheless, if their distribution coincides with other important timber species, they will be affected collaterally (Pérez-López et al. 2020). In addition, some species are illegally extracted because they are confused with others of greater distribution or removed due to a change in land use for grazing or clearing. For example, in *Araucaria angustifolia* (Bert.) O. Kuntze, an endangered Brazilian plant species, forest management reduces the number of alleles and therefore genetic diversity (Medri et al. 2003). Phuong Thuy et al. (2020) observed poor genetic diversity (h = 0.2223) in *Pinus kwangtungensis* Chun ex Tsiang compared to other species of the same genus [*Pinus koraiensis* Sieb. et Zucc H_e = 0.61, (Tong et al. 2020); *P. densiflora* Siebold & Zucc H_e = 0.652, (Ahn et al. 2021)] due to reduction of population size and habitat fragmentation. Additionally, it has been documented that relict populations in fragmented landscapes show low genetic diversity and high differentiation due to spatial isolation and high levels of inbreeding (Young et al. 1996; Szczecińska et al. 2016; Li et al. 2018).

Abies hidalgensis Debreczy, Rácz, & Guízar (Debreczy, Rácz 1995) is endemic to the state of Hidalgo, Mexico, and its distribution is extremely limited. To date, nine relict populations with a total of fewer than 1 000 individuals have been identified (Rosales-Islas et al. 2023). It is therefore on the Red List of threatened species in the Vu D2 category but is not covered by Mexican legislation (SEMARNAT 2010).

Abies hidalgensis is distributed in the municipalities of Agua Blanca de Iturbide, Tenango de Doria, and Acaxochitlán, Hidalgo, in nine relict populations. Several of them have been subjected to forest extraction (Las Águilas, El Tejocote, Ejido San Pedrito, Los Manantiales, and Zacacuautla), either controlled through the Silvicultural Development Method (SDM) or uncontrolled through elimination directed at corpulent trees, with or without the permission of the owners. In most of the stands under forest management, the cover has been replaced with the genus *Pinus* due to its economic importance. In addition, livestock activities were detected in these areas.

Of the nine populations, only two conserve a partially original cover (Ejido San Cornelio and Ejido San Pedrito). In these, extraction activities are not evident, extraction of specimens of commercial size has not been reported, and there is a scarce presence of stumps or evidence of extraction of trees, either of *A. hidalgensis* or the associated species. In these areas, it is possible to observe different development strata, with high densities of individuals. Two other populations have disappeared because of changes in land use.

The extraction activities are an example of collateral damage, since the scarce knowledge of its morphology, distribution and, in general, about its taxonomic status has resulted in the extraction of specimens in areas under forest management. INEGI (2016, 2017) reports the extraction of 250 $m^3 \cdot r^{-1}$ (m^3 of round wood) and 191 $m^3 \cdot r^{-1}$ of *Abies religiosa* (Kunth) Schltdl. & Cham. in the municipality of Agua Blanca, where this species is not distributed, but *A. hidalgensis* is.

The genetic structure of this type of species is evaluated by means of molecular markers to enable the impact of forest management to be estimated at the genetic level and to identify promising tree populations for the delimitation of conservation units, risk assessment, and design of management strategies (González 2003; Godoy 2009).

The objective of this work was to evaluate the effect of logging on the genetic variation of *A. hidalgensis* using nuclear molecular markers that would enable identification of losses associated with the population reduction caused specifically by the documented logging to assist in the design of restoration and conservation strategies.

MATERIAL AND METHODS

Study sites

The populations at Las Águilas and Ejido San Cornelio (managed and conserved, respectively) located at coordinates 20.369°N, −98.361°E and

20.361°N, −98.347°E were selected for the study (Figure 1). Forest extraction in Las Águilas is regulated by SDM, and the individuals of *A. hidalgensis* are distributed in regeneration areas of *Pinus patula.* This population has about 30 specimens; in contrast, the population of Ejido San Cornelio has about 396 individuals. Other populations were not considered for contrast since the number of individuals in the managed conditions was small (fewer than ten adults), distributed in small areas and/or geographically close. In addition, the localities under management are changing, some have even disappeared, and the relict individuals are merely ornamental within stands dedicated to timber production. Therefore, free access to the trees is not possible it requires agreements with the owners of the properties.

The populations are located in pine-oak forest and mountain cloud forest (INEGI 2018), between 2 100 m a.s.l and 2 320 m a.s.l. The region is characterised by a humid temperate climate C(m), with a maximum and minimum temperature of 21 °C and 6 °C respectively, precipitation of 1 200 mm to 1 500 mm per year with typical summer rains and 0% to 18% of the total annual precipitation in the winter (García 1973; INEGI 2008).

Field data recording

The group distribution of individuals makes it difficult to take samples with genetic independence, so we worked based on population conditions. However, it is common to obtain samples of individuals 20 m to 30 m apart within the population (Mosca et al. 2012; Stojnić et al. 2019). In the Ejido San Cornelio (conserved), sampled specimens were at least 30 m apart, while in the Las Águilas population (managed), the number of samples collected represents almost the total number of reproductive specimens in the population, so the distance between some individuals is less.

Leaf samples from the middle to the upper part of the crown of 30 reproductive specimens per condition were collected and placed in sealed bags with silica gel, labelled, and kept refrigerated at −20 °C before extracting DNA (Awad et al. 2014).

Laboratory analysis

DNA was extracted from leaves following the CTAB method of Doyle and Doyle (1987). 2 g of plant tissue were macerated with liquid nitrogen until a fine powder was obtained, and 1 mL of extraction buffer was added (CTAB-PVP 2% -Tris-HCl 100 mM pH 8, NaCl 1.4 M, EDTA 20 mM pH 8, and 1 µL of 2-β-mercaptoethanol) to continue mashing. The mixture was centrifuged at 800 rpm for 8 min. The precipitate was obtained and washed up to three times with the extraction buffer and then incubated at 37 °C for 1 h with 10 µL of RNAse (1 mg·mL^{-1}). Next, 10 µL of proteinase K (10 mg·mL^{-1}) was added and incubated

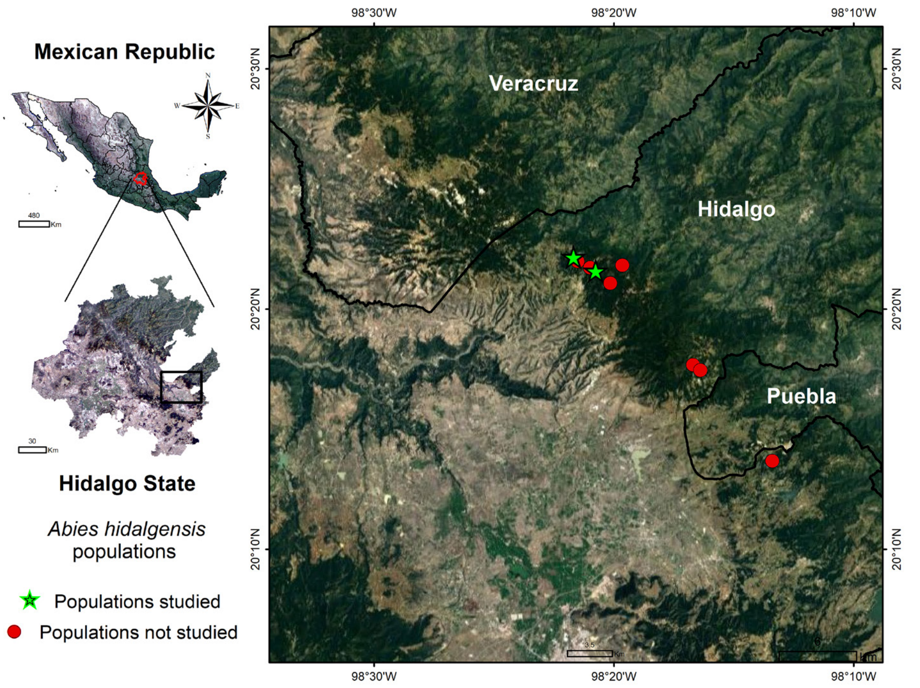

Figure 1. Studied populations of *Abies hidalgensis* in Hidalgo, Mexico

again at 60 °C for 1 h. Subsequently, 600 μL of chloroform : isoamyl : alcohol (25 : 24 : 1) and 250 μL 2% NaCl were added due to the high resin content (Sánchez-Coello et al. 2012), and shaken at 300 rpm for 1 h at 25 °C. Finally, the mixture was centrifuged at 10 000 rpm for 10 min, and the supernatant was recovered and precipitated with 2/3 parts of the final volume (300–500 μL) of cold isopropanol, allowing it to settle for 12–24 h at –20 °C. After 12 h, it was centrifuged at 12 000 rpm for 10 min, the supernatant was removed without losing the precipitated product, and 1 ml of cold absolute ethanol was added and centrifuged at 14 000 rpm for 10 min. Upon completion, the precipitated product was removed from ethanol and allowed to dry to be resuspended in 50 μL of sterile distilled water or TE buffer (10mM Tris-HCl, 1mM EDTA Na_2). The genetic material was quantified by spectrophotometry in a MAPADA nano spectrophotometer (MAPADA instruments, China).

Seven nuclear microsatellite markers developed for *A. guatemalensis* Rehder by Rasmussen et al. (2008) were used (Table 1). The mix for a tested PCR reaction was 2 μL genomic DNA (> 50 ng·μL^{-1}), 1.6 μL $MgCl_2$ (25 mM), 2.4 μL buffer (5×), 0.5 μL dH_2O, 0.7 μL of forward and reverse primers (5 μM), 0.3 μL of deoxynucleotide triphosphates (dNTPs) (10 mM) and 0.3 μL (1U) of Promega® TaqDNA polymerase for a total of 8.5 μL. The PCR technique was performed in a Thermo Scientific Artik endpoint thermal cycler (Thermo, USA).

The PCR program consisted of an initial denaturation step of 9 min at 94 °C; cycling in three steps repeated 30 times consisting of denaturation at 94 °C for 55 s, annealing for 50 s at 57 °C (primer Ab07 and Ab09), 50 s at 59 °C (primer Ab08 and Ab15), 50 s at 58 °C (primer Ab12 and Ab20), and 50 s at 61 °C (primer Ab23) and annealing at 72 °C for 55 s and the final extension at 72 °C for 7 min. PCR products were visualised on 15% acrylamide gels, run at 90 volts × 90 min and stained with ethidium bromide (10 mg·mL^{-1}) for 10 min. The gels were visualised in a transilluminator and recorded photographically.

Data analysis

Genetic diversity. The alleles (bands) were determined with the GelAnalyzer application (Version 19.1). The number of alleles was filtered by Poisson probability; null alleles (frequency less than 5%) were ruled out, which made it possible to eliminate genotyping errors, including non-amplified alleles. Gene frequencies were calculated to determine the average number of amplified samples per locus/condition (N), the average number of alleles (A), the effective number of alleles $N_e = \frac{1}{(\Sigma p_i)^2}$ by locus and by condition, where the p_i are the allele frequencies, the Shannon informative index for each primer $I = -1 \times \Sigma(p_i \times \ln p_i)$, the observed and expected heterozygosity (H_o and H_e respectively), and the fixation index $f = \frac{H_e - H_o}{H_e}$, which is zero if there are Hardy-Weinberg proportions (Hedrick 2005). The GenAlEx package v. 6.5 (Peakall, Smouse 2006) was used for this.

Genetic structure. The genetic structure was evaluated by Wright's (1951) F statistics (total inbreeding coefficient F_{IT}, inbreeding coefficient F_{IS}, differentiation coefficient F_{ST}) within and between study conditions to determine the distribution of genetic variability within the individual, among individuals in localities, and among the analysed localitiess. The number of migrants per generation (Nm) was also determined pairwise. Molecular analysis of variance (AMOVA) was performed, assuming an infinite allele model with 999 permutations. For these analyses, the GenAlEx v. 6.5 software (Peakall, Smouse 2006) was used. Additionally, to analyse the distribution of genetic variation within conditions, a Bayesian assignment analysis was carried out with the STRUCTURE 2.3.4 program (Pritchard et al. 2000). For each hypothetical genetic group (number or hypothetical genetic groups K = 2–5), 50 000 iterations were performed, with 50 000 Markovian repetitions, assuming the Admixture model and including the correlated allele frequencies (Falush et al. 2003). The process was repeated 10 times to calculate the confidence interval. Structure selector was used to determine the number of genetic groups from the ΔK model (Evanno et al. 2005) and obtain the genetic structure graph (Li, Liu 2018).

Bottleneck. A bottleneck analysis was performed considering a stepwise mutation model (SMM) using the Bottleneck v. 1.2.02 program (Cornuet, Luikart 1996) with resampling of 1 000 steps to the recalculation of the heterozygosity. The mutation-drift equilibrium model assumes that the severe reduction in the effective size of a population is reflected in an expected deficiency or excess of the

Table 1. Molecular markers used for analysis of the population structure of *Abies hidalgensis* in Hidalgo, Mexico

Locus	Forward primer 5'→ 3'	Reverse primer 3' ← 5'	Size range	T_a (°C)
Ab07	ACTGGCATTTGTCGCATTC	CCTCGGAGGACAAGATTTGC	219–257	57
Ab08	ATCGAGAGGCCCAGGTAGAC	GACATAGCTGATAGTGACGCAAC	128–161	59
Ab09	AGCTTATTTGCACGCTGAAG	TTTTCTTTATGAGAAACCAAGTTCC	129–174	57
Ab12	AGGTTGTGTAAGCCCGTGTAG	CCCTTTGTTGATAGAGGGAAAC	223–244	58
Ab15	AACTAACTCCTATGTGTCAAAATATCC	GCATGGAGGATAAGTAAAGATGG	239–254	59
Ab20	GATCCAGGTTTAGCGTATCTGAG	CAATGAATCTCTGCAACTGACC	134–169	58
Ab23	GGCATTATTTCCCACTTTTCC	TCAGATACATACTTGGGTTGGTG	181–207	61

T_a – annealing temperature

recalculated heterozygosity relative to the observed heterozygosity and whose intensity will depend on the time in which the reduction occurred and the resulted frequency of heterozygous individuals after the population reduction.

RESULTS

Genetic diversity. The microsatellites used were polymorphic. A total of 35 alleles were detected, with a range of 2 to 10 alleles per locus in both study conditions, and with an average of 5 for the managed condition and 4.86 for the conserved condition. The number of alleles for the Ab07, Ab09, Ab12, Ab15, Ab20 and Ab23 loci were 10, 5, 4, 2, 4, 3, respectively, for both study conditions. The Ab08 locus presented seven alleles for the managed condition, one private allele, and six alleles for the conserved condition.

The frequency of some alleles for the Ab07, Ab08, Ab09, Ab20 and Ab23 loci showed differences between the study conditions [Table S1 in Electronic Supplementary Material (ESM)]. At these loci, the most frequent alleles are different between conditions.

The number of effective alleles is generally low for the loci studied. In particular, the Ab12, Ab15, Ab23 loci present less than three effective alleles. Consistent with this, the Shannon index is low for the Ab15 locus in both conditions, while the Ab23 locus is low only for the managed condition (Table 2).

Table 2. Number of alleles and effective alleles, Shannon index, observed and expected heterozygosity and fixation index by locus

Study condition	Locus	N_a	N_e	I	H_o	H_e	f
Forest management	Ab07	10	7.595	2.139	0.933	0.868^{ns}	–0.075
	Ab08	7	5.732	1.838	0.567	0.826^{***}	0.314
	Ab09	5	3.734	1.456	0.433	0.732^{***}	0.408
	Ab12	4	2.456	1.013	0.600	0.593^{*}	–0.012
	Ab15	2	1.946	0.679	0.833	0.486^{***}	–0.714
	Ab20	4	3.509	1.313	0.500	0.715^{*}	0.301
	Ab23	3	1.972	0.742	0.400	0.493^{ns}	0.188
Conserved	Ab07	10	7.229	2.098	0.800	0.862^{ns}	0.072
	Ab08	6	5.187	1.706	0.433	0.807^{***}	0.463
	Ab09	5	3.854	1.461	0.433	0.741^{***}	0.415
	Ab12	4	3.789	1.358	0.967	0.736^{ns}	–0.313
	Ab15	2	1.897	0.666	0.633	0.473^{ns}	–0.340
	Ab20	4	3.742	1.352	0.333	0.733^{***}	0.545
	Ab23	3	2.605	1.012	0.667	0.616^{ns}	–0.082

$^{ns}P > 0.05$; *,**,*** $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively; N_a – number of alleles; N_e – effective number of alleles; I – Shannon index; H_o – observed heterozygosity; H_e – expected heterozygosity; f – fixation index, significant difference between H_o and H_e

The Ab07 and Ab23 loci for the managed condition are in the Hardy-Weinberg (HW) equilibrium; in contrast, the Ab08, Ab09 and Ab20 loci are heterozygous deficient, and Ab15 locus has an excess of heterozygotes. In the conserved condition, the Ab07, Ab12, Ab15, Ab23 loci are in the HW equilibrium, while the Ab08, Ab09 and Ab20 locus shows a high excess of homozygotes. (Table 2). The fixation index *f* showed that the Ab08, Ab09 and Ab20 loci have greater inbreeding in both study conditions (Table 2).

The H_o mean for both conditions was significantly different from the H_e mean, so they are not under the HW equilibrium. The *f* fixation index generally showed that the species has little inbreeding. In particular, the conserved condition presented more inbreeding (Table 3).

Genetic structure. F_{ST} indicates small but significant genetic differentiation between study conditions ($P < 0.001$), while F_{IS} and F_{IT} indicate significantly different inbreeding between conditions (Table 4). In particular, the Ab08, Ab09 and Ab20 loci for the F_{IS} and F_{IT} statistics indicate an excess of homozygotes. The Ab15 locus indicates an excess of heterozygotes for both statistics; however, it is not statistically different. The F_{ST} statistic for five of the seven evaluated loci indicates small but significant differentiation between the study conditions.

