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Physiological and biochemical adjustments of the assimilatory system of advance regeneration of Norway spruce to cutting-induced changes in the environment

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Abstract Various forestry practices (shelterwood systems, selective felling) promoted for the natural regeneration of Norway spruce can speed up the emergence of the advanced growth. Overstory removal can cause drastic environmental changes, resulting in the death of newly regenerated trees or blocking their growth. We studied changes in the content of photosynthetic pigments, parameters of chlorophyll a fluorescence, activity of antioxidant system enzymes in the needles of 20-year-old advance regeneration of Norway spruce and alteration of xylem radial growth rate in response to abrupt cutting-induced changes in the environment. Cutting led to inhibition of photochemical activity (the maximum photochemical efficiency of PSII (*Fv/Fm*), quantum yield of photosystem II (*Y(II)*) and relative electron transport rate (*ETR*) decreased), rearrangement of the pigment system (the content of chlorophyll *a* (*Chl a*) and chlorophyll *b* (*Chl b*) decreased, chlorophyll *a/b* (*Chl a/b*) ratio increased) and a sharp rise in non-photochemical quenching (*NPQ*). A year later, after clear-cutting, the needles had adapted to the new conditions. Low *NPQ* level was associated with increased *Chl a* content and *Chl a/b* ratio and upregulation of antioxidant enzymes. The activity of photochemical processes increased (*Fv/Fm*, *Y(II),* and *ETR* and the radial xylem increment was promoted significantly. When using forestry practices involving overstory removal, it is necessary to consider that light intensity increase, associated reduction of air and soil humidity, as well as an increase in the air temperature, can have a negative effect on advance regeneration of Norway spruce growth after cutting down, effect that is reversed after one year.

Keywords: reforestation, forest cutting, chlorophyll a fluorescence, photosynthetic pigments, antioxidant enzymes.

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Introduction

Currently, forest management in commercial forestry in Nordic countries is based mainly on clear-cutting and subsequent reforestation by planting or natural regeneration (Nilsson et al. 2010, Kuuluvainen et al. 2012). Alternative forestry practices can help maintain ecosystem services and community stability (Felton et al. 2010, Puettmann et al. 2015) better than clear-cutting.

Norway spruce (*Picea abies* (L.) H. Karst.), along with Scots pine (*Pinus sylvestris* L.), are the prevailing species in commercial forestry in Nordic countries (Szymański 2007, Caudullo et al. 2016). Clear-cuttings with advanced growth retention, shelterwood systems, and selective felling can promote natural regeneration of Norway spruce (Nilsson et al. 2002, Szymański 2007).

However, overstory removal (release cutting) induces abrupt changes in abiotic environmental factors: illumination and the amplitude of air temperature fluctuations increase, soil moisture and air humidity decrease (Palviainen et al. 2005). Limited information exists regarding spruce regeneration after environmental changes (Luguza et al. 2020). Studies have chiefly handled advance regeneration emergence and density (Örlander & Karlsson 2000, Nilsson et al. 2002, Luguza et al. 2020). Adaptability and survival in the first growing seasons determine the survival rate of advance regeneration. Norway spruce survival rate is linked to size and shading intensity (Örlander & Karlsson 2000, Metslaid et al. 2005).

Increased light intensity triggers a response in needles acclimated to shade. When exposed to high light intensity, unadapted needles experience an excessive influx of photons into the PSII reaction centre, potentially causing photoinhibition and increased production of reactive oxygen species (*ROS*) (Takahashi & Murata 2008, Pospíšil 2017). Photoinhibition reduces plant productivity, radial and height growth and biomass accumulation (Yin et al. 2008, Goh et al. 2012, Lapenis et al. 2022). Elevation of antioxidant enzyme activity is a universal plant response to intensive ROS generation caused by alteration of ambient conditions (Mishra et al. 1995). Also, antioxidant system enzymes are very sensitive to growth and development processes (Devireddy et al. 2021). Mechanisms known to prevent photoinhibition include pigment rearrangement and increased non-photochemical quenching (*NPQ*) (Müller et al. 2001, Raven 2011). However, an *NPQ* increase may decrease in photosynthesis and growth rates (Murchie & Ruban 2020). The effects of photoinhibition and photochemical reactions can be evaluated using chlorophyll a fluorescence (Nar et al. 2009, Huang et al. 2010). Decreasing maximum photochemical efficiency of PSII (*Fv/Fm*) indicates, in particular, the phenomenon of photoinhibition (Maxwell & Johnson 2000). Quantum yield of photosystem II (*Y(II))* and relative electron transport rate (*ETR*) related to photosynthetic activity and hence the rate of growth (Maxwell & Johnson 2000). Photodamage and the acclimation rate will determine the growth rate and survival of the seedlings in the new conditions.

