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**Faculty of Forest Sciences** 

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## Abstract

New intensified forest management measures are needed in order to meet the increasing demands of bio products. Intensive fertilization has large potential to enhance wood production in already existing forests. By repeatedly fertilizing young stands of Norway spruce, stem volume growth can increase by up to 350 %. Higher levels of forest exploitation are usually associated with loss of biodiversity and new forest management measures should therefore be carefully evaluated before implemented on larger scale. This study examines the immediate effects of intensive fertilization on the beetle community in an intensive managed forest area in southern Sweden. The results show that fertilized stands had 51% more individuals and 23% more species than unfertilized stands. Even though the total abundance and total species richness did not differ statistically between treatments, the results indicate a trend for short term positive effect at stand scale. The results also suggest that there is a difference in the response between functional groups of beetles based on their nutritional ecology. Herbivorous beetles were significantly more abundant and had higher species richness in fertilized stands. likely favored by the enhanced nutrition availability. It was particularly species that primarily foraging on other plant species than trees that differed in abundance between treatments. Predator beetles were also significantly more abundant in fertilized stands and may have been indirectly affected by greater number of potential prey. Cambium consuming beetles showed a significantly higher species richness in fertilized stands. This result is hard to interpret and may have been interfered by the effects of pre-commercial thinning that was performed in one stand during the monitoring period. The results from this study suggests that the beetle community is immediate affected by fertilization. Long term effects and the implication of large scale application should be evaluated before this management measure is applied on national scale. As this study is part of a large scale and long term experiment, future implications for beetle biodiversity and assemblage composition will be monitored.

## Introduction

The global demand of bio products is expected to increase significantly in a near future. Raunikar et al. (2010) predicts, for instance, a nearly six-fold increase in global demand of biofuel until year 2060. Increasing, high demands of bio products, together with other already existing claims and restrictions, will put our forests under pressure. In addition to forest production, society expects the forest to provide other services such as ecotourism, recreation, wildlife and biodiversity. This means that the global demand of forest products must be met without increasing the area of production forest in Sweden (Bergh et al., 2008). The global interest in replacing fossil fuel with renewable alternatives will contribute to fulfill one of Sweden's sixteen environmental objectives (Anon., 2008), namely "Reduced climate impact". The objective states that Sweden will reduce the emission of greenhouse gases from fossil fuels. The Swedish parliament has as an extension to that objective adopted a goal of zero net emissions of greenhouse gases to the atmosphere in Sweden by 2050 (Anon., 2014a). However, an intensified biomass production in the forest may contradict or cause a conflict with two other Swedish environmental objectives namely "Sustainable forests" and "A rich diversity of plant and animal life". It is therefore important to have a sustainable approach to wood production and to satisfy the increased production demands in already existing production forests (Nabuurs et al., 2007).

Long-term forest experiments have shown that growth rate in most forests are much lower than its potential and that an addition of nutrients or water or a combinations of both could effectively increase growth rate (Bergh *et al.*, 1999). In Sweden, and most northern forests, the single most limiting factor for forest growth is the insufficient amount of plant-available nitrogen in the ground (Tamm, 1991). When applying additional nitrogen in these forests, increased production of biomass far above levels of traditional forest management can be expected (Kenk & Fischer, 1988).

In Sweden, interest for forest fertilization has been fluctuating during recent decades. In the 1970s, the total fertilized area reached its peak notation and about 200 000 ha of forest land was annually fertilized. Since then, the annual area that has been fertilized has varied around 20 000- 50 000 ha, with some peak year notations of 80 000 ha. The most recent figure (2013) on fertilized area in Sweden lies just under 24 000 ha and year (Anon., 2014b). In contrast to traditional fertilization, where approximately 150 kg ha<sup>-1</sup> nitrogen is added to mature stands once or twice before final harvest (Pettersson & Högbom, 2004), Bergh *et al.* (2005) showed that repeated addition of nitrogen fertilizer to young forest stands of Norway spruce (*Picea abies*) can increase stem wood production up to 350 %. A practicable dose of nitrogen in young Norway spruce stands could be 125-150 kg ha<sup>-1</sup> every second year (Bergh *et al.*, 2008). However, an increased biomass production is potentially associated with loss of biodiversity and new forest measures need to be carefully evaluated before implemented on a larger scale (Hartmann *et al.*, 2010).

