Fertilization of Norway spruce forest with wood ash and nitrogen affected both tree growth and composition of chemical defence

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Abstract

We fertilized a Norway spruce (*Picea abies* (L.) Karst.) stand on rich mineral soil with 3 t ha^{-1} of wood ash (ASH), 150 kg ha^{-1} of nitrogen (N) or a combination of wood ash and nitrogen (ASH+N), in addition to unfertilized control plots. After five growing seasons, we remeasured the trees and took core samples. Current- and previous-year needles were sampled and analysed for total nitrogen and carbon, low-molecular weight phenolics and condensed tannins. Annual volume increment and standing volume were significantly higher in the ASH+N treatment than in control plots after five years. N gave a significant positive effect on basal area growth in the third year, after which the effect diminished. The ASH+N treated trees, on the other hand, showed an increasing basal area growth trend throughout the period. ASH reduced the total concentration of low-molecular weight phenolic compounds significantly in current-year needles. Phenolic acids increased under both ASH and ASH+N, while flavonoids decreased significantly under the same treatments compared to N. By including annual growth rate before fertilization in the analyses, the effect of N-treatment on flavonoids was positive only in trees with higher growth rates, and in those trees the concentration was higher than in both ASH-treated plots and controls. An acetophenone, constituting more than half of the total low-molecular weight phenolics concentration, was strongly reduced under all fertilization treatments. These results demonstrate that in addition to effects on tree growth, fertilization of the forest floor also has a strong influence on other metabolic processes of trees, with potential implications for ecosystem functioning.

Keywords

Ash recycling, fertilizers, forest health, nutrients, tree growth, phenolic compounds

Introduction

Boreal forests have an important role in mitigating climate change (IPCC 2014; IEA 2016), and forest fertilization has been put forward as a means to rapidly increase forest growth and thereby CO_2 sequestration (Anon. 2009; Haugland *et al.,* 2014; Rytter *et al.,* 2016; Petaja *et al.,* 2018). Many studies have shown that fertilization may have positive effects on tree growth (Ingerslev *et al.,* 2001; Nilsen 2001; Saarsalmi and Mälkönen 2001; Jacobson and Pettersson 2010; Hedwall *et al.,* 2014) and increase the carbon stocks in trees and soil (Johnson and Curtis 2001; Hyvönen *et al.,* 2008; Jacobson and Pettersson 2010). Fertilization experiments on mineral soil in older coniferous stands in Fennoscandia usually show that nitrogen (N) is the growth limiting element, and that the addition of other elements seldom has noticeable effects on growth (Brantseg *et al.,* 1970; Blingsmo 1986; Pettersson 1994; Tamm *et al.,* 1999; Jacobson and Pettersson 2001; Nilsen 2001). However, in some cases an additional effect has been found with adding for instance phosphorous (P) and potassium (K) together with N (Kukkola and Saramäki 1983; Tveite 1994; Saarsalmi *et al.,* 2012). Kukkola and Saramäki (1983) showed that the effect of P applied together with N became proportionally more important as the fertility of the sites increased.

In addition to mineral fertilizers, elements like P, K, calcium (Ca) and magnesium (Mg) may be supplied through wood ash. The production of ash from wood has greatly increased in the last years, because biofuels are increasingly being used for heating and energy production. In addition to the content of essential nutrients which can be exploited for fertilization, wood ash has a high pH value and acid neutralizing capacity that affects the forest soils (Augusto *et al.,* 2008). Saarsalmi *et al*. (2010; 2012) showed that ash supplied together with N can prolong the effect of N fertilization in forests. The studies of Jacobson (2003) and Sikström *et al.,* (2009) indicated that the addition of wood ash alone may increase stem wood growth somewhat on fertile sites and decrease it on less fertile sites.

69 Even though the effect on growth is most often positive, fertilization may affect metabolic
70 processes in the trees, with indirect implications for both growth and ecosystem functioning.
71 For instance, changes in growing conditions will often affect the production of defensive
72 compounds, the so-called plant secondary metabolites (PSMs) (e.g. Koricheva *et al.,* 1998;
73 Zvereva and Kozlov 2006). One important metabolite group, the phenolics, functions as
74 sunscreens, allelopathics, herbivore deterrents and pest protection (Bryant *et al.,* 1983; Inderjit
75 1996; Close and McArthur 2002; Witzell and Martin 2008). The PSMs also play important
76 roles in slowing down decomposition of forest litter, and thus for sequestration of C in soil.
77 This is mediated both through the slow breakdown of big and complex molecules and also
78 through interaction with microbes (Adamczyk *et al.,* 2019).
79
80 It is well established that when nutrient availability limits growth, plants will invest more in
81 C-based PSMs like phenolics. Correspondingly, Koricheva *et al.* (1998) showed in a meta-
82 analysis that when fertilized, trees in general reduced their concentrations of phenolics and
83 increased growth. However, most fertilization experiments with trees have been performed
84 under controlled conditions with small seedlings or young plants. Thus, little is known about
85 the effect of fertilization of large trees and on the forest ecosystem as a whole. In a recent
86 experiment with chronic N-fertilization (i.e. repeated additions over several years) of mature
87 Norway spruce, we found that levels of phenolics were strongly reduced in current year
88 needles, while one-year-old needles were not much affected (Nybakken *et al.,* 2018).
89
90 In this study, we fertilized a spruce stand on rich mineral soil with wood ash and/or N once
91 and tested its effect on tree growth and on phenolic compounds in needles after five years. We
92 used this experimental set-up to test the following four hypotheses: 1) fertilization with wood
93 ash only will not increase tree growth, 2) there will be no significant differences in growth

between fertilization with N or ash + N, 3) fertilization with wood ash only will not affect the total concentration of PSMs in needles, and 4) fertilization with N or ash + N will reduce the concentration of PSMs. By testing these hypotheses we aim to advance the understanding of fertilization on both growth and ecosystem functioning in forests, using N doses commonly employed in Nordic forestry (Hedwall *et al.,* 2014) and also recommended doses of wood ash (Hanssen *et al.,* 2014).

Materials and methods

A field trial was established in a spruce stand at Bærøe farm in Hobøl municipality, south-eastern Norway (59.56˚N, 10.95˚E (WGS84), 195-215 m a.s.l.) (Figure 1). Normal mean annual temperature and precipitation (1961-1990) at the nearby meteorological station at Ås are 5.3°C and 785 mm respectively (The Norwegian Meteorological Institute, http://www.eklima.no [accessed 15.04.19]). The soil is variable, podzol/cambisol on thin moraine deposits, which in turn cover Precambrian gneiss (http://geo.ngu.no [accessed 15.04.19]). The topography is slightly undulating with nearby steeper slopes. The vegetation zone is southern boreal and vegetation section slightly oceanic (Moen 1999). The experimental site is a Norway spruce forest with Norwegian site index G20-G23 (Tveite 1977), corresponding to a yield capacity of 9.5-12 m^3 ha^{-1} $year^{-1}$. The forest was planted in the 1950s after clear-cutting and thinned in 2006/2007.

