



# **Microalgae and ash as biofertilizer for spruce and pine seedlings.**

A quantitative study exploring the usability of microalgae and wood ash as an alternative to conventional fertilizer.

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## Abstract

The growing demand for sustainable forest management is higher than ever. This has attracted interest in the opportunity to use microalgae and ash as biofertilizer. Today's commonly used conventional fertilizer comes with an environmental cost in the manufacturing process because of its energy intensive process. This study investigates the possibility of using microalgae and/or ash fertilizer in nurseries as an alternative to traditional conventional fertilizer. Growth of diameter and height in Norwegian spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) seedlings was used as quantitative variables to assess treatment effects. The sample was divided into seven treatments with varying compositions of biofertilizer, containing microalgae and ash. These seven treatments were compared with two control treatments containing no-fertilizer and conventional fertilizer. The results indicate that over a short time (10 weeks), height and diameter in the biofertilized treatments do not show a significant difference compared to conventional fertilized treatments. These findings suggest that biofertilizer can perform comparably to conventional fertilizers in terms of promoting growth at a seedling level in the short-term. Further research is needed to evaluate their long-term efficacy and sustainability.

## Keyword

Algae, wood ash, nitrogen, potassium, tree growth, sustainable management, *Pinus Sylvestris*, *Picea Abies*

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## Abbreviations

Ca	Calcium
K	Potassium
Mg	Magnesium
NPK	Nitrogen, Phosphorus, Potassium
N	Nitrogen
P	Phosphorus
PBR	Photobioreactor
S	Sulfur
Spruce	<i>Picea abies</i>
Pine	<i>Pinus sylvestris</i>

# 1. Introduction

Today there is a need for green transition to address environmental challenges, where forestry plays an important role. While other sectors, such as energy and agriculture, have begun adopting renewable and eco-friendly technologies, forestry also has the potential to implement more sustainable practices. Forest nurseries play a crucial role in producing vigorous seedlings that are essential for successful forest regeneration. The choice of fertilization strategy in nursery production can significantly influence both seedling quality and the overall environmental impact of forest management practices.

Since the early 19<sup>th</sup> century, conventional fertilizers rich in nitrogen (N) have been the dominant method to promote seedling development (Lindkvist et al. 2011; Lucander et al. 2021). However, these fertilizers are energy-intensive to produce and can contribute to global emission of CO<sub>2</sub> (Ishaq & Crawford 2024) and environmental problems such as nutrient runoff and biodiversity loss. In contrast, biofertilizers such as wood ash and microalgae can be seen as a more sustainable alternative. The process of producing microalgae is low-cost and eco-friendly alternative (Lage et al. 2021a).

In the beginning of 2025, the Swedish Forest Agency received a government mandate to promote increased and sustainable forest fertilization for the country. The assignment includes evaluating different fertilization systems and their effects on forest growth, carbon storage, biodiversity, and ecosystem services, as well as proposing suitable policy instruments and long-term strategies (Regeringsbrev LI2023/03423 2025).

In line with this national initiative, this project investigates the use of blue-green microalgae, alone and in combination with wood ash, as an alternative for sustainable fertilizer for spruce and pine seedlings. By exploring different bio-based fertilization treatments, the study contributes to the development of more climate adaptive and ecologically responsible practices in Swedish forestry.

## 1.1 Aim

The purpose of the study is to investigate the possibility of using microalgae and/or ash fertilizer in nurseries as an alternative to traditional conventional fertilizer.

### 1.1.1 Research question

- How is growth of diameter and height in spruce and pine seedlings affected by the application of microalgae and ash based biofertilizer?
- How does the growth of diameter and height in spruce and pine seedlings differ when applying microalgae and ash biofertilizer compared to conventional fertilizer?



## 2. Background

To understand whether microalgae and wood ash can act as an alternative to conventional fertilizer in forest nurseries, it is important to investigate the nutritional needs of trees, the characteristics of different fertilizers, and how they influence seedling growth. The background section provides the foundational understanding needed to assess both conventional and bio-based fertilization methods.

### 2.1 Tree nutrition and growth requirement

Several factors influence the growth and survival of tree seedlings. Among these, the most fundamental requirements are access to light (energy), water and nutrition (Wennström et al. 2016).

In total, 14 essential mineral nutrients are required for plant growth, and these are commonly categorized into macronutrients and micronutrients depending on the quantities needed by the plant (Hawkins, 2011; Evert & Eichorn 2013). The six macronutrients - N, P, Ca, Mg, K and S – play a critical role in several process in the plant (Johnson et al. 2022).