Quite many studies have addressed the environmental impact derived from fertilization, e.g. Lilleskov *et al.* (2002) showed a compositional change and a decrease in species richness of ectomycorrhizal fungi after long-term nitrogen fertilization. Another study reviewed articles

about insect responses to fertilization and found that there is a fundamental difference between direct, individual-level effects (e.g. body size, development time, survival) and indirect, population-level effects (e.g. abundance, feeding damage) and that the latter imply a more variable, mostly negative, response to fertilization (Kyto *et al.*, 1996). They also suggested that the negative response on population-level could potentially be explained by top-down regulating forces from predation and parasitism. A handful of studies covers abundance and community structure of different biota after repeated fertilization in young spruce dominated forests, e.g. Hedwall *et al.* (2010) found that ground vegetation in fertilized stands is affected both directly by altered nutrient balance and indirectly by canopy closure. Bird assemblages have been studied by Edenius *et al.* (2011), who conclude that repeated fertilization may increase both species richness and overall abundance of birds at stand scale. Studies on the effect of repeated fertilization on arthropods are quite rare but a follow up study to Edenius *et al.* (2011) addressed the effects of fertilization on arthropods (Edenius *et al.*, 2012).

Most earlier studies focus on specific groups of beetles, e.g. Blake *et al.* (2011) and Wermelinger (2004) and the effects of fertilization are monitored or seen several years after the fertilization was applied, as in e.g. Folkard and Smith (1995). By contrast, the present study is based on the first immediate results from fertilization utilizing a large scale and long term experiment on intensive forest management and its effect on forest living beetles. The experiment is well replicated and performed at stand scale which enables analyses of many organism groups. The sampling method used in this study enables analyzes on the beetle community as a whole. This first catch result allows us to investigate the immediate effect on the beetle community.

The overall aim in the present study is to evaluate how the beetle community in young Norway spruce stands is affected by nitrogen fertilization. The beetle assemblages are measured in fertilized and unfertilized stands and the effects are measured in terms of abundance and species richness. Also, beetles are divided into functional groups based on their nutritional ecology to investigate the effects on different trophic levels. The following questions will be addressed:

- 1) Are there differences in abundance and species richness of beetles between fertilized and unfertilized stands?
- 2) Do the effects of fertilization vary among nutritional functional groups of beetles?

## Materials and methods

#### Study area

The study was conducted in Asa experimental forest about 40 km north of Växjö in Småland, southern Sweden (lat. 57°10'N, long. 14°47'E, 225-250 m a.s.l). The growing season is 190 days and the mean temperature during growing season is 11.5 °C. Mean annual precipitation is ca. 700 mm. Twenty-two stands dominated by Norway spruce were selected for the experiment. Eleven stands were fertilized in 2012 with a fertilizer containing a mixture of nitrogen, magnesium, carbon and boron. The amount of nitrogen that was added to the stands was 150 kg ha<sup>-1</sup>. Eleven control stands were selected to have similar properties as the fertilized stands in terms of stand size, tree height and age. The stand variables were analyzed using t-tests. The stand variables did not differ significantly between treatments. Stands involved in the experiment had a mean size of 4.8 ha. The trees had a mean age of 16.4 years and a mean height of 5.2 m. The site productivity index (SI) ranges from 22-30 (*Table 1*).

Table 1. Stand data of stands included in the study. Stand treatment, mean stand size, mean tree age, mean standing volume, mean tree height, mean diameter at breast height (with associated standard deviation (SD)) and range of site productivity index.

Treatment	Area (ha)	Age	Volume (m <sup>3</sup> )	Height (m)	Diameter (cm)	SI
Fertilized stands	3.5 (3.5)	14.1 (5.9)	15.2 (11.6)	4.5 (1.8)	4.9 (2.0)	22-30
Control stands	6.1 (5.7)	18.7 (4.6)	26.3 (22.5)	5.9 (1.3)	6.0 (1.1)	24-30

#### **Insect sampling**

Within each stand, three large flight intercept traps (IBL2-trap) (see, Stenbacka *et al.* (2010) and Pettersson *et al.* (2007)) were set up May 8-11, 2013, i.e. one year after fertilization. The traps were attached approximately 25 m from the center of the stand with one trap to the south, one to the northwest and on to the northeast from the central point. The traps are triangular shaped with a semitransparent plastic flight intercept of approximate  $0.35 \text{ m}^2$ . A vial filled with 50 percent propylene glycol and some detergent collected the insects. The traps were active during the vegetation period and were emptied in September 2013. Beetles were sorted out and determined to species by the expert entomologist Bengt Andersson.