Little is known about the mechanisms of acclimation following an environmental change. Some researchers have investigated the effect of release on needle properties and growth of Norway spruce advance regeneration (Metslaid et al. 2005a,b, Hökkä & Repola 2012, Niemistö & Valkonen 2021, Lehtonen et al. 2023). Nikolova et al. (2021) have demonstrated that the change in biomass allocation resulted from strip cutting. Others used physiological indicators such as photosynthetic capacity and maximum photochemical efficiency of PSII (*Fv/Fm*) to investigate physiological stress reactions after release (Gnojek 1992, Metslaid et al. 2007). Research indicates that thinning and clear-cutting often lead to suppressed advance regeneration and increased risk of damage.

(Niemistö et al. 2021). It takes up to several years for a full recovery (Gnojek 1992, Metslaid et al. 2005a, b), which is also due to acclimation of the needles (Metslaid et al. 2005, Lehtonen et al. 2023).

By studying the physiological and biochemical changes caused by environmental changes, we can predict how advanced regeneration will react after cutting and choose the best forestry practices for each region based on species characteristics and local abiotic factors. This paper assesses the capacity of Norway spruce trees from advance regeneration to respond to an abrupt change in environmental factors after overstory removal.

We asked (i) how does the content of photosynthetic pigments, the parameters of chlorophyll *a* fluorescence , and the activity of antioxidant enzymes in the needles of 20-year-old advance regeneration of Norway spruce change in response to abrupt cutting-induced changes in the environment? (ii) How are these changes affected by trunk xylem increment as a resultant productivity index? We hypothesized that (i) an abrupt change in external factors, firstly, illumination, caused by cutting would lead to an increase in the activity of antioxidant enzymes and *NPQ*, a rearrangement of the pigment system aimed at protecting of the photosynthetic apparatus; would lead to a decrease in the activity of photochemical processes, and, as a consequence, to a decrease in growth; (ii) due to the rearrangement of the pigment complex and the regulation of activity of antioxidant enzymes acclimation to new growing conditions is possible in the long term.

Materials and Methods

Study area and treatment

The study was conducted in the middle taiga subzone of Karelia (61°50’40” N, 33°53’30” E). The area is situated in the Atlantic-Arctic temperate zone, with an average annual air temperature of about 3°C and average precipitation of about 600 mm annually (Nazarova 2015). The site was a bilberry-type spruce forest (spruce-pine stand) on podzolic soil with mesic moisture conditions (pH = 4.5). The tree stand comprised 30% Norway spruce, 20% Scots pine, 30% birch (*Betula* sp.) and 20% aspen (*Populus tremula* L.).

In 2000, skid roads were cut through the site with some thinning in the remaining strips. Skid roads were spaced 25 m apart, their width was 4-5 m. In November 2018, the remaining strips were clear-cut with the retention of the advance regeneration that has emerged on the skid roads. A 0.35 ha (70×50 m) seed-tree block was kept in the cut-over site as the seed source (Figure 1).

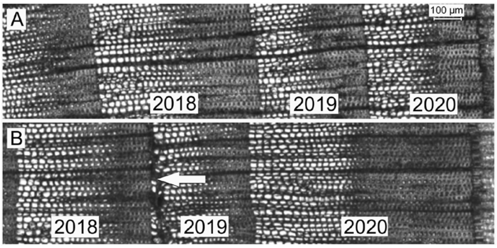

Figure 1 Experimental site design. (A): after skid road cutting in 2000; (B): after clear-cutting in 2018.

