



Doubling of biomass production in European boreal forest trees by a four-year suppression of background insect herbivory

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ABSTRACT

Background insect herbivory, i.e. the minor but chronic plant damage caused by insects, is usually considered 'negligible' for plants when compared with the severe defoliation associated with forest pest outbreaks. We experimentally tested the hypothesis that the impacts of background herbivory on tree growth and mortality accumulate over years, resulting in much larger effects than usually assumed. In boreal taiga forests near Arkhangelsk (Northern Russia), application of insecticide at 10-day intervals for four growth seasons (June–September of 2014–2017) decreased foliage losses to insects in our study species (early successional deciduous *Betula pubescens* and *Populus tremula*; late successional coniferous *Picea abies* and *Pinus sylvestris*) from 2.04–6.35% to 0.72–1.18%. The magnitude of the insecticide treatment effect on plant losses to defoliating insects varied considerably among the study species, with the highest effect observed in white birch and the lowest effects in the two late successional species. Across all tree species, insecticide treatment nearly doubled the increase in tree biomass relative to control plots, demonstrating that background insect herbivory has major negative impacts on tree growth and productivity. The insecticide-treated plots showed the largest increase in biomass in Norway spruce and the smallest increase in European aspen when compared to the control plots. The changes in birch growth following the release from insect herbivory were three times greater than the effects of the same level of simulated herbivory in an earlier experiment, thereby hinting at the importance of herbivore-specific elicitors in the growth suppression of trees damaged by insects. The mortality of late successional species in the treatment plots increased nearly three-fold, whereas the mortality of early successional species did not change relative to controls, suggesting an increase in competitiveness of the early successional trees released from herbivory. Thus, in agreement with an earlier modelling study, we conclude that minor herbivore damage, over the long term, substantially reduces biomass production in North European forest trees. Due to differential effects on coexisting tree species, this damage has a pronounced impact on plant competitiveness and affects both the productivity and the structure of boreal forests.

1. Introduction

The ecological importance of insect herbivory in forest ecosystems is indisputable, and a wealth of studies has documented the adverse impacts of plant-feeding insects on tree growth and productivity (Whitehead, 2011; Flower and González-Meler, 2015; Kozlov and Zvereva, 2017; and references therein). Admittedly, the larger part of these studies has explored the consequences of devastating pest outbreaks, although only a few plant-feeding insects exhibit eruptive population dynamics (Faeth, 1987). Moreover, peaks in densities of herbivorous insects are relatively rare, are usually (with a few notable

exceptions) local and are typically 1–3 years in duration. By contrast, the majority of plant-feeding insects remain permanently at low densities and collectively impose minor, albeit chronic, damage on plants, termed background insect herbivory (BIH hereafter; Kozlov and Zvereva, 2017).

Direct losses of woody plant foliage to BIH are relatively minor (1–15% annually for different regions and plant communities: Coley and Aide, 1991; Turcotte et al., 2014; Kozlov et al., 2015b,c), and the consequences of these losses are often considered 'negligible', especially when compared to the severe defoliation associated with pest outbreaks. However, all insect outbreaks reported from the boreal forests

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of the European part of Russia from 1953 to 1998 jointly caused an annual loss of only 0.02% of the aboveground biomass of forest trees (Selikhovkin, 2009). Keeping in mind that foliar biomass constitutes 7–8% of the aboveground biomass (Reich et al., 2014; Fang et al., 2018), even an annual loss of 1% of the foliar biomass due to BIH at the regional or global scale would greatly exceed the annual loss caused by local outbreaks of forest pests. The challenge, therefore, is to explore the ecosystem role of those plant-feeding insects which collectively remove only a few percent of tree foliage in each growth season (Kozlov and Zvereva, 2017).

Previous defoliation experiments mimicking insect herbivory generally lasted for only one year and used a single removal of 50–100% of foliage (e.g. Honkanen et al., 1994; Anttonen et al., 2002), thereby simulating insect outbreaks. Similarly, about one half of the herbivore exclusion studies established with woody plants also lasted for only one growth season (e.g. Crandall and Knight, 2018; Meiners et al., 2000; Solé et al., 2019). This time scale is likely too short to allow adequate evaluation of the effects of BIH on tree growth and productivity. An earlier study revealed that an annual removal of 8% of the leaf area resulted in a statistically significant decrease in shoot length of mountain birch (*Betula pubescens* var. *pumila*) by the end of the third year of the experiment, whereas decreases in leaf size and in total leaf area were detected only in the sixth year of the experiment. The removal of 4% and 2% of the leaf area caused reductions in vertical growth and shoot length only after seven years of treatments (Zvereva et al., 2012). However, in certain respects simulated herbivory cannot completely mimic the equivalent levels of natural herbivory (Heil, 2010; Quentin et al., 2010), because many plant responses to herbivory are elicited by compounds present in insect oral secretions and thus do not develop following simulated herbivory (Kessler and Baldwin 2002). Therefore the acute shortage of data from long-term, community-wide herbivore exclusion experiments makes the existing estimates of productivity losses from BIH in forests highly uncertain (Kozlov and Zvereva, 2017).

