Norwegian University of Life Sciences

Master's Thesis 2021 60 ECTS

Faculty of Environmental Science and Natural Resource Management

Condensed tannins in senesced foliage and soil organic layer in response to fertilization in boreal forest ecosystem

Zannatul Ferdous

Master of Science in Ecology

Acknowledgement

This dissertation is written as a compulsory part of my master's degree programme in Ecology at the Faculty of Environmental Sciences and Natural Resource Management of the Norwegian University of Life Sciences (NMBU).

I would like to express my gratitude and thanks to my main supervisor, Professor Johan Asplund, and my co-supervisors, Professor Line Nybakken and PhD Fellow Lea-Rebekka Tonjer, who supported me throughout the thesis with guidance and showing the laboratory work statistical analysis and writing. Also, I would like to thank Engineer Annie Aaseen and Claus D Kreibich for laboratory assistance.

Further, my heartful thanks and love to my fellow master student Puspa Subedi for giving me company in the field study and Lisa Åsgård for helping me making location map and proofreading. A special thanks to my friends Dr. Nahmina Begum, Dr. Mahmuda Akter and PhD Fellow Mohammad Ali Noman Reza for helping me with data analysis and proofreading and supporting me when I was helpless and broken. Of course, I am solely responsible for any mistake in this dissertation.

Most of all, I am grateful from the bottom of my heart to have all my family members for their love, support, and encouragement during the entire study period in Norway.

Ås, May 2021

Zannatul Ferdous

Norwegian University of Life Science

Abstract

Anthropogenic nitrogen deposition rate in boreal forests increased over the last century and significantly impacted many parts of these ecosystems. Boreal forests contain a large amount of carbon (C), and nitrogen (N) is a limiting factor for plant growth. Consequently, anthropogenic N deposition is likely to boost productivity and C sequestration in the boreal ecosystem. Some boreal regions are experiencing a significant amount of N deposition. On the other hand, forest fertilization is used in areas with limiting N supply to enhance timber volume and C sequestration as a means of climate change mitigation. As a result, other parts of the boreal ecosystem may be considerably affected due to nutrient enrichment. Condensed tannin (CT) is a carbon-based secondary metabolite known to provide herbivore defence to the plants, available in boreal plant species with a high concentration. Change in CT concentrations with N deposition in green leaves has been reported in earlier studies. This experiment was carried out to comprehend better how the change of resource condition affects tannins concentration in senesced foliage and soil organic layer as well as nitrogen pool and carbon storage in the boreal forests' soil. I conducted this research in an old *Picea abies* forest in South-Eastern Norway, which has been fertilized annually since 2003 with 150 kg N ha^{-1}. The forest has 20 established experimental plots, ten controls, and the other ten are fertilization application plots. I measured CT concentration, C and N concentration in senesced foliage of *Picea abies* and *Vaccinium myrtillus* as well as in soil organic layer and pH value for soil.

Fertilization increased foliar N concentration, decreased C: N ratio, while foliar CT did not change in senesced foliage. Increase of N concentration and decrease of C: N ratio were in accordance with the resource-based ecological theories on plant defence, i.e., a higher resource availability condition causes improved growth rather than improving plant defence. However, no response in CT concentrations to fertilization suggests an inherent phenological accumulation pattern of tannins. As expected, soil organic layer CT declined in fertilized plots, likely due to a shift in ground vegetation from Ericaceae dominance to graminoid dominance. I also found an increase in C and N concentration and decreased corresponding C: N ratio in fertilized plots. N content significantly increased, whereas C content was nonresponsive. Organic layer pH value responded negatively to fertilization, which can be explained by increasing low pH containing *Picea abies* litterfall. Overall, tannins are well known to immobilize nutrients, and N availability in this study reduced the CT concentration of the soil organic layer and increased the N concentration, thereby increasing the decomposition of organic matter. Therefore, my study findings may imply that N deposition and forest fertilization facilitate organic matter decomposition when climate change also speeds up this process.

Table of Contents

1. Introduction

Boreal forests, the largest terrestrial ecosystem, contain a large proportion of global carbon in their soil and play a significant role in climate change mitigation (Carroll and Noss, 2020, IPCC, 2014, Price et al., 2013). These forests soil have a limited supply of nitrogen (N) naturally, and forest productivity is known to be limited by available soil N (Tamm, 1991). Consequently, anthropogenic N addition is likely to boost productivity and sequestration of carbon (C) in those ecosystems (De Vries, 2014, Fernández-Martínez et al., 2014). However, over the last century, the use of anthropogenic N has increased significantly due to fertilizers production and usage, combustion of fossil fuels and land-use intensification, resulting in increasing N deposition, which in turn have changed many parts of this biome. (Galloway and Cowling, 2002, Meunier et al., 2016, Gundale et al., 2014). Anthropogenic N deposition varies regionally. For example, Fennoscandian boreal forests have relatively low N deposition than boreal forests in Central Europe (Dentener et al., 2006, Gundale et al., 2011). However, N deposition generally has a minor effect on the boreal forest ecosystem (Gundale et al., 2011).

Nitrogen plays a pivotal role in the plant life cycle, especially in plant growth (Bergh et al., 2014, Nissinen and Hari, 1998, Wallace et al., 2007), and N fertilization has been proven to show an immediate effect on forest yield in low N environments (Jacobson and Pettersson, 2010, Saarsalmi and Mälkönen, 2001). Therefore, forest management has been using fertilizer applications since the 1960s to increase timber production to meet global demand and as a means of C sequestration (Hedwall et al., 2014, De Vries, 2014, Gundale et al., 2011). Subsequently, in addition to improving tree growth, a substantial rise in ecosystem C storage has been reported as a consequence of N deposition (Maaroufi et al., 2015, Pregitzer et al., 2008). Besides, nutrient enrichment may cause changes in other parts of the ecosystem; for instance, it might cause an increase in foliar N concentration also (Throop and Lerdau, 2004, Booker and Maier, 2001, Nybakken et al., 2018). It is demonstrated that N availability also changes species composition in boreal forest floors, shifting from slow-growing Ericaceous shrubs to fast-growing graminoids (Mäkipää, 1994, Strengbom and Nordin, 2008). Even improved N available condition at the base of forest food webs potentially triggers cascade effects; thus, changes in food web structure and function can significantly affect higher trophic levels (Meunier et al., 2016, Nybakken et al., 2018). Additionally, insect herbivore populations have also been shown to benefit from N deposition by increasing insects population growth and performance (Strengbom et al., 2005, Throop and Lerdau, 2004). Despite several

investigations, the entire effects of fertilizer on forest susceptibility to pathogens and herbivores are still unclear.