#### **Classification into functional groups**

Beetles caught by the IBL-traps were categorized into functional groups based on their nutritional ecology. The classification was based on existing literature and expert opinions, see e.g. Johansson *et al.* (2007). Functional groups used in this experiment were "herbivores" (species that feed on living vegetable tissue), "predators" (predators and ectoparasitoids), "wood-borers" (species that bore into the sapwood), "cambium consumers" (cambium and phloem consumers and consumers of cortex on living trees), "fungivores" (mycetophagous species), "detritus feeders" (necrophagous saproxylic species and coprophagous and ant associated species) and "insufficient knowledge" (species that could not be categorized into a functional group due to insufficient knowledge). Some beetles were classified into multiple functional groups (e.g. *Phyllobius argentatus* was classified as both cambium consumer and

herbivore). Beetles classified in multiple groups were later counted as member in more than one functional group in the data analyses of functional groups. Beetles in the insufficient knowledge group were included in the analyses of total abundance and total species richness but excluded when analyzing functional groups.

#### Data analysis

The data was analyzed in terms of species abundance and species richness both as total and for the different functional groups. The statistical analyses of the fertilization effects on abundance and species richness was carried out using parametric tests (ANOVA). Log- transformations were used to homogenize variances in groups that did not fulfill the Shaprio-Wilk W test for goodness-of-fit for normal distributions. Two groups (species richness of wood-borers and fungivores) were analyzed using the nonparametric Wilcoxon exact test because they did not fulfill the requirements for normal distribution even after transformations. The analyses were performed using the statistical program JMP PRO 12.1.0 (© 2015 SAS Institute Inc.).

#### Results

The IBL-traps catches resulted in a total of 7318 beetles of 356 different species. The most abundant species were *Dryocoetes autographus* (13.4 % of the total catch). *Crypturgus cinereus* (8.1 %) and *Quedius mesomelinus* (6.3 %). The most abundant functional group was cambium consumers (33.3 % of the total catch) followed by fungivores (28.5 %), predators (19.9 %), detritus feeders (7.0 %), wood-borers (6.8 %) and herbivores (4.5 %). The most species rich functional group was predators (93 different species) followed by fungivores (85), cambium consumers (31), detritus feeders (28), herbivores (24) and wood-borers (20). The overall tendency for all groups was that both abundance and species richness was higher in fertilized stands. However, no significant ( $p \le 0.050$ ) difference was found between fertilized and control stands for neither total abundance (p = 0.088) nor total species richness (p = 0.074). By contrast, significant differences was found for three functional groups; abundance of herbivores (p = 0.036), species richness of herbivores (p = 0.039) (*Table 2*).

Table 2. Mean abundance and mean species richness with the associated standard error of themean (SE), difference in percent between treatment means, data transformation, statistical testthat was performed and corresponding test score of beetles categorized into functional groups.Functional groupsFertilized standsControl standsDifference toTransforStatisticalScore

	(n=11)	(n=11)	Control %	mation*	test	(P-value)
Abundance						
All	400.3 (65.5)	265.0 (22.9)	+51	Log.	Anova	0.0879
Herbivores	17.1 (3.4)	9.2 (2.4)	+86	Log.	Anova	0.0364
Predators	67.1 (7.5)	48.6 (4.4)	+38		Anova	0.0465
Cambium consumers	136.0 (47.9)	57.8 (13.5)	+135	Log.	Anova	0.1478
Wood-borers	21.8 (3.1)	17.5 (2.0)	+25	Log.	Anova	0.3525
Fungivores	90.5 (18.4)	75.3 (9.0)	+20	Log.	Anova	0.5950
Detritus feeders	22.5 (4.0)	18.2 (3.0)	+24	Log.	Anova	0.5263
Species richness						
Âll	81.9 (6.8)	66.6 (4.4)	+23		Anova	0.0739
Herbivores	5.6 (0.7)	3.5 (0.6)	+60		Anova	0.0396
Predators	18.5 (1.9)	14.9 (1.4)	+24	Log.	Anova	0.1804
Cambium consumers	8.3 (0.8)	6.3 (0.5)	+32	-	Anova	0.0392
Wood-borers	4.5 (0.5)	3.9 (0.3)	+15		Wilcoxon**	0.7441
Fungivores	26.2 (2.3)	22.0 (1.2)	+19		Wilcoxon**	0.1066
Detritus Feeders	9.0 (0.9)	6.7 (0.7)	+34		Anova	0.0627

\* Natural logarithm transformation was performed to homogenize variance in groups that did not fulfill the requirements for normal distributions.