The estimated number of migrants (*Nm*) per generation is 4.22 between conditions, which indicates a high gene flow, but when we compare by locus, these values are diverse (Table 4). This high gene flow indicates a common origin; therefore, the ob-

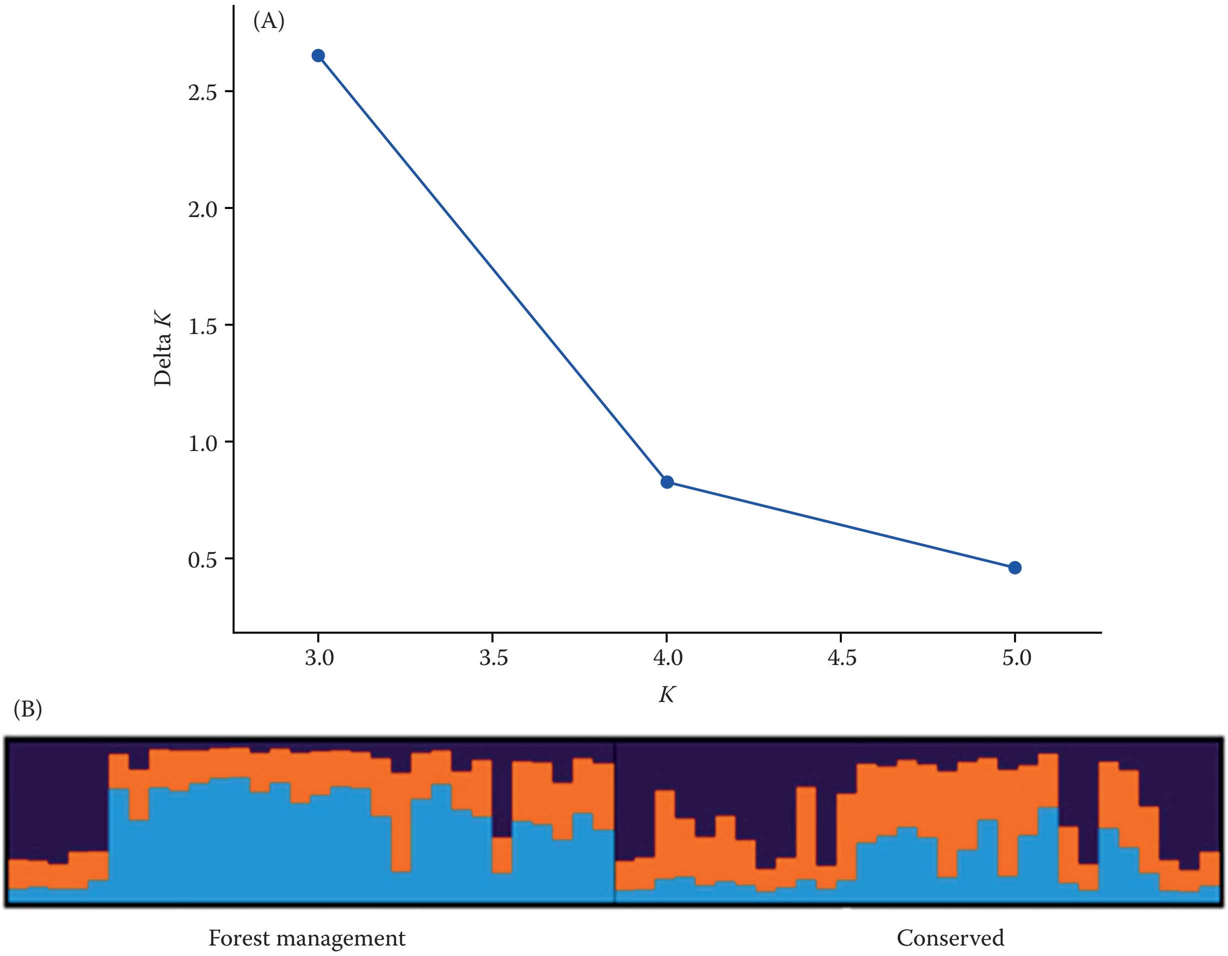

Figure 2. Results of Structure analysis using Structure selector; each vertical bar represents the inferred ancestry in each genetic group (A) estimation of the most probable *K* using the delta *K* method by Evanno et al. (2005); (B) bar plot representing the genetic structure at $K = 3$.

K – number or hypothetical genetic groups; delta *K* (the method) – mean ($|L''(K)|$)/sd($L(K)$); $L(K)$ – likelihood distribution difference; $L''(K)$ – values of the second-order rate of change of the likelihood distribution; sd – standard deviation

Table 3. Shannon index, observed and expected heterozygosity, and fixation index by study condition and population mean

Study condition	I	H_o	H_e	f
Forest management	1.31 ± 0.21	0.61 ± 0.08	0.67 ± 0.06*	0.06 ± 0.14
Conserved	1.38 ± 0.17	0.61 ± 0.09	0.71 ± 0.05*	0.11 ± 0.14
Mean	1.35 ± 0.13	0.61 ± 0.05	0.69 ± 0.04	0.08 ± 0.10

*$P < 0.05$ significant differences of the Hardy-Weinberg equilibrium; I – Shannon index; H_o – observed heterozygosity; H_e – expected heterozygosity; f – fixation index

Table 4. F coefficients and number of migrants by locus and total population

Locus	F_{ST}	F_{IS}	F_{IT}	Nm
Ab07	0.026**	0.015	0.041	11.28
Ab08	0.029**	0.402***	0.419***	9.13
Ab09	0.059**	0.426***	0.459***	5.66
Ab12	0.044*	–0.162	–0.111	8.19
Ab15	–0.006	–0.517	–0.526	215.75
Ab20	–0.015	0.438***	0.430***	52.12
Ab23	0.240***	0.055	0.282***	1.47
Mean	0.056***	0.135***	0.183***	43.37 ± 29.44

*,**,**$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively; F_{ST} – differentiation coefficient; F_{IS} – inbreeding coefficient; F_{IT} – total inbreeding coefficient; Nm – number of migrants per generation

served differences are caused by the different management between the study conditions.

A low Nei's genetic distance (0.195) and high identity (0.823) were observed, which indicates that it is the same species.The AMOVA results indicate that the genetic divergence between populations is 5.6% and among individuals 12.8%, while the divergence within individuals is 81.36% (Table S2 in the ESM). The greater observed divergence within individuals is influenced by a private allele, the 146 allele (frequency = 0.2) for the Ab08 locus, in the managed condition.

The number of genetic groups estimated from the Bayesian analysis in STRUCTURE is three ($K = 3$) (Figure 2A). The managed condition shows a larger predominance of one genetic group, while the rest seem to be more evenly distributed, although for the first evaluated specimens, one of the last two genetic groups is predominant. In the conserved condition, there is apparently a greater predominance of two genetic groups that present less dominance compared to the managed condition (Figure 2B).

Bottleneck. Six loci in the managed condition (Wilcoxon test, $P = 0.007$) and all loci in the conserved condition have excess heterozygosity under the mutation-drift equilibrium model. These results indicate that both conditions show evidence of a recent genetic bottleneck, which is stronger in the conserved condition.

DISCUSSION

Both conditions are in disequilibrium and maintain an apparently high connectivity, high levels of heterozygosity (slightly higher in the conserved condition), and greater genetic homogeneity among individuals under management conditions. This is because forest management activities are aggressive and are fragmenting the landscape, so genetic connectivity is a reflection of the past; a relatively recent history of extraction has eradicated all the individuals in some areas, and the few isolated trees are related adults grouped within a radius of less than 30 m. Similarly, low genetic diversity has been observed for some relict species in fragmented landscapes, but unlike our results, they show high differentiation between populations due to spatial isolation and high levels of inbreeding (Young et al. 1996; Szczecińska et al. 2016; Li et al. 2018) which in these specific cases is due to the time elapsed since fragmentation, since a few generations (one to four) are enough for population genetic divergence to manifest (Lavigne et al. 2001).

However, this can take decades in long-lived trees, like the ones sampled in this work (Eguiarte et al. 2007; Wiberg et al. 2016). Thus, the loss of genetic diversity depends on the degree of fragmentation related to gene flow and dispersal mechanisms, the demographic structure and the time elapsed since the reduction in population size (Bialozyt et al. 2006; Aldrich et al. 1998).

However, even when heterozygosity is high for *A. hidalgensis*, Rasmussen et al. (2008) observed 114 alleles using the same microsatellites as in this study on *A. guatemalensis*, a related species (Cruz-Nicolás et al. 2021), but unlike this study, 18 populations were evaluated in a wide distribution range. In our work, such population representativeness is impossible due to the conservation conditions described. We only obtained between 4 and 5 effective alleles average depending on the condition, so there is a real loss of allelic diversity but an artificial, short-lived extreme increase in the heterozygosity, which is explained by recent bottlenecks, since allelic diversity is much more sensitive to genetic changes (Spencer et al. 2000).

High levels of genetic diversity have also been observed in other conifers with relict populations such as *Picea omorika* (Panč) Purk (Aleksić et al. 2009) and *Picea chihuahuana* Martinez (Jaramillo-Correa et al. 2006), and in other species of the genus *Abies* (Table 5). Therefore, the high levels of H_o and H_e are the product of frequent past events of mixing of genetically differentiated populations and the recent drastic population reduction (Luikart et al. 1998), which decreases the number of alleles in a microsatellite concerning its allelic range (Garza, Williamson 2001), although such effects are temporary.

For example, in the particular case of *A. ziyuanensis*; another highly threatened fir species endemic to China, which like *A. hidalgensis* has low population numbers and declining population size mainly due to human activities, the species has a reduced total number of nuclear alleles (29) (Tang et al. 2008), similar to *A. hidalgensis* (35). The number of polymorphic loci found in *A. ziyuanensis* was five; at three of the eight evaluated loci ,only one allele was identified. Despite being polymorphic in *A. hidalgensis*, the Ab15 locus only had two alleles identified, so it is evident that the reduction in genetic diversity is related to the loss of alleles.

In *A. nebrodensis*, a highly threatened species with a single population, Vendramin et al. (1995), observed a higher H_o than H_e, and indicated that it could be due to selection in favour of heterozygotes. In contrast, in this study, four loci were observed with an excess of homozygotes, which, based on the population structure, could be due to the Wahlund effect, which homogenises diversity within populations and ends up generating high inbreeding in later stages of succession (Litrico et al. 2005). All tested loci would be expected to exhibit the same trend in the balancing selection hypothesis, still both excess and deficiency can be observed in both conditions. Considering that selection is unlikely to act on more than one locus, the observed deviations are consistent with the drastic reduction in population size that occurs stochastically and randomly.

On the other hand, based on the *f* fixation index, *A. hidalgensis* shows little inbreeding. However, if the reduction in effective population size is recent and since only adult trees were assessed,

Table 5. Comparison of genetic diversity of *A. hidalgensis* with other species of the genus *Abies*

Species	Molecular marker	Mean N_e	Mean *Ar*	H_o/H_e	H_e	F_{ST}	F_{IS}	Reference
Abies guatemalensis	SSR	28.600	–	0.822	0.862	0.051	0.045	Rasmussen et al. (2010)
Abies guatemalensis	isoenzyme	–	–	–	0.069	–	–	Aguirre-Planter et al. (2000)
Abies religiosa		–	–	–	0.108	–	–	
Abies flinckii		–	–	–	0.113	–	–	
Abies hickelii		–	–	–	0.100	–		
Abies pinsapo	nSSR	2.825	–	0.528	0.596	–	0.120	Cobo-Simón et al. (2020)
	ISSR	1.058	–	–	0.035	–	–	
Abies ciclica	SSR	–	2.77	0.578	0.595	–	0.029	Awad et al. (2014)
Abies marocana		–	2.86	0.405	0.572	–	–	
Abies bornmuelleriana		–	4.22	0.668	0.799	–	–	
Abies cephalonica		–	4.17	0.713	0.821	–	–	
Abies alba		–	3.93	0.663	0.808	–	–	
Abies ziyuanensis	SSR	3.600	–	–	0.437	0.250	–	Tang et al. (2008)

N_e – effective number of alleles; *Ar* – rarefied allelic richness; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{ST} – differentiation coefficient; F_{IS} – inbreeding coefficient; nSSR –nuclear microsatellites; ISSR – inter-microsatellites; SSR – Simple Sequence Repeats it is unlikely to be observed. In this regard, Leding et al. (2006), found significantly different levels in the inbreeding coefficient in *A. bracteata* D. Don ex Poiteau between samples of adult trees and seeds, so that in the next generations of *A. hidalgensis* more significant inbreeding could be expected, which would be a critical factor for the design of management and conservation strategies. Consequently, the level of inbreeding concerning Wright's *F* statistics is low (Wright 1951).

Implications for conservation. The patterns of genetic diversity detected in this study can be used in designing management and conservation strategies for *A. hidalgensis*. The total of the populations known up to now was not analysed. However, two populations are evaluated, where the most significant distance between specimens occurs and where one has a good degree of conservation.

Conserving alleles in the context of populations is a fundamental strategy because, as already mentioned, they are more susceptible to loss due to bottlenecks. Since they are unique, it is important to obtain representative seeds of all populations for breeding purposes, especially of those specimens with the 146 allele. Given the climate fluctuations that imply new adverse scenarios, any allele is a potential benefit. Increasing the population size with the greatest possible diversity is urgently necessary since its ability to adapt to changes depends on it (Gargiulo et al. 2019).

In the case of *A. hidalgensis*, conservation efforts should focus on preventing habitat loss due to human impacts and future climate change impacts, as well as assessing the feasibility of carrying out *ex situ* conservation of genetic resources or assisted migration, given its limited ability to migrate. In addition, the seeds are difficult to obtain because they are found in isolated and tall individuals; the species does not form a seed bank, the seedlings emerge infrequently (Rosales-Islas, personal observation), and most of them die before reaching the juvenile stage. So, seeds and seedlings are a reservoir of genetic variability (Mosseler et al. 2003) where all recovery, propagation, and management efforts should be directed.

CONCLUSION

Abies hidalgensis has a high heterozygosity but suffers a negative effect of logging associated with a reduction of the population size and show a limited allelic richness distributed differently in response to forest management. However, its populations maintain a common genetic base that has not yet been differentiated because fragmentation and reduction in population size are very recent. We are observing a corpse that still does not know it is dead, since the total eradication of individuals in some areas generates a total loss of connectivity, which increases the level of threat to the species and accelerates its extinction rate; therefore, conservation actions are paramount.

REFERENCES

Aguirre-Planter E., Furnier G.R., Eguiarte L.E. (2000): Low levels of genetic variation within y high levels of genetic differentiation among populations of species of Abies from Southern Mexico and Guatemala. American Journal of Botany, 87: 362–371

Ahn J.Y., Lee J.W., Hong K.N. (2021): Genetic diversity and structure of *Pinus densiflora* Siebold & Zucc. populations in Republic of Korea based on microsatellite markers. Forests, 12: 750.

Aldrich P.R., Hamrick J.L., Chavarriaga P., Kochert G. (1998): Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. Molecular Ecology, 7: 933–944.

Aleksić J.M., Schueler S., Mengl M., Geburek T. (2009): EST-SSRS developed for other *Picea* species amplify in *Picea omorika* and reveal high genetic variation in two natural populations. Belgian Journal of Botany, 142: 89–95.

Aravanopoulos F.A. (2018): Do silviculture and forest management affect the genetic diversity and structure of long-impacted forest tree populations? Forests, 9: 355.

Awad L., Fady B., Khater C., Roig A., Cheddadi R. (2014): Genetic structure and diversity of the endangered fir tree of Lebanon (*Abies cilicica* Carr.): Implications for conservation. PLoS ONE, 9: e90086.

Bialozyt R., Ziegenhagen B., Petit R.J. (2006): Contrasting effects of long-distance seed dispersal on genetic diversity during range expansion. Journal of Evolutionary Biology, 19: 12–20.

Cobo-Simón I., Méndez-Cea B., Jump A.S., Seco J., Gallejo F.J., Linares J.C. (2020): Understanding genetic diversity of relict forests. Linking long-term isolation legacies and current habitat fragmentation in *Abies pinsapo* Boiss. Forest Ecology and Management, 461: 117947.

Conord C., Gurevitch J., Fady B. (2012): Large-scale longitudinal gradients of genetic diversity: A meta-analysis across six phyla in the Mediterranean basin. Ecology and Evolution, 2: 2600–2614.

Cornuet J.M., Luikart G. (1996): Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144: 2001–2014.

Cruz-Nicolás J., Villarruel-Arroyo A., Gernandt D.S., Fonseca R.M., Aguirre-Planter E., Eguiarte L.E., Jaramillo-Correa J.P. (2021): Non-adaptive evolutionary processes governed the diversification of a temperate conifer lineage after its migration into the tropics. Molecular Phylogenetics and Evolution, 160: 107125.

Debreczy Z., Rácz I. (1995): New species and varieties of conifers from Mexico. Phytologia, 78: 217–243.

Doyle J.J., Doyle J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11–15.

Eguiarte L.E., Souza V., Aguirre X. (2007): Ecología Molecular. Mexico City, Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad: 1142. (in Spanish)

Evanno G., Regnaut S., Goudet J. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology, 14: 2611–2620.

Falush D., Stephens M., Pritchard J.K. (2003): Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics, 164: 1567–1587.

García E. (1973): Modificaciones al sistema de clasificación climática de Köppen (para adaptarlo a las condiciones climáticas de la república mexicana). Segunda edición. Mexico City, Universidad Nacional Autónoma de México, Instituto de Geografía: 246. (in Spanish)

Gargiulo R., Saubin M., Rizzuto G., West B., Fay, M.F., Kallow S., Trivedi C. (2019): Genetic diversity in British populations of *Taxus baccata* L.: Is the seedbank collection representative of the genetic variation in the wild?. Biological Conservation, 233: 289–297.

Garza J.C., Williamson E.G. (2001): Detection of reduction in population size using data from microsatellite loci. Molecular Ecology, 10: 305–318.

Gautam S., Timilsina S., Shrestha M. (2021): The effects of forest management activities on genetic diversity of forest trees. Indonesian Journal of Social and Environmental Issues, 2: 110–118.

Godoy J.A. (2009): La genética, los marcadores moleculares y la conservación de especies. Ecosistemas, 18: 23–33. (in Spanish)

González E.G. (2003): Microsatélites: Sus aplicaciones en la conservación de la biodiversidad. Graellsia, 59: 377–388. (in Spanish)

Gordon S.P., Sloop C.M., Davis H.G., Cushman J.H. (2012): Population genetic diversity and structure of two rare vernal pool grasses in central California. Conservation Genetics, 13: 117–130.

Hedrick P. (2005): Genetics of Populations. Sudbury, Jones and Bartlett Publishers: 737.

INEGI (Instituto Nacional de Estadística y Geografía) (2008): Conjunto de datos vectoriales, escala 1 : 1 000 000. Unidades climáticas. Available at: https://www.inegi.org.mx/temas/climatologia/ (in Spanish).

INEGI (Instituto Nacional de Estadística y Geografía) (2016): Anuario estadístico y geográfico de Hidalgo 2016. Available at: http://internet.contenidos.inegi.org.mx/contenidos/Productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2017/702825095093.pdf (in Spanish).

INEGI (Instituto Nacional de Estadística y Geografía) (2017): Anuario estadístico y geográfico de Hidalgo 2017. Available at: https://www.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2016/702825083748.pdf (in Spanish)

INEGI, Instituto Nacional de Estadística y Geografía (2018): Conjunto de datos vectoriales de uso de suelo y vegetación. Escala 1 : 250 000. Serie VII. Available at: https://www.inegi.org.mx/temas/usosuelo/ (in Spanish).