Tannins are an important group of phenolics, a carbon-based plant secondary metabolite (PSM) of vascular plants, traditionally considered as defensive compounds against herbivores. Their variation in chemistry and the massive concentration within species makes them the fourth most abundant biochemical compound in plants (Hernes and Hedges, 2000), indicating their importance in plants function and evolution (Zucker, 1983). According to Kuiters (1990), 40% of bark and leaves dry weight in woody species are made up of tannins, where foliar tannins account for up to 25% of dry weight (Kraus et al., 2003). Tannins are divided into two major categories: condensed tannin (CT) and hydrolyzable tannin (HT) (Kraus et al., 2003). The type of tannin varies by plant species, such as gymnosperms and monocots produce only CTs (Bate-Smith, 1977). Condensed tannins are particularly in attention because of their diverse structural variation and unique protein binding ability, which was thought to be effective for herbivory defence. However, contradictory empirical evidence about tannin's effectiveness in herbivore defence leads to an increasing interest in tannin's role in the plant-litter-soil interactions (Close and McArthur, 2002, Feeny, 1970, Kraus et al., 2004). CTs concentration and composition vary across plant species and within plants in response to changes in environmental conditions, including nutrient availability, CO2, pH, light intensity, temperature, water availability, and ozone (Gonzalez-Hernandez et al., 2003, Close and McArthur, 2002, Hofland-Zijlstra and Berendse, 2009, Kraus et al., 2003, Nabeshima et al., 2001, Northup et al., 1998, Rivero et al., 2001, Wam et al., 2017, Salminen and Karonen, 2011). Several theories have been proposed to explain differences in foliar tannin levels (Kraus et al., 2003). The carbon-nutrient balance hypothesis (Bryant et al., 1983) and growth-differentiation balance hypothesis (Herms and Mattson, 1992) suggest that N deficiency inhibits growth; as a result, photosynthates accumulate in the plant that potentially redirected to PSM production. Moreover, the protein competition model explained that protein and phenolic compound production compete for the same precursor, phenylalanine (Jones and Hartley, 1999, Nybakken et al., 2018). Endara and Coley (2011) supported these hypotheses in a meta-analysis and showed, species that grow in high fertility lead plants to spend more on growth than defence. Tannins concentration also have reported to change by genotype (Driebe and Whitham, 2000), season (Ganthaler et al., 2017), and phenology (green versus senescent leaves) (Covelo and Gallardo, 2001), in addition to the environmental factors.

Nonetheless, in addition to CTs' importance in green leaves for herbivore defence, after leaves senescence, they may have afterlife effects on decomposition and nutrient cycling. CTs potentially affect nutrient cycling by decelerating decomposition and N mineralization process through direct microbial toxicity or by creating recalcitrant complexes with organic N, thereby immobilizing nutrients and making them unavailable for plants and microbial uptake (Fierer et al., 2001, Kraus et al., 2003), which may have knock-on effects on soil C sequestration and nutrient turnover (Adamczyk et al., 2019, Madritch and Lindroth, 2015). Certain boreal forest plants, such as coniferous trees (e.g., *Picea abies*) and Ericaceous dwarf shrubs (e.g., *Vaccinium myritillus*), naturally contain high levels of CTs. Because tannins are known to enter the soil through both above- and below-ground litter, those ecosystems also contain a high CT concentration in the soil organic layer, where foliage litter contribute a significant part (Smolander et al., 2012, Adamczyk et al., 2014, Kuiters and Sarink, 1986, Adamczyk et al., 2013). Species composition changes are, therefore, likely to change ecosystem-level CT concentration, as CTs production varies across species. Thus, quantifying the CT level in senesced foliage is important and interesting in order to comprehend their role in the ecological processes more clearly.

However, unfortunately, investigations on PSM production response to different influential factors mainly focused on only green leaves. Among them, very few have examined the effects of fertilization on PSM production in green foliage of tree species (Nybakken et al., 2018) and on the total phenolic concentration (Blodgett et al., 2005), while trees senesced foliage tannins level has not been studied. Nybakken et al. (2018) demonstrated that fertilization decreases total phenolics in current-year needles of *Picea abies* but did not affect the previous year needles. Another study by De Long et al. (2016) showed that N fertilization decreased CT level in both green and senesced leaves in subarctic heath species. Although this study tested both green and senesced leaves in heath species, still we lack sufficient scientific knowledge on how the improved N available situation may affect trees senesced foliage and ground vegetation tannins level and the forest ecosystem.

This study aimed to investigate CT concentration's response with fertilizer application in senesced foliage of two different plant species, *Picea abies* and *Vaccinium myrtillus*, as well as soil organic layer. The research was carried out in an old *Picea abies* dominated boreal forest in South-Eastern Norway, which has been fertilized annually since 2003 with 150 kg N ha^{-1}. I measured CT level, C, N, C: N ratio of both senesced foliage and soil as well as pH value for soil. I used this setup to test the following hypotheses: fertilization will (1) increase N

concentration in senesced foliage; therefore, C: N ratio will decrease, resulting in a decrease of CT level; (2) decrease CT concentration in soil organic layer due to a shift in the field layer from *Vaccinium myrtillus* dominance to the dominance of graminoids and forbs; and (3) increase N and C concentration, correspondingly C: N ratio will decrease in soil organic layer. Understanding CT's response with N input is vital as they play a significant role in ecosystem processes, including hampering decomposition and, therefore, influencing nutrient dynamics.

2. Material and Methods

2.1. Study site

The study site is located in Gausdal Vestfjell (61°10'N, 09°90'E), near Kittilbu, South-Eastern Norway, at an elevation of approximately 800 m (Gauslaa et al., 2008, Davey et al., 2017). The annual mean temperature and mean precipitation is -0.1°C and 810mm, respectively, from the period 1961-1990 (based on information from the Kittilbu meteorological station; source: www.met.no) (Bach et al., 2009, Gauslaa et al., 2008). The bedrock consists of cambro-Ordovician-age sedimentary rocks (shale) covered by dense moraine layers (Source: www.NGU.no) (Bach et al., 2009, Gauslaa et al., 2008). The forest is a subalpine old boreal forest dominated by Norway Spruce (*Picea abies*) (Gauslaa et al., 2008). The dwarf shrub *Vaccinium myrtillus* and bryophytes, for instance, *Pleurozium schreberi*, *Hylocomium splendens*, *Polytrichum commune*, and *Sphagnum girgensohnii*, dominate the field vegetation (Gauslaa et al., 2008). There are also several flowering plants, including *Vaccinium vitis-idaea* and *Avenella flexuosa* (Gauslaa et al., 2008).