We therefore designed the present study to test the hypothesis coined by Wolf et al. (2008) that the impacts of BIH on forest productivity accumulate over years and cause much larger effects than could be expected from direct annual losses of foliar biomass to insects. Based on the results of a seven-year-long defoliation experiment (Zvereva et al., 2012), we predicted that suppression of BIH over several consecutive years would substantially increase biomass production by boreal forest trees. Furthermore, since BIH in cold climates is more extensive in early successional trees than in late successional trees (Kozlov et al., 2015d), we predicted that BIH suppression would benefit early successional deciduous trees more than late successional coniferous trees. To test these predictions, we compared the levels of plant damage by herbivorous insects and the growth and mortality of tree saplings between insecticide-treated and control plots in a four-year-long field experiment.

2. Material and methods

2.1. Field experiment

We explored the community-level impacts of BIH by exclusion of plant-feeding insects with insecticides. The experiment was conducted at three sites (Talagi: 64°36' N, 40°43' E; Yuras: 64°32' N, 40°46' E; Babonegovo, 64°26' N, 40°57' E) in boreal taiga forests near Arkhangelsk, northern Russia. Study plots (5 × 5 m size; three pairs per site) were selected in habitats at early stages of post-cutting forest regeneration, where saplings of at least three of the four study species (white birch, *Betula pubescens*; European aspen, *Populus tremula*; Norway spruce, *Picea abies*; Scots pine, *Pinus sylvestris*) were common. White birch and European aspen are broadleaved, deciduous, early successional trees, whereas Norway spruce and Scots pine are coniferous, evergreen, late successional trees. The plots were not fenced, because mammals that could damage tree saplings were virtually

absent from the study sites: during the observation period, we recorded browsing on a single sapling.

In spring of 2014 (from 23 May to 13 June), we tagged all saplings of our study species within each of the 18 plots, and we measured their basal diameters and heights. These measurements reflected the size of saplings at the end of the growth season of 2013. A total of 849 saplings (with basal trunk diameters exceeding 2 mm) were tagged; their average height at the beginning of the experiment was ca. 60 cm. One pair of study plots was vandalised in 2015 and was therefore excluded from our experiment. This unfortunate event decreased our sample sizes to 16 plots and 654 saplings.

Within each pair (= block) of plots, we randomly assigned one plot to insecticide treatment while the other served as a control. We used a commercially available systemic insecticide (Actara, produced by Syngenta International AG, Switzerland; active ingredient: thiamethoxam; dose: 0.02 g in 100 g of water per m² of study plot) that affects both above- and belowground invertebrate herbivores. The insecticide was applied on windless days with a hand pump sprayer at approximately 10-day intervals (depending on weather conditions) to all saplings in the treatment plots during the entire growth seasons of 2014–2017 (a total of 34 treatments). The distance between the paired plots (20–120 m) was sufficient to prevent occasional transfer of insecticide to the control plots, which were sprayed with tap water on the same dates.

2.2. Greenhouse experiment

The chemical exclusion of plant-feeding insects requires demonstration of the absence of direct effects of the insecticides on tree growth (Silfver et al., 2013). Therefore, we established a greenhouse experiment on Khabarka Island at the outskirts of Arkhangelsk at the end of May in 2014. Saplings of the four study species (average height ca. 30 cm) were collected near the experimental plots and planted individually in 2.5 L pots filled with commercially available garden soil. The saplings were randomly arranged in 12 blocks of 4 saplings each (one of each of the four species) and placed inside a 10 m² unheated greenhouse to prevent their infestation by insects. We examined plants growing in a greenhouse at regular intervals, but found (and removed) only a couple of insect herbivores during the entire experiment. The saplings were watered once or twice per week, depending on the air temperatures. Insecticide was applied to half of the blocks 12 times during the growth seasons of 2014 and 2015, at the same doses and using the same procedures as in the field experiment. Control saplings were sprayed with tap water.

2.3. Data collection

In the field experiment, we measured leaf/needle losses to chewing insects and the sizes of alive saplings at the ends of the growth seasons of 2014–2017. Possible effects of sap- and root-feeding insects on our plants were quantified at the end of the experiment (in late July of 2017). In the greenhouse experiment, we measured sapling size prior the beginning of treatments (in May 2014) and again at the end of the second growth season (in September 2015).

Sapling growth was quantified by increases in height and basal stem diameter. Height (h) was measured with a ruler to the nearest 1 cm, and basal diameter (d) was measured with electronic callipers to the nearest 0.1 mm. Measurements were conducted blindly relative to the results of previous measurements; however, when the difference between the two subsequent measurements hinted at some error (e.g. a sapling height decrease), the tree was re-measured and the reason for the discrepancy (e.g. a broken apical shoot) was recorded. The biomass of each sapling was approximated as $d^2 \times h$. This combined variable allows a reasonably accurate prediction of tree biomass across a range of species and environments (Overman et al., 1994; Radtke et al., 2017). The difference between the estimated sapling biomass at the end (2017) and

before the beginning of the experiment (2013), reflecting tree growth rate, was calculated by averaging the data on saplings for which (1) both diameter and height were measured in 2017, and (2) whose diameter in 2013 did not exceed 21 mm (96.7% of saplings which satisfy the first condition).