In particular, the concentration with  $K^+$ -ions in the soil solution between ground and plant is critical for the roots ability to take up nutrients (Johnson et al. 2022). Deficiency in any of these nutrients can limit growth. A deficiency of one nutrient cannot be compensated by an excess of another (Hawkins 2011).

#### 2.1.1 Nitrogen

It is crucial for the plant how much N is available. However, there is difference between organic N and inorganic N. Organic N is found in composted organic material, as dead vegetation. This N is bound to C in organic compounds such as proteins, amino acids, and humic substances. For the plants to be able to assimilate the organic N needs to be broken down by microorganisms through mineralization. However, Näsholm et al. (1998) demonstrated that plants can take up organic N as amino acids (Näsholm et al. 1998).

In contrast, inorganic N exists as free ions for example ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ). The inorganic N is readily available for the plants. This gives a fast and direct uptake to plants (Nasholm et al. 2000).

Furthermore, spruce needle nutrient concentrations must reach certain critical thresholds to maintain optimal growth. For N, this critical concentration has been identified as approximately 20.3 mg/g in juvenile needles (Ericsson et al. 1994).

### 2.1.2 Potassium

One of the most important macronutrients for plants is potassium (K) and is important for the ability to photosynthesize (Johnson et al. 2022). Once absorbed,  $\text{K}^+$ -ions are transported through the whole plant in the cellular fluid, where they help regulate salt concentration. This regulates the osmotic potential and maintains the osmotic pressure in the cells (Johnson et al. 2022).

Moreover, K also is essential for energy transfer within the plant and regulates the opening- and closing of the stomata, which is vital for maintaining water losses at as low level as possible (Johnson et al. 2022).

In addition to its role in osmotic regulation, K also contributes significantly to the plant's tolerance against abiotic stresses such as drought, salinity, and extreme temperature. Research has shown that K supports ion homeostasis, stabilizes cellular structures, and activates various enzymes that are crucial for plant metabolism. (Johnson et al. 2022).

## 2.2 Conventional fertilizer

Conventional fertilizer contains inorganic N, which makes the N easily available for the plants (Nasholm et al. 2000). Production of commercial fertilizer started in the 20<sup>th</sup> century with the discovery of the Haber-Bosch method (Abdi Onsäter 2021). This process changed the whole global nourishment industry with its ability to convert N from the atmosphere to  $\text{NH}_3$ , that's the main component to produce commercial fertilizer (Elding 2025).

However, the process of producing conventional fertilizer is demanding, as it is both costly and energy-intensive (Ammar et al. 2022), and moreover it relies on fossil methane gas (natural gas) (Abdi Onsäter 2021).

### 2.2.1 Manufacturing process using the Haber-Bosch method.

Today, 80% of all ammonia is produced by the Haber-Bosch method, and 1918 Fritz Haber was awarded the Nobel Prize for his discovery of converting  $\text{N}_2$  from the atmosphere into ammonia (Elding 2025). In this method, N is extracted from the air, while the hydrogen gas is obtained from hydrocarbons such as natural gas

and naphtha. The process of synthesizing ammonia is made under high pressure and temperature. By reacting hydrogen and N under 200 atm and about 400 ° C in the presence of an iron-based catalyst. In the following stage, the synthesized gas is purified, compressed, and passed over catalysts to form ammonia, which then separated by cooling and condensation (Elding 2025).

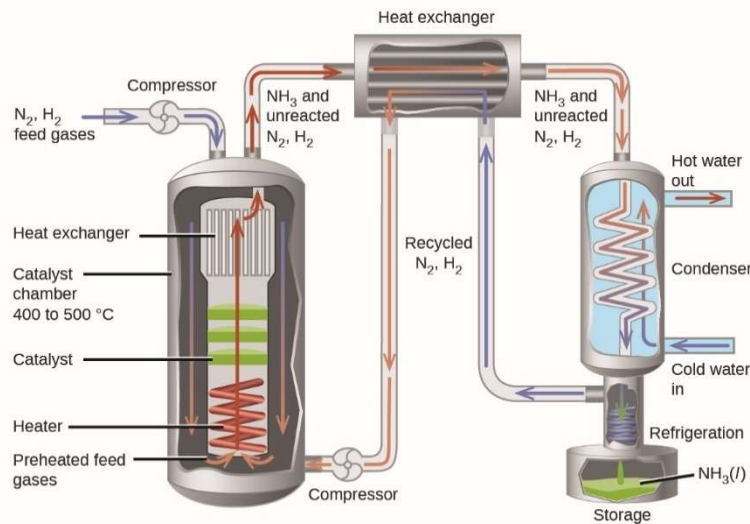


Figure 1 Manufacturing process of Haber-Bosch method (Moore et al. 2021)

### 2.2.2 Fertilization in plant-nursery

Plant nursery has been important for the development of Swedish forestry. The research around plants started in the 1800-century when the The Royal Swedish Academy of Agriculture and Forestry started (Andréasson Sjögren 2007). Currently the research involves many aspects of agriculture and forestry. Around the 1950-century the bare-root pant started showing up and nursery become a natural part in the Swedish forestry (Rytter 2007). In the early 1970<sup>th</sup> the nursery and forestry started using container-grown plants (Wennström et al. 2016).