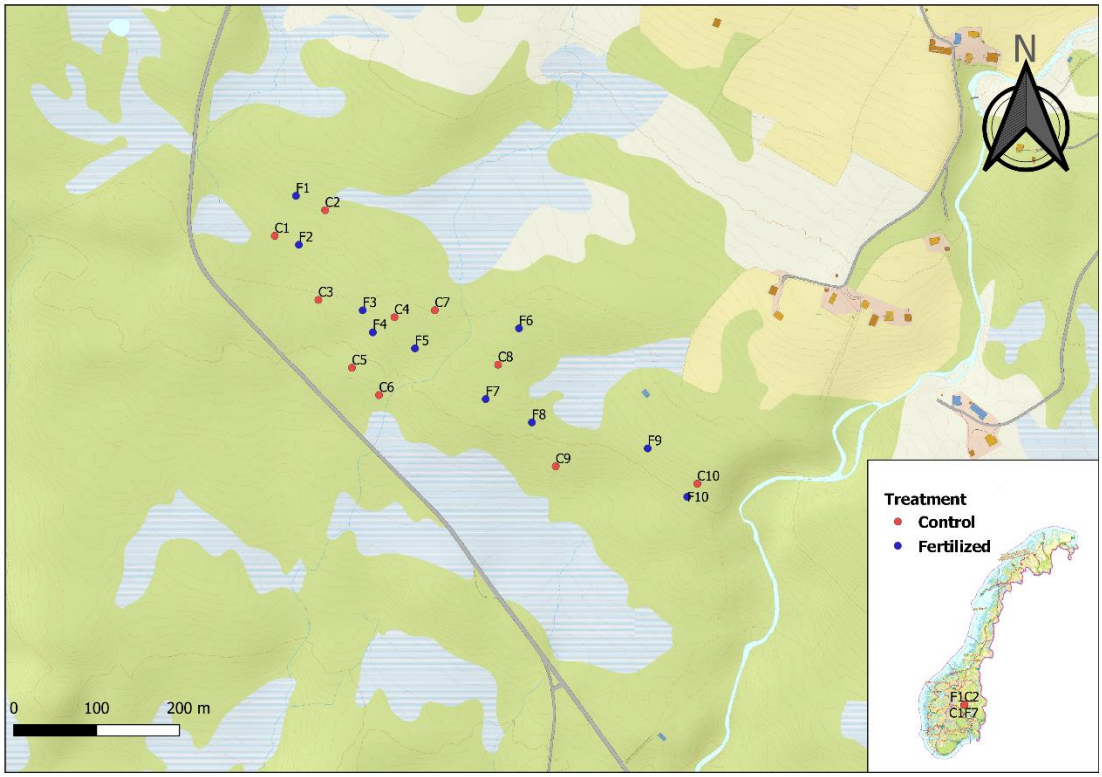

Figure 1: Location map of the study site in Gausdal Vestfjell, South-Eastern Norway.

There are 20 established 15 x 15 m^2 experimental plots, 10 controls, and the other 10 are fertilization application plots (Fig 1). The plots are approximately 50 to 350 m in distance from each other (Davey et al., 2017). Fertilized plots have been treated since 2003 with granulated pellets consisting of 24% N, 2%P, 6% K with other trace elements (Ca, Mg, S, B, and Cliorine-Containing) with the amount of 150 kg ha^{-1} (YaraMila® FULLGJØDSEL® by Yara, Norway)(Davey et al., 2017).

2.2. Sample collection and preparation

2.2.1. soil

I collected soil samples in early summer, 15-16 June 2020**.** I took a 5x5 m^2 area within each plot for the soil sample and divided it into five 1 x1 m^2 subplots. Plots contained one subplot in each corner and one in the centre. Therefore, I have set up a total of 100 subplots with five subplots inside each of the ten control and ten fertilized plots. First, I collected soil cores of 10 cm in diameter and split the cores into two; one half I placed into plastic bags and another half was used for another project. The organic layer depth was measured on the hole. The next day, I weighed wet soil cores, let them dry for 48 hours in an oven at 30°C temperature, and weighed the dry cores to measure the soil's water holding capacity.

For the extraction process, at first, I homogenized soil cores by mixing well in a two-litre plastic box using hand so that there were no chunks, picked out roots and twigs with a diameter larger than approximately 2mm, and removed living bryophytes and other plant parts. I filled those homogenized samples in two steel containers of 50 ml capacity using a clean spoon, ground them in a ball mill (MM 400, Retsch, Haag, Germany) with 30 Rpm for 1.45 minutes to get a fine powder, mixed them into one plastic container and kept in the freezer for further analysis. This powdered soil was used for measuring tannin, pH, and C and N concentrations.

2.2.2. Senesced foliage

Senesced foliage samples were collected in early autumn 12-13 September 2020. I collected senesced foliage (needles and leaves) randomly from all over the plots by shaking trees for *Picea abies* needles and cutting leaves with stem using scissors for *Vaccinium myrtillus.* Then

placed them into paper bags and air-dried for approximately 15 days. For preparing foliage samples for analysis, I sorted the dried needles and leaves from the stem before grinding and then milled approximately 0.5 to 1gm of foliage out of them. The grinding process was the same for foliage as the soil samples.

2.3. Extraction of condensed tannins

The concentration of CT from both soil and plant material was measured by following the acid butanol assay described for proanthocyanidins by Hagerman (2002). 100 mg of powdered soil sample and 10 mg of foliage sample were dissolved with 4 ml of 70% acetone in a test tube, close the lid immediately, and vortex in a planner shaker (KS 501 digital, IKA-WERKE, Germany) for 1 hour (200 rpm). Immediate after shaking, I centrifuged them at 4000 rpm for 10 minutes and then discarded the supernatant in 15 ml test tubes. The same extraction process was repeated twice and then evaporated to dryness in a vacuum centrifuge (Eppendorf concentrator plus; Eppendorf, Hamburg, Germany), lid tightly and stored in the freezer with foil-covered to keep them away from sunlight and not over the room temperature until further analysis.

2.4. Analysis of condensed tannins

The dried extracts were redissolved in 2 ml MeOH using an ultrasonic cleaner (VWR, Malaysia), and I took out 0.5 ml extract from it to10 ml glass tubes for analysis. Then added 3ml butyric acid (95% butanol,5% HCL) and 0.1 ml of iron reagent (2% ferric ammonium sulfate in 2 M HCL) with the extract, lid tightly the tubes and placed them for boiling in water at 99°C for one hour. Similar two control samples (without sample extract) were made along with the original samples to compare the absorbance. After cooling down, the light absorption at 550 nm was measured with a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). The same procedure was followed for both type of samples (soil and foliage).