Losses of leaf/needle area to defoliating insects were measured in situ at the end of growth seasons, but before the beginning of the autumnal leaf fall (on 18–27 September). These measurements were conducted on three saplings of each species which were located closest to the centre of each plot. Each leaf/needle on a haphazardly selected branch (which, on average, contained 119 ± 3 leaves/needles, $n = 705$ branches), was assigned to a damage class based on the percentage of the leaf area consumed or damaged by insects: intact leaves, 0.01–1%, 1–5%, 5–25%, 25–50%, 50–75% and 75–100%. All measurements were performed by A.L. Sh. The percentage of leaf area lost to insects was calculated as follows: the numbers of leaves in each damage class were multiplied by the respective median values of the damaged leaf area (i.e. 0 for intact leaves, 0.5% for the damage class 0.01–1%, 3% for the damage class 1–5%, etc.); the obtained values were then summed for all damage classes and divided by the total number of leaves (including undamaged ones) in a sample.

Plant losses to sap-feeding insects, in contrast to defoliators, cannot be reliably measured from traces of their damage. Therefore, we assessed the density of sap-feeding insects by sampling one branch about 50 cm in length from one sapling of each species that was closest to the centre of each plot. One of two collectors placed a mesh bag attached to a ring (60 cm in diameter) under the selected branch, while the second collector cut the branch in such a way that it fell into the bag, together with the insects that dropped from the branch when disturbed. The bag was immediately closed, labelled and transported to the laboratory, where all invertebrates were collected and preserved in alcohol. The insect dry weight was estimated as described previously by Kozlov et al. (2015a). The foliage from the collected branches was dried at $+ 105^\circ\text{C}$ for 48 h and weighed. The load of sap-feeders was expressed as the insect dry biomass per unit of dry weight of foliage.

The effects of insecticide treatment on soil macroinvertebrates were quantified by collecting two samples (20×20 cm, 30 cm depth) with a flat shovel from each of 16 study plots. We placed the litter and organic soil layer from each sample into a 10 L plastic bag and the mineral soil into a 28 L plastic bag, closed both bags tightly and transported them to the Arkhangelsk laboratory. The samples were kept at room temperature and hand-sorted within 3 days of the collection date. Soil was manually broken into small pieces and sieved (4 mm grid); all discovered invertebrates were captured with tweezers and stored in 70% ethanol. They were later weighed (to the nearest 0.1 mg) and identified based on morphological characters.

The difference in fine root biomass between insecticide-treated and control plots can be used to quantify root losses to insects, because these insects generally damage the distal lower-order roots (Sun et al., 2011). The samples of fine roots (two per plot) were collected using a cylindrical metal corer with an inner diameter of 36 mm. We measured the thicknesses of the organic layer and mineral soil horizons and divided each sample into the organic layer and 0–10 cm, 10–20 cm and 20–30 cm mineral soil layers. The deepest sampled layer was often incomplete (i.e. its thickness was < 10 cm) due to the high stone content of the soil. All living fine roots (i.e. roots not exceeding 2 mm in diameter), including mycorrhizae and rhizomes, were separated by hand under laboratory conditions and washed with water to remove the adhered soil. The separation of living and dead roots was based on root branching patterns, periderm colour, surface structure, root elasticity and toughness (Persson, 1983). The root samples were dried for 48 h at $+ 105^\circ\text{C}$ and weighed to the nearest 0.1 mg. The fine root density in each layer was estimated by dividing the root biomass by the sample volume (Finér et al., 2019).

To compare our results with those from an earlier defoliation experiment (reported by Zvereva et al., 2012), in the mid-summer of 2017

we collected additional data from birch saplings. Birches have two different types of shoots: short shoots that produce two to five leaves and grow less than 2 mm per year and long shoots that may bear more leaves and grow tens of millimetres long. From each of two birch saplings (again, growing closest to the centre of each plot), we measured (with a ruler, to the nearest 1 mm) the lengths of up to 10 long shoots, starting from the top of a sapling, and we collected the largest leaf from 10 haphazardly selected short shoots. The leaves were transported to the laboratory, where they were mounted on strong paper using adhesive tape and press-dried as ordinary herbarium specimens.

Following the protocol used in our earlier studies (Kozlov et al., 1996; Kozlov and Zverev, 2018), for each leaf, we measured the length of lamina and width of the left and right sides from the midrib to the leaf margins (at the midpoint between the base and the tip) perpendicular to the midrib (WL and WR, respectively). The measurements were conducted with a ruler to the nearest 0.5 mm; the perpendicularity of the measurement line to the midrib was controlled visually. All measurements were performed twice, by different persons who had no knowledge of either the hypotheses being tested, the plant origin or the results of previous measurements. Leaves in which the absolute difference between the first and second measurement of the same side exceeded 2 mm were remeasured to exclude an occasional error. The values of leaf fluctuating asymmetry (FA hereafter) were calculated as follows: $FA = 2 \times \text{abs}(WL - WR)/(WL + WR)$.