Jaramillo-Correa J.P., Beaulieu J., Leding F.T., Bousquet J. (2006): Decoupled mitochondrial and chloroplast DNA population structure reveals Holocene collapse and population isolation in a threatened Mexican-endemic conifer. Molecular Ecology, 15: 2787–2800.

Lavigne C., Reboud X., Lefranc M., Porcher E., Roux F., Olivieri I., Godelle B. (2001): Evolution of genetic diversity in metapopulations: *Arabidopsis thaliana* as an experimental model. Genetics Selection Evolution, 33: S399.

Leding F.T., Hodgskiss P.D., Johnson D.R. (2006): Genetic diversity and seed production in Santa Lucia fir (*Abies bracteata*), a relict of the Miocene broadleaved evergreen forest. Conservation Genetics, 7: 383–398.

Li Y.L., Liu J.X. (2018): STRUCTURE SELECTOR: A web based software to select and visualize the optimal number of clusters using multiple methods. Molecular Ecology Resources, 18: 176–177.

Li S., Gan X., Han H., Zhang X., Tian Z. (2018): Low within-population genetic diversity and high genetic differentiation among populations of the endangered plant *Tetracentron sinense* Oliver revealed by inter-simple sequence repeat analysis. Annals of Forest Science, 75: 74.

Litrico I., Ronfort J., Verlaque R., Thompson J.D. (2005): Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Réunion. Molecular Ecology, 14: 1575–1584.

Lowe A.J., Cavers S., Boshier D., Breed M.F., Hollingsworth P.M. (2015): The resilience of forest fragmentation genetics – no longer a paradox – we were just looking in the wrong place. Heredity, 115: 97–99.

Luikart G., Sherwin W.B., Steele B.M., Allendorf F.W. (1998): Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology, 7: 963–974.

Mosca E., Eckert A.J., Di Pierro E.A., Rocchini D., La Porta N., Belletti P., Neale D.B. (2012): The geographical and environmental determinants of genetic diversity for four alpine conifers of the European Alps. Molecular Ecology, 21: 5530–55045.

Mosseler A., Major J.E., Rajora O.P. (2003): Old-growth red spruce forests as reservoirs of genetic diversity and reproductive fitness. Theoretical and Applied Genetics, 106: 931–937.

Medri C., Ruas P.M., Higa A.R., Murakami M., de Fátima Ruas C. (2003): Effects of forest management on the genetic diversity in a population of *Araucaria angustifolia* (Bert.) O. Kuntze. Silvae Genetica, 52: 202–205.

Paffetti D., Travaglini D., Buonamici A., Nocentini S., Vendramin G.G., Giannini R., Vettori C. (2012): The influence of forest management on beech (*Fagus sylvatica* L.) stand structure and genetic diversity. Forest Ecology and Management, 284: 34–44.

Peakall R., Smouse P.E. (2006): GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288–295.

Pérez-López R.I., González-Espinosa M., Ramírez-Marcial N., Toledo-Aceves T. (2020): Efectos del "Método de Desarrollo Silvícola" sobre la diversidad arbórea en bosques húmedos de montaña del norte de Chiapas, México. Revista Mexicana de Biodiversidad, 91: e913326. (in Spanish)

Phuong Thuy M.T., Thu Ha T.T., Quang T.H. (2020): Analysis of genetic diversity in Pa Co pine (*Pinus kwangtungensis* Chun ex Tsiang) using RAPD and ISSR markers. Vietnam Journal of Science, Technology and Engineering, 62: 62–68.

Poelchau M.F., Hamrick J.L. (2013): Comparative phylogeography of three common Neotropical tree species. Journal of Biogeography, 40: 618–631.

Pritchard J.K., Stephens M., Donnelly P. (2000): Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

Rasmussen K.K., Andersen U.S., Frauenfelder N., Kollmann J. (2008): Microsatellite markers for the endangered fir *Abies guatemalensis* (Pinaceae). Molecular Ecology Resources, 8: 1307–1309.

Rasmussen K.K., Strandby U., Kollmann J. (2010): High genetic diversity within but limited differentiation among populations of the vulnerable Guatemalan fir. Journal of Tropical Forest Science, 22: 247–259.

Rosales-Islas E., Barrera-Tello D., Sánchez-González A., Galván-Hernández D.M., Hernández-León S., Octavio-Aguilar P. (2023): Morphological and genetical characterization of *Abies*' populations in Hidalgo, México: importance of the taxonomical identity to the harvesting. Botanical Sciences, 100: 417–434.

Rungis D.E., Lībiete Z., Korica A., Katrevičs J., Jansons A., Veinberga I., Jansons J. (2019): Genetic diversity and differentiation of even-aged Norway spruce stands in Latvia. Baltic Forestry, 25: 45–51.

Sánchez-Coello N.G., Luna-Rodríguez M., Vázquez-Torres M., Sánchez-Velásquez L.R., Santana-Buzzy N., Octavio-Aguilar P., Iglesias-Andreu L.G. (2012): Optimización de un protocolo del aislamiento del ADN y de un sistema de amplificación ISSR-PCR para *Ceratozamia mexicana* Brongn. (Zamiaceae). Revista Chapingo Serie Ciencias Forestales y del Ambiente, 18: 123–133. (in Spanish)

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) (2010): NOM-059-SEMARNAT-2010, Protección ambiental, Especies nativas de México de flora y fauna silvestres, Categorías de Riesgo y especificaciones para su inclusión, exclusión o cambio – Lista de especies en riesgo. México, Diario Oficial de la Federación. Available at: https://www.gob.mx/profepa/documentos/norma-oficial-mexicana-nom-059-semarnat-2010 (in Spanish)

Spencer C.C., Neigel J.E., Leberg P.L. (2000): Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Molecular Ecology, 9: 1517–1528.

Stojnić S., Avramidou E.V., Fussi B., Westergren M., Orlović S., Matović B., Trudić B., Kraigher H., Aravanopoulos F.A., Konnert M. (2019): Assessment of genetic diversity and population genetic structure of norway spruce (*Picea abies* (L.) Karsten) at its southern lineage in Europe. Implications for conservation of forest genetic resources. Forests, 10: 258.

Szczecińska M., Sramko G., Wołosz K., Sawicki J. (2016): Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. PLoS ONE, 11: e0151730.

Tang S., Dai W., Li M., Zhang Y., Geng Y., Wang L., Zhong Y. (2008): Genetic diversity of relictual and endangered plant *Abies ziyuanensis* (Pinaceae) revealed by AFLP and SSR markers. Genetica, 133: 21–30.

Tong Y.W., Lewis B.J., Zhou W.M., Mao C.R., Wang Y., Zhou L., Yu D.P., Dai L.M., Qi L. (2020): Genetic diversity and population structure of natural *Pinus koraiensis* populations. Forests, 11: 39.

Vendramin G.G., Michelozzi M., Lelli L., Tognetti R. (1995): Genetic variation in *Abies nebrodensis*: A case study for a highly endangered species. Forest Genetics, 2: 171–175.

Vranckx G., Jacquemyn H., Muys B., Honnay O. (2012): Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conservation Biology, 26: 228–237.

Wiberg R.A.W., Scobie A.R., A'Hara S.W., Ennos R.A., Cottrell J.E. (2016): The genetic consequences of long term habitat fragmentation on a self-incompatible clonal plant, *Linnaea borealis* L. Biological Conservation, 201: 405–413.

Wright S. (1951): The genetical structure of populations. Annals Eugenesic, 15: 323–354.

Wu F.Q., Shen S.K., Zhang X.J., Wang Y.H., Sun W.B. (2015): Genetic diversity and population structure of an extremely endangered species: The world's largest Rhododendron. AoB PLANTS, 7: plu082.

Wyatt G.E., Hamrick J.L., Trapnell D.W. (2021): The role of anthropogenic dispersal in shaping the distribution and genetic composition of a widespread North American tree species. Ecology and Evolution, 11: 11515–11532.

Young A., Boyle T., Brown T. (1996): The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution, 11: 413–418.

Zhang X., Shi M.M., Shen D.W., Chen X.Y. (2012). Habitat loss other than fragmentation per se decreased nuclear and chloroplast genetic diversity in a monoecious tree. PLoS ONE, 7: e39146.

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The effect of thinning intensity on sap flow and growth of Norway spruce

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Abstract: Forest thinning can be used as an adaptive measure to improve the growth and resistance of Norway spruce forests affected by climate change. The impact of different thinning intensities on sap flow, growth, and tree water deficit of 40-year-old Norway spruce was tested. High thinning intensity (–61% of basal area) resulted in increased tree-level sap flow compared to the control (+27%), but it caused a decrease in the stand-level transpiration (–34%) due to reduced leaf area index. Low-intensity thinning (–28% basal area), high-intensity thinning, and control showed similar responses of sap flow to vapour pressure deficit and global radiation, suggesting unchanged isohydric behaviour. Both low- and high-intensity treatments displayed greater radial growth than the control. There were no differences in tree water deficit between the treatments. The low-intensity treatment can be considered the best water utilisation treatment with increased growth and unchanged transpiration at the tree level. The high-intensity treatment had similar radial growth as the low-intensity but lower stand-level transpiration, implying improved soil water availability. The study expands the ecophysiological understanding of thinning as a valuable silvicultural practice for adapting forest management of Norway spruce to the effects of climate change.

Keywords: increment; *Picea abies*; silviculture; transpiration; tree water deficit

Thinning is a common silvicultural technique used to manage growth and development of forests. Alteration of stand density and structure can improve growth and stress resistance of remaining trees due to reduced competition (Krajnc et al. 2019). Norway spruce (*Picea abies* (L.) Karst.) in Central Europe is especially affected by a combination of abiotic and biotic stresses which threaten

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its productivity and stability (Bosela et al. 2021; Sedmáková et al. 2022). The decrease in precipitation and higher evaporative demand in the past decades are already negatively affecting the growth of Norway spruce and pushing its ecological optimum to higher altitudes and latitudes (Krejza et al. 2021). Modern forestry should utilise thinning techniques as an adaptive measure to stabilise the existing Norway spruce stands. The use of thinning at a relatively young tree age can improve growth, allometry, and soil water availability, which can make the trees more resistant and resilient to expected climate change (Pretzsch, Mette 2008; Bhandari et al. 2021; Dušek et al. 2021). Heavy thinning can also improve understory conditions for the survival of natural regeneration or planted seedlings, with the aim of transforming a pure Norway spruce stand to a more heterogenous mixed stand (Lin et al. 2012; Reventlow et al. 2021).

Thinning affects the growth of Norway spruce in several ways. By reducing competition for resources such as light, water, and nutrients, thinning allows the remaining trees to grow more efficiently and with improved vigour (Jiménez et al. 2019; Houtmeyers, Brunner 2020). This can increase the trees' overall size and radial growth, as well as their resistance to environmental stressors and pathogens (Houtmeyers, Brunner 2020). Thinning from below (reduction of understory trees) increases the mean diameter and height of the remaining trees (Eliasson, Lageson 1999). Removal of the trees from this layer has a greater impact on soil-zone competition than on light competition (Kenkel 1988; Fernandez et al. 2012). Regardless, the understory trees exhibit a higher probability of mortality due to competitive pressure imposed by the trees in the uppermost canopy layer (Powers et al. 2010). Therefore, thinning from below can be seen as a great management tool to improve resistance and growth of the large trees from the canopy layer. The thinning intensity is also an important factor affecting the response of remaining trees to the silvicultural intervention. Higher-intensity thinning can have a more pronounced effect on the remaining large trees but can negatively affect total stand biomass production in the short-term (Mäkinen, Isomäki 2004).

Sap flow is the movement of water and nutrients from the roots to the branches and leaves, driven mostly by changes in the water pressure through the soil-plant-atmosphere continuum (Zhang et al. 1997; Yang et al. 2022). Thinning can increase sap flow by reducing competition for resources and allowing for increased light penetration and greater leaf area per tree (Kellomäki et al. 2023). Greater nutrient availability due to lower competition can also lead to formation of more efficient tracheids capable of greater sap flow (Ward et al. 2008). At the same time, thinning can also reduce stand-level respiration as the total leaf area or leaf area index is reduced and leads to less water being lost in the process (Forrester et al. 2013; Wang et al. 2019). Lower stand-level transpiration in stands with higher thinning intensity can lead to greater soil water reserves under higher evaporative demand, compared to unthinned stands (Rimal et al. 2022). In addition to measuring tree water status through sap flow, the automatic dendrometer that records stem radial variations (SRV) is a valuable tool for monitoring tree growth patterns and diurnal water changes in the stem (Krejza et al. 2021; Szatniewska et al. 2022). Dendrometers can provide a useful method for assessing tree water status (i.e. tree water deficit; TWD), which can indicate stress and potentially compromised vitality status of forest trees (Zavadilova et al. 2023). During prolonged droughts, trees use more of their stored water to maintain their essential functions (Salomón et al. 2022). As the stored water is depleted, TWD becomes more pronounced, leading to reduced growth, hydraulic constraints, and even death in extreme cases (Preisler et al. 2021). Changes in stand density (i.e. thinning) may have favourable effects on soil water availability and consequently improve water status and reduce the remaining trees' dependence on stored trunk water (Jiménez et al. 2019). Investigation of multiple environmental factors can improve current ecophysiological knowledge, which can enable us to use proper management strategies to achieve higher resistance and productivity of forest ecosystems.

In this study, we analysed stem radial growth and sap flow dynamics in *Picea abies* trees two years after different thinning intensities. We aimed to validate the following hypotheses: (*i*) thinning will have a positive impact on radial growth and a tree-level sap flow, and (*ii*) will cause a decrease in stand-level transpiration. Moreover, we wanted to test if (*iii*) the unthinned plot will experience a greater TWD due to higher competition for soil water sources than the thinning treatments and will use more stored stem water for the sap flow. The relevance of these objec-

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tives is enhanced by the site conditions that represent a lower altitude edge (625 m a.s.l.) for Norway spruce cultivation in the region.

MATERIAL AND METHODS

Study site description. The study site was located near Rajec, Czech Republic (49°26'45"N, 16°41'49"E; 625 m a.s.l.). The soil type was modal podzol with maximum soil depth of 60–80 cm. The stand was established in 1979 by afforestation of a previously clear-cut Norway spruce forest. Three-year-old seedlings were planted during the spring of 1979 in a 2 m × 1 m spread (Krejza 2016). The pure Norway spruce stand was divided into three equally large plots of 625 m^2. Two plots were thinned from below during the spring of 2018 with two different thinning intensities (30% and 60% of basal area removed). The third plot was left unthinned as a control treatment. The diameter at breast height (*DBH*) measurements were conducted during the 2020 vegetation season, two years post-thinning on each plot (Figure 1, Table 1). The leaf area index (*LAI*) was measured in July 2020 by the SS1 SunSCAN (Delta-T Devices Ltd., the United Kingdom) at five different positions at each plot to cover the heterogeneity of the canopy stands (Table 1). The broader area is covered mostly by coniferous species (77%), of which Norway spruce is most abundant (54%), but based on phytocenological classification, the natural cover was historically dominated by European beech (Světlík 2016). According to climatic conditions, altitude, and the southern slope of the stand, the site is not suitable for Norway spruce under the prospects of climate change. The evidence of that is a massive bark beetle infestation in the neighbouring old Norway spruce stand that was clear-cut and is being afforested with mixed broadleaved and coniferous species.

Meteorological measurements. Meteorological data were measured on the site above the canopy at 40 m height on a meteorological tower with the sensor for relative air humidity and air temperature (EMS 33, Czech Republic), incoming global radiation (CNR 1, Kipp & Zonen, the Netherlands), and precipitation (386 C, MetOne, USA; Figure 2). The mean annual temperature in 2020 was 9 °C which is 1.2 °C above the long-term yearly average (7.8 °C; Krejza et al. 2022). The amount of precipitation in 2020 was 879 mm, 200 mm more than the long-term annual average (679 mm; Krejza et al. 2022).

Sap flow measurements. We measured the sap flow using EMS81 (EMS Brno, Czech Republic) modules on four trees per treatment during

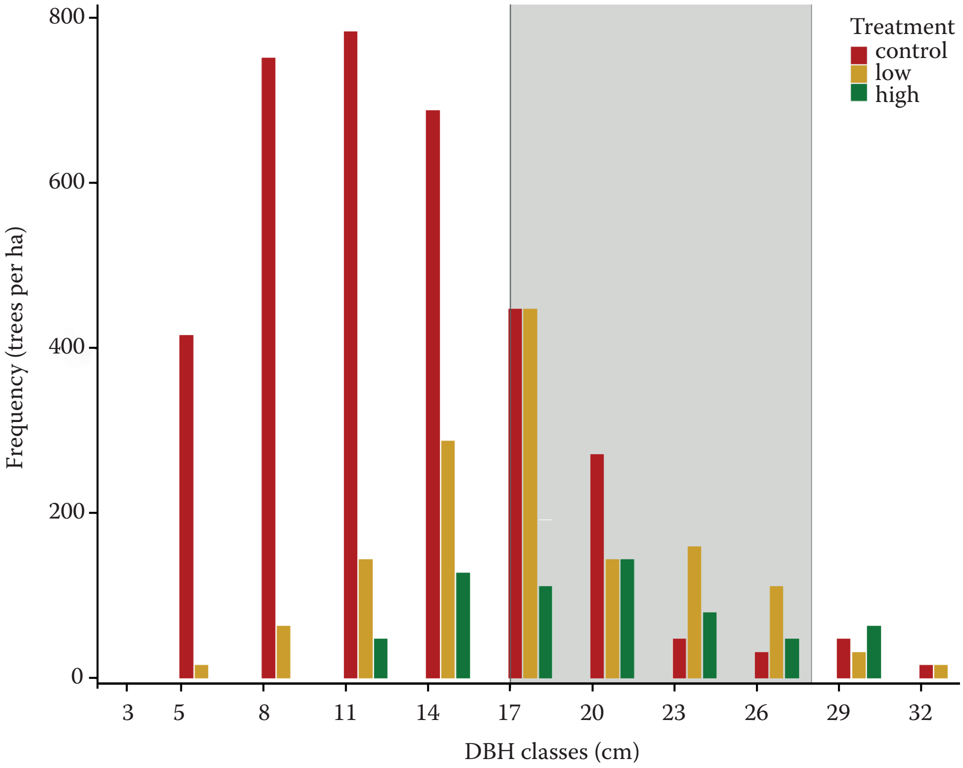

Figure 1. Tree diameter structure of the stands; grey area indicates DBH classes of selected trees for sap flow measurements

DBH – diameter at breast height

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Table 1. Overview of the stand structure, Leaf Area Index and average (mean ± SE) sap flow at tree and stand-level for all three treatments

Thinning intensity	Stand density (tree·ha^{-1})	Stand *BA* (m^2·ha^{-1})	Mean *DBH* (cm)	*LAI*	Mean sap flow (sampled trees) (kg·year^{-1}·tree^{-1})	Stand transpiration (mm·year^{-1})
Control	3 504	3.506	13 ± 5.2	5.02 ± 0.90	1 900 ± 375	227
Low	1 424	2.514	18 ± 5.1	3.95 ± 1.42	1 889 ± 813	219
High	624	1.358	20 ± 5.2	3.08 ± 0.75	2 507 ± 888	149

BA – basal area; *DBH* – diameter at breast height; *LAI* – Leaf Area Index

the 2020 growing season. The measurement principle is based on the Tissue Heat Balance method (Čermák et al. 2004). The post-processing of data includes establishing a baseline that eliminates noise caused by heat losses in the system under zero-flow conditions (Kučera et al. 2020). Moreover, the 23 days (12% of data) where precipitation exceeded 10 mm·day^{-1} were excluded according to the declining regression curve between sap flow and rainfall. The collected 10-min data were averaged to hourly and then aggregated to daily values. The tree sap flow (Q_{tree}) was standardised per one cm of the tree circumference (Q, kg·day^{-1}·cm^{-1}). Tree-level sap flow was also upscaled to stand-level sap flow via a scaling factor calculated from the ratio of the sampled trees' sap flow and the sap flow of all trees in the selected diameter classes (Čermák et al. 2004). The scaling curves are visualised for all three plots in supplementary files [Figure S1 in the Electronic Supplementary Material (ESM)].