CT analysis of half of the soil samples was done using the same procedure with some modification in redissolving the dry extract with 0.5 ml MeOH and vortex vigorously. The residue colour turned very dark during this process; therefore, I used the method described above to avoid the error due to diluting the residue repeatedly.

2.5. Analysis of soil pH

For pH measurement, 5 ml of each subplot's powdered soil were mixed very nicely with 12.5 ml of deionized water in a glass test tube and left overnight. After 24 hours, shaken again and measured the pH value using a pH meter (WTW GmbH, Weilheim, Germany).

2.6. Analysis of carbon and nitrogen

For both soil and senesced foliage, total carbon and nitrogen concentration was measured using 5-7 mg of powdered sample, wrapped in tin foil, with a Micro cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

2.7. Statistical analysis

Data arrangement and calculation of CT concentration, mean and standard error of the variables were performed using Microsoft Excel, version 365 (Microsoft Corporation 2019). Linear mixed-effect model analysis was performed for soil samples to test the correlation between response variables and fixed and random factors using the lmer () function of package "lme4" in R, as there were five sub-samples per plot. On the other hand, foliage samples had only one value per plot; hence, linear model analysis was performed using the lm () function of the "stat" package in R. Treatment (control and fertilized) was used as the main explanatory variable for all analyses, and PlotId (sub-plots) used as a random factor when using linear-mixed effect analysis for soil sample. Response variables were CT concentration, N and C concentration and C: N ratio for both foliage and soil samples and pH value for soil. Other cofactors such as water holding capacity (WHC), depth of the organic layer, total graminoids and *Vaccinium myrtillus* were used as fixed effects to see interaction effects between treatment. Each covariate was analyzed in a separate analysis, and results have shown in the appendix. The soil organic layer's N and C content was calculated by dividing the product of concentration and dry weight of the soil core in kg by the area in m^2. The soil sample analyses, including vegetation data, were done at the plot level using averages of the sub-plots from a previous master thesis (Lorentzen, 2017). The normality assumption and whether the data meets homoscedastic or not is checked visually by Q-Q (quantile-quantile) plots. Statistical analysis is considered significant when $p < 0.05$. These p-values were obtained from the t-statistic using Satterthwaite's approximation to the denominator degrees of freedom as a default function of the "lmerTest" package for soil samples. For senesced foliage, Welch's t-test was performed to

obtain the p-value. All statistical analyses and the graphical illustration of data were performed in R version 4.0.3 (2020-10-10, Bunny-Wunnies Freak Out) and Rstudio version 1.3.1039. Package "tidyverse" was used for graphic illustration, and QGIS version 3.16.3 was used to create a location map of the study area.

3. Result

3.1. Senesced foliage response

Condensed tannins (CT) concentration of neither *Picea abies* nor *Vaccinium myrtillus* was affected by fertilization (Table 1) (Fig 2a). Nitrogen concentration of *Picea abies* and *Vaccinium myrtillus* was significantly higher by (27%) and (24%) in fertilized plots compared to the control plots (Table 1) (Fig 2b). C to N ratio differed significantly between treatments with a decrease of 26% for *Picea abies* and 24% for *Vaccinium myrtillus* in fertilized plots (Table 1) (Fig 2d). Carbon concentration was not different between plots (Table 1) (Fig 2c).

Table 1: T-value and P-value of the Soil CT (CT concentration; mg g^{-1}), pH (pH value), N (nitrogen concentration), C (carbon concentration), CN (carbon to nitrogen ratio), N Content and C Content, and foliar CT, N, C, CN of *Picea abies* and *Vaccinium myrtillus* test results of each of the two treatments control and fertilized. The T-value obtained from the basic linear mixed model represents the quotient of the estimated mean and standard error of the respective response variable. Significant P-values from Satterethwaite's t-test for soil sample and Welch's t-test for foliar samples are presented by bold and symbols: * p<0.05; ** p<0.01; *** p<0.001.

Material	Responses	T value	P-Value
Soil	CT	**-2.23**	**0.039***
	pH	**-2.61**	**0.0178***
	N concentration	**7.00**	**0.000*****
	C concentration	**2.55**	**0.012***
	CN	**-5.00**	**0.000*****
	N Content	**2.647**	**0.016***
	C Content	1.63	0.121
Vaccinium myrtillus	CT	-0.97	0.35
	N	**11.15**	**0.000*****
	C	-0.012	0.991
	CN	**-10.27**	**0.000*****
Picea abies	CT	-0.40	0.70
	N	**5.669**	**0.000*****
	C	-0.253	0.803
	CN	**-8.867**	**0.000*****