2.4. Data analysis

The proportions of saplings that died during the experiment were analysed using a generalised linear mixed model with binomial error distribution and a logit link function with the event/trial syntax (procedure GLMMIX; SAS Institute, 2009). For each plot by plant species combination, a trial was the number of saplings at the start of the experiment and an event was the number of saplings that died by the end of the experiment. Sources of variation in other response variables were explored with a repeated (whenever appropriate) linear mixed model (SAS GLMMIX procedure, type III tests: SAS Institute, 2009). The analysis of data from the field experiment considered the treatment (insecticide vs. control), tree species, study site, year of data collection (a repeated factor with compound symmetry as a variance-covariance structure; only for growth and herbivory data) and all their interactions as fixed effects, whereas a block (nested within site) was treated as a random intercept effect. The analysis of growth data also included pre-treatment (2013) measurements. The differences between early and late successional species were sought by adding tree successional status (a fixed factor) to these models.

To facilitate accurate F tests of the fixed effects, we adjusted the standard errors and denominator degrees of freedom in all our analyses by the latest version of the method described by Kenward and Roger (2009). The significance of the random factor was evaluated by calculating the likelihood ratio and testing it against the chi-squared distribution (as described in Littell et al., 2006).

3. Results

3.1. Aboveground herbivory

In control plots, losses of foliage to defoliating insects were higher in early successional leaf-bearing tree species than in late successional needle-bearing species (estimated marginal means \pm S.E.: $5.83 \pm 0.27\%$ and $2.36 \pm 0.28\%$, respectively; $F_{1, 672.9} = 67.44$, $P < 0.0001$). The effect of the insecticide treatment varied among tree species (Table 1, Fig. 1); the decrease in herbivory was greater in early successional species than in late successional species (to 20% and 33% of the control, respectively; $F_{1, 669.4} = 44.71$, $P < 0.0001$). On average, insecticide application reduced BIH four-fold, from $4.04 \pm 0.27\%$ in control plots to $1.01 \pm 0.27\%$ in treatment plots

Table 1
Sources of variation in losses of plant foliage to insects (repeated linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Test statistics	P-value
Fixed	Treatment	$F_{1, 603.6} = 180.93$	< 0.0001
	Year	$F_{3, 603.1} = 4.97$	0.0020
	Species	$F_{3, 606} = 23.20$	< 0.0001
	Site	$F_{2, 4.14} = 2.90$	0.16
	Treatment × Year	$F_{3, 603.1} = 12.11$	< 0.0001
	Treatment × Species	$F_{3, 605.9} = 17.74$	< 0.0001
	Treatment × Site	$F_{2, 603.6} = 5.37$	0.0049
	Species × Year	$F_{9, 603.1} = 3.45$	0.0004
	Site × Year	$F_{6, 603.1} = 1.88$	0.0820
	Site × Species	$F_{6, 604.8} = 3.52$	0.0020
	Treatment × Species × Year	$F_{9, 603.1} = 1.31$	0.23
	Treatment × Site × Year	$F_{6, 603.1} = 0.95$	0.46
	Treatment × Species × Site	$F_{6, 605.5} = 2.47$	0.0230
	Species × Site × Year	$F_{18, 603.1} = 1.08$	0.37
	Treatment × Species × Site × Year	$F_{18, 603.1} = 1.69$	0.0367
Random	Block	$\chi^2_1 = 6.11$	0.0067

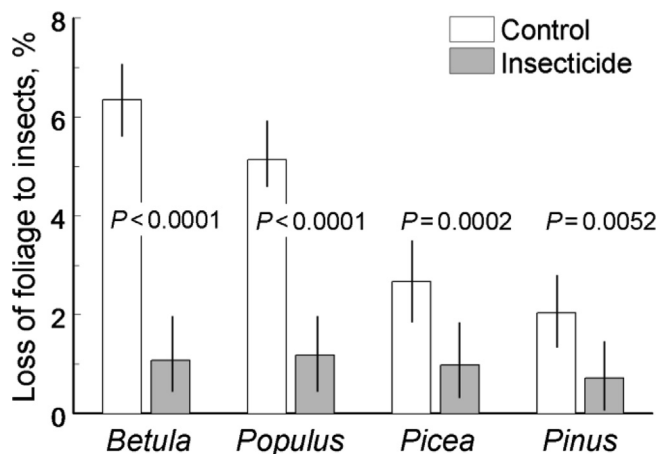


Fig. 1. Losses of foliage to defoliating insects in the field experiment (2014–2017). Values shown are the estimated marginal means, i.e. the least-square means (with 95% confidence limits) produced by SAS GLIMMIX procedure. *P* values refer to the comparisons between control and experimental plots.

(Table A1).

From 58 branches, we collected only three sap-feeding insects (two true bugs and one leafhopper). Their load did not differ significantly between treatments (data not shown).