[Figure 1]

Treatment plot size was 25 m × 25 m, including a 5 m buffer zone. All sampling was carried out in the inner 15 m × 15 m area, and there were between 13 and 23 trees in each of these inner plots. Before treatment, diameter at breast height and height of all trees were measured with a pi tape and a Vertex III (Haglöf, Sweden), respectively. Stem volume per treatment plot was calculated using the volume functions of Vestjordet (1967). The average standing volume at the time of fertilization was 302 m^3 ha^{-1}, while the basal area was 30 m^2 ha^{-1} and the number of stems 850 ha^{-1}. Four treatments were applied in a block design: 3 t ha^{-1} ash (ASH), 150 kg ha^{-1} of N given as ammonium nitrate (N), 3 t ha^{-1} ash + 150 kg ha^{-1} of N given as ammonium nitrate (ASH + N), and an unfertilized control (Control). There were three replicates for each treatment. The forest was fertilized manually with ammonium nitrate at the end of May 2013 and with ash at the end of June 2013. Treatments were applied on the soil surface. The ammonium nitrate fertilizer was Opti-KAS Skog (Yara) and contained 27% N (13.5% as NO_3^- and 13.5% as NH_4^+), 5% Ca, 2.4% Mg and 0.2% B. The wood ash was granulated hardened bottom ash from the sawn timber producer Bergene Holm. The concentrations of various elements in the ash are given in Table 1.

Soil chemistry near the start of the experiment is given in Table 2. A soil profile, 1 × 1 × 1 m, was dug in an untreated area in the middle of the experimental site in September 2013. Soil samples were separated by horizon, dried and sieved (2 mm), after which they were analysed for pH potentiometrically in a water extract (25 ml water: 10 ml soil) using a glass membrane combination electrode, and for total C and N after grinding the sample, by combustion at 950°C using an Elementar Vario EL with TCD detection (Ogner et al. 1999). Concentrations of base cations (Ca, K, Mg and Na) and other elements such as Al were determined by ICP-AES (AtomComp 1100, Thermo Jarrell-Ash, MA, USA) in a 1 M NH_4NO_3 extract according to Ogner *et al.,* (1999). This method is assumed to reflect plant-available element

concentrations. Cation exchange capacity (CEC) and base saturation (BS) were calculated from the element concentrations. Cation exchange capacity is the number of exchangeable cations per dry weight that a soil is capable of holding, at a given pH value, and available for exchange with the soil solution. Base saturation is the fraction of exchangeable cations that are base cations.

The C:N ratio in the humus layer was around 27 before treatment (Clarke *et al.,* 2018). Based on this ratio, the soil chemistry data and the site index, the site can be classified as nutrient rich.

Growth effects

In November 2017, five growing seasons after fertilization, height and diameter at breast height of all trees were remeasured and standing volumes and average annual increment were calculated (Vestjordet 1967). In addition, one increment core per tree was taken at breast height (130 cm above ground). The cores were taken from different compass angles, depending on the direction from which the tree was approached. The width of the year rings was measured with TSAP-Win™ (Rinntech, Germany) at least ten years back.

A neighbouring stand at the corner of the experimental site was harvested in 2015. This could potentially affect tree growth inside the nearest plot (an ASH-treated plot), even though the clear-cut was outside the buffer zone. However, growth measurement data showed no diverging effects on the trees in this plot. Thus, data from all trees was used in the analyses.

Chemical analyses of needles

The current- and previous-year (1 year old) needles from 10 of the largest dominant trees per plot were sampled on May 29 and 30 2017. Because of the neighbouring clear-cut, we chose to

168 decrease the size of the ASH-treatment plot closest to it, and thus got only five sample trees
169 there. This resulted in 230 samples from 115 trees altogether. The chosen trees were as similar
170 as possible regarding height and crown size, and the samples were taken from a twig in the
171 outer part of the crown, on the north side of the tree, and at 8-10 m height. We put the needles
172 in paper bags with silica gel immediately and in a drying oven at 30 °C the same evening. After
173 48 h drying, the paper bags were packed in plastic bags and frozen at -20 °C until further
174 handling.
175
176 Before chemical analyses, the needles were ground to powder on a ball mill (Retsch MM400,
177 Haag, Tyskland) at 30 revolutions s^{-1} for 180 s. From the resulting powder, we determined total
178 carbon (C) and nitrogen (N) with a Micro Cube (Elementar Analysen, Hanau, Germany), using
179 5-6 mg plant material. For phenolic analysis, further sub-samples of c. 10 mg were extracted
180 with 400 µl methanol (MeOH) and homogenised at 5000 rpm for 20 s on a Precellys 24
181 homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France). Samples were then
182 cooled on ice for 15 min before being centrifuged at 15000 rpm for 3 min (Eppendorf centrifuge
183 5417C, Eppendorf, Hamburg, Germany). The supernatant was transferred to a 10 ml glass tube,
184 and the residue was again dissolved in 400 µl MeOH, homogenised, and centrifuged in the same
185 manner as above; the supernatant was removed, and the same extraction process was conducted
186 two more times until both the residue and the supernatant were completely colourless. The
187 combined supernatants were evaporated in a vacuum centrifuge (Eppendorf concentrator plus;
188 Eppendorf, Hamburg, Germany), sealed, and stored in a freezer (-18°C) until high performance
189 liquid chromatography (HPLC). The residues were also stored in a freezer for further analysis
190 of MeOH-insoluble condensed tannins. Low molecular weight phenolics were analysed using
191 a HPLC system (Agilent Series 1200, Agilent Technologies, Waldbronn, Germany) with a
192 G1312A binary pump, a G1329A autosampler, a G1316A thermoregulated column heater, and