For plants to grow successfully in nursery it's important with the correct light program, temperature, storage and fertilization. In the beginning of plant-nursery the main use of fertilizer was in powder form and the same amount for the whole season. Later on fertilizer became developed into liquid form, and that's today's most used form. It's easier to handle and you have the ability to adjust the amount of fertilizer to the plants optimal growth (Rytter 2007).

## 2.3 Biofertilizer

Biofertilizer has become an increasing interest as fertilizer in the areas of sustainable agriculture and forestry. In the present study, the two current types of biofertilizer are microalgae and wood ash.

### 2.3.1 Microalgae

Algae can be divided into macro- and microalgae, and they both have variety of use depending on which type of algae are used. Due to their ability to enhance soil nutrient content, microalgae have significant potential to be used as biofertilizers. (Ammar et al. 2022). Macroalgae are multicellular organisms, while microalgae are unicellular. Both types of algae are naturally found in aquatic environments such as oceans, rivers, ponds, and even wastewater from industry (da Rosa et al. 2023).

As fertilizers, microalgae consist of living organisms that improve plant nutrient uptake through natural biological processes. One group, the blue-green microalgae (cyanobacteria) has the ability to fix  $N_2$  from the atmosphere to a form that can be taken up by plant (Cao et al. 2023; Gonçalves et al. 2023). Microalgae also have significantly higher photosynthetic efficiency than terrestrial plants, allowing them to convert sunlight into biomass at much faster rates (Abdelfattah et al. 2023).

Although research on the use of microalgae in forestry is still limited, most existing studies focus on agricultural applications. Still, many of the insights gained may also be applicable to forest nurseries. Taking this together, the properties of microalgae make it environmentally friendly alternative to conventional fertilizers. (Ammar et al. 2022).

#### *Manufacturing process*

Microalgae have been increasingly used to detoxify various pollutants released from industrial, agricultural and domestic sectors. By taking up heavy metals and nutrients such as N and P from wastewater process of conventional purification methods, which can decrease the environmental impact from these processes (Abdelfattah et al. 2023).

To be used as biofertilizer, microalgae must go through several production steps, including cultivation, harvesting, and dehydration. Cultivation can take place in two main systems: open ponds and closed photobioreactors (PBR). A key difference between these two systems is that open ponds are more cost-effective, while PBRs offer a more controlled environment with significantly lower risk of contamination and having higher productivity. The choice between systems often depends on the intended application, production scale, and resource availability (Ammar et al. 2022; Gonçalves et al. 2023). One advantage of microalgae cultivation is the ability to take advantage of natural sunlight as a free energy source, significantly lowering the energy demand of the production process.

Once the microalgae have reached a high biomass concentration during cultivation, the next step is harvesting. In this step the microalgae are separated from the growth medium. Several methods are available, including flocculation, flotation, centrifugation, and filtration (Ammar et al. 2022). Each method has its advantages and limitations depending on the algal species, cell size, and desired product.

After harvesting, the biomass is dehydrated to extend shelf life and prevent spoilage. Common drying methods include spray drying, sun drying, and freeze drying (Ammar et al. 2022).