3.2. Fine root biomass and soil macrofauna

At the end of the experiment, fine root biomass (Table A2; corrected for the depth of the soil layer) did not differ ($F_{1, 89.9} = 2.03$, $P = 0.16$) between the insecticide-treated and control plots. Similarly, fine root density did not differ between these plots, either in the organic (humus) layer (mean \pm S.E.: 5.36 ± 0.91 and 5.69 ± 0.86 mg cm⁻³, respectively; $F_{1, 20.2} = 0.12$, $P = 0.73$) or in the upper 10 cm of the mineral soil horizon (4.42 ± 1.38 and 4.81 ± 1.36 mg cm⁻³, respectively; $F_{1, 18.9} = 0.43$, $P = 0.52$).

From the 30 soil samples, we extracted 385 specimens of macroinvertebrates, including 176 earthworms, 67 larvae of Diptera, 44 larvae of the Elateridae, 28 millipedes/centipedes and 30 spiders. The dry weight of macrofauna in the insecticide-treated plots (0.93 ± 0.66 g/m²) was about a half that in the control plots (1.88 ± 0.88 g/m²), but this difference did not reach statistical significance (Table 2). Separate analyses of data on the most abundant groups of macrofauna (listed above) also did not reveal any statistically

Table 2
Sources of variation in dry weight of soil macrofauna (linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Test statistics	P value
Fixed	Treatment	$F_{1, 20.2} = 1.56$	0.23
	Site	$F_{2, 5.2} = 3.21$	0.12
	Treatment × Site	$F_{2, 20.2} = 0.91$	0.42
Random	Block	$\chi^2_1 = 3.86$	0.0247

Table 3
Sources of variation in mortality of study plants (generalized linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Test statistics	P-value
Fixed	Site	$F_{2, 4.63} = 0.17$	0.85
	Treatment	$F_{1, 50} = 2.36$	0.13
	Species	$F_{3, 50} = 6.09$	0.0013
	Treatment × Species	$F_{3, 50} = 3.46$	0.0231
Random	Block	$\chi^2_1 = 6.91$	0.0043

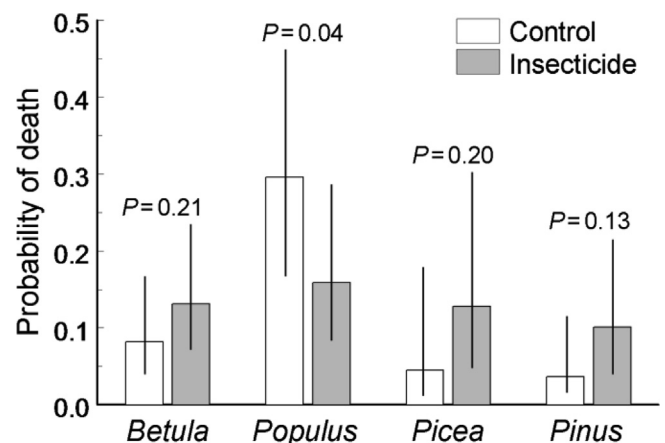


Fig. 2. The probability of death of a sapling in a study plot during the field experiment (2014–2017). Values shown are the back-transformed (to the original data scale) estimated marginal means (with 95% confidence limits), i.e. the least-square means produced by SAS GLIMMIX procedure. *P* values refer to comparisons between control and experimental plots.

significant effects of insecticide application (data not shown).

3.3. Sapling mortality in field experiment

Overall, 92 of 654 saplings died during the experiment (Table A3). The effect of treatment varied among tree species (Table 3, Fig. 2): insecticide application increased the mortality of late successional species nearly three-fold, thereby eliminating the differences in mortality between early and late successional species that were observed in control plots (treatment × successional status: $F_{1, 52} = 4.29$, $P = 0.04$). However, white birch and European aspen responded differently to insecticide treatment ($F_{1, 24} = 6.05$, $P = 0.02$), whereas Norway spruce and Scots pine showed similar responses to insecticide ($F_{1, 24} = 0.30$, $P = 0.59$).

3.4. Sapling growth in the greenhouse experiment

The average height of our saplings increased by a factor of 1.7 during the experiment, from 32.2 ± 1.8 cm ($n = 42$) in the spring of 2014 to 55.2 ± 3.7 cm ($n = 42$) in the autumn of 2015 (Table A4). In the absence of herbivory, application of insecticide had no effect on tree growth. The effect of interest, namely the Treatment × Year interaction, was far from being significant, indicating that the differences in

Table 4

Sources of variation in basal diameter and height of saplings in greenhouse experiment (repeated linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Basal diameter		Height	
		Test statistics	P value	Test statistics	P value
Fixed	Treatment	$F_{1, 3.60} = 0.55$	0.50	$F_{1, 14.6} = 1.32$	0.27
	Year	$F_{1, 23.5} = 262.88$	< 0.0001	$F_{1, 34} = 229.91$	< 0.0001
	Species	$F_{3, 14.8} = 4.79$	0.0158	$F_{3, 27.9} = 58.37$	< 0.0001
	Treatment × Year	$F_{1, 24.4} = 0.51$	0.48	$F_{1, 34} = 0.24$	0.63
	Treatment × Species	$F_{3, 15.3} = 0.50$	0.68	$F_{3, 27.9} = 1.01$	0.40
	Species × Year	$F_{3, 23.1} = 20.71$	< 0.0001	$F_{3, 34} = 41.74$	< 0.0001
	Treatment × Species × Year	$F_{3, 22.7} = 0.43$	0.73	$F_{3, 34} = 1.45$	0.25
	Block	$\chi^2_1 = 2.96$	0.09	$\chi^2_1 = 1.22$	0.27
Random					

both basal stem diameter and height between the control and experimental trees did not change during the experiment (Table 4). This conclusion was valid for all study species, as indicated by non-significant Treatment × Species and Treatment × Species × Year interactions (Table 4).