193 a G1315D diode array detector. As the stationary phase a Thermo Scientific column type was
194 used (Thermo Fisher Scientific Inc, Waltham, USA), with a 50 × 4.6 mm internal diameter and
195 filled with ODS Hypersil (3 µm) particles. The mobile phase consisted of two solvents that
196 eluted the samples by way of a gradient as in Julkunen-Tiitto and Sorsa (2001). The injection
197 volume was 20 µl. The absorption spectra at 270 and 320 nm, along with respective retention
198 times, were used to identify the chemical compounds and to calculate concentrations by
199 comparing with commercial standards. The analyses of the MeOH-soluble and -insoluble
200 condensed tannins followed the procedures described in Hagerman (2002).
201
202 *Data analyses*
203 The annual growth of the year rings was first averaged for each treatment and block and
204 calibrated against the growth five years prior to 2013, to adjust for the differences in growing
205 conditions between the plots before treatment. The formula used for each treatment and block
206 was
207
208 $$P_{ty} = \frac{A_{ty}}{\bar{X}_{2008-2012}} * 100 \qquad (1)$$
209
210 where P_{ty} is the adjusted annual growth for a specific year after fertilization, A_{ty} is the annual
211 growth for a specific year and $\overline{X}_{2008\text{-}2012}$ is the average growth in the five years before
212 fertilization. The effects of the fertilization treatments on the adjusted annual basal growth, and
213 on the average annual volume increment and standing volume five years after treatment, were
214 tested with a linear mixed model analysis. The calculations were done using the GLM and
215 Glimmix procedures in SAS™. The treatments were regarded as fixed effects whereas blocks
216 were considered to be random effects. For the annual volume increment and standing volume
217 five years after treatment, the standing volume in 2013 was used as a covariate.

The effect of treatment and needle age on needle C, N and C:N ratio as well as PSMs was tested with linear mixed effects models with block as random. We also ran mixed effects models including mean annual increment for the five years prior to the start of the experiment. All analysis on biochemical data was performed with R v 3.5.2. The value 0.05 was used as significance level for all analyses.

Results

3.1. Growth effects

After five years, both current annual increment and standing volume were highest in the ASH + N treatment and least in the Control plots. The ASH + N treatment was significantly different from the Control for adjusted annual increment (Figure 2) as well as for the adjusted standing volumes, which were 364, 371, 373 and 387 m^3 ha^{-1} for Control, ASH, N and ASH + N, respectively.

[Figure 2]

The increment cores (Figure 3) showed that N gave a modest but positive effect which was significantly different from the Control in 2015, three years after fertilization, and thereafter diminished. The effect of ASH only was smaller than for N and not statistically different from Control. The ASH + N treatment, on the other hand, was significantly different from Control from 2015 and onwards and from ASH and N in 2016 and 2017, showing an increasing growth trend throughout the period.

[Figure 3]

3.2. Total needle C and N concentrations

Previous-year needles had almost three times as high concentrations of N as the current year ones, but the difference between the cohorts was not affected by the treatments (Table 3). The ASH-only treatment significantly reduced N concentration in previous-year needles compared with controls, while for current-year needles there were only significant differences between the ASH-only (decrease) and N-only (increase) treatments. The C:N ratios were correspondingly affected, differing between ASH- and control-needles from the previous year, and between ASH- and N-treated needles from the current year.

The treatments did not affect the total carbon concentrations in needles in any of the two cohorts.

3.3 Plant secondary metabolites

The total concentration of low molecular weight phenolics did not differ between the two needle cohorts (Figure 4). However, the concentrations for some compound groups differed strongly, as current-year needles contained four times as much flavonoids as those from the previous year, while stilbenes were almost not present in the current-year needles but constituted almost half of the total concentrations in the previous-year ones (Figure 4, c and d).

[Figure 4]

265 The composition of individual compounds also differed between the needle cohorts, and only
266 some few compounds were found in both. Of the two hydroxycinnamic acid derivatives found
267 in both needle types (hydroxycinnamic acid 1 and 3), the first was present in higher
268 concentrations in the previous-year needles, while the second was highest in the current-year
269 ones. Gallocatechin and monocoumaroyl astragallin 2, on the other hand, were found in
270 higher amounts in the previous-year needles, while acetophenone and both fractions of
271 condensed tannins were higher in the current-year ones (Table 4).

272 With some few exceptions among the individual phenolic compounds, the treatments only
273 affected the chemical defence of current year needles (Figure 4, Table 4). ASH reduced the
274 total concentration of low molecular weight phenolic compounds in these needles. Phenolic
275 acids increased under ASH and ASH + N fertilization, while needles from N-only plots did
276 not differ significantly from those from control plots, although the mean concentration of
277 phenolic acids was highest in the N-only plots. The larger variation between samples meant
278 that the values did not differ significantly from the controls. Flavonoids, on the other hand,
279 were higher after N treatment compared with needles from ASH-treated plots, but none of the
280 treatments differed significantly from the controls. However, by including annual growth rate
281 before fertilization in the analyses, we saw that the effect of N-treatment on flavonoids was
282 positive only in trees with higher growth rates, and in those trees the concentration was higher
283 than in both ASH-treated plots and controls (Table 5, Fig. 5). Among the individual
284 compounds, acetophenone was strongly reduced by all treatments in current-year needles
285 (Table 4), while condensed tannins were lower under ASH + N than under N. Kaempferol-3-
286 galactoside and hydroxycinnamic acid 2 increased under addition of ASH, while kaempferol-
287 3-glucoronide was lower in ASH-treated needles than in those treated with N.

288 In previous-year needles, piceatannol glucoside increased in needles from N-treated plots
289 compared with controls, while gallocatechin decreased in ASH-treated plots.

290

291 [Figure 5]

292

293 **Discussion**

294 In this mature spruce stand on rich soil, both fertilization treatments containing N had a
295 positive effect on basal area growth, but only the ASH + N combination gave a significant
296 effect on volume. Earlier Norwegian N fertilization trials over a span of site indices typically
297 showed increment increases in the range of 1–2 m^3 ha^{-1} yr^{-1} for a period of 6–8 years after
298 application of 150 kg N ha^{-1} (Sture 1984; Nilsen 2001). In our study the effect of N only on
299 volume increment was within this range, though it was not significantly different from the
300 control. The analyses of the increment cores showed that growth levelled off after 4-5 years
301 only. This is not unexpected at a site with a rather high site index. The growth effect of N
302 fertilization is usually best on low to average site indices (Kukkola and Saramäki 1983) and
303 may endure for a shorter time on rich soils (Pettersson 1994).

304

305 The application of wood ash is shown to decrease soil acidity and increase the base saturation
306 in forested mineral soils (Saarsalmi *et al.,* 2001; Brunner *et al.,* 2004; Jacobson *et al.,* 2004;
307 Saarsalmi *et al.,* 2010; Clarke *et al.,* 2018). The pH effect of the ash increases microbial
308 activity (Perkiömäki and Fritze 2005), stimulating carbon mineralization (Moilanen *et al.,*
309 2002; Perkiömäki and Fritze 2002). It may also activate N-cycling in the topmost forest floor,
310 but this effect is less clear and more often found on rich soils with low C:N ratios than on
311 poor soils (Persson *et al.,* 1989; Jacobson 2003; Rosenberg *et al.,* 2010). Even if the C:N ratio
312 was relatively low at our site, the growth effect of ASH was small and of short duration. This
313 is, after all, in agreement with most studies on ash amendment in mature Norway spruce

314 stands on mineral soil (Jacobson and Pettersson 2001; Nilsen 2001), supporting our first
315 hypothesis.