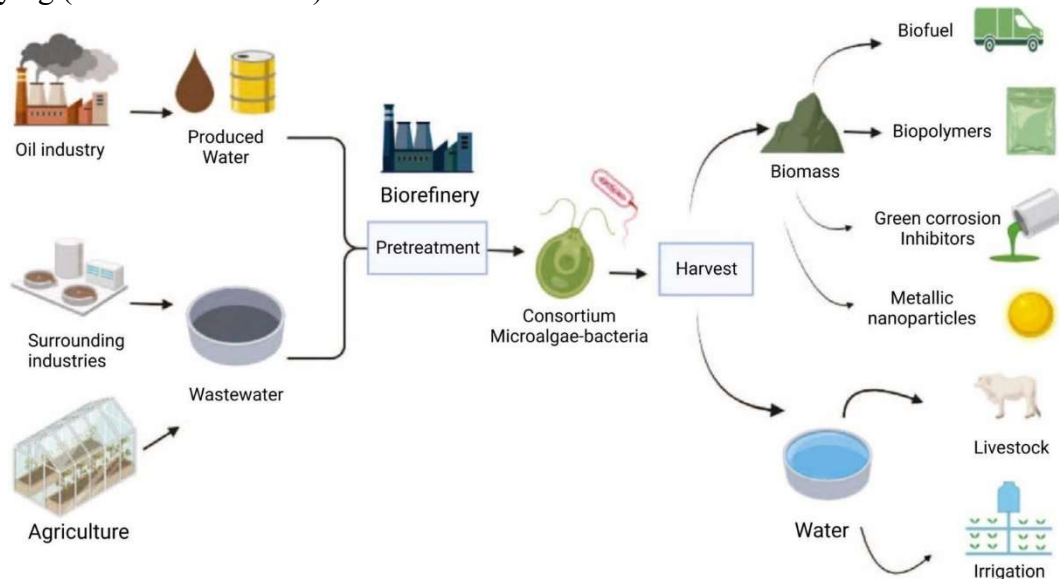


Figure 2 Manufacturing process of microalgae (Cavalcanti Pessoa et al. 2022).

### 2.3.2 Wood ash

Today's forestry and the harvest of biomass can cause forest soils to become more acidic. Ash includes the most common nutrients as K, Ca, P except N. The presence of K, Ca and P make the ash alkaline, which make the return of ash important for the whole pH- value of the forest soil (Reichel et al. 2013). In peat and nutrient rich soils the high value of pH in ash positively affect N availability for the plants. There is a risk that ash could slower the growth if its added to a N-poor soil (Jacobson et al. 2017).

Ash as a biofertilizer has been used for a long time and is a quite studied subject, for growing forests. But there's a knowledge gap in how ash affects plant nursery, and in particular when combined with microalgae.

#### *Manufacturing process*

Wood ash is a byproduct formed after combustion of bioproducts, for example when burning biofuels in the heating plants. The ash is mostly minerals that cannot be combusted, but it can depend on what type of wood that's combusted.

Before ash can be recycled back to the forest, it needs to go through an analysis in the lab to investigate the chemical composition. After the chemical investigation the ash can be mixed with organic materials if the ash misses important nutrients. When this is done, the ash is milled and sieved (Skogsforsk 2024).

## 3. Method

This research constitutes an empirical case study, grounded in the specific context of the Dåva combined heat and power facility, located outside Umeå in the north of Sweden. Dåva has two combined heat power plants “dåva 1” and “dåva 2”. The project of growing microalgae takes place in “dåva 1” (Lage et al. 2021b; Lage & Gentili 2023). The microalgae grew in open ponds using wastewater from Dåva (Umeå Energi) and from Umeå wastewater treatment plant (Vakin). Microalgae produce biomass by converting nutrients and pollutants, reducing emissions and treating wastewater (Blomquist Bergman et al. 2012).

### 3.1 Experimental design

#### 3.1.1 Microalgae preparation

The microalgae was harvested through centrifugation at Dåva where the algae was separated from the water. The harvested microalgae biomass was a polyculture consisting of several naturally occurring strains and contained approximately 44% C and 6.6% N. After harvest, the microalgae were frozen until the start of the application process.

Before application, the frozen microalgae were mixed with the peat and watered, to start the process of breaking down the microalgae so that they will release nutrients to be taken up by the seedlings. This microalgae biomass will be used in treatment 3.1-4.4 that is shown from Table 1.

#### 3.1.2 Ash preparation

The ash used for this study was prepared in two forms one pH adjusted and one not pH-adjusted, powder and liquid form. Powder form was used for treatment 4.1 and 4.2 (Table 1) and consist of pure ash that was not pH-adjusted but analyzed in the lab for K content. The powder was peppered in two concentrations of 120mg ash that consisted of 12 mg K for treatment 4.1, and 240 mg powder ash contained 24 mg K for treatment 4.2.

In liquid ash the pH was adjusted. The ash was diluted in deionized water, ca 26 g ash per liter water. When the ash is dissolved in the water the pH was adjusted with concentrated sulfuric acid to pH 6.

### 3.1.3 Seedling preparation

The seedling used in this study consisted of spruce and pine, received from Holmen Plantskola at Gideå. In total, 180 seedlings were included in the study, obtaining 90 individuals of each species. The seedlings were randomly assigned to nine different treatment groups, specified in Table 1. Each treatment group consisted of ten spruce and ten pine seedlings.