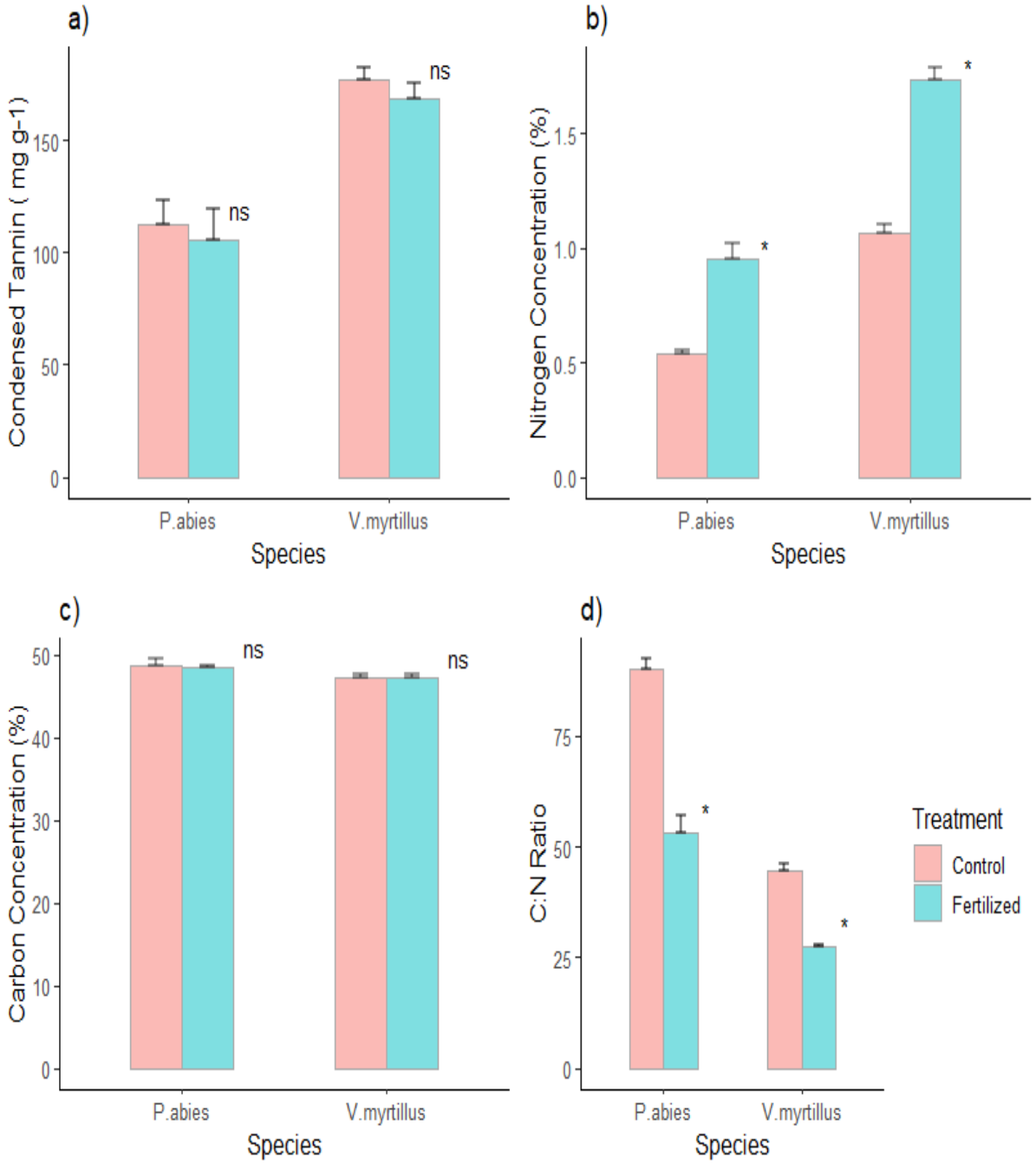

Figure 2: Mean value of response variables of *Picea abies* and *Vaccinium myrtillus* with standard error (a) Condensed Tannin concentration, (b) Nitrogen concentration, (c) Carbon concentration, (d) C: N (carbon to nitrogen) ratio for the two treatments. The mean value was calculated by dividing the total sum of the values by the number of plots, including subplots (n= 50 for each treatment). The significant p-value for each response is shown by '*' and nonsignificant p-values by 'ns' beside the respective column.

3.2. Soil response

Condensed tannin concentration was significantly lower by an average of 13% in fertilized plots compared to control (Table1) (Fig:3a). CT concentration was not affected by any of the variables (WHC, depth of the soil core, total graminoids, and *Vaccinium myrtillus* cover) individually. However, a substantial interaction effect between treatment and total *V. myrtillus* cover was found (Table 2) (Appendix).

Carbon and nitrogen concentration increased in fertilized plots, but C: N ratio and pH value decreased significantly. C and N concentration increased by 12% and 7%, respectively (Table1) (Fig 3d) (Fig 3c). pH value decreased by 2% (Table1) (Fig 3b) and C:N ratio by 8% (Table1) (Fig 3e). Nitrogen concentration and C: N ratio was affected significantly only by water holding capacity (WHC), and C concentration only affected *Vaccinium myrtillus* cover (Table 2) (Appendix) among all the variables. Nitrogen content significantly increased by 18% (Table 1) (Fig 4a), but C content was not affected (Table 1) (Fig 4b).