Stem variation measurements. Stem radial variation (*SRV*) was obtained by measuring changes in tree circumference by automatic band dendrometers (DRL26, EMSBrno, Czech Republic) on four trees per treatment. The tree periderm was carefully removed before installing dendrometers to reduce the impact of hydroscopic shrinkage and swelling of the tree's outer bark. To measure the water-related changes in the stem, *TWD* (in mm) was calculated using dendrometer records that were de-trended for growth (Ehrenberger et al. 2012; Zweifel 2016). In the first step, a growth line was constructed by drawing lines between the daily maximum stem circumference values and the next equal stem circumference value, ignoring periods of incomplete stem circumference recov-

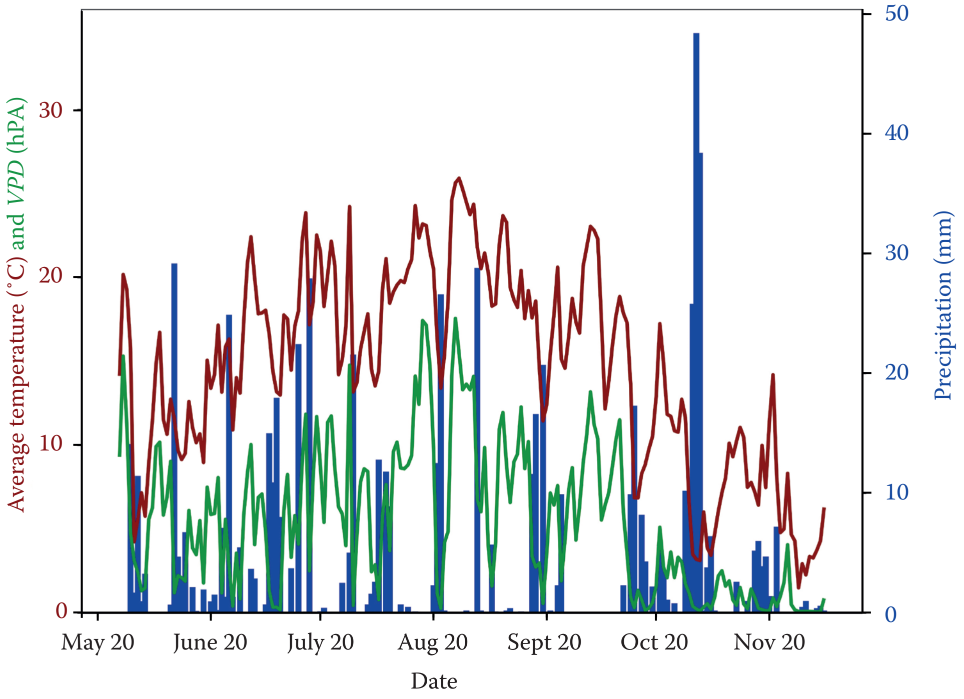

Figure 2. Daily dynamics of mean air temperature (red), vapour pressure deficit (*VPD*) (green) and precipitation (blue) during the vegetation season of 2020 at the experimental site

ery due to stem shrinkage induced by water shortage. In the second step, the *TWD* was calculated as a difference between the actual *SRV* and growth line. The normalisation of the dendrometer data was not necessary as the difference in the average *DBH* of all the sampled trees was found to be statistically insignificant among plots.

Statistical analyses. All statistical analyses were conducted with R software (Version 4.2, 2022). Two-way ANOVA was used to test the sap flow differences between treatments and DBH classes. As the assumptions of homogeneity and normality were not met for the dendrometer data, we used Kruskal-Wallis' test for the analysis of variance. Differences between treatments and DBH classes were further analysed with nonparametric Fisher's LSD (least significant difference) test for multiple pairwise comparisons and the parametric Tukey's HSD (honest significant difference) test for mean comparisons. Both post-hoc tests were conducted with the Holm method (Holm 1979) for adjustment of the *P*-value. All statistical analyses were conducted at a significance level of $\alpha = 0.05$. Relationships between environmental conditions and sap flow were tested by linear and logarithmic regressions.

RESULTS AND DISCUSSION

Effect of thinning on standardised sap flow and stand transpiration. The effects of thinning on sap flow and transpiration in Norway spruce are complex and can depend on various factors such as the intensity of thinning, the age and size of the remaining trees, and local environmental conditions (Lagergren et al. 2008; Clausnitzer et al. 2011). In this study, the thinning treatment had a significant impact on standardised sap flow (*Q*) in stands with high-intensity thinning but not low-intensity thinning (Figure 3A, Table S1 in the ESM). High-intensity treatment showed overall higher maximum values of *Q* throughout the vegetation season compared to control and low-intensity treatment (Figure 3B), comparable with results observed by McJannet and Vertessy (2001) and Park et al. (2018).

The main factor which could explain the increase of individual tree *Q* in the high-intensity treatment is the lower competition for soil water and nutrient availability due to the removal of smaller trees. Another explanatory factor could be increased light availability. However, most of the trees removed during thinning were chosen from the understory; hence, the soil-water competition alternations are probably a more dominant factor affecting the *Q* changes. Greater access to soil water resources could be directly reflected in higher transpiration and, therefore, higher *Q* rates (Clausnitzer et al. 2011; Szatniewska et al. 2022). Higher competition leads to lower *Q* values and reduced competition via thinning can lead to a rapid increase of *Q* in the following vegetation season (Lagergren, Lindroth 2004; Gebauer et al. 2011). Trees with the dominant social position and reduced competition pressure can show larger xylem conductivity, as indicated by Zhang et al. (2019). Given the warmer and wetter vegetation season in 2020 compared to the long-term average, changes in *Q* may have been notably influenced by the higher nutrient availability. Nutrient limitation of Norway spruce can lead to the reduction of total leaf area and, consequently, lower transpiration and tree level *Q* (Phillips et al. 2001). Better access to nutrients can also lead to the development of wider tracheids of Norway spruce which could support larger *Q* rates (Ward et al. 2008). The lower-intensity thinning had no significant effect on tree level *Q*, which corresponds to the non-significant effect of similar thinning (50% stand density) on tree level *Q* observed in Gebauer et al. (2011).

The results of *Q* per DBH classes showed that 23 cm and 26 cm DBH classes of high-intensity treatments had significantly higher *Q* than corresponding DBH classes from the control treatment (Figure 4). The smaller 17 cm and 20 cm DBH classes showed no significant difference between high-intensity and control treatments. Moreover, all four DBH classes showed no significant difference in *Q* between low-intensity thinning and control treatment. The high-intensity thinning effect had a stronger impact on the larger trees than the smaller trees. As the taller trees with greater *DBH* occupy higher canopy layers, they might exert higher competitive pressure on smaller trees after thinning. The *Q* results represent the state of the stand two years after thinning. Therefore, the reduction of stand density can have a long-lasting effect on the larger trees via changes in competition.

The linear regression analysis between vapour pressure deficit (*VPD*), global radiation (*GR*), and *Q* revealed that all three treatments reacted similarly to the changing *VPD* and *GR* during the vegetation season (Figure 5). There were no significant chang-

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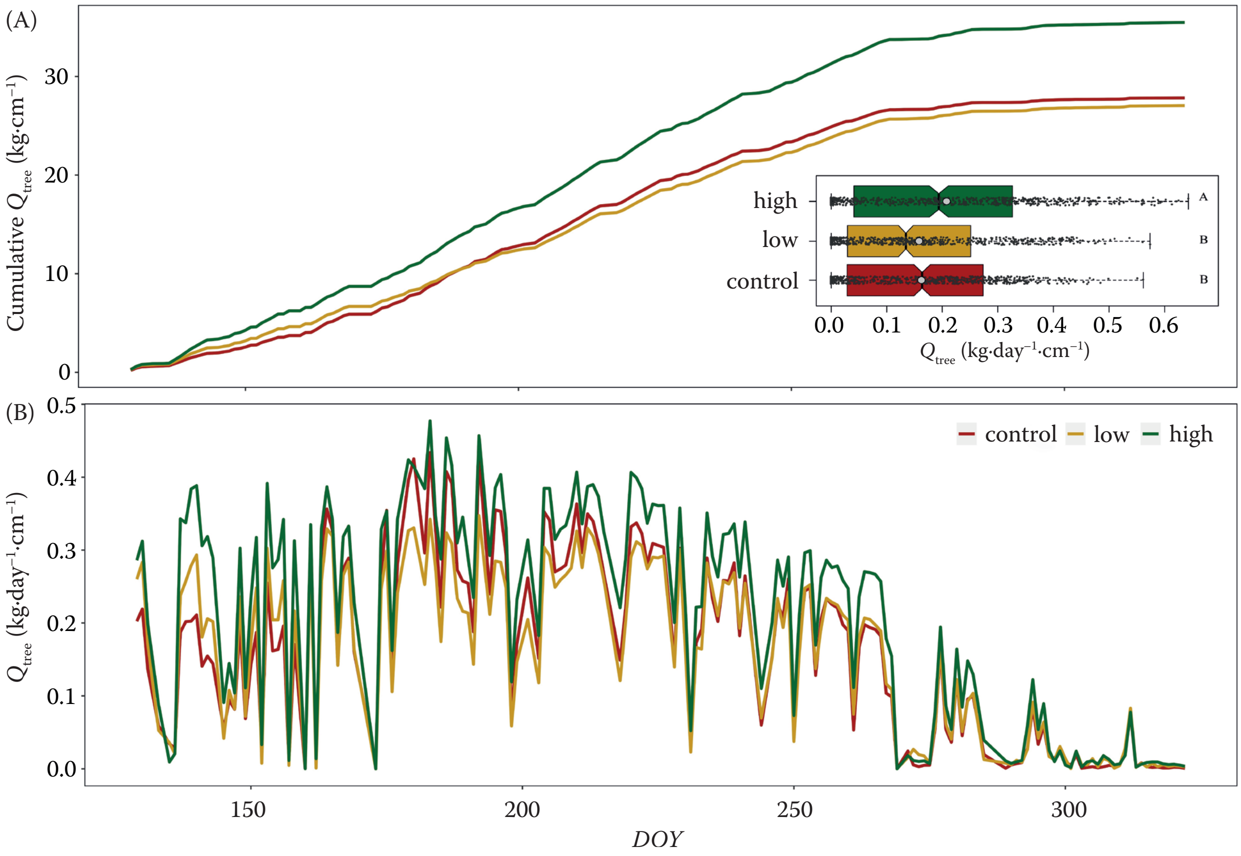

Figure 3. (A) Cumulative standardized sap flow (Q) and (B) daily dynamics of Q for control, low thinning intensity and high thinning intensity; the embedded graph in the upper section shows boxplots of the Q with capital letters showing the results of the post-hoc test; white dot in the boxplot refers to the mean value and the black line to the median

DOY – day of the year

es among slopes or intercepts of *VPD* and *GR* response between the treatments. All three treatments showed a relative reduction of sap flow after the 10 hPa *VPD* threshold captured by logarithmic function (Figure 5A) and responded linearly to increasing *GR* (Figure 5B). The results match the overall charac-

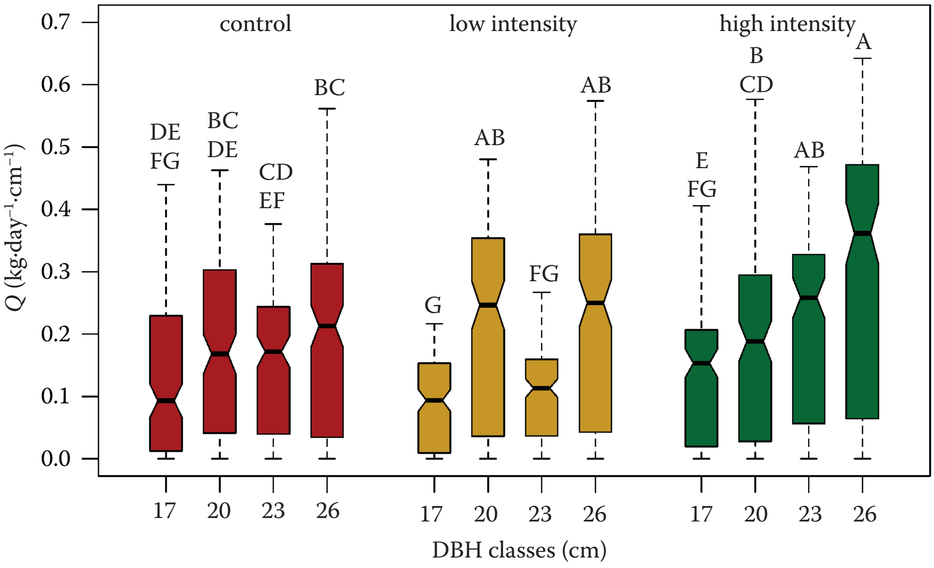

Figure 4. Box plots of standardized sap flow (Q) for respective diameter at breast height classes (*DBH*) and thinning treatments; capital letters represent significantly distinguishable groups based on post-hoc tests

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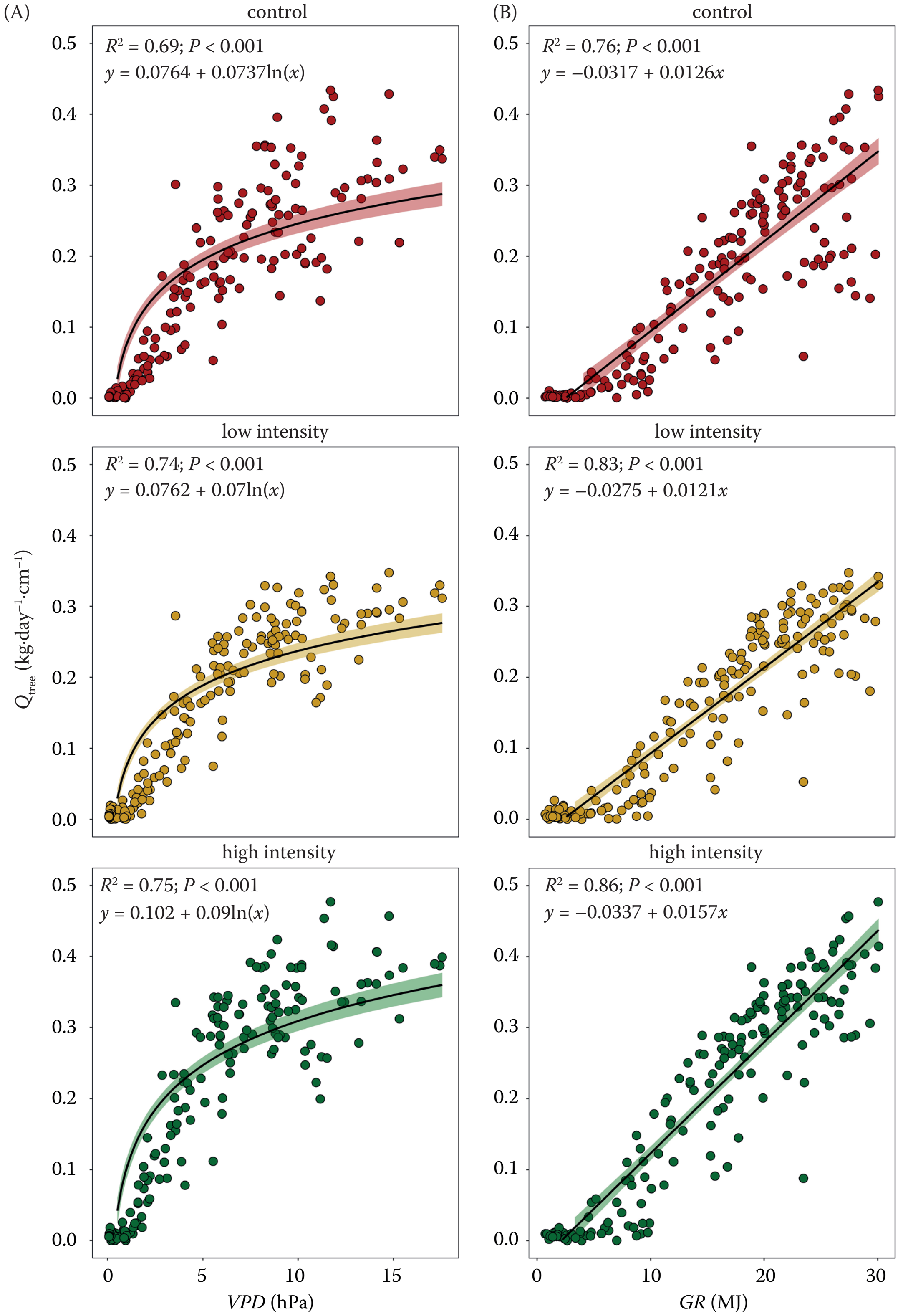

Figure 5. Regression between standardized sap flow and (A) vapour pressure deficit, and (B) global radiation for all thinning treatments (red – control, yellow – low thinning intensity, green – high thinning intensity)

Q – sap flow; *VPD* – vapour pressure deficit; *GR* – global radiation terisation of Norway spruce as an isohydric species (Pashkovskiy et al. 2019; Zavadilová et al. 2023).