The seedlings were planted in individual pots filled with a standardized peat substrate. Depending on the treatment the microalgae biomass, with or without wood ash, or conventional fertilizer, was added to achieve the targeted nutrient concentrations according to Table 1. All experimental (preparation and monitoring) work was conducted in a controlled greenhouse environment to ensure consistent environmental conditions with a temperature of 20 °C

At the time of planting, treatments 4.3 - 4.4 received applications of liquid ash, while seedlings in treatment 2 were given their initial weekly dose of the conventional fertilizer ArGrow. To minimize spatial variability within the greenhouse, the position of each pot was randomized. Additionally, the placement of the seedlings was rotated weekly following a rotation plan to avoid potential environmental inequality in sunlight. This rotation coincided with the weekly growth measurements.

<b>Treatment</b>	<b>Type</b>	<b>Description</b>
<b>1.</b>	Negative control	Peat substrate only (no fertilization).
<b>2.</b>	Positive control	Fertilized with the standard conventional fertilizer used in forest nurseries (arGrow) 60 mg/N per plant
<b>3.1.</b>	Microalgae biofertilizer	Fertilized with microalgae extract 60mg/N per plant
<b>3.2.</b>	Microalgae biofertilizer	Fertilized with microalgae extract 90mg/N per plant
<b>3.3.</b>	Microalgae biofertilizer	Fertilized with microalgae extract 120mg/N per plant
<b>4.1.</b>	Microalgae biofertilizer + ash	Fertilized with a combination of microalgae extract 60mg/N per plant + 120 mg ash (ca 12 mg K)
<b>4.2.</b>	Microalgae biofertilizer + ash	Fertilized with a combination of microalgae extract 60mg/N per plant + 240 mg ash (ca 24 mg K)

4.3.	Microalgae biofertilizer + ash	Fertilized with a combination of microalgae extract 60mg/N per plant + 120 mg ash (ca 12 mg K) pH adjusted
4.4.	Microalgae biofertilizer + ash	Fertilized with a combination of microalgae extract 60mg/N per plant + 240 mg ash (ca 24 mg K) pH adjusted

*Table 1 Overview of the different treatment groups*

## 3.2 Conventional fertilization

Conventional fertilizer was prepared every week for the fertilization of the plants in treatment 2 (Table 1). The conventional fertilizer, consisting of ArGrow (Arevo), was diluted in tap water to reach the right concentration. ArGrow is a common N fertilizer used in plant nurseries in Sweden. The seedlings in treatment 2 received the fertilizer weekly through a pipette.

Date	Amount of fertilizer
7 February	10 ml/plant
14 February	5 ml/plant
20 February	5 ml/plant
28 February	5 ml/plant
6 Mars	6 ml/plant
13 Mars	6 ml/plant
20 Mars	6 ml/plant
28 Mars	7 ml/plant
4 April	10 ml/plant
11 April	10 ml/plant

*Table 2 amount of conventional fertilizer that's given to the plants in treatment 2.*

## 3.3 Data collection

The data was weekly collected over 10 weeks. During this period the measurements of height and diameter were taken and documented in an Excel file to facilitate the data analysis later on.



### 3.3.1 Height and diameter measurement

Height measurements were taken with a ruler. The ruler was placed in the soil along the stem on the plant, the value was then taken from the bottom of the stem to the shoot.

The diameter measurements were taken with a digital caliper. The caliper was placed at the bottom on the stem.

## 3.4 Data analysis

To compare the measurements from the different treatments the difference in growth over 10 weeks was analyzed. Because of the different normal distribution between height and diameter for spruce and pine seedlings, different analyses were needed. Kruskal Wallis test was used for height, where the data were not normally distributed, with a Pos Hoc Dunn-test necessary for both pine and spruce to see if there was any significant difference. One way ANOVA was used for the diameter data that were normally distributed, with a following Tukey test for both pine and spruce to see if there occurred any significant difference. All data analysis was made in R-studio.

## 3.5 Limitations

For this study to be possible some limitations were necessary. The study was selected to only measure spruce and pine - seedling, for 10 weeks. Also, a limitation for the measurements was implemented, to only measure height and diameter.

## 4. Results

### 4.1 Height

The result for the height measurement shows that for both pine and spruce unfertilized Treatment 1 has grown the least. The greatest variation for the different treatments is found for spruce, as shown in figure 3. Of the treatment with only microalgae as biofertilizer Treatment 3.1 (Fertilized with microalgae extract 60mg/N per plant) gives the highest height growth.