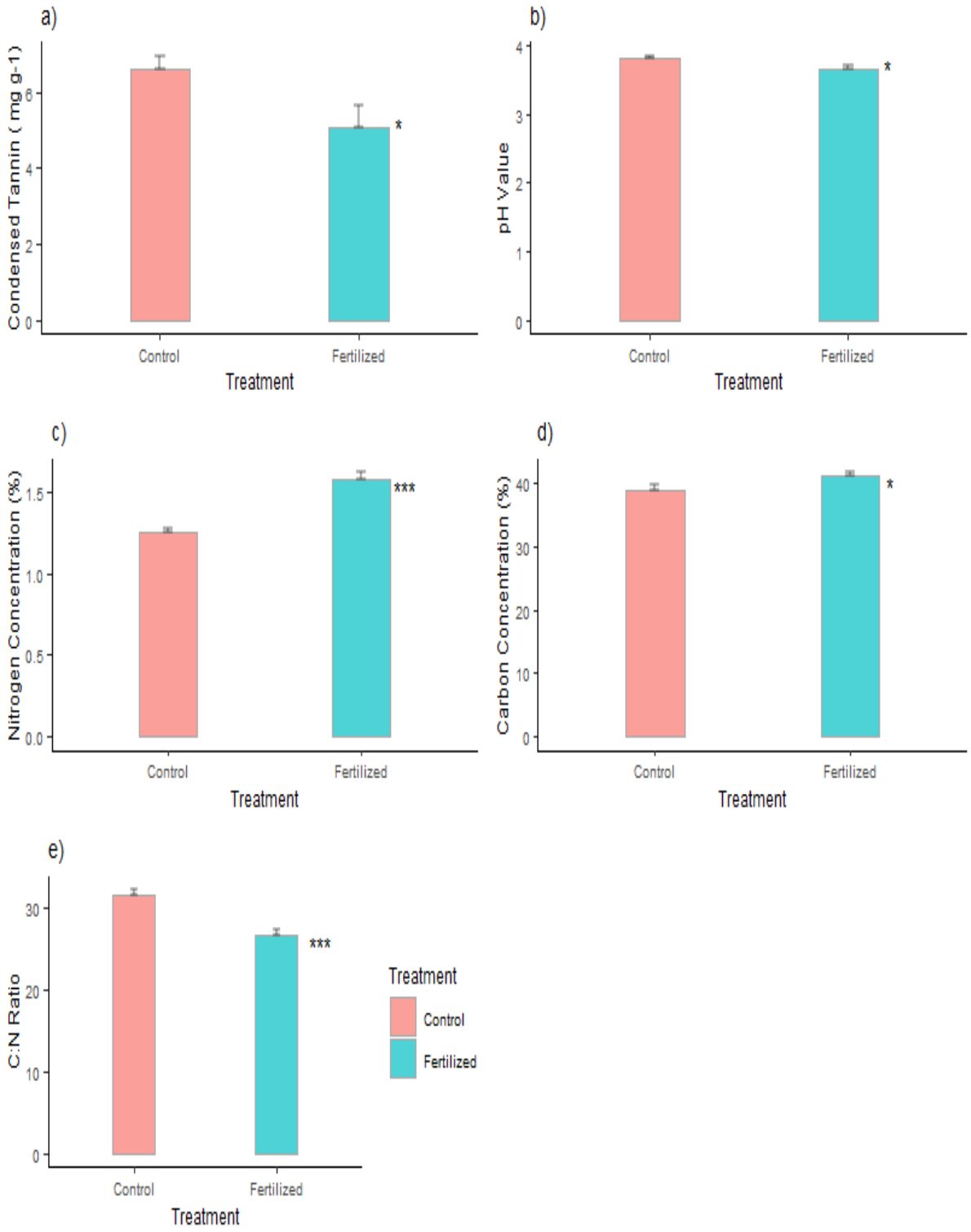

Figure 3: Mean values of response variables of soil with standard error (a) condensed Tannin Concentration, (b) pH value, (c) Nitrogen Concentration, (d) Carbon Concentration), (e) C: N Ratio (carbon to nitrogen ratio), for the two treatments. The mean value was calculated by dividing the total sum of the values by the number of plots, including subplots (n=50 for each treatment). Significant p-values for each response are shown by the asteroid ('*' and '***') beside the respective column.

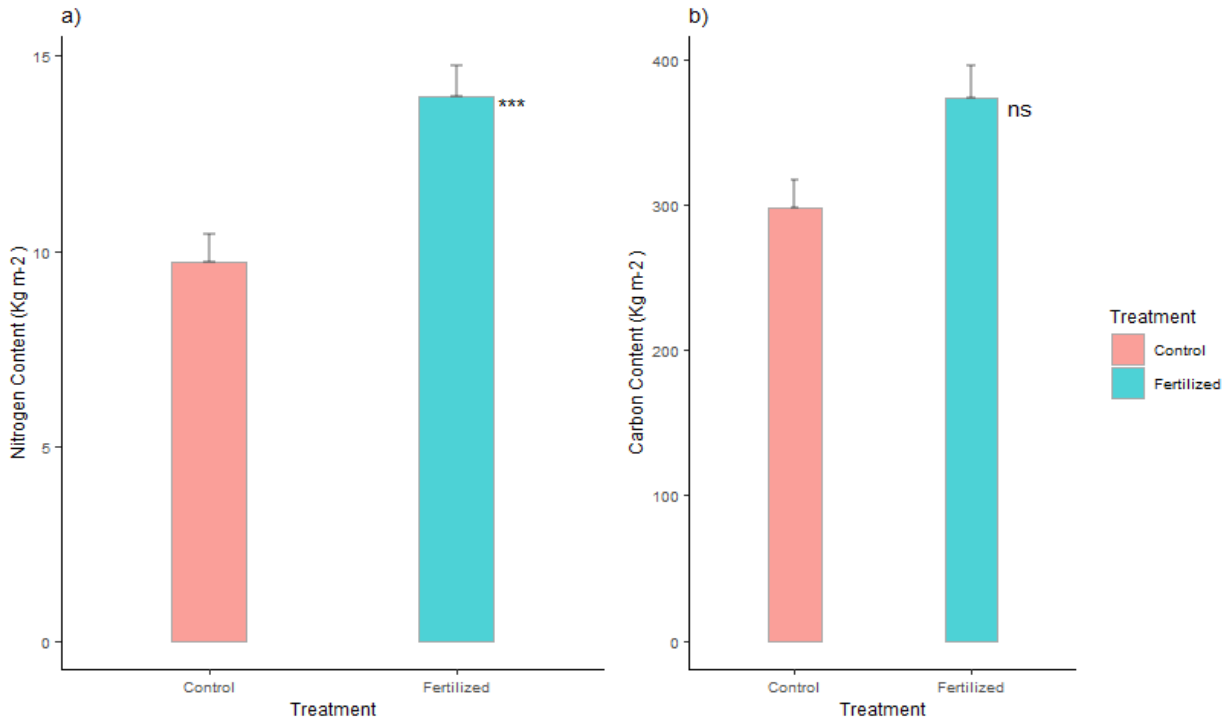

Figure 4: Mean value with standard error a) nitrogen and b) carbon content in control and fertilized plots. The significant p-value for each response is shown by '***' and nonsignificant p-values by 'ns' beside the respective column.

4. Discussion

4.1. Senesced foliage responses with increased nitrogen

I found mixed support for my first hypothesis. That is, fertilization increased foliar N concentration and lowered the C: N ratio. Foliar CT concentrations were, however, not responsive to the fertilization treatment. The increase of foliar nitrogen concentration of both species to N addition is in accordance with the result of the previous thesis of Lorentzen (2017), who measured C and N concentration in fresh green foliage. This result also is in line with earlier studies (Booker and Maier, 2001, Nybakken et al., 2018) and reflects consistency with ecological theories on carbon and nitrogen balance (Bryant et al., 1983, Jones and Hartley, 1999). Because N is an essential element of chlorophyll, and chlorophyll content is roughly proportional to leaf N content (Evans, 1983), plants with a higher foliar N concentration are likely to have higher chlorophyll levels (Foulkes et al., 2009). Thus high N concentration with increased N availability may indicate increased chlorophyll content that escalates photosynthesis and, as a result, C fixation for plant growth. Booker and Maier (2001) found that N fertilization increased needle N concentration of *Pinus taeda* by 23%. Strengbom and Nordin (2008) and Gundale et al. (2014) also showed *V. myrtillus* had a rise in N concentration with fertilizer addition.

Moreover, my results in both species, C concentration, did not show any significant difference between treatments, consistent with the decrease of C: N ratio with fertilization, suggesting that the plant growth possibly increased compared to the carbon storage (Ågren, 2004). It may be that I performed this study with senesced foliage, and the metabolic process of senesced foliage is different from green foliage. In senesced foliage, the metabolic processes undergo through nutrient conservation mechanism in which the breakdown of chloroplast leads to the redistribution of amino acids, a major nitrogen source (Buet et al., 2019). In this way, plants store nutrients to use other parts of the plant later and reduce their nutrient dependency at the nutrition-poor site (Gregersen et al., 2008, Buet et al., 2019). Thus, in light of the stated reasoning, findings of increased N concentration and decreased C: N ratio are in accordance with the carbon-nutrient balance hypothesis (Bryant et al., 1983) and the protein competition model (Jones and Hartley, 1999); i.e. a better resource availability condition should result in improved growth and decreased plant defence. It should be mentioned that plant growth was not measured in this experiment. However, an exception to this idea, Strengbom and Nordin

(2008) found significantly higher C concentration in a grass species, *Avenella flexuosa*, after fertilizer addition.