316 The short-term effect of adding ASH + N was positive and still increasing in the fifth year
317 after fertilization. The annual volume increment and the standing volume were not
318 significantly different from N or ASH treatment after five years, but the basal area increment
319 was higher for ASH + N in the fourth and fifth year after fertilization (Figure 3). Thus, we
320 must conclude that our second hypothesis was at least partly rejected. Saarsalmi *et al.,* (2006)
321 also found positive growth effects of adding ash together with N, but in contrast to our results
322 this effect became evident only after about 10 years. Their study was conducted in a relatively
323 poor Scots pine stand. It is possible that better initial soil nutrient conditions at our rather rich
324 spruce site contributed to an earlier on-set of the positive growth effects. This highlights the
325 importance of initial soil nutrient conditions in determining the effects of different
326 fertilization treatments. Our study was performed in a rich Norway spruce stand, and the
327 results cannot necessarily be extended to poorer soils. In accordance with Jacobson (2003),
328 caution should be exercised in applying wood ash on low site indices. The effects on tree
329 growth of adding both ash and N under different nutrient conditions are not yet sufficiently
330 understood.

331 Saarsalmi *et al.* (2012) showed that changes in soil chemical properties and microbial
332 processes in C and N cycling gave some explanations for the positive response in tree growth
333 after ash + N fertilization. In our study, adding ash both with and without N increased pH,
334 cation exchange capacity and base saturation, while exchangeable acidity was reduced
335 (Clarke *et al.,* 2018). Jacobson (2003) suggested that adding wood ash to fertile sites with N-
336 rich forest soils may increase the net rate of mineralization of N in the soil organic layer, and
337 lead to a positive growth response. However, this does not explain why adding ASH only did
338 not show the same positive effect on basal increment as adding ASH + N. A simple

339 explanation, in agreement with Liebig's "Law of the Minimum", could be that adding both
340 types of fertilizer increased the supply of all the main nutrient resources in a more balanced
341 way, raising the production to a higher level than by adding just one of them.

342 Interestingly, the ASH treatment reduced the N concentration in previous-year needles, but as
343 stated above, this did not affect the growth negatively. Most previous experiments with wood-
344 ash fertilization have only measured the effects on soil nutrient status, not needle
345 concentrations. In their long-term study, Saarsalmi *et al.* (2006) found no effects of ash
346 fertilization in needles after 23 years, but any effects may have disappeared after so many
347 years. It should be noted that our measurements were done on needles sampled in the early
348 growing season, while Saarsalmi *et al.* (2006) sampled when the trees were dormant, as
349 usually recommended (e.g. Brække 1994). The N-concentrations in our current-year needles
350 were still significantly higher in N-fertilized plots than in controls five years after fertilization,
351 but the differences were small (0.99 compared to 1.03%) and may as such reflect the
352 decreasing effect on growth. Previous-year needles, on the other hand, had almost three times
353 as high concentrations of N as the current-year ones, and this was not affected by N-
354 fertilization. The explanation for the big differences between the cohorts may be the timepoint
355 of sampling, just after the current-year needles were fully grown, which is probably when the
356 use of N is at its highest and the needle N concentrations at their lowest. We earlier found
357 corresponding results in needles from mature spruce sampled at the same time of the year
358 (Nybakken *et al.,* 2018).

359 Fertilization effects on chemical defence are potentially tightly connected with the effects on
360 growth, as both metabolic processes require C. When N limits growth, more C may be
361 available for building C-based phenolic defence compounds (Bryant *et al.,* 1983). After
362 fertilization, more C is used for growth, and production of phenolic compounds is usually
363 reduced (Koricheva *et al.,* 1998). In our previous study of chronically fertilized spruce on a

low fertility site, we found large reductions in low-molecular weight phenolics in current-year needles, while there was no effect on those from the previous year (Nybakken *et al.,* 2018). The present experiment is more realistic concerning forest fertilization as it is practiced in northern Europe today, with a one-time addition of 150 kg N ha^{-1} to a mature forest stand. The total concentration of low-molecular weight phenolics was not significantly reduced five years after N fertilization. Phenolic acids, on the other hand, increased under ASH, while flavonoids increased under N in trees with high growth rates prior to fertilization. In our previous study (Nybakken *et al.,* 2018), both phenolic acids and flavonoids were reduced in current-year needles. Together, our findings suggest that the effect of N-fertilization on flavonoid and phenolic acid concentrations is context-dependent; under productive conditions, the spruce trees might have enough resources for both growth and defence. Consequently, in a less productive forest, N addition is likely to give lower concentrations of these defences when C is largely allocated to growth (Bryant *et al.,* 1983; Herms and Mattson 1992).

On the other hand, an acetophenone that constituted more than half of the total phenolic concentration in current year needles was strongly reduced by N fertilization in the present study. Acetophenones have been related to spruce budworm resistance as constitutive defence in white spruce (*Picea glauca* (Moench) Voss) (Delvas *et al.,* 2011) and to fungitoxicity in Norway spruce (Osswald and Benz 1989; Boufalis and Pellissier 1994). The acetophenone in the current year´s needles was even more strongly reduced under the ASH treatment, while the ASH+N treatment showed least reduction. In our previous study of spruce at a low fertility site (Nybakken *et al.,* 2018), the current-year needles had very low concentrations of acetophenones, which were unaffected by fertilization. In addition, the total concentrations of low-molecular weight phenolics in unfertilized trees were almost three times as high in previous-year needles compared with the current-year ones (Nybakken *et al.,* 2018), while in this study there were no significant differences. This indicates that soil fertility, but also the

389 genetically and possibly ontogenetically decided composition of PSMs, may play a role in
390 how tree defence is affected by fertilization.

391

392 **Conclusions and practical implications**

393 Forest fertilization may contribute positively to climate change mitigation and satisfying the
394 increasing demand for timber resources. Our results and former studies show that fertilization
395 with wood ash, in addition to nitrogen, may further increase growth on rich mineral soil types.
396 This could also contribute to sensible recycling of nutrients from a growing bioenergy sector.
397 However, we showed that addition of both N and ash also affected the chemical defence of
398 trees, and as such potentially reduces the resistance against pests. Further, changed needle
399 chemistry may also affect decomposition and soil ecology with possible feedbacks on tree
400 growth. Our results underline the need for further studies on ecophysiological effects of forest
401 fertilization to evaluate its potential as a climate mitigation tool.