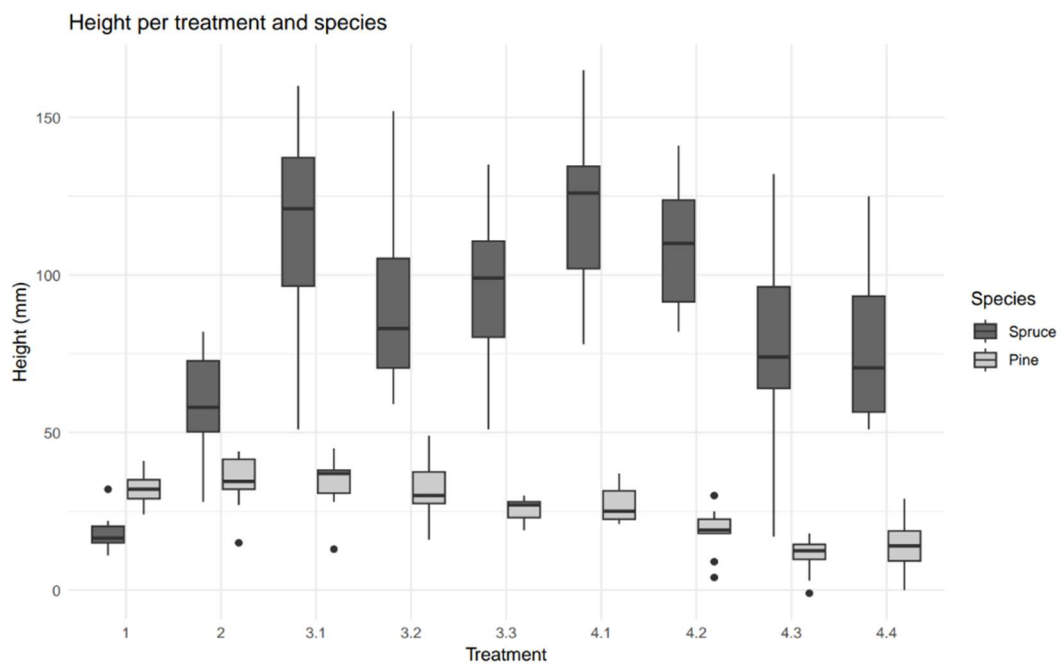


Figure 3 Height per species and treatment.

For  $p\text{-value} < 0,05$  the result shows a significant difference between 14 combinations for spruce and 15 combinations for pine seedlings.

Spruce		Pine	
Compared treatments	P-value	Compared treatments	P-value
1 - 3.1	$5.3 \cdot 10^{-6}$	1 - 4.2	$1.1 \cdot 10^{-2}$
1 - 3.2	$1.3 \cdot 10^{-3}$	1 - 4.3	$2.7 \cdot 10^{-4}$
1 - 3.3	$6.4 \cdot 10^{-4}$	1 - 4.4	$1.7 \cdot 10^{-3}$
1 - 4.1	$1.3 \cdot 10^{-6}$	2 - 4.2	$2.6 \cdot 10^{-3}$
1 - 4.2	$1.1 \cdot 10^{-5}$	2 - 4.3	$4.1 \cdot 10^{-5}$
1 - 4.3	$1.3 \cdot 10^{-2}$	2 - 4.4	$3.6 \cdot 10^{-4}$
1 - 4.4	$2.1 \cdot 10^{-2}$	3.1 - 4.2	$2.4 \cdot 10^{-3}$
2 - 3.1	$1.9 \cdot 10^{-3}$	3.1 - 4.3	$4.8 \cdot 10^{-5}$
2 - 3.3	$4.8 \cdot 10^{-2}$	3.1 - 4.4	$2.8 \cdot 10^{-4}$
2 - 4.1	$7.5 \cdot 10^{-4}$	3.2 - 4.2	$1.9 \cdot 10^{-2}$
2 - 4.2	$3.8 \cdot 10^{-3}$	3.2 - 4.3	$3.1 \cdot 10^{-4}$
3.1 - 4.4	$4.4 \cdot 10^{-2}$	3.2 - 4.4	$3.0 \cdot 10^{-3}$
4.1 - 4.3	$3.2 \cdot 10^{-2}$	3.3 - 4.3	$1.6 \cdot 10^{-2}$
4.1 - 4.4	$1.9 \cdot 10^{-2}$	4.1 - 4.3	$4.1 \cdot 10^{-3}$
		4.1 - 4.4	$2.5 \cdot 10^{-2}$

Table 3 P-value for pine and spruce seedlings in height

## 4.2 Diameter

The result in figure 4 shows the differences in diameter between pine and spruce plotted on a scale in mm. For pine, the biggest difference is shown between treatment 1 (un-fertilized) and treatment 4.1 (Fertilized with a combination of microalgae extract 60mg/N per plant + 120 mg ash (ca 12 mg K))

For spruce the biggest difference is shown between treatment 1 (un-fertilized) and treatment 3.1 (Fertilized with microalgae extract 60mg/N per plant) and 4.1 (Fertilized with a combination of microalgae extract 60mg/N per plant + 120 mg ash (ca 12 mg K)).