Upscaled *Q* data showed that the high-intensity treatment had 34% lower than the control and the low-intensity treatment had 4% lower stand transpiration (Figure 6). The lower stand-level *Q* of the high-intensity treatment can be explained by the smaller leaf area, which was also reflected in lower *LAI* (3.1), compared to low-intensity (4.0) and the control (5.0) (Table 1). Rimal et al. (2022) similarly showed that thinning of Norway spruce led to reduced *LAI* of remaining trees and corresponded to lower transpiration derived from the model. The individual-level increase of *Q* for the high-intensity treatment is balanced at the stand level due to a decrease of *LAI*, reducing total water use of the stand. Tsamir et al. (2019) similarly found that stand transpiration decreased with thinning intensity while tree transpiration increased. The study by Gebhardt et al. (2014) also demonstrated that high-intensity thinning reduced total stand evapotranspiration and led to greater soil water availability even three years after thinning. The proportional decrease of stand transpiration to a reduction of the basal area through thinning indicates that our findings align with Wang et al. (2019).

Thinning in a pine-spruce forest led to a significant reduction of the stand-level canopy transpiration but quickly recovered in the following year (Clausnitzer et al. 2011). Our results contradict this previous observation as results were measured two years after thinning took place, and we still observe significant differences for the high-intensity treatment.

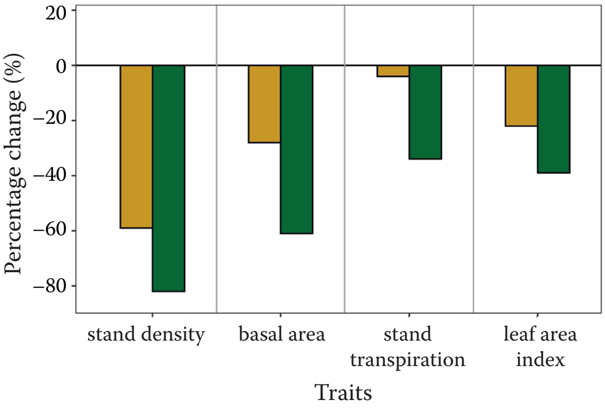

Figure 6. Reduction in thinning treatment of stand density, basal area, annual stand transpiration and Leaf Area Index compared to the control plot (Table 1), values represent means of percentual changes

Effect of thinning on growth and tree water deficit. Both low-intensity and high-intensity treatments showed significantly greater radial growth compared to the control treatment when measured two years after the thinning (Figure 7A). Improved radial growth after thinning is a general fact stemming from lower competition, better light conditions, and possibly greater nutrient access for the remaining trees (Jaakkola et al. 2005; Gspaltl et al. 2013; Bianchi et al. 2022). These positive effects for Norway spruce span both short-term and long-term periods (Sohn et al. 2013). Reduced stand density of Norway spruce forests can also improve the growth resistance during drought periods (Laurent et al. 2003). The post-drought recovery of radial growth can be further improved by a higher-intensity thinning regime (Kohler et al. 2010). As the total tree density decreases in thinned stands, the total wood production will be lowered, but the remaining canopy layer trees provide larger economic value than wood from the understory layer (Cao et al. 2008; Ara et al. 2022). Higher-intensity thinning can reduce the total stand wood production compared to unthinned stands, but the difference decreases over time due to excessive tree mortality in high-density unthinned plots (Mäkinen, Isomäki 2004). It is pertinent to note that the older Norway spruce stands in this region are negatively affected by bark beetle infestation. Therefore, thinning alone can be ineffective if the trees cannot reach maturity (70–130 years); transformation of such forests to mixed/broadleaved forests is needed (Hillayová et al. 2022).

We observed no significant differences in tree water deficit between the treatments (Figure 7B). The high-intensity treatment experienced slightly greater *TWD* at the start of the vegetation season, and the low-intensity treatment experienced a greater *TWD* during the end of the vegetation season. It is possible the spring and autumn phenology were slightly altered, but we likely did not have a sample size large enough to recognise the shift. Norway spruce has a shallow root system; it is likely that all three treatments used the internal stem water storage similarly (Schäfer et al. 2019). The year of the measurements was colder and received more precipitation than the long-term average; therefore, the differences in *TWD* could be more pronounced if the trees experienced drought.

Comparison of sap flow and growth reaction to thinning. The low-intensity thinning showed

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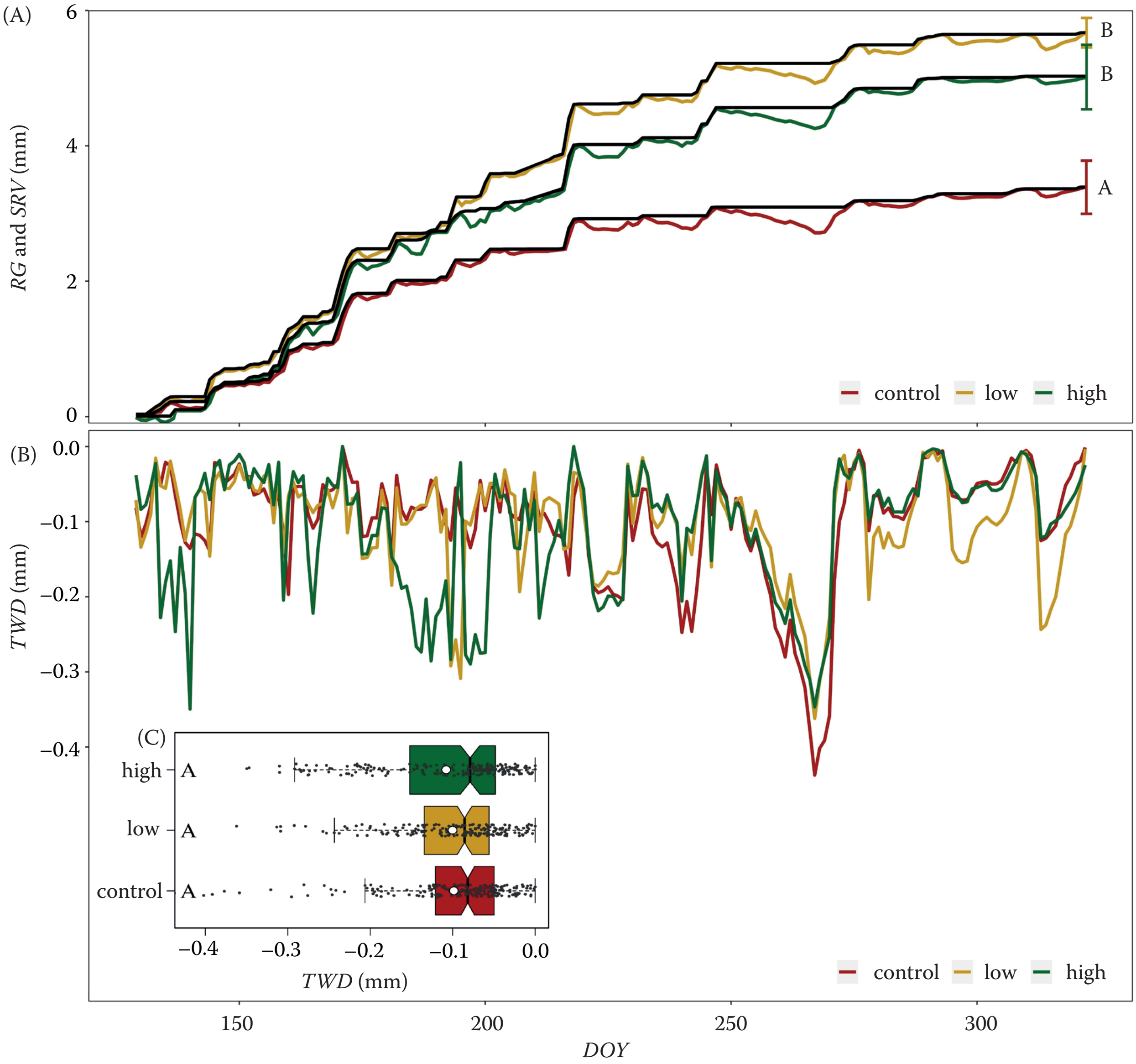

Figure 7. Hourly dynamics of (A) radial growth (*RG*; black lines), stem radial variation (*SRV*), and (B) extracted tree water deficit (*TWD*) and (C) treatment level variability of *TWD* throughout the measurement period; the last value of radial growth includes a 95% confidence interval around it; capital letters represent significantly distinguishable groups based on the Fisher's LSD (parts A, B) and post-hoc test (part C); white dot in the boxplot refers to the mean value and the black line to the median

DOY – day of the year

greater radial growth but no significant change in sap flow compared to the control (Figure 3A, Figure 7A). This could suggest that the low-intensity treatment improved its water use efficiency for radial growth (Gebhardt et al. 2014; Niccoli et al. 2020). For example, Fernandes et al. (2016) found that thinning increased the water use efficiency of *Pinus halapensis*. On the other hand, the radial growth increased proportionally to sap flow in the high-intensity treatment. The treatments could have altered carbon allocation patterns; the high-intensity treatment might invest more carbon into crown expansion (higher light availability) or root growth (lower soil-zone competition), and the low-intensity treatment might retain carbon allocation patterns more similar to the control (Skovsgaard et al. 2006; Campbell et al. 2009). Low-intensity thinning can have an immediate effect on the water utilisation of Norway spruce trees for radial growth, but high-intensity thinning could alter the overall growth efficiency in the long term. Additional research focused on both water use efficiencies (carbon isotopes or eddy-covariance) and carbon allocation patterns of thinning intensity treatments could provide valuable information for adaptive forestry. The higher-intensity treatment might also improve the conditions for the regeneration and growth of understory seedlings (Lin et al. 2012). The best results for conversion of the Norway spruce stands would be achieved by their transformation to mixed forests with a heterogenous stand structure (Reventlow et al. 2021). Mixed heterogeneous forests are much more resilient under drought stress compared to Norway spruce monoculture stands (Pardos et al. 2021). Heavy thinning can improve the conditions in the forest for the establishment of other intermixed species.

CONCLUSION

Both low-intensity and high-intensity thinning had a positive impact on the radial growth of Norway spruce. Moreover, the high-intensity treatment showed greater tree-level sap flow due to the lower competition but reduced stand-level transpiration as a result of the lower leaf area index. The low-intensity treatment could be considered the most water-efficient management strategy at the individual tree level due to higher growth and unchanged transpiration compared to the control. On the other hand, the high-intensity thinning lowers stand-level transpiration, which could improve the overall soil water availability during a drought. Thinning practices should also be supported by actions that transform the pure Norway spruce forests into more resilient mixed forests. Further research, including an investigation into water use efficiency and soil water content, can be beneficial for adaptive forestry under climate change.

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REFERENCES

Ara M., Berglund M., Fahlvik N., Johansson U., Nilsson U. (2022): Pre-commercial thinning increases the profitability of Norway spruce monoculture and supports Norway spruce-birch mixture over full rotations. Forests, 13: 1156.

Bhandari S.K., Veneklaas E.J., McCaw L., Mazanec R., Whitford K., Renton M. (2021): Effect of thinning and fertilizer on growth and allometry of *Eucalyptus marginata*. Forest Ecology and Management, 479: 118594.

Bianchi S., Huuskonen S., Hynynen J., Siipilehto J., Niemistö P. (2022): Tree-level differences in Norway spruce and Scots pine growth after extreme thinning treatments. Scandinavian Journal of Forest Research, 37: 109–118.

Bosela M., Tumajer J., Cienciala E., Dobor L., Kulla L., Marčiš P., Popa I., Sedmák R., Sedmáková D., Sitko R., Šebeň V., Štěpánek P., Büntgen U. (2021): Climate warming induced synchronous growth decline in Norway spruce populations across biogeographical gradients since 2000. Science of The Total Environment, 752: 141794.

Campbell J., Alberti G., Martin J., Law B.E. (2009): Carbon dynamics of a ponderosa pine plantation following a thinning treatment in the northern Sierra Nevada. Forest Ecology and Management, 257: 453–463.

Cao T., Valsta L., Härkönen S., Saranpää P., Mäkelä A. (2008): Effects of thinning and fertilization on wood properties and economic returns for Norway spruce. Forest Ecology and Management, 256: 1280–1289.

Čermák J., Kučera J., Nadezhdina N. (2004): Sap flow measurements with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands. Trees, 18: 529–546.

Clausnitzer F., Köstner B., Schwärzel K., Bernhofer C. (2011): Relationships between canopy transpiration, atmospheric conditions and soil water availability – Analyses of long-term sap-flow measurements in an old Norway spruce forest at the Ore Mountains/Germany. Agricultural and Forest Meteorology, 151: 1023–1034.

Dušek D., Novák J., Kacálek D., Slodičák M. (2021): Norway spruce production and static stability in IUFRO thinning experiments in the Czech Republic. Journal of Forest Science, 67: 185–194.

Ehrenberger W., Rüger S., Fitzke R., Vollenweider P., Günthardt-Goerg M., Kuster T., Zimmerman U., Arend, M. (2012): Concomitant dendrometer and leaf patch pressure probe measurements reveal the effect of microclimate and soil moisture on diurnal stem water and leaf turgor variations in young oak trees. Functional Plant Biology, 39: 297–305.

Eliasson L., Lageson H. (1999): Simulation study of a single-grip harvester in thinning from below and thinning from above. Scandinavian Journal of Forest Research, 14: 589–595.

Fernandes T.J.G., Del Campo A.D., Herrera R., Molina A.J. (2016): Simultaneous assessment, through sap flow and stable isotopes, of water use efficiency (WUE) in thinned pines shows improvement in growth, tree-climate sensitivity and WUE, but not in WUEi. Forest Ecology and Management, 361: 298–308.

Fernandez I., Álvarez-González J.G., Carrasco B., Ruíz-González A.D., Cabaneiro A. (2012): Post-thinning soil organic matter evolution and soil CO_2 effluxes in temperate radiata pine plantations: Impacts of moderate thinning regimes on the forest C cycle. Canadian Journal of Forest Research, 42: 1953–1964.

Forrester D.I., Collopy J.J., Beadle C.L., Baker T.G. (2013): Effect of thinning, pruning and nitrogen fertiliser application on light interception and light-use efficiency in a young *Eucalyptus nitens* plantation. Forest Ecology and Management, 288: 21–30.

Gebauer R., Volařík D., Urban J., Børja I., Nagy N.E., Eldhuset T.D., Krokene P. (2011): Effect of thinning on anatomical adaptations of Norway spruce needles. Tree Physiology, 31: 1103–1113.

Gebhardt T., Häberle K.H., Matyssek R., Schulz C., Ammer C. (2014): The more, the better? Water relations of Norway spruce stands after progressive thinning. Agricultural and Forest Meteorology, 197: 235–243.

Gspaltl M., Bauerle W., Binkley D., Sterba H. (2013): Leaf area and light use efficiency patterns of Norway spruce under different thinning regimes and age classes. Forest Ecology and Management, 288: 49–59.

Hillayová M.K., Holušová K., Báliková K., Holécy J. (2022): Comparison of the approach to determination of the rotation period of forest stands in the Czech Republic and in the Slovak Republic. Journal of Forest Science, 68: 413–422.

Holm S. (1979): A Simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics, 6: 65–70.

Houtmeyers S., Brunner A. (2020): Thinning responses of individual trees in mixed stands of Norway spruce and Scots pine. Scandinavian Journal of Forest Research, 35: 351–366.

Jaakkola T., Mäkinen H., Saranpää P. (2005): Wood density in Norway spruce: Changes with thinning intensity and tree age. Canadian Journal of Forest Research, 35: 1767–1778.

Jiménez M.N., Navarro F.B., Sánchez-Miranda A., Ripoll M.A. (2019): Using stem diameter variations to detect and quantify growth and relationships with climatic variables on a gradient of thinned Aleppo pines. Forest Ecology and Management, 442: 53–62.

Kellomäki S., Strandman H., Kirsikka-Aho S., Kirschbaum M.U., Peltola H. (2023): Effects of thinning intensity and rotation length on albedo- and carbon stock-based radiative forcing in boreal Norway spruce stands. Forestry: An International Journal of Forest Research, 2023: cpac058.

Kenkel N.C. (1988): Pattern of self-thinning in jack pine: Testing the random mortality hypothesis. Ecology, 69: 1017–1024.

Kohler M., Sohn J., Nägele G., Bauhus J. (2010): Can drought tolerance of Norway spruce (*Picea abies* (L.) Karst.) be increased through thinning? European Journal of Forest Research, 129: 1109–1118.

Krajnc L., Farrelly N., Harte A.M. (2019): The effect of thinning on mechanical properties of Douglas fir, Norway spruce, and Sitka spruce. Annals of Forest Science, 76: 3.

Krejza J. (2016): Vliv probírkového zásahu na sekvestraci uhlíku a strukturu porostu [Ph.D. Thesis.] Brno, Mendel University in Brno. (in Czech)

Krejza J., Cienciala E., Světlík J., Bellan M., Noyer E., Horáček P., Štěpánek P., Marek M.V. (2021): Evidence of climate-induced stress of Norway spruce along elevation gradient preceding the current dieback in Central Europe. Trees, 35: 103–119.

Krejza J., Haeni M., Darenova E., Foltýnová L., Fajstavr M., Světlík J., Nezval O., Bednář P., Šigut L., Horáček P., Zweifel R. (2022): Disentangling carbon uptake and allocation in the stems of a spruce forest. Environmental and Experimental Botany, 196: 104787.

Kučera J., Vaníček R., Urban J. (2020): Automated exponential feedback weighting method for subtraction of heat losses from sap flow measured by the trunk heat balance method. Acta Horticulturae. 1300: 81–88.

Lagergren F., Lindroth A. (2004): Variation in sapflow and stem growth in relation to tree size, competition and thinning in a mixed forest of pine and spruce in Sweden. Forest Ecology and Management, 188: 51–63.

Lagergren F., Lankreijer H., Kučera J., Cienciala E., Mölder M., Lindroth A. (2008): Thinning effects on pine-spruce forest transpiration in central Sweden. Forest Ecology and Management, 255: 2312–2323.

Laurent M., Antoine N., Joël G. (2003): Effects of different thinning intensities on drought response in Norway spruce (*Picea abies* (L.) Karst.). Forest Ecology and Management, 183: 47–60.

Lin C.J., Laiho O., Lähde E. (2012): Norway spruce (*Picea abies* L.) regeneration and growth of understory trees under single-tree selection silviculture in Finland. European Journal of Forest Research, 131: 683–691.

Mäkinen H., Isomäki A. (2004): Thinning intensity and growth of Norway spruce stands in Finland. Forestry: An International Journal of Forest Research, 77: 349–364.