402

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406

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556 Table 1. Element concentrations and pH in the ash used in the field experiment.

Element	Concentration	Element	Concentration	Element	Concentration	Element	Concentration
C (%)	0.3	Cd (mg/kg)	3.0	Mg (g/kg)	37.3	Sc (mg/kg)	3.9
N (%)	<0.1	Cl (mg/kg)	0.1	Mn (g/kg)	33.1	Se (mg/kg)	12.0
pH	11.6	Co (mg/kg)	18.6	Mo (mg/kg)	6.5	Si (g/kg)	40.7
Al (g/kg)	8.9	Cr (mg/kg)	127.9	Na (g/kg)	0.2	Sr (g/kg)	2.1
As (mg/kg)	0.6	Cu (mg/kg)	20.7	Ni (mg/kg)	50.3	Ti (mg/kg)	367.5
Ba (g/kg)	10.5	Fe (g/kg)	4.6	P (g/kg)	24.2	V (mg/kg)	10.1
Be (mg/kg)	4.6	K (g/kg)	8.2	Pb (mg/kg)	11.9	Y (mg/kg)	3.9
Ca (g/kg)	437.2	Li (mg/kg)	19.9	S (g/kg)	0.9	Z (g/kg)	0.1

557 Note: Data from Dibdiakova and Horn (2014) and email from J Dibdiakova; unreferenced.

558 All concentrations are on a dry weight basis.

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560 Table 2. Soil chemistry at Bærøe. CEC = cation exchange capacity, BS = base saturation,
561 Exch. = exchangeable. For the methods of determination used, see the text.

Horizon	C (%)	N (%)	pH(H_2O)	Exch. Ca (mmol/kg)	Exch. Mg (mmol/kg)	Exch. K (mmol/kg)	CEC (mmol(+)/kg)	BS (%)
L	50	1.7	5.0	133	37	34	488	79
F	48	1.8	4.3	123	26	37	480	72
H	30	1.1	3.8	65	15	11	270	66
Ae	13	0.49	3.7	11	4.1	2.7	96	37
E	1.2	0.06	3.9	1.7	0.74	0.55	23	28
Bh	1.2	0.06	4.8	0.31	0.06	0.16	12	11
Bh2	0.8	0.05	4.7	0.26	0.06	0.11	10	13
B1	2.9	0.14	4.2	2.5	1.2	0.34	69	13
B2	2.1	0.09	4.6	0.49	0.19	0.27	31	7.7
C	0.3	0.02	4.8	0.16	0.05	0.10	5.0	16

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Table 3. Total nitrogen (N) and carbon (C) concentrations in current and previous year needles and results from the mixed effects model. Different lowercase letters following the concentrations denote significant differences ($P < 0.05$, Tukey). Significant effects ($P < 0.05$) in bold typeface.

					***F* (*P*)**		
	Control	N	ASH	ASH+N	Year	Treatment	Interaction
Carbon (%)							
Current	47.2±0.17	47.2±0.14	47.1±0.27	47.6±0.20	1.11	1.10	0.560
Previous	47.4±0.42	47.6±0.20	47.3±0.09	47.5±0.12	(0.293)	(0.352)	(0.629)
Nitrogen (%)							
Current	0.99±0.016ab	1.03±0.025a	0.92±0.026b	0.99±0.021ab	**3160.16**	**9.81**	0.32
Previous	2.82±0.081a	2.96±0.083a	2.51±0.071b	2.72±0.077ab	**(<0.001)**	**(<0.001)**	(0.812)
C:N							
Current	47.9±0.75ab	46.4±1.04b	52.2±1.04a	48.9±1.03ab	**3283.42**	**9.65**	0.23
Previous	17.1±0.45b	16.5±0.55b	19.2±0.53a	17.9±0.52ab	**(<0.001)**	**(<0.001)**	(0.877)

573 Table 4. Concentrations of detected individual phenolic compounds (mg g^{-1} ± 1 SE) under different treatments and years (C=current, P=previous)

574 and results from the mixed effects model. Bold values represent significant effects at $P<0.05$.

						F (*P*)		
	Y	Control	N	ASH	ASH+N	Year	Treatment	Interaction
Phenolic acids								
Hydroxycinnamic acid 1	C	0.15±0.05	0.19±0.04	0.25±0.05	0.10±0.04	**55.11 (<0.001)**	1.96 (0.122)	1.51 (0.213)
	P	0.91±0.31	0.97±0.32	1.66±0.36	1.67±0.52			
Hydroxycinnamic acid 2	C	0.17±0.05a	0.21±0.07ab	0.19±0.05b	0.51±0.13a	–	**3.88 (0.011)**	–
Hydroxycinnamic acid 3	C	0.32±0.10	0.84±0.23	0.65±0.11	0.51±0.15	**34.62 (<0.001)**	2.02 (0.112)	1.74 (0.160)
	P	0.09±0.02	0.11±0.02	0.14±0.02	0.09±0.02			
Hydroxycinnamic acid 4	P	0.059±0.010	0.092±0.013	0.068±0.012	0.073±0.011	–	1.65 (0.183)	–
Flavonoids								
Apigenin-7-glucoside	C	0.64±0.13a	0.36±0.08ab	0.36±0.09ab	0.27±0.05b	–	**3.03 (0.033)**	–
Apigenin-7-glucoside	C	0.29±0.06	0.24±0.06	0.16±0.02	0.20±0.04	–	1.35 (0.262)	–
Apigenin aglycon	P	0.17±0.04	0.12±0.04	0.11±0.02	0.11±0.02	–	2.02 (0.116)	–
Dicoumaroyl astragalin 1	P	0.24±0.04	0.18±0.05	0.19±0.02	0.17±0.02	–	1.19 (0.319)	–
Dicoumaroyl astragalin 2	P	0.24±0.08	0.20±0.05	0.24±0.11	0.17±0.03	–	1.01 (0.391)	–
Dicoumaroyl astragalin 3	P	1.05±0.27	0.72±0.22	0.77±0.23	0.77±0.25	–	**3.05 (0.032)**	–
Dicoumaroyl astragalin 4	P	0.22±0.08	0.69±0.23	0.31±0.11	0.36±0.14	–	1.42 (0.242)	–
Dihydroquercetion	P	0.85±0.38	1.49±0.53	0.93±0.16	0.86±0.14	–	0.490 (0.690)	–
Gallocatechin	C	1.06±0.79	2.23±1.01	0.67±0.12	0.84±0.16	**10.96 (0.001)**	**3.05 (0.030)**	2.55 (0.057)
	P	5.19±7.60a	4.71±2.26ab	0.94±0.28b	5.00±1.52a			