The tree stands in the seed-tree block was composed of two generations of Norway spruce (80-100 and 50-60 years old), Scots pine (80-100 years old), aspen and birch (both about 50-60 years old) and comprised 50% spruce, 31% pine, 12% birch, and 7% aspen, totalling 289 stems. The mean height of spruce was 22.9 m and 6.7 m for the first and second generation respectively (the height range was 15.6–28.0 m and 4.2–13.6 m respectively), pine – 24 m (21–27 m), birch – 21.5 m (16–28 m), aspen – 23 m (21–24 m). The average diameter at breast height (DBH) of spruce was 25.0 cm and 9.1 cm for the first and second generation respectively (the diameter range was 16–40 cm and 4–14 cm respectively), pine - 29.7 cm (16–44 cm), birch - 24.5 cm (14–46), and aspen - 31.0 cm (22–40). The growing stock of stand was 288 m^3ha^{-1}, the canopy closure varied within 52.7–70.4%, averaging 64.7%.

The focus of the study was the growth

of 20-22-year-old Norway spruce advance regeneration in skid roads. We focused on two sample plots: (i) cut-over area (advance regeneration that emerged on skid roads and kept in clear-cut area, experimental saplings); (ii) seed-tree block (advance regeneration in skid roads within the seed-tree block, control saplings).

Ten advance-regeneration seedlings were randomly selected in each plot (cut-over area, seed-tree block). The average height of the saplings was 1.28±0.04 m in the seed-tree block and 1.17±0.04 m on cut-over area, the stem base diameter was 2.40±0.13 cm and 2.55±0.11 cm in the seed-tree block and on cut-over area, respectively.

We explored the following variants of acclimation of needles to change in the environment: (i) short-term acclimation (Experiment 1); (ii) acclimation over the year (Experiment 2).

Experiment 1: To assess short-term acclimation needles were collected from saplings grown on a skid road in shade and abruptly exposed to high light after clear-cutting. As a control, we selected needles from saplings grown on a skid road in the shade and found in seed-tree block (in which the adult trees were maintained). One-year-old needles were collected in late May 2019. Thus, we compared two groups of needles: (i) formed in all sampled saplings on a skid road in the shade (shade-grown needles) and exposed to high light after clear-cutting (further in the text and in the figures: "cut-over area 2019") with (ii) shade-grown needles from the control ("seed-tree block 2019").

Experiment 2: To assess long-term acclimation over the year one-year-old needles were collected in early June 2020 (after one full growing season has passed since clearcutting) from the same saplings as in 2019. Thus, we compared two groups of needles: (i) needles formed in high light in cut-over area in 2019 (light-grown needles) ("cut-over area 2020") with (ii) shade-grown needles from the control ("seed-tree block 2020").

Chlorophyll *a* fluorescence parameters

Chlorophyll *a* fluorescence parameters were measured in the field with a pulse amplitude modulated fluorimeter (JUNIOR-PAM, Walz, Germany) after kept needles in darkness for 30-min. The measurements were carried out at 10-13 a.m. local time (UTC+3). The following parameters were determined: initial - (F_0), maximal - (F_m), and variable fluorescence (F_v) (saturating pulse PPFD was 10 000 μmol×m^{-2}×s^{-1}), maximum photochemical efficiency of PSII (*Fv/Fm*). Rapid light curves (RLC) were recorded using 8 levels of photosynthetically active radiation (PAR): 66, 90, 125, 190, 285, 420, 625, and 820 μmol×m^{-2}×s^{-1}. The following parameters were measured for the different PAR levels: relative electron transport rate (*ETR*), quantum yield of photosystem II (*Y(II)*), non-photochemical quenching (*NPQ*). The measurements were performed on the needles of 5 seedlings of each experimental group. Needles were collected from all sides of the middle part of the crown. Measurements were taken in three replications from each seedling.

Pigment content

For the analysis, about 50 g of needles from 10 seedlings of each experimental group were collected from all sides of the middle part of the crown. The needles were put into black plastic bags with ice and then taken to the laboratory. Analysis was performed the next day. Needles were stored in a freezer prior to analysis (the temperature was -70°C). Photosynthetic pigments were extracted from fresh needles (5g) by 80% acetone. The extract was analyzed in the 410-700 nm wavelength range (spectrophotometer SF 2000, Russia). The contents of chlorophyll a (*Chl a*), chlorophyll *b* (*Chl b*), and carotenoids (car) were calculated from formulas suggested by H.K. Lichtenthaler (1987). The share of chlorophylls within light-harvesting complexes (*LHC*) in total chlorophyll was determined, keeping in mind that all chlorophyll *b* is within

LHC and the *a/b* ratio in *LHC* is 1.2. That this ratio, compared with total *a/b* ratio is used for calculation of the share of chlorophyll in *LHC* (Lichtenthaler 1987). The analysis was done in three analytical replications for each seedling.