\*\* The nonparametric Wilcoxon exact test was used in groups that did not fulfill the requirements for normal distribution even after transformation.

Roughly, 60 % (4403) of all caught beetles were caught in fertilized stands and 40 % (2915) were caught in control stands. The overall most abundant species, *Dryocoetes autographus*, was the most abundant species in both control stands and fertilized stands but the total number was almost three times higher in fertilized stands (*Figure 1*). All but one functional group had the same species as most abundant in both treatments. The exception was within the group of detritus feeders where *Aphodius rufipes* was most abundant in control stands and *Tachinus laticollis* was most abundant in fertilized stands. The species that had the greatest proportional difference between treatments (when only counting species that had a total number larger than five and was represented in both treatments) were *Pityogenes chalcographus* (88.6 %) and *Cyphon coarctatus* (86.7 %), where *Pityogenes chalcographus* and *Pityophthorus micrographus* belong to the cambium consumer group and *Cyphon coarctatus* represents both detritus feeders and predators. There were 185 species that were only present in one treatment. The most numerous among them were *Stephostethus rugicollis*, belonging to the fungivores, with a total of sixteen beetles caught in six different fertilized stands and none in control stands.

Fertilized stands (n=11)

Control stands (n=11)





*Figure 1*. Rank-abundance diagrams showing the ten highest mean abundance of caught beetle species across stands divided into treatment and functional groups. The error bars represents  $\pm$  1 SE. The highest mean abundance has the highest rank (leftmost stack).

### Discussion

All functional groups of beetles included in this study showed a tendency for greater mean value of both abundance and species richness in stands that had been fertilized than in stands that served as control. Despite the overall greater mean values in the fertilized stands, the statistical test did not find a significant difference between treatments when analyzing total abundance and total species richness. However, the p-values were marginally significant, indicating a trend towards a positive beetle response to fertilization. High variance in mean values between stands could have reduced the power in the analyses and potentially explain the

lack of a general significant response in the beetle community to fertilization. It is possibly the higher productivity derived from fertilization that causes the tendency for the general increase in abundance and species richness. The relationship between productivity and species richness is of fundamental importance to understanding of the underlying elements of biodiversity. The productivity-species richness relationship has attracted much attention and been studied for many years. Yet the relationship is heavily debated and remains controversial among ecologists worldwide (Mittelbach *et al.*, 2001). Most studies addressing the relationship have so far focused on terrestrial plants (Mittelbach *et al.*, 2001) and relationship patterns seem to be scale dependent and varying with what type of biome that is studied (Waide *et al.*, 1999). Srivastava and Lawton (1998) tested the "More Individuals Hypothesis" by studying insect communities in water-filled tree holes with varying levels of productivity. According to the hypothesis, sites with high productivity can support higher total abundance. Species richness is a function of total abundance, and thereby also a function of productivity. Their result suggested that more productive sites had higher species richness but the abundance did not differ between sites with different productivity.