Isorhamnetin (quercetin)	P	0.60±0.09	0.62±0.18	0.72±0.07	0.57±0.06	–	0.99 (0.400)	–
Kaempferol-3-galactoside	C	6.97±0.67b	7.62±0.73b	10.35±0.63a	8.52±0.63ab	–	**4.45 (0.005)**	–
Kaempferol-3-glucoronide	C	3.61±0.59ab	7.28±2.79a	1.10±0.11b	2.76±0.59ab	–	**3.25 (0.025)**	–
Kaempferol-3-glucoside	C P	0.83±0.52 0.22±0.03	0.06±0.02 0.34±0.10	0.11±0.03 0.39±0.20	0.07±0.03 0.27±0.16	0.06 (0.810)	1.47 (0.223)	2.48 (0.062)
Kaempferol-3-glucoside	C	0.84±0.52	0.06±0.02	0.11±0.03	0.07±0.03	–	2.05 (0.111)	–
Luteolin glycoside	C	0.85±0.19	1.01±0.24	0.36±0.12	0.73±0.19	–	1.95 (0.127)	–
Monocoumaryl astragalin 1	C P	0.25±0.12 0.26±0.09	0.11±0.03 0.11±0.03	0.09±0.03 0.15±0.02	0.14±0.04 0.15±0.03	0.18 (0.669)	2.42 (0.067)	0.13 (0.941)
Monocoumaryl astragallin 2	C P	0.093±0.027 0.17±0.05	0.067±0.026 0.23±0.05	0.092±0.022 0.24±0.02	0.14±0.03 0.31±0.05	**29.39 (<0.01)**	2.64 (0.051)	0.65 (0.583)
Myricetin-3-glucoside	C	0.21±0.07	0.07±0.02	0.07±0.03	0.09±0.03	–	2.06 (0.110)	–
Quercetin-3-galactoside	C	1.16±0.36	1.60±1.04	0.52±0.15	0.64±0.16	–	0.84 (0.476)	–
Quercetin-3-glucoronide	C	0.91±0.18	1.15±0.25	0.56±0.20	1.17±0.24	–	2.08 (0.108)	–
Quercetin-3-glucoside	C	0.12±0.04	0.10±0.05	0.16±0.03	0.09±0.02	–	0.67 (0.575)	–
Quercetin glycoside	C	0.49±0.08	0.69±0.12	0.42±0.08	0.35±0.11	–	2.20 (0.092)	–
Stilbenes								
Isorhapontin	P	0.52±0.13	0.50±0.09	0.34±0.09	0.27±0.06	–	2.33 (0.078)	–
Methyl piceatannol glucoside	P	1.60±0.34	1.02±0.17	1.38±0.25	0.95±0.15	–	0.82 (0.484)	–
Piceatannol glucoside	P	4.90±0.48b	8.21±0.78a	6.15±0.88ab	5.74±0.78ab	–	**3.84 (0.012)**	–
Piceatannol glucoside	P	2.85±0.28	2.37±0.27	2.14±0.37	2.39±0.22	–	1.12 (0.345)	–
Procyanidin	C	0.58±0.36	0.19±0.05	0.40±0.11	0.20±0.06	–	0.92 (0.435)	–
Resveratrol aglycon	P	8.52±1.40	9.06±1.25	11.88±1.74	8.84±2.12	–	0.56 (0.644)	–

Resveratrol aglycon	P	0.59±0.12	0.32±0.06	0.66±0.29	0.22±0.07	–	2.68 (0.051)	–
Others								
Acetophenone	C	36.55±11.07a	18.65±5.05b	12.47±3.15b	27.79±11.16b	**138.70 (0.001)**	**3.01 (0.031)**	**3.17 (0.025)**
	P	0.21±0.05	0.17±0.03	0.26±0.07	0.25±0.05			
Coumarin	P	0.18±0.05	0.15±0.06	0.30±0.08	0.36±0.09	–	3.34 (0.022)	–
Lignan	P	9.64±1.24	10.74±3.19	8.32±3.66	11.54±3.52	–	1.96 (0.125)	–
Condensed tannins								
Methanol soluble	C	60.2±4.9	67.9±5.8	67.7±4.6	55.1±3.9	**42.90 (<0.001)**	**4.52 (0.004)**	0.24 (0.865)
	P	43.3±1.8	46.9±2.2	52.0±2.0	39.6±2.0			
Methanol insoluble	C	43.1±3.9	45.4±3.8	37.7±2.6	36.7±2.5	**134.7 (<0.001)**	2.31 (0.078)	0.90 (0.443)
	P	18.9±1.3	22.3±1.2	18.9±1.4	19.5±1.5			
Total	C	104.2±6.1ab	113.0±7.9a	102.9±4.5ab	93.8±4.2b	**139.2 (<0.001)**	**4.43 (0.005)**	0.62 (0.605)
	P	63.1±2.3	70.1±2.4	71.4±2.4	57.8±2.4			

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576

577 Table 5. Mixed effects model [F (*P*)-values] testing for the effect of pre-treatment growth rate
578 of single trees and fertilization treatment (Control, ASH, N and ASH + N) on low molecular
579 weight plant secondary metabolites in spruce needles. Bold values represent significant
580 effects at $P<0.05$.

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	Tree growth	Treatment	Interaction
Current-year needles			
Phenolic acids	0.27 (0.602)	0.34 (0.795)	0.31 (0.815)
Flavonoids	0.11 (0.741)	**34.25 (0.007)**	**7.93 (<0.001)**
Stilbenes	2.51 (0.116)	0.85 (0.472)	1.49 (0.223)
Total	2.48 (0.118)	0.84 (0.476)	1.59 (0.196)
Previous-year needles			
Phenolic acids	0.27 (0.604)	1.09 (0.355)	1.30 (0.280)
Flavonoids	0.07 (0.796)	1.38 (0.253)	2.66 (0.053)
Stilbenes	1.13 (0.291)	0.70 (0.553)	0.46 (0.709)
Total	0.86 (0.356)	1.85 (0.144)	2.08 (0.107)

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Figures and captions

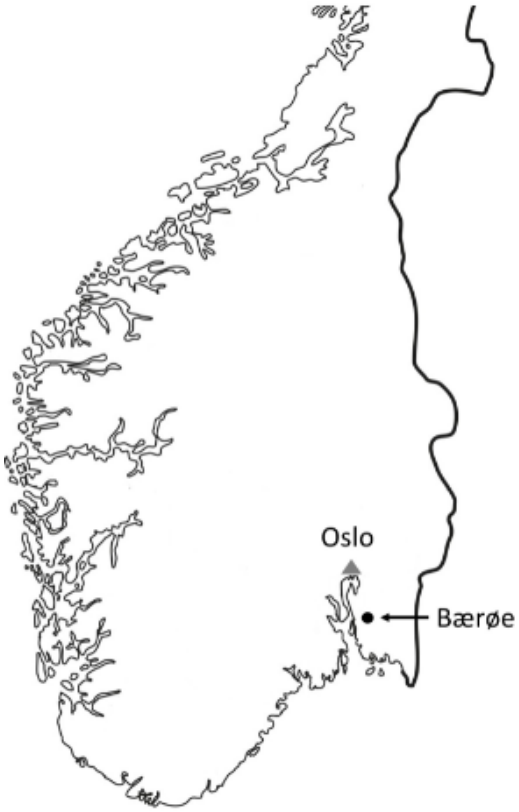


Figure 1. Map of southern Norway with the location of the field trial at Bærøe.