Activity of antioxidant enzymes

For the analysis, needles from 10 seedlings of each experimental group were collected from all sides of the middle part of the crown. The needles were put into black plastic bags with ice and then taken to the laboratory. Needles for the determination of antioxidant enzyme activity were frozen in liquid nitrogen and stored until analysis in a low-temperature chamber at -70°C. Then 100 mg of needles were taken for the analysis.

Needles were ground in liquid nitrogen and homogenized at 4°C in a buffer comprising 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM EGTA, 3 mM DTT, 5 mM $MgCl_2$, 0.5 mM PMSF. After 20-min extraction, the homogenate was centrifuged at 12000 g for 20 minutes (MPW-351R centrifuge, Poland). The supernatant was desalted by chromatography through a Sephadex G-25 (medium) column at 4°C. The supernatant was collected and checked for total soluble protein by the Bradford method.

Inhibition of photoreduction of nitro blue tetrazolium was used to measure SOD activity. The incubation medium for determining the activity of *SOD* contained 50 mm K, Na-phosphate buffer (pH 7.8), 172 µm NBT, 210 µm methionine, 24 µm riboflavin, 0.1% Triton X-100. *SOD* activity was determined by measuring optical density reduction at 560 nm after 30 minutes of incubation in fluorescent light. *SOD* activity was expressed as units per mg protein per 30 minutes (unit/mg protein) (Nikerova et al. 2019).

The indicator used for catalase (*CAT*) activity was hydrogen peroxide decomposition by the enzyme. The incubation time was 4 minutes. The incubation medium contained 50 mm K, Na-phosphate buffer (pH 7.8), and 14.7 mm hydrogen peroxide. *CAT* activity was determined by measuring optical density reduction at 240 nm, H_2O_2 content was calculated from the calibration line produced in advance (Nikerova et al. 2018). *CAT* activity was expressed as µmol reduced hydrogen peroxide per 1 mg protein (µmol H_2O_2/mg protein).

The activity of peroxidase (*POD*) was estimated with guaiacol as hydrogen donor, and with hydrogen peroxide as substrate. The incubation medium for determining the activity of POD contained 50 mm K, Na-phosphate buffer (pH 5), 2.6 mm hydrogen peroxide, and 21.5 mm guaiacol. The incubation time was 30 minutes. *POD* activity was determined from the rate at which the product of the reaction – tetraguaiacol (TG), formed. The amount of TG produced was determined by measuring the increase in optical density at 470 nm and calculating TG amount, considering the extinction coefficient (ε470 nm = 0.0266 μM^{-1} cm^{-1}). *POD* activity was expressed as µmol of TG produced per 1 mg protein (µmol TG/mg protein) (Nikerova et al. 2018).

The substrate for polyphenol oxidase (*PPO*) activity determinations was pyrocatechol. The incubation medium for determining *PPO* activity contained 50 mm K, Na-phosphate buffer (pH 5), and 16.4 mm pyrocatechol. We measured the increase in optical density at 420 nm wavelength, which is absorbed by pyrocatechol oxidation products. The reaction was observed for 20 minutes. *PPO* activity was expressed as units per mg protein per minute (unit/mg protein) (Nikerova et al. 2019).

The analysis was done in two analytical replications for each seedling.

Study of trunk tissue anatomy

To determine radial increments of the xylem, advance-regeneration saplings were sawn down in the fall of 2020. Samples were cut out of the mid-trunk area on the north-facing side. Xylem samples for microscopy were fixed in glutaraldehyde. Slices 15 µm thick were made with freezing microtome Frigomobil (R.Jung, Germany). The slices were stained with 1% safranin solution in water.

Micrographs were made through light microscope AxioImagerA1 (CarlZeiss, Germany) withADFPRO03camera (ADF, China), and processed with ImageJ software (NIH, USA).

Xylem increments were measured for the past five years (2016-2020). The 2016-2018 data were averaged to eliminate the effect of weather variation among the years.

Statistical analyses

The data followed a normal distribution (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test). The content of photosynthetic pigments and their ratio, *Fv*/*Fm*, the changes in the activity of antioxidant enzymes in the needles as well as xylem increment of the advance regeneration growing on cut-over area and in the seed-tree block (control) were compared using the Student's test. Data were statistically processed with STATISTICA software (StatSoftInc.). For all analysis differences were considered statistically significant at $p<0.05$.