3.5. Sapling growth in field experiment

The average height of our saplings nearly doubled during the experiment, from 59.2 ± 1.2 cm ($n = 849$) in 2013 to 117.4 ± 2.8 cm ($n = 539$) in 2017 (Table A5). The effect of insecticide on tree growth (Treatment × Year interaction) was highly significant, indicating that the differences in both basal stem diameter and height between the control and experimental trees changed with the study year (Table 5). All four study species showed similar responses to the treatment, as indicated by non-significant Treatment × Species and Treatment × Species × Year interactions (Table 5).

Early successional and late successional species did not differ in their responses to BIH suppression (basal diameter: $F_{4, 2240} = 1.40$, $P = 0.23$; height: $F_{4, 2196} = 1.71$, $P = 0.15$). Similarly, the effect of the treatment was consistent among sites (Table 5). At the beginning of the experiment, the saplings in the treatment plots were slightly (non-significantly) smaller than the saplings in the control plots, whereas at the end of the experiment, the experimental saplings were larger than the control ones (Fig. 3). The shoot length, leaf length and leaf FA for white birch (Table A6) at the end of the experiment did not differ between the treatment and control plots (Table 6).

The reduction in plant losses to insects caused by application of the insecticide resulted in a 65% increase in radial increment and by 30% increase in vertical increment relative to the control trees (Table A7). As a result, the estimated biomass of trees treated with insecticide increased during the experiment nearly twice the increase in biomass observed in the control trees. This effect was highest in Norway spruce and lowest in European aspen (treatment/control ratio of 3.25 and

1.08, respectively; Table A7).

4. Discussion

The levels of BIH in northern boreal forests are generally low: from 4 to 5% in leaf-bearing trees and shrubs (Kozlov, 2008; Kozlov et al., 2015b,c) to below 1% in conifers (Larsson and Tenow, 1980; Galasjeva and Pisareva, 1991). Thus, plant losses to insects in our control plots from 2014 to 2017 were typical for the study region. Nevertheless, despite the small absolute levels of herbivory, our experiment confirmed the ecological importance of BIH: the reduction in annual losses to defoliating insects from 4% to 1% during four consecutive years was associated with nearly two-fold increase in tree biomass relative to the control plots.

Our findings support the conclusion of an earlier modelling study (Wolf et al., 2008) showing that the long-term, indirect losses in tree biomass due to growth suppression are much greater than the direct losses, i.e. the removal of foliage by insects. The multiplicative effect of a minor decrease in herbivory may arise from the reduced supply of assimilates for growth processes due to multiple factors that include the considerable loss of photosynthetic capacity in the undamaged tissues of the damaged leaves (Zangerl et al., 2002; Nability et al., 2009), from the water loss through injured plant leaves (Aldea et al., 2005), from the premature abscission of damaged leaves (Zvereva and Kozlov, 2014), and/or from the cost of production of induced antiherbivore defences (Herms and Mattson, 1992; Koricheva, 2002). The relative contributions of these processes to the growth suppression caused by BIH remain to be studied; but—regardless of the mechanisms involved—the present herbivore exclusion experiment demonstrated that BIH has substantial impacts on forest productivity.

The insecticide that we used in our study could, potentially, facilitate plant growth. For example, Tang et al. (2017) found that plants, whose seeds were treated by thiamethoxam, grow more vigorously than control plants. However, other studies reported no effect of

Table 5

Sources of variation in diameter and height of saplings in field experiment (repeated linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Basal diameter		Height	
		Test statistics	P value	Test statistics	P value
Fixed	Treatment	$F_{1, 545.5} = 0.52$	0.47	$F_{1, 546.8} = 0.17$	0.68
	Year	$F_{4, 2224} = 867.8$	< 0.0001	$F_{4, 2180} = 765.6$	< 0.0001
	Species	$F_{3, 546.2} = 16.11$	< 0.0001	$F_{3, 530.1} = 15.15$	< 0.0001
	Site	$F_{2, 4.68} = 3.48$	0.11	$F_{2, 4.63} = 7.05$	0.0393
	Treatment × Year	$F_{4, 2224} = 45.19$	< 0.0001	$F_{4, 2180} = 10.35$	< 0.0001
	Treatment × Species	$F_{3, 546} = 0.37$	0.77	$F_{3, 547.1} = 0.09$	0.96
	Treatment × Site	$F_{2, 547.7} = 1.11$	0.33	$F_{2, 547.3} = 0.41$	0.67
	Species × Year	$F_{12, 2224} = 10.43$	< 0.0001	$F_{12, 2180} = 16.74$	< 0.0001
	Site × Year	$F_{8, 2224} = 2.42$	0.0134	$F_{8, 2180} = 2.98$	0.0025
	Site × Species	$F_{6, 545.8} = 9.34$	< 0.0001	$F_{6, 529.6} = 10.55$	< 0.0001
	Treatment × Species × Year	$F_{12, 2224} = 1.13$	0.33	$F_{12, 2180} = 0.87$	0.58
	Block	$\chi^2_1 = 10.15$	0.0007	$\chi^2_1 = 2.65$	0.0516
	Random				