McJannet D., Vertessy R. (2001): Effects of thinning on wood production, leaf area index, transpiration and canopy interception of a plantation subject to drought. Tree Physiology, 21: 1001–1008.

Niccoli F., Pelleri F., Manetti M.C., Sansone D., Battipaglia G. (2020): Effects of thinning intensity on productivity and water use efficiency of *Quercus robur* L. Forest Ecology and Management, 473: 118282.

Pardos M., del Río M., Pretzsch H., Jactel H., Bielak K., Bravo F., Brazaitis G., Defossez E., Engel M., Godvod K., Jacobs K., Jansone L., Jansons A., Morin X., Nothdurft A., Oreti L., Ponette Q., Pach M., Riofrío J., Ruíz-Peinado R., Tomao A., Uhl E., Calama R. (2021): The greater resilience of mixed forests to drought mainly depends on their composition: Analysis along a climate gradient across Europe. Forest Ecology and Management, 481: 118687.

Park J., Kim T., Moon M., Cho S., Ryu D., Kim H.S. (2018): Effects of thinning intensities on tree water use, growth, and resultant water use efficiency of 50-year-old *Pinus koraiensis* forest over four years. Forest Ecology and Management, 408: 121–128.

Pashkovskiy P.P., Vankova R., Zlobin I.E., Dobrev P., Ivanov Y.V., Kartashov A.V., Kuzentson V.V. (2019): Comparative analysis of abscisic acid levels and expression of abscisic acid-related genes in Scots pine and Norway spruce seedlings under water deficit. Plant Physiology and Biochemistry, 140: 105–112.

Phillips N., Bergh J., Oren R., Linder S. (2001): Effects of nutrition and soil water availability on water use in a Norway spruce stand. Tree Physiology, 21: 851–860.

Powers M.D., Palik B.J., Bradford J.B., Fraver S., Webster C.R. (2010): Thinning method and intensity influence long-term mortality trends in a red pine forest. Forest Ecology and Management, 260: 1138–1148.

Preisler Y., Tatarinov F., Grünzweig J.M., Yakir D. (2021): Seeking the “point of no return” in the sequence of events leading to mortality of mature trees. Plant, Cell & Environment, 44: 1315-1328.

Pretzsch H., Mette T. (2008): Linking stand-level self-thinning allometry to the tree-level leaf biomass allometry. Trees, 22: 611–622.

Reventlow D.O.J., Nord-Larsen T., Biber P., Hilmers T., Pretzsch H. (2021): Simulating conversion of even-aged Norway spruce into uneven-aged mixed forest: effects of different scenarios on production, economy and heterogeneity. European Journal of Forest Research, 140: 1005–1027.

Rimal S., Djahangard M., Yousefpour R. (2022): Forest management under climate change: A decision analysis of thinning interventions for water services and biomass in a Norway spruce stand in south Germany. Land, 11: 446.

Salomón R.L., Peters R.L., Zweifel R., Sass-Klaassen U.G., Stegehuis A.I., Smiljanic M., Poyatos R., Babst F., Cienciala E., Fonti P., et al. (2022): The 2018 European heatwave led to stem dehydration but not to consistent growth reductions in forests. Nature Communications, 13: 28.

Schäfer C., Rötzer T., Thurm E.A., Biber P., Kallenbach C., Pretzsch H. (2019): Growth and tree water deficit of mixed Norway spruce and European beech at different heights in a tree and under heavy drought. Forests, 10: 577.

Sedmáková D., Sedmák R., Kúdela P., Ďurica P., Saniga M., Jalovíar P., Kucbel S. (2022): Divergent growth responses of healthy and declining spruce trees to climatic stress: A case study from the Western Carpathians. Dendrochronologia, 76: 126023.

Skovsgaard J.P., Stupak I., Vesterdal L. (2006): Distribution of biomass and carbon in even-aged stands of Norway spruce (*Picea abies* (L.) Karst.): A case study on spacing and thinning effects in northern Denmark. Scandinavian Journal of Forest Research, 21: 470–488.

Sohn J.A., Gebhardt T., Ammer C., Bauhus J., Häberle K.H., Matyssek R., Grams T.E. (2013): Mitigation of drought by thinning: Short-term and long-term effects on growth and physiological performance of Norway spruce (*Picea abies*). Forest Ecology and Management, 308: 188–197.

Světlík J. (2016): Struktura dospělého vrchovinného smrkového porostu [Ph.D. Thesis.] Brno, Mendel University in Brno. (in Czech)

Szatniewska J., Zavadilova I., Nezval O., Krejza J., Petrik P., Čater M., Stojanović M. (2022): Species-specific growth and transpiration response to changing environmental conditions in floodplain forest. Forest Ecology and Management, 516: 120248.

Tsamir M., Gottlieb S., Preisler Y., Rotenberg E., Tatarinov F., Yakir D., Tague C., Klein T. (2019): Stand density effects on carbon and water fluxes in a semi-arid forest, from leaf to stand-scale. Forest Ecology and Management, 453: 117573.

Wang Y., Wei X., del Campo A.D., Winkler R., Wu J., Li Q., Liu W. (2019): Juvenile thinning can effectively mitigate the effects of drought on tree growth and water consumption in a young *Pinus contorta* stand in the interior of British Columbia, Canada. Forest Ecology and Management, 454: 117667.

Ward E.J., Oren R., Sigurdsson B.D., Jarvis P.G., Linder S. (2008): Fertilization effects on mean stomatal conductance are mediated through changes in the hydraulic attributes of mature Norway spruce trees. Tree Physiology, 28: 579–596.

Yang J., He Z., Lin P., Du J., Tian Q., Feng J., Liu Y., Guo L., Wang G., Yan J., Zhao W. (2022): Variability in minimal-damage sap flow observations and whole-tree transpiration estimates in a coniferous forest. Water (Switzerland), 14: 2551.

Zavadilová I., Szatniewska J., Petrík P., Mauer O., Pokorný R., Stojanović M. (2023): Sap flow and growth response of Norway spruce under long-term partial rainfall exclusion at low altitude. Frontiers in Plant Science, 14: 317.

Zhang H., Simmonds L.P., Morison J.I.I., Payne D. (1997): Estimation of transpiration by single trees: Comparison of sap flow measurements with a combination equation. Agricultural and Forest Meteorology, 87: 155–169.

Zhang X., Wang Y., Wang Y., Zhang S., Zhao X. (2019): Effects of social position and competition on tree transpiration of a natural mixed forest in Chongqing, China. Trees, 33: 719–732.

Zweifel R. (2016): Radial stem variations–a source of tree physiological information not fully exploited yet. Plant, Cell & Environment, 39: 231–232.

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Estimation of land cover changes and biomass carbon stock in north-eastern hill forests of Bangladesh

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Abstract: Forests are atmospheric CO_2 sinks, but their losses and degradation accelerate the emissions of carbon stored as a sink. Deforestation and forest degradation are widespread in Bangladesh, but their impact on greenhouse gas (GHG) emissions is unknown. We assess land use and land cover (LULC) change and forest loss in this study by classifying different Landsat satellite imagery with a focus on forest cover loss from 1989 to 2020. Tier 1 standards were used to estimate the carbon removal and emissions from a small-scale tropical forest. Over the last three decades, the forest area has decreased by 2.40%, 3.74% and 7.52%, respectively. The primary causes of forest loss are large-scale tea garden and homestead expansion, as well as increase in agricultural activities. Because of a reduction in the forest area, the annual gain of carbon in forest biomass has also decreased. Although overall carbon emission was a net gain for the Maulvibazar hill forest, it has decreased from 331.24 Gg·yr^{-1} in the first decade (1989 to 2000) to 307.7 Gg·yr^{-1} in the most recent decade (2011 to 2020), which is an alarming trend. As a result, this research will contribute to leaders' commitment to "halt and reverse forest loss and land degradation by 2030" at the 26th United Nations Climate Change Conference of the Parties (COP 26) in 2021 to improve carbon sequestration, combat climate change and conserve biodiversity.

Keywords: carbon storage; emission; land transformation; remote sensing; trees

Climate change – the outcome of anthropogenic global warming – is the single biggest environmental crisis the Earth is facing (Mal et al. 2018). Carbon emissions from deforestation are the most significant source of climate change, and they are the dominant driver of global warming (Ahmed et al. 2017). But trees can also act as a carbon sink to combat the climate change. Terrestrial systems, predominantly plants, represent an important carbon store, estimated globally at 638 Gt, of which 44% is present in plant biomass (FAO 2015). Carbon stock varies across forest types. While an average of 303 tons of carbon per ha are retained in tropical forests (Lü et al. 2010), 66 tons of carbon per ha and

44 tons of carbon per ha are retained in temperate and boreal forests, respectively (Thurner et al. 2014). Land cover change has significant effects on carbon emission. Roughly 1.1 $\text{Pg(C)}\cdot\text{yr}^{-1}$ was emitted due to tropical land use change (Pan et al. 2011). The vast majority of forest losses is the result of agricultural and habitation-related land-use changes (Akinyemi 2017) and human interventions (Ahmed et al. 2020). Forest land has deteriorated as a result of development and other anthropogenic activities, resulting in biodiversity loss and increased CO_2 levels in the atmosphere. As a result, determining regional and national carbon stocks is critical for developing policies and programs for reducing CO_2 emissions from the forest (Salunkhe et al. 2018).

Satellite data is widely used to analyze land use land cover (LULC) changes in a given region over time (Alam et al. 2020). In Bangladesh, remote sensing data can be used to assess the state of spatial development, and developing appropriate policy plans may be a cost-effective approach (Rahman et al. 2019). Besides, satellite remote sensing data and GIS techniques can be used to identify decadal trends in forest cover and the source of deforestation (Chen et al. 2013; Vanonckelen et al. 2015; Islam et al. 2021). Avoiding the forest loss and lowering carbon emissions are critical in this era of climate change. To mitigate climate change, various frameworks such as the Reducing Emissions from Deforestation and Degradation (REDD+) and Clean Development Mechanism (CDM) may be more effectively implemented (Sangermano et al. 2012; Potapov et al. 2014).

The countrys forest resources are heavily exploited, but their restoration efforts are limited. Between 2000 and 2014, total tree canopy coverage increased slightly but natural forest acreage decreased dramatically (Potapov et al. 2017). Otherwise, global greenhouse gas emissions will rise due to deforestation in developing countries, particularly in tropical and subtropical regions (Bustamante et al. 2016). Natural hill forests are being destroyed as a result of illegal logging, shifting farming, and other land uses (Hansen et al. 2013; Islam et al. 2017). Although remotely sensed imagery was used to stratify the forest in Bangladesh, the method used to estimate forest carbon release was different. Hoque et al. (2019) used methods developed by Islam et al. (2011) and Turner et al. (1995) to estimate carbon release from the Teknaf and Rajapalong Hill Tracts in Cox's Bazar and Khadimnagar in Sylhet. In this work, changes in the carbon stock in forest biomass were quantified using the gain-loss approach in accordance with IPCC 2006 guideline. greenhouse gas (GHG) estimation is required for national-level carbon inventories. Taking these facts into account, the present study was conducted (*i*) to correctly identify and quantify the magnitude of land use land cover change from 1989 to 2020 in the Maulvibazar region as a proxy for the hill forest of Bangladesh, and (*ii*) to quantify the amount of carbon removal and emission from forest biomass.

MATERIAL AND METHODS

Study area. The study area includes the entire Maulvibazar region composed of seven upazilas: Barlekha, Juri, Kamalganj, Kulaura, Maulvi Bazar Sadar, Rajnagar, and Sreemangal (Figure 1). Maulvibazar District, located at 24.3095°N latitude and 91.7315°E longitude, has a land area of 2 799 km^2. It is bounded by the Indian states of Tripura and Assam in the south and east, and by the Bangladesh districts of Habiganj and Sylhet in the west and north. The temperature ranges from a low of 26.8 °C in February to a high of 36.1 °C in June. The average monthly humidity ranges from 74% in March to 89% in July (Kawsar et al. 2015). This area is divided into three agroecological zones: the Old Eastern Surma Kusiyara Floodplain, the Northern and Eastern Piedmont Plains, and the Northern and Eastern Hills (FAO 1988). This district is home to more than 70% of Bangladesh tea gardens. The most common forests in Maulvibazar are Lawachara National Park, Rajkandi Reserve Forest, Muraichara Eco Park, Madhovkundo Eco Park, and Lathitila Forest. Top tree species in northeastern hill forests of Bangladesh are *Tectona grandis*, *Artocarpus chaplasha*, *Lagerstromia speciosa*, *Chikrassia tabularis*, *Xylia dolabriformis* etc.

Data acquisition. Four Landsat satellite images (1989, 2000, 2011, and 2020) were obtained from Earth Explorer (earthexplorer.usgs.gov) to assess the land cover change of Maulvibazar over a 31-year period. Table 1 summarizes the Landsat data used in the study. The month of January was chosen for image selection because there was no cloud cover. The winter season in the area lasts from November to February. Because vegetation phenology occurs throughout the year, it was intended to collect images from the same month.

Image preprocessing and LULC classification. Layer-stacking multiband images were created us-

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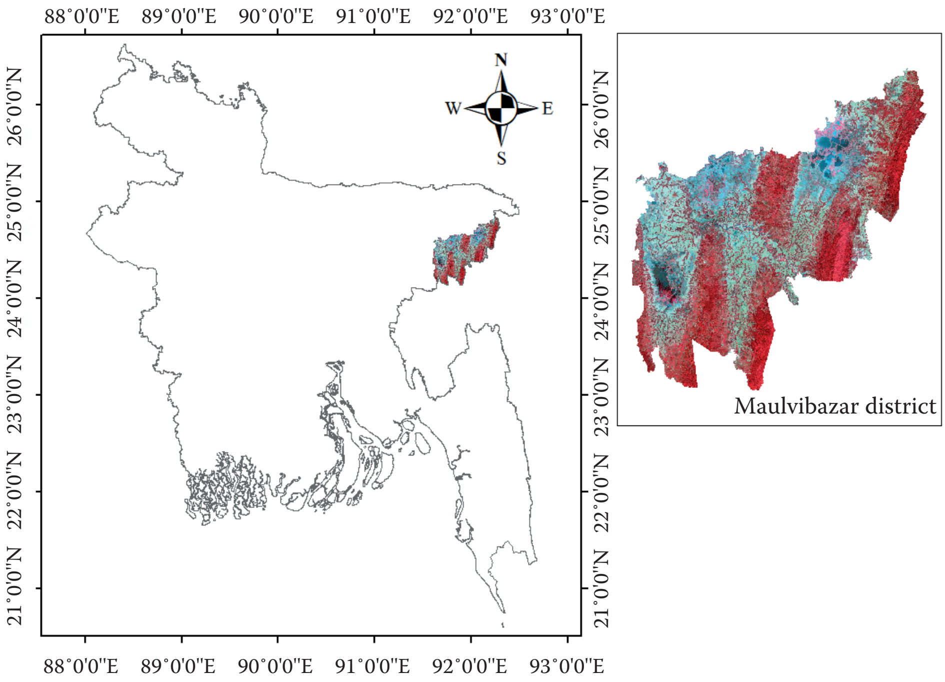


Figure 1. Study area map of Maulvibazar district of Bangladesh

ing QGIS software (QGIS 2021). All of the multiband images were visualized using a false-colour composite. The study area was clipped through using a shapefile. Following that, a training shapefile was created. A number of 65 training points for each (1989, 2000, 2011, and 2020) year and for each class were taken using QGIS to ensure appropriate classification. The R statistical package (Version 4.2.1., 2021) and the "Random Forest" (RF) algorithm were employed to categorize spectral images. For building decision trees, the RF algorithm employs a bootstrap approach (Pavanelli et al. 2018) and a non-parametric machine learning method frequently utilized in satellite image classification (Zhang et al. 2018). The RF classification was carried out in R using the programs "raster", "maptools", "biomod2", "rgdal", "plyr" and "Random Forest". The training data and validation data were split into 0.75 : 0.25. The variables that went into the algorithm were band 1, band 2, band 3, band 4, band 5, band 6, band 7, band 8, band 9, *NDVI* (Normalised Difference Vegetation Index), *NDWI* (Normalised Difference Water Index). The training data and validation data were split into 0.75 : 0.25. The variables (covariates) were surface reflectance band 1, band 2, band 3, band 4, band 5, band 6, band 7, band 8, band 9, *NDVI*, *NDWI*. The area of the LULC map was calculated using ArcGIS. In QGIS-SAGA, cross-tabulation was used to accomplish the change detection analysis. LULC is classified into six groups (built-up, crop/fallow, forest, homestead, tea garden, and water), as shown in Table 2. For an extended period, cropland and

Table 1. Characteristics of satellite images

Sensor	Path/Row	Image acquisition date	Resolution
Landsat 4-5 TM	137/43	Jan 13, 1989	30 m
Landsat 4-5 TM		Jan 12, 2000	
Landsat 4-5 TM		Jan 26, 2011	
Landsat 8 OLI		Jan 19, 2020	

Table 2. Land use land cover classification scheme

Land use/cover types	Description
Built-up	industrial, residential, transportation, road, urban, commercial
Crop/fallow	agriculture area, crop fields, vegetable lands, fallow lands
Homestead	a home consists of a tree, pond/crop field
Forest	mainly Lawachara National Park, Madhabkundo Eco-park, Rajkandi Reserve Forest, Muraichara Eco-park etc.
Tea garden	scattered shade trees with tea
Water	river, permanent water, lakes, ponds

fallow land were put together since so much cropland had been fallow (awaiting harvest or seed sowing), even though that area is still cropland. Figure 2 displays all the methodologies used in the study in a single diagram.

Accuracy assessment. According to the classification accuracy results, for 1989, 2000, 2011 and 2020, the overall classification accuracy was 97%, 93%, 88% and 94%, and overall kappa statistics were 0.95, 0.90, 0.89 and 0.91, respectively (Table 3). These estimates indicate that the classification accuracies were of substantial agreement. This level of agreement is acceptable for the classification of land use and land cover changes.