Results

Photosynthetic pigment content in needles

Overstory removal altered the content of photosynthetic pigments. Shade-grown needles in saplings abruptly exposed to high light demonstrated a reduction in the content of *Chl a* (by 21.0%) and *b* (by 37.3%) simultaneously with an increase in *Chl a/b* ratio (by 19.8%) and *LHC* size reduction (by 14.2%), as well as an elevation of *car* content (by 16.2%) compared to shade-grown needles of the control saplings still growing in shade (Figure 2A).

A year after clear-cutting, the content of *Chl a* and *car* in light-grown needles of saplings developing on cut-over area increase by 32.4% and 37.1% respectively compared to shade-grown needles of the control saplings still growing in shade. An increase in *Chl a/b* ratio (by 18.5%) and *LHC* size reduction (by 13.2%) was also noted (Figure 2B).

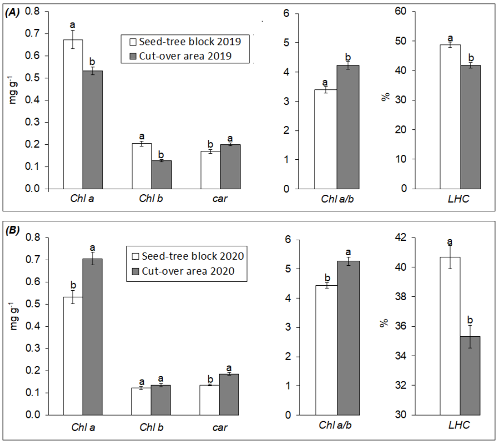

Figure 2 Changes in the content of photosynthetic pigments (chlorophyll *a* and *b* (*Chl a* and *b*) and carotenoids (*car*), their ratio and the share of chlorophylls within light-harvesting complexes (*LHC*) in needles of advance-regeneration saplings after overstory removal (A) in the first growing season (experiment 1) and (B) one year later (experiment 2). Different letters denote the reliability of differences between saplings on the cut-over area and in seed-tree block ($p<0.05$).

Chlorophyll *a* fluorescence parameters

The abrupt change in ambient conditions after overstory removal resulted in a 13.4% reduction in Fv/Fm (Figure 3A) for saplings exposed to high light as a result of cutting, compared to control saplings growing in shade.

Also, we noted a decrease in *Y(II)*, *ETR*, and an increase in *NPQ*. *NPQ* immediately

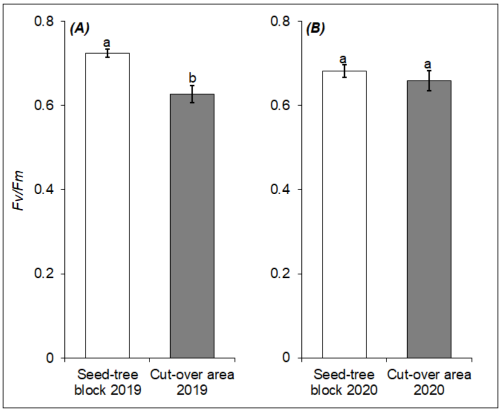

Figure 3 Changes in the maximum photochemical efficiency of photosystem II (*Fv/Fm*) in advance regeneration after overstory removal (A) in the first growing season (experiment 1) and (B) one year later (experiment 2). Different letters denote the reliability of differences between saplings on the cut-over area and in seed-tree block ($p<0.05$).

after unshading grew sharply even where PAR increased only slightly (Figure 4).

A year later, *Fv/Fm* in light-grown needles of saplings developing on the cut-over area was the same as in the control saplings growing in the seed-tree block (Figure 3B). *Y(II)* and *ETR* in needles formed on the cut-over area increased, while *NPQ* decreased (Figure 4).