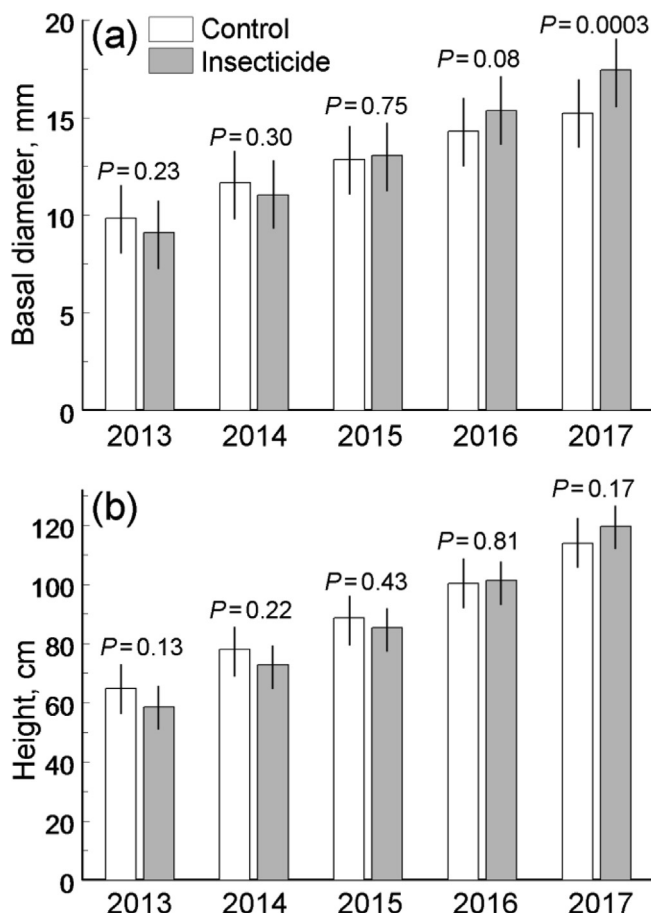


Fig. 3. Basal stem diameter (a) and height (b) of saplings prior the beginning of the field experiment (2013) and at the end of growth seasons 2014–2017. Values shown are the estimated marginal means (with 95% confidence limits), i.e. the least-square means produced by SAS GLIMMIX procedure. P values refer to the comparisons between control and experimental plots in a single year (see Table 5 for the results of repeated linear mixed model).

thiamethoxam on plant yield (Lanka et al., 2017) or even decrease in shoot growth following insecticide application (Macedo et al., 2013). We did not find any positive effects of insecticide treatment on the plant growth in the greenhouse experiment either. Therefore, we attribute the increase in tree growth observed in the field experiment to BIH suppression.

Interestingly, we did not find any statistically significant differences among our study species in terms of the effects of BIH suppression on growth. This was the case even though the white birch and European poplar in our control plots suffered three times greater foliar losses to insects than did the Scots pine and Norway spruce. Nevertheless, if we (as currently suggested: Hurlbert et al., 2019) focus on the magnitudes of the effects rather on their statistical significance, then we get a clear indication that that herbivore removal accelerated sapling growth to a

greater extent in Norway spruce than in the other study species. At the same time, BIH suppression decreased only the mortality of European aspen, whereas it increased mortality in the other study species. These findings jointly suggest that the effects of BIH on plants may be idiosyncratic, and that even tree species that share many life history traits, including successional status, may respond differently to changes in herbivory.

This is the first experimental demonstration that minor changes in BIH may differentially affect coexisting tree species at the early stages of forest succession. Thus far, the reported impacts of herbivory have primarily focused on interactions between plants in grassland communities (Rees & Brown, 1992; Schadler et al., 2007; Graff et al., 2007; Stein et al., 2010; and references therein). By contrast, studies conducted in forests have been relatively infrequent and have concentrated on mammalian browsing. In those studies, the exclusion of ungulate herbivory facilitated the growth and recruitment of quaking aspen, *Populus tremuloides*, which suffered from competition with conifers in the grazed plots (Maxwell et al., 2019). In line with that study, we revealed that herbivory suppression benefits early successional trees, both through greater increases in growth (expressed mainly in birch) and decreases in mortality (expressed mainly in European aspen). Consequently, we attribute the increased mortality observed in the late successional conifers to the increased competitiveness of the early successional species in plots released from insect herbivory. This finding confirms the results of an earlier modelling study, which predicted an increase in the proportion of Norway spruce and Scots pine (late successional species) in boreal taiga forests at the expense of birch (early successional species) following an expected increase in BIH impacts on birches as the climate warms (Wolf et al., 2008). More generally, differential BIH, which has stronger effects in white birch and European poplar than in Norway spruce and Scots pine, may be an overlooked driver of secondary successions in boreal forests.