Opposing my hypothesis, foliar CT concentration had no significant difference in response to the N-treatments. Previous empirical studies have shown conflicting effects of fertilization on foliar CTs. The majority of studies found a significant decrease in CT concentration with fertilizer application (Nybakken et al., 2018, Kraus et al., 2004, Osier and Lindroth, 2001), whereas some also found nonsignificant effects of fertilization on foliar CT concentration (Booker and Maier, 2001, Iason et al., 1993, Iason and Hester, 1993, Hättenschwiler et al., 2003). These contradictory outcomes may occur for various reasons, including differences in environmental conditions, the experimental setup of those studies, sample collection and handling and differences in samples (Kraus et al., 2004). Due to these reasons, some factors may not exceed the fertility level or caused significant differences in the carbon-nutrient balance required for the foliar compositional change (Kraus et al., 2004). In the present study, I measured CT concentration in senesced foliage. Nybakken et al. (2018) studied fertilization effect on chemical defence in *Picea abies* green needles at the same study site and found that fertilization decreased CT level in current year needles but increased in previous year needles. There are some other studies that might not be linked to the N addition rather related to the seasonal variation or age variation of PSM accumulation patterns. For example, Ganthaler et al. (2017) studied seasonal and infection-induced accumulation patterns of phenolic compounds in *Picea abies* and found that catechin (the precursor of CT) concentration increased during bud swelling and in the needle maturation, and the previous year needles have a high concentration of CT. Again, this study showed no significant differences in the accumulation pattern of catechin in healthy and infected needles, suggesting an inherent phenological accumulation pattern of CT, regardless of stress condition. Wam et al. (2017) investigated compositional change with plant age in *Betula Pubescens,* reported that CT level increased with plant age, and at older age, they remained fairly constant. In light of the findings of these mentioned studies, my investigated result of *Picea abies* is pointing to the prioritization of phenological accumulation pattern of CT rather than nitrogen availability in senesced foliage.

Furthermore, Iason et al. (1993) found nonresponsive CT concentration to fertilization in *Calluna vulgaris,* an Ericaceous species. The author explained this result by that fertilization increases plant growth initially, but during the time of flowering, there may still be high photosynthesis while plants do not need fixed carbon for further development of growth and

hence, use that carbon to produce phenolics. Because I sampled litter in mid-September and at that time plants have already passed the growing and flowering stage and started litterfall, this explanation may also be applied for my data of *Vaccinium myrtillus*. It might be that the high concentration of CT that accumulated during flowering remained in the leaves until they fall off after-senescence. Thus, my CT results cannot be explained by either the carbon-nutrient balance hypothesis (Bryant et al., 1983) or the protein competition model (Jones and Hartley, 1999); instead, it challenges the generality of these hypotheses. Therefore, my result of increasing N concentration with not responding CT level in senesced foliage could be interpreted in a way that fertilization promotes plant growth, but the chemical response may vary with the phenological stage of the plant according to the need and availability of photosynthates. However, to have a more precise understanding of plants' underlying mechanism on compositional change with increased N input, investigation on CT responses to fertilization in both green and senesced leaves along with N analysis in tissue level should be emphasized further.

4.2. Soil organic layer response with increased nitrogen

As I hypothesized, condensed tannin in the soil organic layer decreased with N addition. Unfortunately, little is known about tannin concentration in response to nitrogen fertilization in forest soil, especially in the organic soil layer. However, I measured soil organic layer tannin concentration, and the primary source of organic layer tannin is mostly from litter inputs. Thus, species compositional change likely to affect the organic layer tannins levels significantly. The reason may be that different plant species produce different level of tannins, and subsequently, different tannin-containing litter input might influence organic layer tannins concentration. In addition, it is well established that tannins are produced in abundance by Ericaceous species but not by graminoid species. (Jung et al., 1979). In my study site, the shift of vegetation cover from Ericaceae dominated to graminoid dominated with fertilization application was reported by Lorentzen (2017). Therefore, the interpretation for the decline of CT concentration in fertilized plots might be the effect of species turnover. Moreover, I found a significant interaction effect between treatment and *Vaccinium myrtillus* on CT concentration, whereas the individual effect of *Vaccinium myrtillus* was not significant when not accounting for treatment (Table 2) (Appendix), suggesting intraspecific variation might not be the reason for the change in soil CT instead, possibly driven by species turnover in ground vegetation.

Supporting my third hypothesis, I found an increase of C and N concentration and a reduction of the C: N ratio in the soil organic layer in response to the fertilization. These findings are in line with the fertilization experiment of Mäkipää (1994), who found that nitrogen addition increased N concentration and the amount of organic matter in the humus layer. Forest fertilization increases litterfall by increasing leaf production and decreasing leaf turnover time, thus increases organic matter in the forest soil (Linder and Axelsson, 1982). I also found a significant positive relationship of N concentration with soil WHC when not accounting for treatments (Table 2) (Appendix), indicating more available N for plants to uptake and increase plant productivity. It is well known that N mineralization and soil moisture are generally positively correlated (Gonçalves and Carlyle, 1994). Moreover, Ilek et al. (2015) showed that the organic horizons of *Picea abies* stands have a higher water holding capacity. Thus, higher N concentration in the organic layer can be reasonably explained by the abundance of N rich foliage litter, especially needle litter, due to fertilization and the increase of soil moisture. Soil C concentration increased in fertilized plots, which could also be due to increased organic matter in the organic layer. Here, it should be mentioned that soil organic matter content or litterfall was not calculated in this experiment. However, above-ground litter can only account for a part of the organic matter, where below-ground litter also significantly contribute to this. Vogt et al. (1990) and Haynes and Gower (1995) observed that N deposition reduces root biomass in mature forest. Ahlström et al. (1988) also reported that root production and the extent of decaying roots declined after fertilization. Thus, to get a more precise understanding, both above- and below-ground litter input should be included in the analysis.