Estimating emissions/removals of carbon from forest biomass. Tiers 1–3 are three general methodologies for evaluating greenhouse emissions and removals. The number of tiers represents the amount of information required and the level of complexity. This study used Tier 1 of the IPCC 2006 methodology. Furthermore, in many tropical countries, forest inventories are scarce. Using the Gain-Loss Method, we estimate carbon discharges from changes in carbon stocks in a living biomass pool in this study. The area of the forest was extracted from classified satellite images. After the classification of satellite images, the dark green colour represents the forest area (hill forest in the northeastern region was considered instead of individual tree species) (Figure 3). The area of forest land and non-forest land was calculated using the land-use conversion matrix. We collected timber and fuelwood extraction data from the Forest Department and through review literature (GoB 2019; FAO 2020). This calculation uses pre-determined default settings for the Tropical Forest. The carbon in forest biomass was estimated using the formulas shown below [Equations (1–7)]:

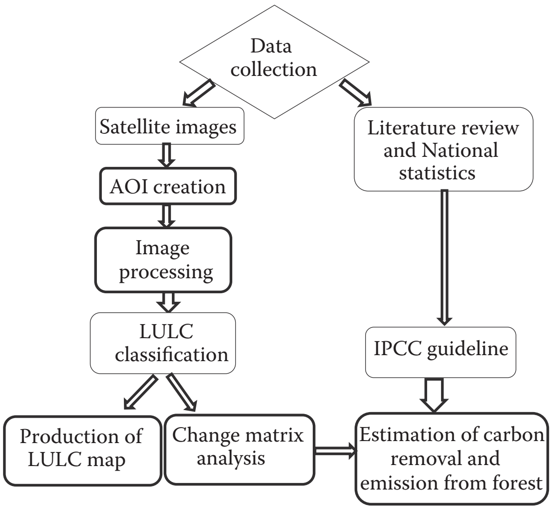


Figure 2. Flow chart of the methodology

Table 3. Accuracy assessment for the classified images

Reference year	Classified image	Overall classification accuracy	Overall kappa statistic
		(%)	
1989	Landsat 4-5 TM	97	0.95
2000	Landsat 4-5 TM	93	0.90
2011	Landsat 4-5 TM	88	0.89
2020	Landsat 8 OLI	94	0.91

$$\Delta C_B = \Delta C_G - \Delta C_L \tag{1}$$

where:

ΔC_B – annual change in carbon stocks in biomass (the sum of aboveground and belowground biomass terms), considering the total area [tonnes(C)·yr^{-1}];

ΔC_G – annual increase in carbon stocks due to biomass growth for each land subcategory, considering the total area [tonnes(C)·yr^{-1}];

ΔC_L – annual decrease in carbon stocks due to biomass loss, considering the total area, [tonnes(C)·yr^{-1}].

$$\Delta C_G = A \times G_{\text{TOTAL}} \times CF \tag{2}$$

where:

A – area of land remaining in the same land-use category (ha);

G_{TOTAL} – mean annual biomass growth [tonnes dry matter (d.m.)·ha^{-1}·yr^{-1}];

CF – carbon fraction of dry matter [tonnes(C)·(tonne d.m.)$^{-1}$]

$$\Delta C_L = L_{\text{wood-removals}} + L_{\text{fuelwood}} + L_{\text{disturbances}} \tag{3}$$

where:

$L_{\text{wood-removals}}$ – annual carbon loss due to wood removals [tonnes(C)·yr^{-1}];

L_{fuelwood} – annual biomass carbon loss due to fuelwood removals [tonnes(C)·yr^{-1}];

$L_{\text{disturbance}}$ – annual biomass carbon losses due to disturbances [tonnes(C)·yr^{-1}].

$$G_{\text{TOTAL}} = [G_W \times (1 + R)] \tag{4}$$

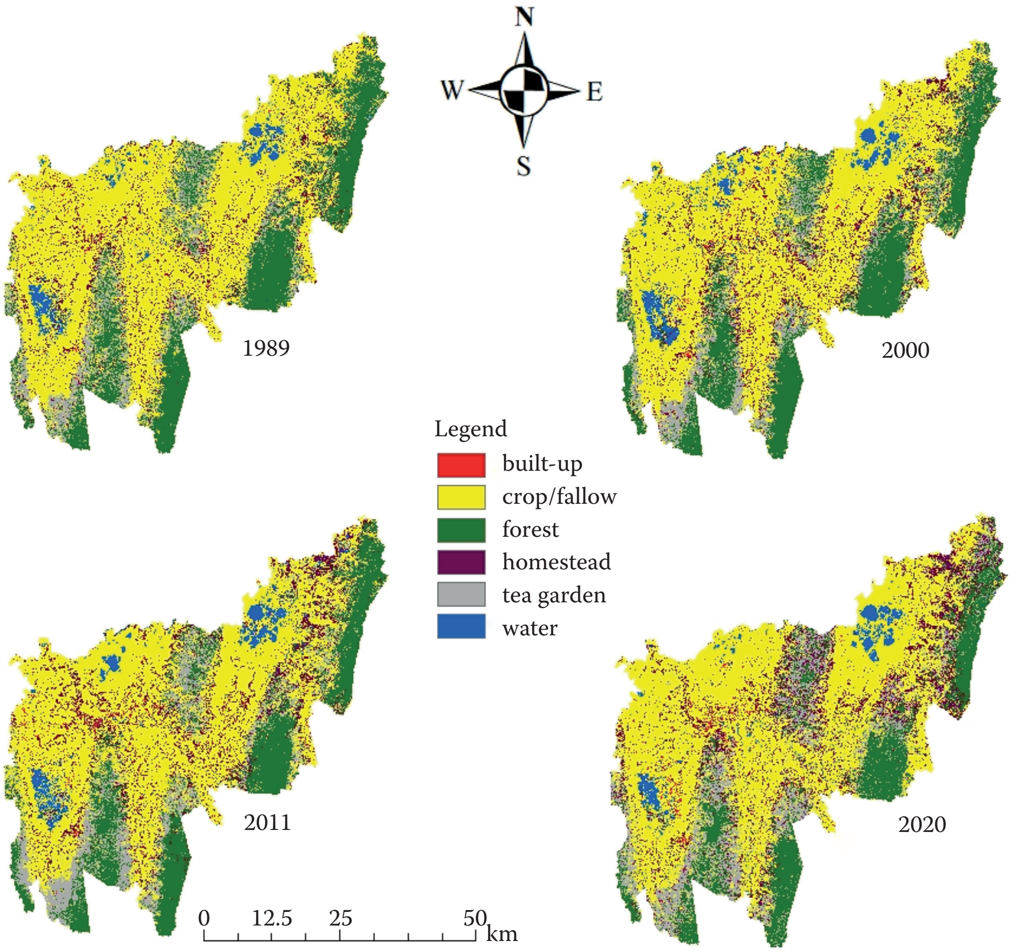


Figure 3. Deacadal changes of LULC during 1989–2020 periods

LULC – land use and land cover

where:

G_W – average annual aboveground biomass growth (tonnes d.m.·ha^{-1}·yr^{-1});

R – ratio of belowground biomass to aboveground biomass, in tonne d.m. belowground biomass (tonnes d.m. aboveground biomass)$^{-1}$.

$$L_{\text{wood-removals}} = H \times BCEF_R \times (1 + R) \times CF \tag{5}$$

where:

H – annual wood removals, roundwood (m^3·yr^{-1});

$BCEF_R$ – biomass conversion and expansion factor for the conversion of removals in merchantable volume to total biomass removals (including bark) [tonnes of biomass removals·(m^3 of removals)$^{-1}$].

$$L_{\text{fuelwood}} = [FG_{\text{trees}} \times BCEF_R \times (1 + R) + FG_{\text{part}} \times \times D] \times CF \tag{6}$$

where:

FG_{trees} – annual volume of fuelwood removal of whole trees (m^3·yr^{-1});

FG_{part} – annual volume of fuelwood removal as tree parts (m^3·yr^{-1});

D – basic wood density (tonnes d.m.·m^{-3}).

$$L_{\text{disturbances}} = A \times B_W \times (1 + R) \times CF \times fd \tag{7}$$

where:

B_W – average aboveground biomass of areas affected;

fd – fraction of biomass lost in disturbance.

RESULTS

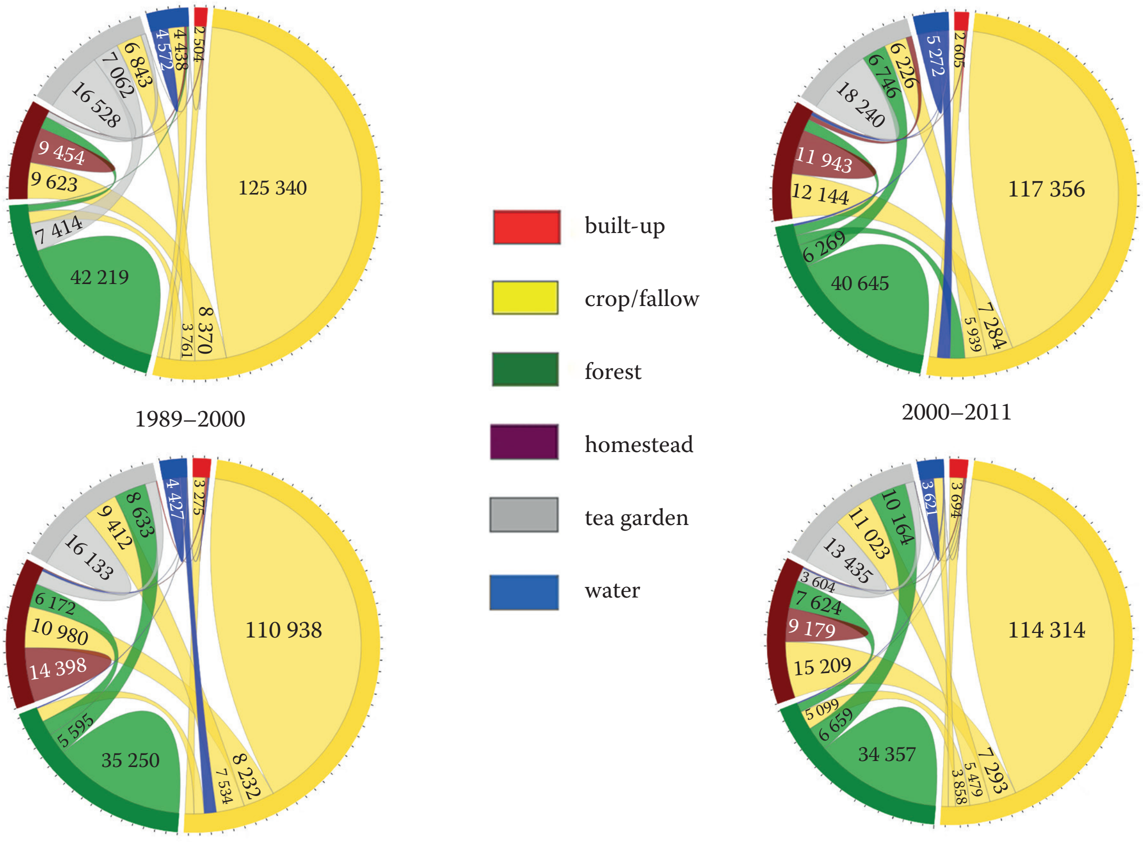
Land use and land cover (LULC) changes

The map of LULC was created to properly identify and adjust different classes in the research area, with a particular emphasis on forest change. Six LULC classes (built-up, crop/fallow, forest, homestead, tea garden, and water) are pointed out in the LULC map (Figure 3) which was classified.

LULC change from 1989 to 2000. In general, all of the LULC categories in Maulvibazar have changed. The transition matrix illustrates the processes and patterns of land-use change (Figure 4). From 1989 to 2000, built-up, homestead, and tea garden increased, while crop/fallow, forest, and water decreased (Table 4). The built-up area increased by 26.80%, with most crop/fallow land converted to built-up. The permanent crop/fallow land area was 125 340 ha, with the majority of crop/fallow land transferred to homestead. Otherwise, the forest area shrank by 2.40%. In the homestead case, most crop/fallow (9 623 ha) area was converted to homestead, with 9 454 ha remaining. The tea garden was also increased by 8.57%, with the conversion of the most significant areas of forest and crop/fallow land. The area of persistent water bodies was 4 527 ha between 1989 to 2000.

LULC change from 2000 to 2011. Land use and land conversion have been ongoing since the early 20th century. From 2000 to 2011, the built-up area grew by 9.30% (Table 4). The persistent crop/fallow area was 117 356 ha, and it decreased at a rate of 2.64%. The forest was reduced by 3.74%, with an annual decrease rate of 185.96 ha·yr^{-1}, and the majority of the forest was transferred to a tea garden. Between 2000 and 2011, homestead and tea gardens increased by 23.72% and 3.79%, respectively. In the homestead case, most of the crop/fallow land was converted to homestead, followed by others. Tea gardens also increased by 3.79%. Water bodies were retained at 5 272 ha between 2000 and 2011 (Figure 4).

LULC change from 2011 to 2020. Between 2011 and 2020, built-up, homestead, and tea garden increased by 32.70%, 21.10%, and 12.96%, respectively, compared to the previous decade (Table 4). The built-up area grew due to converting the maximum crop/fallow area to built-up. The homestead was close to the built-up area, which has seen a significant increase, with the largest intrusion of crop/fallow to the homestead. Tea gardens expanded as 9 412 ha of crop/fallow and 8 633 ha of forest were converted to tea gardens, followed by oth-



1989–2000

2000–2011

2011–2020

1989–2020

Figure 4. Chord diagram showing land conversion due to land use land conver change of the study area in different years (in ha)

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Table 4. Changes in land use and land cover in Maulvibazar

Class	Change (%)			
	1989–2000	2000–2011	2011–2020	1989–2020
Built-up	26.80	9.30	32.70	83.90
Crop/fallow	–5.19	–2.64	–3.84	–11.24
Forest	–2.40	–3.74	–7.52	–13.10
Homestead	10.44	23.72	21.10	65.47
Tea garden	8.57	3.79	12.96	27.29
Water	60.21	–13.48	–26.42	1.99

ers (Figure 4). Crop/fallow, forest, and water all decreased by 3.84%, 7.52%, and 26.42% (Table 4). Instead, the annual water loss was 256.5 ha·yr^{-1}, leaving 4 427 ha of water.

LULC change from 1989 to 2020. Significant changes have occurred over the last 31 years in the research area. The total built-up area has increased by 83.90%. The greatest amount of crop/fallow land is converted to the built-up land. Between 1989 and 2020, crop/fallow and forest declined by 11.24% and 13.10%, respectively (Table 4). The majority of the forest has been converted into a tea garden and homestead. Simultaneously, 15 209 ha of crop/fallow land and 7 624 ha of forest land were transferred to the homestead. Even though water levels fluctuated over time, overall water levels increased by approximately 1.99%. From 1989 to 2020, the figure depicts the temporal trend and relative variations in LULC in the Maulvi Bazar region (Figure 4).

Deacadal LULC changes. The built-up area was increased every year. It expanded from 0.90% in 1989 to 1.66% in 2020. The homestead area was also increased drastically from 8.14% in 1989 to 13.47% in 2020. The increased size of the tea garden was documented at 13.9% in 2020 compared to 10.92% in 1989. Crop/fallow has dropped from 56.7% to 50.33% in the last 20 years. Forest land showed a decreasing trend. In the last three decades, the trend of changing of water has been inconsistent (Figure 5).

Change of forest biomass carbon stock

Nature's carbon-removal machines, forests, are a precious natural resource. The benefits to society, economy, and climate they give cannot be avoided. The annual increase in biomass carbon stocks due to biomass growth from persistent forest land and the afforested area was 6.05 tonnes(C)·ha^{-1}·yr^{-1}. Removal of wood and fuelwood resulted in the total annual carbon loss of 105.69 tonnes(C)·yr^{-1}. Other disturbances (insect/pest, landslide, overgrazing, encroachment, etc.) were not considered. Finally, the net biomass carbon stocks were estimated to be 331 242 tonnes(C)·yr^{-1} between 1989 and 2000 (Table 5).

Between 2000 and 2011, the total amount of persistent forest land and the afforested area was 52 719 ha. Carbon stores in biomass were documented at 318.83 Gg(C)·yr^{-1} after removing wood and fuelwood from the forest (Table 5). Carbon stock in biomass was decreased by around 3.74%

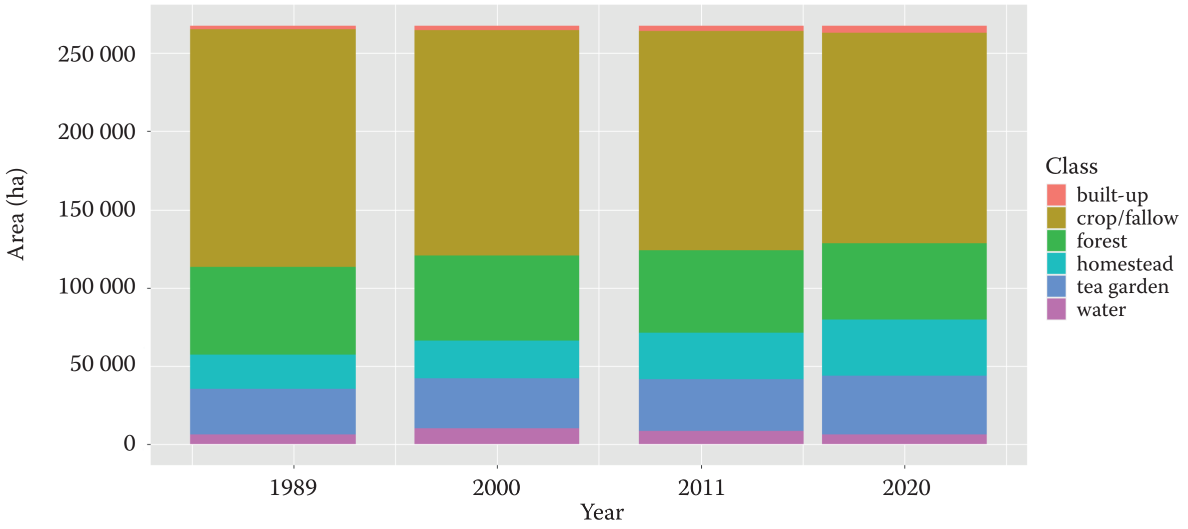

Figure 5. Land use land cover change in Maulvibazar district in 1989 to 2020

Table 5. Net carbon stock change in biomass from forest

Year	Land use category	Area	ΔC_G	$L_{\text{wood−removals}}$	L_{fuelwood}	ΔC_L	ΔC_B
		(ha)	[tonnes(C)·yr^{-1}]				
1989–2000	FL–FL	42 219	202 442	13.70	2.23	15.93	202 426
	non FL–FL	12 546	128 906	74.41	15.35	89.76	128 816
	total [t(C)·yr^{-1}]	54 765	331 348	88.11	17.58	105.69	331 242
	total [Gg(C)·yr^{-1}]	54 765	331.35	0.08	0.01	0.11	331.24
2000–2011	FL–FL	40 645	194 894	17.12	3.12	20.24	194 874
	non FL–FL	12 074	124 061	85.86	11.23	97.09	123 964
	total [t(C)·yr^{-1}]	52 719	318 955	102.98	14.35	117.33	318 838
	total [Gg(C)·yr^{-1}]	52 719	318.96	0.10	0.01	0.12	318.83
2011–2020	FL–FL	35 250	169 023	15.52	2.53	18.05	169 005
	non FL–FL	13 507	138 788	78.23	10.63	88.86	138 699
	total [t(C)·yr^{-1}]	48 757	307 811	93.75	13.16	106.91	307 704
	total [Gg(C)·yr^{-1}]	48 757	307.81	0.09	0.01	0.11	307.70

FL–FL – forest land remaining forest land; non-FL – non-forest land (built-up, crop/fallow, homestead, tea garden, water); ΔC_B – annual change in carbon stocks in biomass; $L_{\text{wood-removals}}$ – annual carbon loss due to biomass removals [tonnes(C)·yr^{-1}]; L_{fuelwood} – annual carbon loss due to fuelwood removals [tonnes(C)·yr^{-1}]; ΔC_G – annual increase in carbon stocks due to biomass growth; ΔC_L – annual decrease in carbon stocks due to biomass loss

along with the decrease of forest land compared to the previous decade.