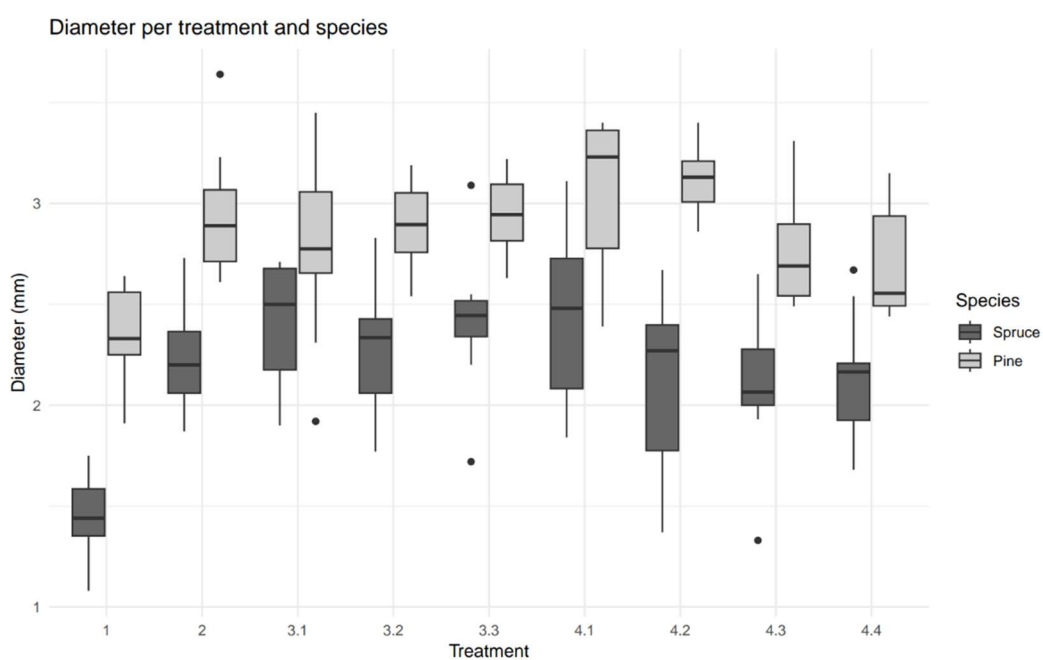


Figure 4 Diameter per species and treatment.

For  $p\text{-value} < 0.05$  the result shows a significant difference between 8 combinations for spruce and 7 combinations for pine seedlings.

Spruce		Pine	
Compared treatments	P-value	Compared treatments	P-value
1 - 2	$5.3 \cdot 10^{-5}$	1 - 2	$7.0 \cdot 10^{-4}$
1 - 3.1	$8.3 \cdot 10^{-7}$	1 - 3.1	$4.1 \cdot 10^{-2}$
1 - 3.2	$3.9 \cdot 10^{-5}$	1 - 3.2	$3.7 \cdot 10^{-3}$
1 - 3.3	$6.1 \cdot 10^{-7}$	1 - 3.3	$8.7 \cdot 10^{-4}$
1 - 4.1	$2.3 \cdot 10^{-7}$	1 - 4.1	$1.9 \cdot 10^{-5}$
1 - 4.2	$9.4 \cdot 10^{-4}$	1 - 4.2	$3.1 \cdot 10^{-6}$
1 - 4.3	$1.7 \cdot 10^{-3}$	4.2 - 4.4	$3.2 \cdot 10^{-2}$
1 - 4.4	$1.2 \cdot 10^{-3}$		

Table 4 P-value for pine and spruce seedlings in diameter

## 5. Discussion

Conventional fertilizer was one of the biggest revolutions in history. When Fritz Harber and Carl Bosch found a way to fix N from the atmosphere with H and convert it into ammonia (Elding 2025). One drawback of this method is that hydrogen gas often comes from nature gas that's a fossil resource. The Haber-Bosch method is very demanding for the climate and emits a lot of CO<sup>2</sup> (Ishaq & Crawford 2024). The whole world is in need of a green future, and a more environmentally friendly way of growing seedlings is a start. Instead of using the Haber-Bosch method to produce N fertilizer, microalgae can absorb nutrients thus producing fertilizers in a natural way (Cao et al. 2023; Gonçalves et al. 2023).