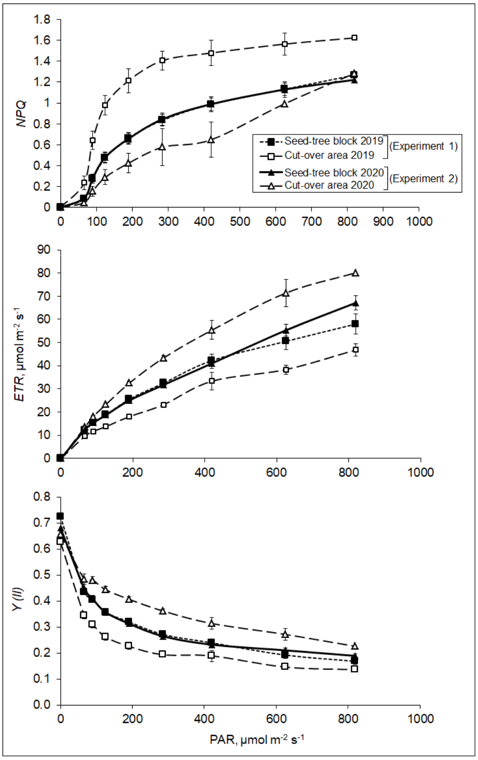

Figure 4 Rapid light curves (*RLCs*) for advance regeneration after overstory removal in the first growing season (experiment 1) and one year later (experiment 2). *NPQ* – non-photochemical quenching, *ETR* – relative electron transport rate, *Y(II)* – quantum yield of PSII.

Activity of antioxidant enzymes in needles

As compared to shade-grown needles in the control group, shade-grown needles that had formed under the canopy and were exposed to high light after cutting had PPO activity reduced by 27.5%, whereas the activity of SOD, CAT, and POD did not change (Figure 5A).

A year later, in light-grown needles on cut-over area, *SOD*, *CAT* and *PPO* activity decreased by 31.4%, 54.6% and 18.4% respectively compared to control saplings growing in shade (Figure 5B).

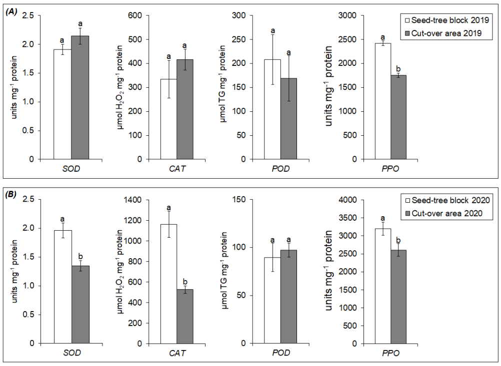

Figure 5 Changes in the activity of antioxidant enzymes in needles of advance-regeneration saplings after overstory removal (A) in the first growing season (experiment 1) and (B) one year later (experiment 2). *SOD* – superoxide dismutase, *CAT* – catalase, *POD* – peroxidase, *PPO* – polyphenol oxidase. Different letters denote the reliability of differences between saplings on the cut-over area and in seed-tree block (p<0.05).

Xylem increment

Xylem increments in the saplings in the first growing season after overstory removal were similar and roughly the same as in 2016–2018. A year after the cutting, xylem increment in advance regeneration on cut-over area was much higher (by 50.8%) than in advance regeneration in the seed-tree block as well as compared to previous years (Figure 6).

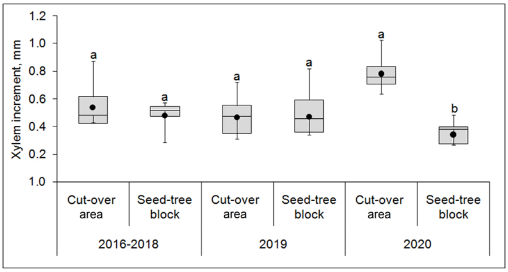

Figure 6 Box plots for xylem increment in advance regeneration saplings before (average over 2016-2018) and after overstory removal in the first growing season (2019) (experiment 1) and one year later (2020) (experiment 2). Cut-over area in 2016-2018: showing xylem increment in advance regeneration occupying skid roads in 2016-2018 and was then unshaded. Midlines and black circles indicate the median and mean values, respectively. Boxes represent the interquartile range. Whiskers represent all values within 1.5 times the interquartile range. Different letters denote the reliability of differences between saplings on the cut-over area and in seed-tree block across years (*p<0.05*)

Eight out of ten trees sampled from cut-over area featured collapsed earlywood tracheids in the xylem corresponding to the beginning of the growing season 2019 (Figure 7).