Herbivorous beetles are among the first species that are affected by an alteration in nutrient availability from a bottom-up perspective in an ecosystem, see e.g. (Chen et al., 2010; Vince et al., 1981). The higher abundance and species richness of herbivorous beetles in fertilized stands seen in this study could possibly be explained by the direct effect of enhanced nutritional quality in the consumed vegetative tissue caused by the fertilization. Several studies show a positive correlation between insect performance and the nitrogen content in consumed food, e.g. (Myers & Post, 1981; Mattson, 1980). Another possible effect could be a fertilization caused reduction in trees defense system which makes the needles more palatable to needle consuming beetles (Chapin et al., 1987; Vitousek et al., 1985), or a combination of both these effects. However, herbivorous beetles are not restricted to foraging on trees. As the traps catches all flying insects, beetles that primarily feed on field and shrub-layer vegetation are also counted. Previous studies have shown that ground vegetation is also affected by nitrogen fertilization (Hedwall et al., 2010). Thus, compositional changes and increased nutrient content in ground vegetation on the forest floor could potentially explain the higher abundance of some herbivorous beetles. In addition to the beetle catches in the present study, branches from trees within the stands were cut off and examined to quantify insect damage. The branches showed none or very few traces of feeding damage (unpublished data), indicating that the positive effects on abundance and species richness are primarily due to beetle species that feeds on other vegetative tissues than needles. The direct fertilization effect on nutrient content in vegetative tissues may be why herbivorous beetles do so well even after this relatively short time after fertilization. The result contradicts the conclusion of Kyto et al. (1996), a review that summarize 79 articles dealing with the effects of soil fertilization on phytophagous insects and mites. They conclude that population-level effects on herbivorous insect are variable and mostly negative. They explain the negative effect with top-down processes like predation and parasitism. The combined results of Edenius et al. (2011) and Edenius et al. (2012) indicate that the effect of forest fertilization in young stands of Norway spruce may alter the food resource for forest living birds by alterations in composition and abundance in different arthropod groups (mainly Hemiptera), and that these changes may be driven by alteration in nutrient quality in leaves and needles. Effects of fertilization can be expected in multiple tropic levels, starting at the bottom of a food web and affect higher tropic levels with a certain time

lag (Chen *et al.*, 2010). It is possible that top-down processes like predation from e.g. beetles and/or birds would push the abundance and species richness of herbivorous beetles back to its original state if the effect were monitored over a longer time period. The higher abundance of herbivorous beetles in fertilized stands seen in this study may just be a temporal pattern after one fertilization event. The abundance of herbivorous beetles may in a later state decrease with increasing predator abundance. If nitrogen fertilizer are applied every second year, which is the planned fertilization frequency in this study site, and also suggested by Bergh et al. (2008), we might observe a cyclic frequency pattern of herbivorous beetles cascading to higher trophic levels. Another possibility is that the frequency rate of applied nitrogen causes a stabilized high abundance of herbivorous beetles that instead of being bottom-up controlled are top-down regulated by predation. The difference in abundance among herbivores between treatments are primary due to the higher abundance of Serica brunnea and Meligethes denticulatus, these species occurred in much elevated numbers in fertilized stands (Figure 1). Serica brunnea thrives in sandy soil on roots of different grasses, bushes and conifers (Koch et al., 1989). The higher abundance is likely driven by changes in the ground vegetation in fertilized stands. Meligethes denticulatus primarily lives on flowering shrubs and develops in Rubus species, most preferably in Rubus caesius (Koch et al., 1989). Rubus species are likely to be positively affected by fertilization and may be one reason to the higher abundance of Meligethes *denticulatus* seen in fertilized stands. None of these species feeds primarily on needles which thus suggest that the higher abundance and species richness among herbivores likely is driven by the enriched nutrient content in other vegetable tissues than needles. The difference in species richness is due to several small or single-individual observations of herbivorous beetles in fertilized stands.

Predatory beetles were significantly more abundant in fertilized stands and may act as topdown regulating forces on lower trophic level insects as suggested by Kyto et al. (1996). The abundance of most low trophic level insects is probably higher in fertilized stands. These insects may act as an excess of available resources to predators and can possibly explain the higher number of predatory beetles. Previous fertilization studies have shown that bottom-up effects first enhanced herbivore population growth before natural enemy impacts are realized, e.g. (Forkner & Hunter, 2000; Vince et al., 1981). The immediate response on predator beetle abundance is however noteworthy, as mentioned, responses to alteration in nutrient availability in higher trophic levels could be expected after a time lag. Ostfeld and Keesing (2000) emphasizes that the time required for a predator species to react to a bottom-up influenced increase in available prey is determined by the predators life history, population growth and dispersal rate. Furthermore, if predators respond to such bottom-up caused increase in prey availability, there is a certain time lag between the state when bottom-up forces determines the herbivore abundance and the state when top-down forces makes an impact on the herbivore abundance. Another factor that might be applicable here is seasonal shifting, a seasonal shift between strong bottom-up forces from the start of the summer and eventually strong top-down forces later in the season. Gratton and Denno (2003) studied populations of planthoppers in fertilized plots of Spartina (a genus in the grass family) and found that the planthopper population initially was promoted by bottom-up forces and later in the season experienced a shift to strong top-down suppression. Unfortunately, traps in the present study were active during the whole summer (May to September) and were emptied only once, thus seasonal variation could not be addressed. In the present study, the predator species that seem to have