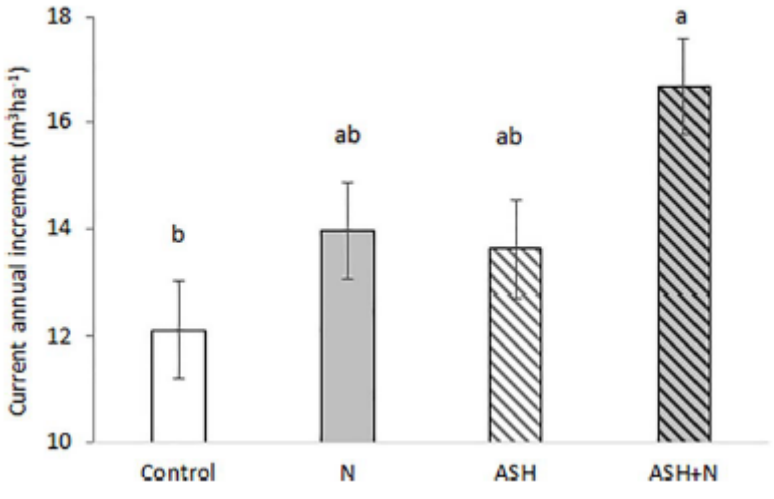
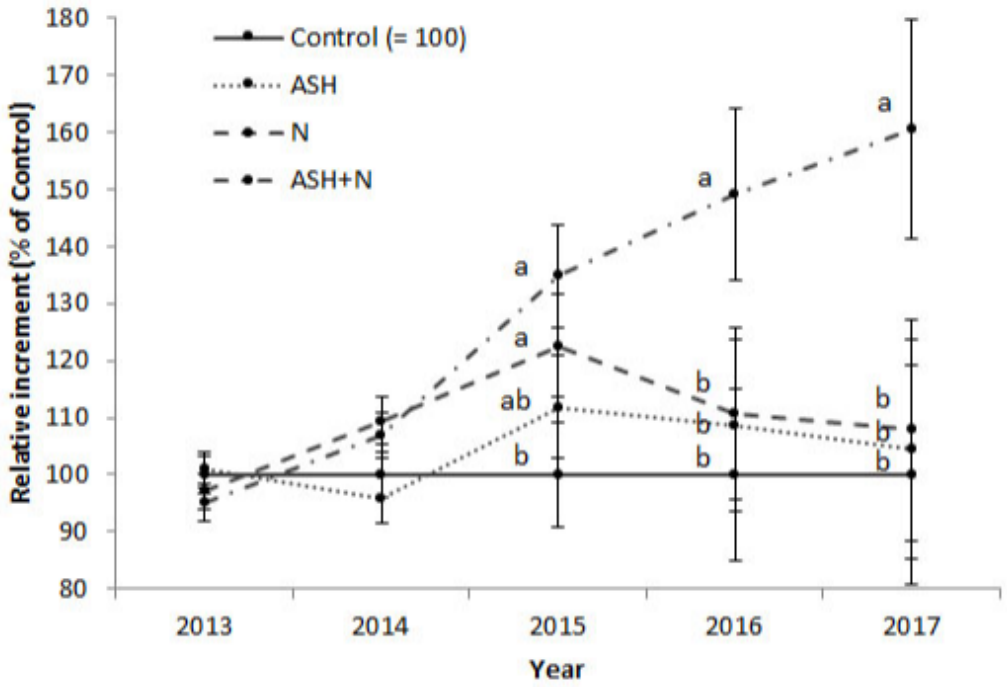


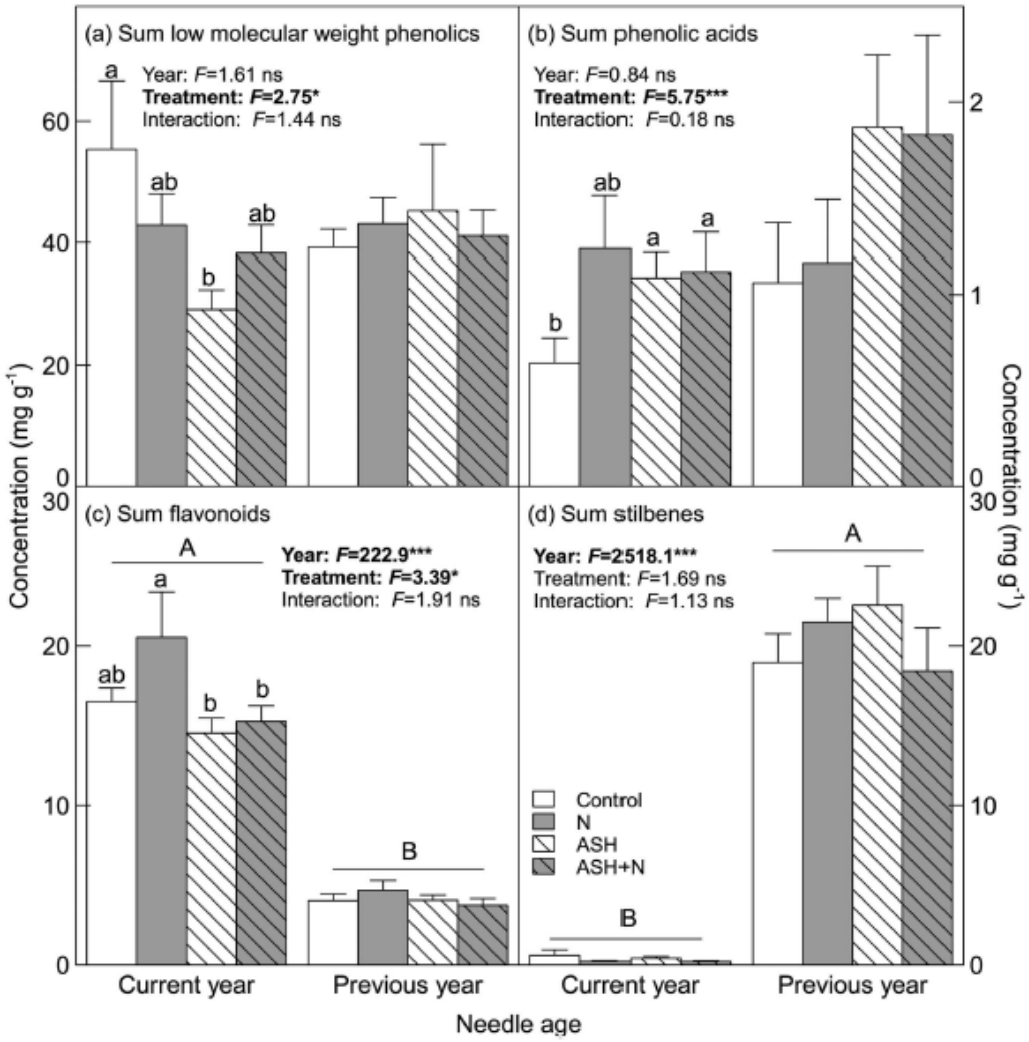
Figure 2. Mean values for annual increment (m^3 ha^{-1}) 2013-2017, adjusted for initial volumes before treatment in 2013 (± 1 SE). Different letters denote significant differences ($P < 0.05$) between treatments.