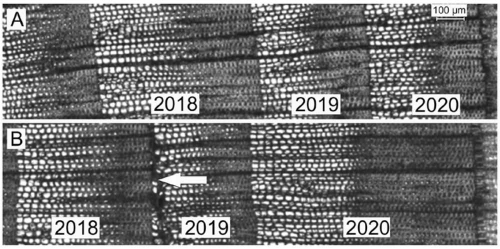

Figure 7 Cross-section of three annual xylem increments (2018-2020) in advance regeneration saplings from the seed-tree block (A) and cut-over area (B). White arrow points to the layer of crumpled envelopes of dead cells.

Discussion

The effectiveness of forestry operations has been studied using metrics such as regeneration survival and growth rates (Holgén & Hånell 2000, Örlander & Karlsson 2000, Nilsson et al. 2002). The oversight of the physiological condition of advanced-regeneration saplings occurs in the process. The evaluation of advance-regeneration saplings' wellbeing and ability to acclimate to new environments allows for quicker assessment of silvicultural methods and their effects on the local ecology and tree physiology.

Short-term acclimation (experiment 1)

An abrupt change in environmental conditions, first increase in light intensity after clear-cutting, led to a change in the functional activity of PSII. The *Fv/Fm* decreased in shade-grown needles of trees finding themselves in a cut-over site, showing a decrease in photochemical activity and CO_2 uptake (Maxwell & Johnson 2000, Baker 2008).

High light stress can cause *ROS* hyperaccumulation, which slows down PSII repair (Takahashi & Murata 2008, Pospíšil 2017). The antioxidant system's components act as protective mechanisms within a network to minimize the harmful impact of ROS on cells (Blokhina et al. 2003, Møller et al.2007). Despite clear-cutting, there were no changes in antioxidant enzyme activity in shade-grown saplings needles after overstory removal, compared to the control, except for reduced *PPO* activity. However, there was a tendency to increase the activity of *SOD* and then the activity of *CAT*, which together likely cope with the utilisation of reactive oxygen species generated after clear-cutting. Thus, oxygen converted into ROS - superoxide radical and hydrogen peroxide - was neutralised by *SOD* and *CAT*. This is confirmed by the tendency for *POD* activity to decrease, indicating the inappropriateness of using excess hydrogen peroxide, and by the significant decrease in *PPO* activity, indicating that there is less free oxygen as a substrate for the reaction of this enzyme after clear-cutting, as since most of it has probably been converted into ROS. Thus, we confirm the compensatory role of *SOD* and *POD* in the utilisation of oxygen and formed ROS, as well as the indicator role of *PPO* in these processes (Fukai & Ushio-Fukai 2011, Nikerova et al. 2023).

That said, saplings exposed to high light on cut-over area showed a sharp rise in *NPQ*. The augmented *NPQ* helps dissipate photon energy, preventing photodamage by ROS production reducing (Zhao et al. 2017). *NPQ* increase, which was accompanied by a decrease in *Y(II)* and *ETR*, may lead to a decrease in the rate of photosynthesis (Murchie & Ruban 2020).

In addition, as *NPQ* increased, clearcutting caused the *Chl a* and *Chl b* content of shade-grown needles to decrease simultaneously with an increase in the *Chl a/b* ratio. In chloroplasts, the bulk of chlorophyll is within *LHC*, which acts as an antenna and transmits energy to PSI and PSII reaction centers (Kitajima & Hogan 2003, Kunugi et al. 2016). The share of chlorophyll in *LHC* decreased after clear-cutting, which can be regarded as an adaptive response to avoid photoinhibition by reducing the amount of light absorbed (Dymova et al. 2010, Kreslavskij et al. 2012).

Acclimation over the year (experiment 2)

A year later after clear-cutting needles were acclimated to the new conditions. *Fv/Fm* values in light-grown needles rose, showing a recovery of photochemical activity in needles. Seedlings in changed conditions produce new acclimated needles, resulting in a decrease in negative cutting influence over time (Velasco & Mattsson 2020). The photosynthetic pigments of 20-year-old spruce underwent significant rearrangements as a result of transitioning from shade-grown to light-grown needles. Needles formed in high light on cut-over area featured an increase in *Chl a* content and *Chl a/b* ratio – a fact which, coupled with a reduction in pigment involvement in *LHC*, may evidence pigment build-up in reaction centers. It has been shown that *Chl a/b* ratio close to 5 was optimal, and plants with this light-harvesting antenna size have the greatest whole plant photosynthetic performance above ground biomass accumulation (Friedland et al. 2019). In our study *Chl a/b* ratio increased from 4.4±0.10 in shade-grown needles of the control saplings to 5.3±0.14 in light-grown needles an one year after clear-cutting.