Our study also shed some light on the relative importance of above- and below-ground herbivory for tree growth and productivity. Neither the biomass of soil macrofauna (including root-feeding insects) nor the density of fine roots (the food for these insects) differed between the insecticide-treated and control plots. The latter result can be explained by our earlier estimate (Kozel et al., 2017) that insects in European forests consume as little as 0.5% of the available fine root biomass: this value is likely too small to allow detection of the effects of suppression of root-feeding insects by measurements of fine root density. Similarly, the abundance of above-ground sap-feeding insects was too low to cause measurable losses of plant biomass, even in the control plots. These results jointly suggest that the increase in tree growth detected in our experiment was driven primarily by the release of insecticide-treated saplings from damage imposed by externally feeding defoliators, such as lepidopteran and hymenopteran larvae and coleopteran larvae and adults.

From a methodological perspective, our findings once again stress the importance of long-term experiments when endeavouring to study the impacts of realistic levels of insect herbivory on plant communities and ecosystem processes. We also confirmed the conclusion by Zvereva et al. (2012) that whole-tree characters reflecting tree size are the first to show a response to changes in BIH. At the same time, we

Table 6

Sources of variation in shoot length, leaf length and leaf FA in *Betula pubescens* during the final (2017) year of the experiment (linear mixed model: SAS GLIMMIX procedure, type III tests).

Effect	Explanatory variable	Shoot length		Leaf length		Leaf FA	
		Test statistics	P value	Test statistics	P value	Test statistics	P value
Fixed	Treatment	$F_{1, 12.9} = 0.06$	0.81	$F_{1, 9.35} = 0.44$	0.52	$F_{1, 6.91} = 0.43$	0.53
	Site	$F_{2, 7.20} = 0.91$	0.45	$F_{2, 4.26} = 0.48$	0.65	$F_{2, 2.89} = 0.18$	0.84
	Treatment × Site	$F_{2, 13.5} = 0.08$	0.92	$F_{2, 9.74} = 0.55$	0.59	$F_{2, 7.22} = 0.22$	0.81
Random	Block	$\chi^2_1 = 2.06$	0.08	$\chi^2_1 = 1.24$	0.13	$\chi^2_1 = 0.74$	0.20

demonstrated that the four-fold decrease in herbivory, which (according to Grime, 1979) can be classified as a stressor due to its adverse impact on tree growth, did not change the leaf FA in white birch. This finding adds to the ‘negative’ experimental results (e.g. Zverev et al., 2018) that question the opinion (e.g. Freeman et al., 1993; Cuevas-Reyes et al., 2018) that FA is a universal indicator of stress.

At the end of our field experiment, the estimated biomass of the birches was 51% greater in the insecticide-treated plots than in the plots exposed to natural herbivory. This effect was nearly three times as large as the effect of a similar level of simulated herbivory: at the end of the fourth year of the defoliation experiment, the estimated biomass of undamaged birches was only 21% greater than that of birches from which 4% and 8% of leaf area have been removed annually (data from Zvereva et al., 2012, and unpublished). The greater effect of herbivore exclusion relative to simulated herbivory contradicts the conclusion of a previous meta-analysis, which revealed no quantitative differences in the responses of woody plants to natural versus simulated herbivory (Nykänen and Koricheva, 2004). We suggest that this discrepancy may have resulted from the high proportion of studies that addressed high levels of plant damage. In this situation, the plant response is primarily driven by the physical loss of leaf area. By contrast, at low levels of damage, the plant response depends on the presence (natural herbivory) or absence (simulated herbivory) of herbivore-specific elicitors.

In conclusion, BIH has a major negative impact on tree growth and productivity. Our findings confirm the prediction of a previous modelling study (Wolf et al., 2008), whereby the indirect losses in tree biomass due to growth suppression over the long term are much greater than the direct losses, i.e. the removal of foliage by insects. In addition, in line with the previous study, our data suggest that an increase in herbivory with climate warming will also change the structure of boreal taiga forests due to differential responses of forest forming tree species to insect herbivory.

CRedit authorship contribution statement

Aleksandr L. Shestakov: Data curation, Funding acquisition, Investigation, Writing - review & editing. **Boris Yu. Filippov:** Funding acquisition, Investigation, Writing - review & editing. **Natalia A. Zubrii:** Investigation, Writing - review & editing. **Tero Klemola:** Formal analysis, Writing - review & editing. **Ilya Zezin:** Investigation, Writing - review & editing. **Vitali Zverev:** Investigation, Methodology, Visualization, Writing - review & editing. **Elena L. Zvereva:** Conceptualization, Methodology, Writing - original draft. **Mikhail V. Kozlov:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing - original draft.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.117992>.

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