From 2011 to 2020, the total annual carbon gain was estimated to be 6.3 tonnes(C)·ha^{-1}·yr^{-1} from 48 757 ha of land. In the case of carbon losses, maximum carbon loss from wood removal [93.75 tonnes(C)·yr^{-1}] was followed by fuelwood removal [13.16 tonnes(C)·yr^{-1}]. Finally, the net annual biomass carbon stock was estimated to be 307 704 tonnes(C)·yr^{-1} (Table 5).

DISCUSSION

The land cover change in Bangladesh Maulvibazar landscape was assessed using medium-resolution satellite data from 1989 to 2000, 2000 to 2011, and 2011 to 2020. A loss in forest cover was revealed to be related to significant changes in land use and land cover change. During the last three decades, the forest has shrunk by 13.10% (Table 4). Murshed et al. (2021) also reported greater deforestation in Bangladesh, which was linked to higher population growth rates and agricultural land expansion. This shift in forest cover is also closely tied to human activities and management on a local scale (Ahammad et al. 2019). South Asia also lost 29.62% of its forest cover (Reddy et al. 2018). As the forest cover area shrinks day by day, this scenario will face the immense challenge posed by the Glasgow Leaders' Declaration on Forests and Land Use, which pledges to halt forest loss in less than a decade (UN Climate Change UK 2021). As a result, the persistent forest land should be declared as protected area, as 71% of the world's protected areas have contributed to the prevention of forest loss (Yang et al. 2021).

Looking at the intensity of change at the categorical level of the six transformed land use/land cover categories, homestead, crop/fallow, and tea garden conversion to forest land has contributed significantly to major forest loss in the northeastern region of Bangladesh over the past three decades (Figure 4). Deforestation caused by the permanent land use change is responsible for 27% of global forest loss (Curtis et al. 2018). Cropland expansion in South East Asia, such as conversions of coffee, tea, upland rice, and other commodities, was responsible for 88% of total forest loss (Zeng et al. 2018). Otherwise, large-scale land acquisitions (LSLAs) are a significant contributor to forest loss in the Global South (Davis et al. 2020). Finally, the land-use change, primarily due to conventional agricultural expansion and deforestation, accounts for roughly 17% of global greenhouse-gas emissions (Barker et al. 2007).

The values for changes in biomass carbon stock are positive, indicating that there is a biomass gain in the carbon stock of Maulvibazar hill forest. However, the net gain has decreased from 331.24 Gg(C) in the first decade (1989 to 2000) to 307.7 Gg(C) in the recent decade (2011 to 2020) due to forest area loss (Table 5). Mukul et al. (2014) noticed 179.1 million Mg of carbon in forest biomass in Bangladesh, whereas the tree carbon stock is associated with anthropogenic disturbance and stand characteristics. According to Saimun et al. (2021), the carbon storage capacity of tropical forest ecosystems is gradually declining, which is a major concern to us because forest deterioration affects the structure, composition, and variety of forests and carbon stocks, functioning, and biological processes (Gao et al. 2020). As a result, the government of Bangladesh is taking steps to collect national carbon stock data and develop the REDD+ Readiness Roadmap.

Although annual carbon loss increased from the first decade to the second decade (0.11 Gg to 0.12 Gg) due to increased wood and fuelwood collection from logging activities, it then decreased to 0.11 Gg (Table 5). Gathering of wood and fuelwood has a long-term effect on the deterioration of forests (Ahammad et al. 2019). According to Pearson et al. (2017), land use change, including deforestation, emitted 1.3 ± 0.7 $\mathrm{Gt(C)\cdot yr^{-1}}$, with timber collection accounting for 53%, fuelwood harvesting accounting for 30%, and forest fires accounting for 17%. Tropical deforestation is expected to increase from 0.467 $\mathrm{Pg\cdot yr^{-1}}$ in the 2010s to 0.628 $\mathrm{Pg\cdot yr^{-1}}$ in the 2090s (+35%), making tropical forests a major carbon source in the 21st century (Vieilledent et al. 2022).

The most notable contribution to the global environmental change is LULC change. The Maulvibazar region has grown in size over time. An increase in the population was a primary driver of built-up expansion, which had a negative impact on the ecology, environment, and biodiversity in the surrounding area (Rahman et al. 2019; Chakroborty et al. 2020). Crop/fallow land in the research area was diminishing year after year. Because of agricultural land depletion, biodiversity and ecological services have suffered, and food insecurity has increased (Kafy et al. 2021). Homesteads in the Maulvibazar region grew rapidly, ranking second only to agriculture in terms of national food supply and income while also preserving biodiversity (Mattsson et al. 2018). Many fallow lands were converted to tea cultivation areas, and the government encouraged the development of tea gardens rather than rubber gardens in the Sylhet Division of Bangladesh, which produces 96% of the country's tea. Maulvibazar produces 63% of the tea, while Sylhet and Habiganj districts produce 33% (Islam, Al-Amin 2019).

CONCLUSION

We present a comprehensive assessment of LULC change with a special focus on the forest change, whereas the IPCC's Tier 1 default parameters are used to calculate the annual change of carbon stock in forest biomass. The forest land area was shrinking as most forest land was converted to homesteads, tea gardens, and crop/fallow land. Although tropical forests have a high capacity for carbon storage, forest degradation, such as wood and fuelwood collection, appears to contribute to carbon emissions into the atmosphere. Our findings highlight the immediate risk of carbon stock depletion linked to Bangladesh's rapid deforestation, which will assist the forest department and legislators in making forest protection and conservation decisions by determining the current state of forest carbon. It is also helpful in mapping the country's hill forest areas for long-term development, biodiversity conservation, and environmental protection.

REFERENCES

Ahammad R., Stacey N., Eddy I.M., Tomscha S.A., Sunderland T.C. (2019): Recent trends of forest cover change and ecosystem services in eastern upland region of Bangladesh. Science of the Total Environment, 647: 379–389.

Ahmed N., Cheung W.W., Thompson S., Glaser M. (2017): Solutions to blue carbon emissions: Shrimp cultivation, mangrove deforestation and climate change in coastal Bangladesh. Marine Policy, 82: 68–75.

Ahmed N., Mahbub R.B., Hossain M.M., Sujauddin M. (2020): Modelling spatio-temporal changes of tropical forest cover in the north-eastern region of Bangladesh. Journal of Tropical Forest Science, 32: 42–51.

Akinyemi F.O. (2017): Land change in the central Albertine rift: Insights from analysis and mapping of land use-land cover change in north-western Rwanda. Applied Geography, 87: 127–138.

Alam A., Bhat M.S., Maheen M. (2020): Using Landsat satellite data for assessing the land use and land cover change in Kashmir valley. GeoJournal, 85: 1529–1543.

Barker T., Bashmakov L., Bernstein J.E., Bogner J.E. (2007): Forestry. In: Metz B., Davidson O., Bosch P., Dave R., Meyer L.A. (eds): IPCC Climate Change 2007. Mitigation. Cambridge. Cambridge University Press: 543–578.

Bustamante M.M.C., Roitman I., Aide T.M., Alencar A., Anderson L.O., Aragao L., Asner G.P., Barlow J., Berenguer E., Chambers J., Costa M.H., Fanin T., Ferreira L.G., Ferreira J., Keller M., Magnusson W.E., Morales-Barquero L., Morton D., Ometto J.P.H.B., Palace M., Peres C.A., Silvério D., Trumbore S., Vieira I.C.G. (2016): Toward an integrated monitoring framework to assess the effects of tropical forest degradation and recovery on carbon stocks and biodiversity. Global Change Biology, 22: 92–109.

Chakroborty S., Al Rakib A., Al Kafy A. (2020): Monitoring water quality based on community perception in the Northwest Region of Bangladesh, In: 1st International Student Research Conference – 2020, Dhaka, Dec 2020: 8.

Chen C.F., Son N.T., Chang N.B., Chen C.R., Chang L.Y., Valdez M., Centeno G., Thompson C.A., Aceituno J.L. (2013): Multi-decadal mangrove forest change detection and prediction in Honduras, Central America, with Landsat imagery and a Markov chain model. Remote Sensing, 5: 6408–6426.

Curtis P.G., Slay C.M., Harris N.L., Tyukavina A., Hansen M.C. (2018): Classifying drivers of global forest loss. Science, 361: 1108–1111.

Davis K.F., Koo H.I., Dell'Angelo J., D'Odorico P., Estes L., Kehoe L.J., Kharratzadeh M., Kuemmerle T., Machava D., Pais A.J.R., Ribeiro N., Rulli M.C., Tatlhego M. (2020): Tropical forest loss enhanced by large-scale land acquisitions. Nature Geoscience, 13: 482–488.

FAO (1988): Land Resources Appraisal of Bangladesh for Agricultural Development. Report 2. Agro-Ecological Regions of Bangladesh. Rome, United Nations Development Programme, Food and Agricultural Organization: 20.

FAO (2015): Global Forest Resources Assessment 2015: How Are the World's Forests Changing? Rome, Food and Agriculture Organization: 54.

FAO (2020): Global Forest Resources Assessment 2020 – Country Report: Bangladesh. Rome, Food and Agriculture Organization: 72.

Gao Y., Skutsch M., Paneque-Gálvez J., Ghilardi A. (2020): Remote sensing of forest degradation: A review. Environmental Research Letters, 15: 103001.

GoB (2019): Tree and Forest Resources of Bangladesh: Report on the Bangladesh Forest Inventory. Dhaka, Forest Department, Ministry of Environment, Forest and Climate Change, Government of the People's Republic of Bangladesh: 213.

Hansen M.C., Potapov P.V., Moore R., Hancher M., Turubanova S.A., Tyukavina A., Thau D., Stehman S.V., Goetz S.J., Loveland T.R., Kommareddy A., Egorov A., Chini L., Justice C.O., Townshend J.R.G. (2013): High-resolution global maps of 21st-century forest cover change. Science, 342: 850–853.

Hoque S.N.M., Ahmed M.M.M., Bhuiyan M.I. (2019): Investigating rapid deforestation and carbon dioxide release in Bangladesh using geospatial information from remote sensing data. Ecocycles, 5: 97–105.

Islam M.N., Al-Amin M. (2019): Life behind leaves: Capability, poverty and social vulnerability of tea garden workers in Bangladesh. Labor History, 60: 571–587.

Islam M.J., Wagner T.W., Xie Y., Mahboob M.G. (2011): Tropical deforestation in Bangladesh and global warming. In: Proceedings of the International Conference of Environmental Aspects of Bangladesh (ICEAB 2011): 115–118.

Islam M., Deb G.P., Rahman M. (2017): Forest fragmentation reduced carbon storage in a moist tropical forest in Bangladesh: Implications for policy development. Land Use Policy, 65: 15–25.

Islam M.R., Khan M.N.I., Khan M.Z., Roy B. (2021): A three decade assessment of forest cover changes in Nijhum dwip national park using remote sensing and GIS. Environmental Challenges, 4: 100162.

Kafy A.A., Naim N.H., Khan M.H.H., Islam M.A., Al Rakib A., Al-Faisal A., Sarker M.H.S. (2021): Prediction of urban expansion and identifying its impacts on the degradation of agricultural land: A machine learning-based remote-sensing approach in Rajshahi, Bangladesh. In: Singh R. (ed.): Re-envisioning Remote Sensing Applications. Boca Raton, CRC Press: 85–106.

Kawsar M.H., Pavel M.A.A., Uddin M.B., Rahman S.A., Mamun M.A.A., Hassan S.B., Alam M.S., Tamrakar R., Wadud M.A. (2015): Quantifying recreational value and the functional relationship between travel cost and visiting national park. International Journal of Environmental Planning and Management, 1: 84–89.

Lü X.T., Yin J.X., Jepsen M.R., Tang J.W. (2010): Ecosystem carbon storage and partitioning in a tropical seasonal forest in Southwestern China. Forest Ecology and Management, 260: 1798–1803.

Mal S., Singh R.B., Huggel C., Grover A. (2018): Introducing linkages between climate change, extreme events, and disaster risk reduction. In: Mal S., Singh R.B., Huggel C. (eds): Climate Change, Extreme Events and Disaster Risk Reduction. Cham, Springer: 1–14.

Mattsson E., Ostwald M., Nissanka S.P. (2018): What is good about Sri Lankan homegardens with regards to food security? A synthesis of the current scientific knowledge of a multifunctional land-use system. Agroforestry Systems, 92: 1469–1484.

Mukul S.A., Biswas S.R., Rashid A.Z.M.M., Miah M.D., Kabir M.E., Uddin M.B., Alamgir M., Khan N.A., Sohel I.M.S., Chowdhury M.S.H., Rana M.P., Rahman S.A., Khan M.A.S.A., Hoque M. A.A. (2014): A new estimate of carbon for Bangladesh forest ecosystems with their spatial distribution and REDD+ implications. International Journal of Research on Land-use Sustainability, 1: 33–41.

Murshed M., Ferdaus J., Rashid S., Tanha M.M., Islam M. (2021): The Environmental Kuznets curve hypothesis for deforestation in Bangladesh: An ARDL analysis with multiple structural breaks. Energy, Ecology and Environment, 6: 111–132.

Pan Y., Birdsey R.A., Fang J., Houghton R., Kauppi P.E., Kurz W.A., Phillips O.L., Shvidenko A., Lewis S.L., Canadell J.G., Ciais P., Jackson R.B., Pacala S.W., McGuire A.D., Piao S., Rautiainen A., Sitch S., Hayes D. (2011): A large and persistent carbon sink in the world's forests. Science, 333: 988–993.

Pavanelli J.A.P., Santos J.R.D., Galvão L.S., Xaud M., Xaud H.A.M. (2018): PALSAR-2/ALOS-2 and OLI/LANDSAT-8 data integration for land use and land cover mapping in northern Brazilian Amazon. Boletim de Ciências Geodésicas, 24: 250–269.

Pearson T.R., Brown S., Murray L., Sidman G. (2017): Greenhouse gas emissions from tropical forest degradation: An underestimated source. Carbon Balance and Management, 12: 1–11.

Potapov P.V., Dempewolf J., Talero Y., Hansen M.C., Stehman S.V., Vargas C., Rojas E.J., Castillo D., Mendoza E., Calderón A., Giudice R., Malaga N., Zutta B.R. (2014). National satellite-based humid tropical forest change assessment in Peru in support of REDD+ implementation. Environmental Research Letters, 9: 124012.

Potapov P., Siddiqui B.N., Iqbal Z., Aziz T., Zzaman B., Islam A., Pickens A., Talero Y., Tyukavina A., Turubanova S., Hansen M.C. (2017): Comprehensive monitoring of Bangladesh tree cover inside and outside of forests, 2000–2014. Environmental Research Letters, 12: 104015.

QGIS Development Team (2021). QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: http://qgis.osgeo.org

Rahman M.S., Mohiuddin H., Kafy A.A., Sheel P.K., Di L. (2019): Classification of cities in Bangladesh based on remote sensing derived spatial characteristics. Journal of Urban Management, 8: 206–224.

Reddy C.S., Saranya K.R.L., Pasha S.V., Satish K.V., Jha C.S., Diwakar P.G., Dadhwal V.K., Rao P.V.N., Murthy Y.V.N.K. (2018): Assessment and monitoring of deforestation and forest fragmentation in South Asia since the 1930s. Global and Planetary Change, 161: 132–148.

Saimun M.S.R., Karim M.R., Sultana F., Arfin-Khan M.A. (2021): Multiple drivers of tree and soil carbon stock in the tropical forest ecosystems of Bangladesh. Trees, Forests and People, 5: 100108.

Salunkhe O., Khare P.K., Kumari R., Khan M.L. (2018): A systematic review on the aboveground biomass and carbon stocks of Indian forest ecosystems. Ecological Processes, 7: 1–12.

Sangermano F., Toledano J., Eastman J.R. (2012): Land cover change in the Bolivian Amazon and its implications for REDD+ and endemic biodiversity. Landscape Ecology, 27: 571–584.

Thurner M., Beer C., Santoro M., Carvalhais N., Wutzler T., Schepaschenko D., Shvidenko A., Kompter E., Ahrens B., Levick S.R., Schmullius C. (2014): Carbon stock and density of northern boreal and temperate forests. Global Ecology and Biogeography, 23: 297–310.

Turner D.P., Koerper G.J., Harmon M.E., Lee J.J. (1995): Carbon sequestration by forests of the United States. Current status and projections to the year 2040. Tellus B: Chemical and Physical Meteorology, 47: 232–239.

UN Climate Change UK (2021): Glasgow leaders' declaration on forests and land use. Available at: https://go.nature.com/3GSEMgm

Vanonckelen S., Lhermitte S., Van Rompaey A. (2015): The effect of atmospheric and topographic correction on pixel-based image composites: Improved forest cover detection in mountain environments. International Journal of Applied Earth Observation and Geoinformation, 35: 320–328.

Vieilledent G., Vancutsem C., Achard F. (2022): Forest refuge areas and carbon emissions from tropical deforestation in the 21st century. Available at: https://www.biorxiv.org/content/10.1101/2022.03.22.485306v3

Yang H., Viña A., Winkler J.A., Chung M.G., Huang Q., Dou Y., McShea W.J., Songer M., Zhang J., Liu J. (2021): A global assessment of the impact of individual protected areas on preventing forest loss. Science of the Total Environment, 777: 145995.

Zeng Z., Estes L., Ziegler A.D., Chen A., Searchinger T., Hua F., Guan K., Jintrawet A., Wood, E.F. (2018): Highland cropland expansion and forest loss in Southeast Asia in the twenty-first century. Nature Geoscience, 11: 556–562.

Zhang C., Sargent I., Pan X., Gardiner A., Hare J., Atkinson P.M. (2018): VPRS-based regional decision fusion of CNN and MRF classifications for very fine resolution remotely sensed images. IEEE Transactions on Geoscience and Remote Sensing, 56: 4507–4521.

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