One year after clear-cutting, the activity of *SOD*, *CAT* and *PPO* is significantly lower in the clear-cut areas than in the seed-block areas, indicating an adaptation to the new conditions. The activity of *POD* showed no difference as oxidative stress was removed and there was no need to utilise the excess hydrogen peroxide formed during the *SOD* reaction – *CAT* took over this role. In the work of the *POD* and the *PPO*, we note the compensatory role. The oxidation of polyphenols is much more intense in the tree-seed block. Clear cutting led to more intensive growth processes, as evidenced by the removal of the load on secondary metabolism, confirmed by a significant decrease in the activities of *SOD*, *CAT* and *PPO* (König et al. 2002, Nikerova et al. 2021).

The saplings whose needles have formed in high light on cut-over area showed low *NPQ* levels associated with a reduction in excessive light energy intake, probably because of rearrangement of pigment apparatus (increase of *Chl a/b* ratio and the share of chlorophyll in *LHC*) and upregulation of antioxidant enzymes. Photochemical processes in needles recovered a year after cutting, showing acclimation to new conditions. At this point, *Y(II)* and *ETR* were even higher than before the cutting. The decrease in the share of chlorophyll in *LHC*, and the corresponding increase in *Chl a/b*, is a response to optimise the ability to capture light under high irradiance conditions (Sato et al., 2015) and leads to a decrease in *NPQ* (Ruban 2016, Wang et al. 2022). The decrease in *NPQ* leads to an increase in light use efficiency, which can be reflected in a significant increase in *Y(II)* and *ETR* (Melis 2009, Wang et al. 2022).

A change in photochemical activity following an abrupt alteration of the environment induced by cutting was expected to be accompanied by a decrease in assimilate flux to acceptor organs, which would in turn affect increments.

We found no difference in xylem increment rate in 2019 between trees in the seed-tree block and the “released” trees, suggesting that the inhibition of photochemical activity (the *Fv/Fm*, *Y(II)* and *ETR* decreased) was probably limited to the beginning of the growing season. A year later, the activity of photochemical processes had increased (*Fv/Fm*, *Y(II)* and *ETR* increased) and radial xylem increment was significantly promoted.

In the cut-over area, 80% of sampled trees had collapsed earlywood tracheids in the xylem during the start of the 2019 growing season. Cell collapse can be caused by exposure to adverse climatic factors (particularly droughts), fungal or insect infestations, and by mechanical impacts or other causes (Schweingruber 2007, Rosneret al. 2018, Schweingruber & Börner 2018).

The needles were completely adjusted to the new lighting conditions. One year later, the activity of photochemical processes increased, resulting in higher xylem increments due to increased light availability. Norway spruce saplings acclimated to high light have higher photosynthetic rates than shade-adapted saplings (Grassi & Bagnaresi 2001). Also, we noted *POD* activity reduced with xylem increments. *CAT* activity facilitates in the development of active cells and their respiratory functions. The significantly decreasing *SOD* activity, which determines the adaptability of saplings, the reduction of oxidative stress, and the redistribution of the oxygen pool with the help of *PPO* confirms this.

Conclusions

The study demonstrated that the Norway spruce photoassimilation system performed well in various conditions. Abrupt change in external factors, first, illumination, caused by cutting, entailed a decline in the activity of photochemical processes.

There were no changes in the activity of antioxidant enzymes in needles immediately after overstory removal except for reduced *PPO* activity. However, the sensitive marker was the activity of *PPO*, which decreased already in the first year of the experiment and took over the role of using the excess oxygen.

It is likely that the decrease and redistribution of photosynthetic pigments, along with *NPQ* elevation, protected the shade-grown needles' photosynthetic machinery against excessive light energy absorption and photoinhibition.

A year later, the photoassimilatory system of the needles formed under the new conditions was already fully acclimated. Excessive light energy intake decreased, likely due to rearrangement of photosynthetic machinery. Photochemical activity has recovered and xylem increment has also been augmented.

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Conflict of interest

The authors declare no financial or personal interests could influence the work presented in this paper.

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