been affected the most from fertilization is *Bibloporus bicolor* (*Figure 1*). *Bibloporus bicolor* is a saproxylic species and lives under bark and in decayed wood (Koch *et al.*, 1989). *Bibloporus bicolor* was found in traps from all stands and it seems like none of the fertilized stands were particularly favorable. *Bibloporus bicolor* is possibly favored by higher abundance of potential prey species.

Among cambium consuming beetles, there was significant difference in terms of species richness between treatments. Cambium consumers generally live on dead trees and branches and are usually favored by newly cut wood (Sauvard, 2004). Therefore, the presence of cambium consuming beetles are strongly related to the availability of slash and dead wood after thinning and pre-commercial thinning. In one stand included in this study, precommercial thinning was performed in May 2013, thus during the sampling period. Traps within that stand caught over one quarter (26.2 %) of all caught cambium consuming beetles and may thereby interfere with the results of abundance of cambium consuming beetles. However, the number of different cambium consuming species caught within that stand was not particularly high (10 different species were caught in that stand and the mean number of different species caught across all stands was 7.3). Because only one stand was pre-commercial thinned, the effect could not be included as a co-variable in the analysis. Effects on cambium consumers are perhaps of particularly interest to forestry and forest owners, that is because this group contains species that are considered potential pest species which can cause economic damage to the forest. Even though the pre-commercial thinning has interfered with the results, the quick and massive response in some species is somewhat noteworthy. It was in particular three species that showed a positive response: Dryocoetes autographus, Crypturgus cinereus and Pityogenes chalcographus (Figure 1). Previous studies suggest that Pityogenes chalcographus and Ips typographus compete when coexisting and that Pityogenes chalcographus then is restricted to parts of trees where the bark is too thin for Ips typographus (Gothlin et al., 2000). Ips typographus was also more abundant in traps from fertilized stands and showed a high proportional difference in number between treatments (48 out of the total 54 individuals were caught in fertilized stands). However, these species are bark beetles that generally thrive in more mature forests, especially *Ips typographus*. Bark beetles generally have a high dispersal range and can fly quite far distances if they are attracted by the smell of newly cut wood, wind thrown trees or timber piles (Nilssen, 1984). These are all factors that may have led to the high number of bark beetle species caught in the traps. It is most unlikely that there is a difference in species richness among cambium consuming beetles derived from fertilization.

The mean abundance and mean species richness of all functional groups showed a tendency for being higher in fertilized stands, indicating that the beetle community, at least after one season, is favored by fertilization. The effects may be different when the same site is monitored the second or third time. The time from when the fertilization was added to the time when the traps was emptied may have been too short for some species or some functional groups to react to the enhanced nutrition availability. This study was performed at stand scale, the same short term effect can be expected even on larger scale application. The long term effects are still uncertain and continued monitoring of this experiment site should be done before intensive fertilization programs are carried out on larger scale. This study has focused on common beetles species to try illuminate fertilization responses on the beetle community as whole. Since intensive forest fertilization programs most likely will be used in forests that already are heavily affected by forestry and in generally have small conservation values, species of special conservation concern, like red-listed species, are not addressed.

#### Conclusions

My results suggest that there is a trend for higher abundance and higher species richness in fertilized than in unfertilized stands. The fertilization effect seems to vary among functional groups of beetles. The effect of fertilization is most pronounced for herbivorous beetles, especially species foraging on other vegetation than trees are favored. The effect is likely driven by the enhanced nutrient availability. The higher abundance of predatory beetles could be a response to a greater number of potential prey species. This first result from the ongoing long term experiment indicates that intensive forest fertilization have potential to increase biomass production in already exploited forests without having negative implications on the beetle community. Continued monitoring of these sites should be done before this forest measure is applied on national scale. Future results from this site can potentially lead to the conclusion if intensive forest measure can increase biomass production without having negative effects on biodiversity and interfering with the national environmental goals.

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