A distinctive difference between the quantitative variables is, that the diameter of pine has a greater growth than spruce, and of the height for spruce has a greater growth than pine. In following discussion, it is adopted that the same assumption is applied for diameter and height, if nothing else is mentioned.

What we can see for spruce, there is a significant difference between negative control (treatment 1) and all other treatments, except positive control (treatment 2), in the quantitative variable of height. In contrast, pine shows this only for treatments 4.1-4.3. This indicates that for spruce seedling microalgae can be used as fertilizer to a similar extent as conventional fertilizer in nursery because there is no significant difference between positive control and the different microalgae treatments (3.1-4.4). Treatment 3.1, 3.3, 4.1 and 4.2 also show that there is a significant higher growth in height when compared to the positive control for spruce seedlings, which further supports the assumption that microalgae-based fertilizer, alone or in combination with ash, may not only match but even exceed the performance of conventional fertilizer.

The results show some interesting differences between the treatments for both spruce and pine. We can see in figure 3 that treatment 3.1 and 4.1 for spruce has the most pronounced height and diameter growth after 10 weeks. Both have a concentration of 60 mg/l N, and for treatment 4.1 also 120 mg ash (ca 12 mg K). While the findings are promising, it would be an overstatement to generalize these results without further long-term trials. We can speculate that the lower results of treatment 3.2 and 3.3 is due to an excess of N, even if there is no lack in other nutrients (Wang et al. 2021). Overapplication of N can be an explanation for the lower results even for pine in treatment 3.2 and 3.3 both for height and diameter.

There is also a risk that the results are affected by external factors like light availability. To minimize this risk, we had a rotation program for the plants so every week we rotated the seedling in a specific pattern.

A general observation for the results in treatments with a combination of ash and microalgae shows that the growth is higher in treatments 4.1 and 4.2 compared to the treatments that is pH-adjusted, treatment 4.3 and 4.4. In treatment 4.1 with the concentration 60 mg N + 12 mg K has the greatest growth compared to 4.2 with the concentration of 60 mg N + 24 mg K. One possible explanation of this result could be that treatment 4.3 and 4.4 got less liquid concentration of K then what was planned, because of an miscalculation.

Treatment 4.3 and 4.4 (microalgae with ash pH adjusted) shows low results in both height and diameter. One explanation of this could be the adjusted pH for these treatments. Because of the low pH-value in these two treatments the microbial activity reduces and due to that the mineralization slows down. This will make the availability of N low in the soil solution (Barrow & Hartemink 2023).

Even though the conventional fertilizer is applied weakly, to simulate the current standard method used in plant nursery, the diameter growth was not significantly higher than in the treatments where microalgae-based fertilizer was applied only once at planting. This suggests that microalgae-based fertilization could reduce the amount of practical handling and management routines in plant nurseries. In a large-scale perspective, this may contribute to both economic savings and more efficient resource use, since fewer applications are needed while still achieving effective plant growth.

One explanation for the lasting effect of the microalgae treatments is that microalgae are a natural component of the plants microbiome and may support plant growth beyond nutrient supply (Lee & Ryu 2024). However, previous study by Bylund highlights that even if microalgae is a natural component for the plants, they can build a toxic environment if the composting is not properly developed before application (Bylund 2015). This due to the fact that microalgae have ability to accumulate heavy metal (Lage et al. 2021a; Gonçalves et al. 2023).

One of our potential sources of error in this trial is that the peat mix is mixed by hand and that can lead to some difference in concentration within the treatments. Our healthy microalgae treatment- seedlings can indicate that we have been able to mix the microalgae properly and the composting was properly developed before application, and this did not create a toxic environment for our seedling. Because the biofertilizer only is applied when the seedlings are planted this makes it less demanding from a resource perspective compared to conventional fertilizer that's need application weekly.

However, more research in this area is necessary before the comparison between biofertilizer and conventional fertilizer can be understood more deeply.

## 5.1 Conclusions

In our study we can show that biofertilizer in the form of microalgae and ash can be an alternative to conventional fertilizer. The results can prove that some combination of biofertilizer is better or at least has the same potential as conventional fertilizer when it comes to height and diameter growth for spruce and pine seedling. This can support the forest sector to develop sustainable fertilization program for plant-nursery.

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