597

598 Figure 3. Annual relative basal area increment (based on increment cores) for the different
599 treatments. For illustration, the effects of the different fertilization treatments are adjusted
600 against the Control plots (= 100). Mean values (± 1 SE). Different letters denote significant
601 differences ($P < 0.05$) between treatments according to the analysis of variance.

602

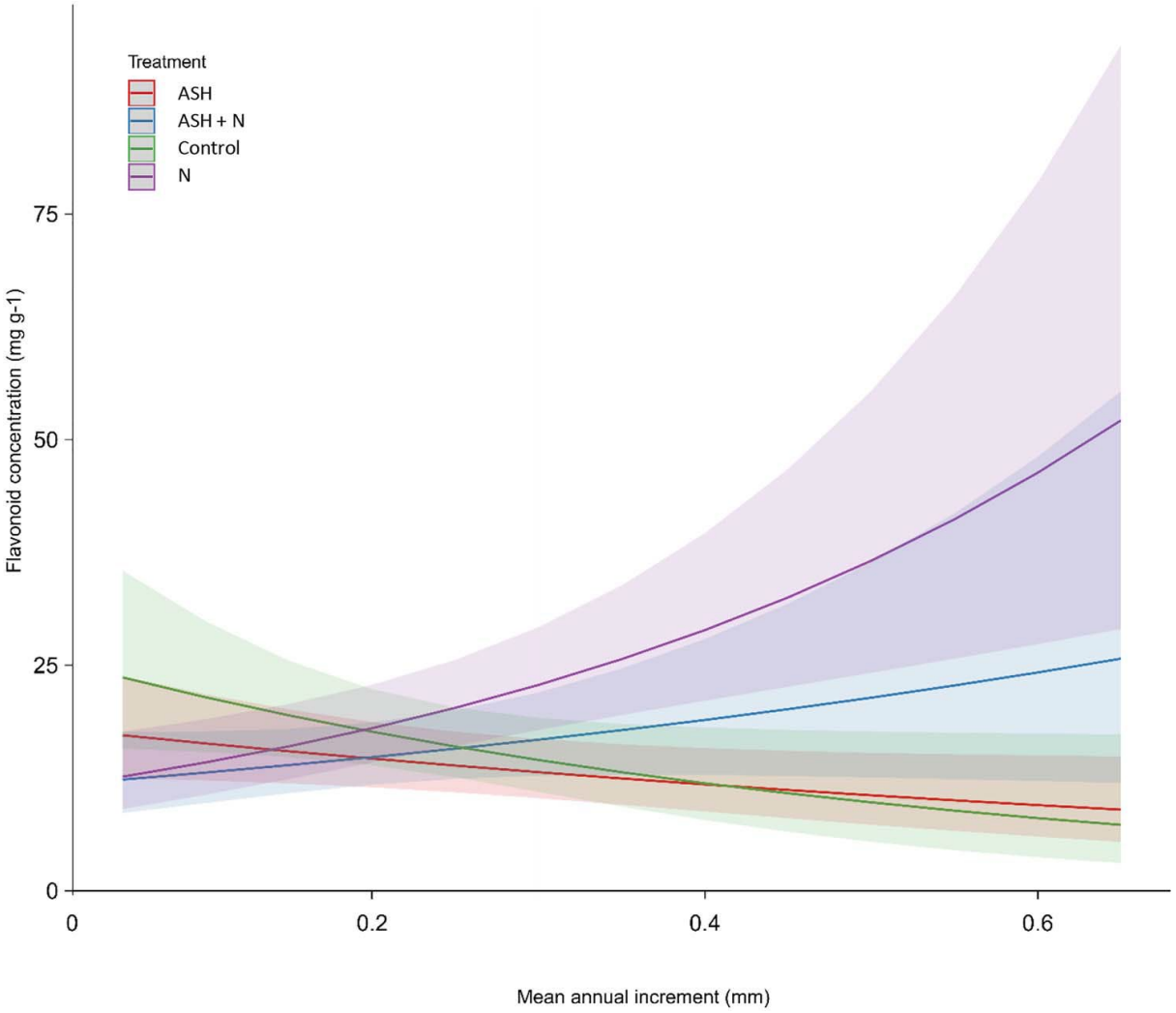


603

604 Figure 4. Concentrations of low molecular weight phenolics (a), phenolic acids (b), flavonoids
605 (c) and stilbenes (d) in current and previous year needles for the different treatments. Open
606 white bars are the average concentrations for control needles, open grey bars for needles from
607 N-plots, hatched white bars for needles from ASH-plots, and hatched grey bars for needles
608 from ASH+N-plots. Different capital letters above the bars denote statistically significant
609 differences ($P < 0.05$) between needle cohorts (current- and previous-year needles), while
610 different lowercase letters above bars signify statistically significant differences ($P < 0.05$)
611 between treatments. Analysis of phenolic acids was performed on log-transformed data.

612

613



614

615 Figure 5. Predicted values from a linear mixed effects model testing for the effect of pre-
616 treatment growth rate and fertilization treatment (Control, ASH, N and ASH + N) on
617 flavonoid concentration in the current-year spruce needles.

618