In line with the previous studies, the corresponding C to N ratio significantly declined in fertilized plots, reflecting increasing soil organic matter decomposition. A potential reason for this could be that fertilization improves litter quality with high N concentration and lower PSM concentration, subsequently facilitate microorganisms' efficiency to boost decomposition. Additionally, increasing graminoid abundance, which has rapid turnover time, probably makes this environment more conducive to decomposers. This explanation is in accordance with Prescott et al. (1992), who showed that fertilization increased decomposition. When N and C content is calculated as per unit area, the data showed a significant increase of N in fertilized plots. This data is consistent with earlier studies from boreal forest ecosystems (Mälkönen, 1990, Nohrstedt et al., 2000, Tamm et al., 1995) and indicates an increase of soil N pool with fertilizer addition. Contrary to most studies, C content did not differ among treatments (Control and fertilized), suggesting no fertilization effect on soil carbon storage. According to Lorentzen

(2017), the application of fertilizers did not change the total amount of biomass, which may explain the similarity of carbon storage between treatments.

Contrary to earlier studies, pH level significantly reduced in fertilized plots compared to controls. It could be that fertilization increases the amount of *Picea abies* needle litter in the organic matter, leading to a further decrease of pH in the soil organic layer. Pallant and Riha (1990) studied the influence of individual trees on the spatial variability in soil pH and concluded that *Picea abies* could significantly contribute to soil acidification. Thus, following this line of reasoning, the above explanation for this decrease of pH value in fertilized plots seems likely to be true. However, since I have only measured the pH of the organic layer, it is important to examine the broadscale by adding mineral layer pH response to understand better the effects of nitrogen addition on ecosystem processes.

5. Conclusion

The present study demonstrated that nitrogen input affects ecosystem processes in the boreal forest. I found that fertilization affected senesced foliage chemical composition by increasing N concentration and decreasing the C to N ratio, but unexpectedly, CT concentration did not respond. On the other hand, in the soil organic layer, both C and N increased, but C to N ratio and CT decreased with fertilizer addition. C and N results for both foliage and soil are consistent with previous studies on fertilization effects in boreal forests. The findings of foliar N concentration and corresponding C: N ratio response to fertilizer is in accordance with the resource-based ecological theories. However, senesced foliage CT concentration results can be well explained by CT's inherent phenological accumulation pattern. In terms of forest soil, the decrease of CT in fertilized plots could be due to vegetation coverage shifting from Ericaceae to graminoid and forbs. I also found an increase of N content in fertilized plots, reflecting an increase of soil N pool. C content did not differ between treatments, suggesting soil C storage was not affected by N fertilizer. Even though only MeOH soluble CT, C, N and C: N ratio of foliage and soil organic layer and pH of soil have been measured in this experiment, the results provide a good overview of the ecosystem responses to fertilizer addition.

In conclusion, tannins are well known to immobilize nutrients, and N availability in this study reduced the CT concentration of the soil organic layer and increased the N concentration, thereby increasing the decomposition of organic matter. Therefore, my study findings may imply that N deposition and forest fertilization facilitate organic matter decomposition when climate change also speeds up this process.

6. References

Adamczyk, B., Kiikkilä, O., Kitunen, V. & Smolander, A. (2014). Can we measure condensed tannins from tannin–protein complexes? – A case study with acid–butanol assay in boreal forest soil organic layer. *European Journal of Soil Biology,* 64**,** 40-45.

Adamczyk, B., Kitunen, V. & Smolander, A. (2013). Response of soil C and N transformations to condensed tannins and different organic N-condensed tannin complexes. *Applied Soil Ecology,* 64**,** 163-170.

Adamczyk, B., Sietiö, O. M., Biasi, C. & Heinonsalo, J. (2019). Interaction between tannins and fungal necromass stabilizes fungal residues in boreal forest soils.

Ågren, G. I. (2004). The C: N: P stoichiometry of autotrophs–theory and observations. *Ecology Letters,* 7**,** 185-191.

Ahlström, K., Persson, H. & Börjesson, I. (1988). Fertilization in a mature Scots pine (Pinus sylvestris L.) stand—effects on fine roots. *Plant and Soil,* 106**,** 179-190.

Bach, L. H., Frostegård, Å. & Ohlson, M. (2009). Site identity and moss species as determinants of soil microbial community structure in Norway spruce forests across three vegetation zones. *Plant and soil,* 318**,** 81-91.

Bate-Smith, E. (1977). Astringent tannins of Acer species. *Phytochemistry,* 16**,** 1421-1426.

Bergh, J., Nilsson, U., Allen, H. L., Johansson, U. & Fahlvik, N. (2014). Long-term responses of Scots pine and Norway spruce stands in Sweden to repeated fertilization and thinning. *Forest Ecology and Management,* 320**,** 118-128.

Blodgett, J. T., Herms, D. A. & Bonello, P. (2005). Effects of fertilization on red pine defense chemistry and resistance to Sphaeropsis sapinea. *Forest Ecology and Management,* 208**,** 373-382.

Booker, F. L. & Maier, C. A. (2001). Atmospheric carbon dioxide, irrigation, and fertilization effects on phenolic and nitrogen concentrations in loblolly pine (Pinus taeda) needles. *Tree Physiology,* 21**,** 609-616.

Bryant, J. P., Chapin Iii, F. S. & Klein, D. R. (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos***,** 357-368.

Buet, A., Costa, M. L., Martínez, D. E. & Guiamet, J. J. (2019). Chloroplast Protein Degradation in Senescing Leaves: Proteases and Lytic Compartments. *Frontiers in Plant Science,* 10.

Carroll, C. & Noss, R. F. (2020). Rewilding in the face of climate change. *Conservation Biology*.

Close, D. C. & Mcarthur, C. (2002). Rethinking the role of many plant phenolics–protection from photodamage not herbivores? *Oikos,* 99**,** 166-172.

Covelo, F. & Gallardo, A. (2001). Temporal variation in total leaf phenolics concentration of Quercus robur in forested and harvested stands in northwestern Spain. *Canadian Journal of Botany,* 79**,** 1262-1269.

Davey, M. L., Skogen, M. J., Heegaard, E., Halvorsen, R., Kauserud, H. & Ohlson, M. (2017). Host and tissue variations overshadow the response of boreal moss-associated fungal communities to increased nitrogen load. *Molecular ecology,* 26**,** 571-588.

De Long, J. R., Sundqvist, M. K., Gundale, M. J., Giesler, R. & Wardle, D. A. (2016). Effects of elevation and nitrogen and phosphorus fertilization on plant defence compounds in subarctic tundra heath vegetation. *Functional ecology,* 30**,** 314-325.

De Vries, W. (2014). Nutrients trigger carbon storage. *Nature Climate Change,* 4**,** 425-426.

Dentener, F., Drevet, J., Lamarque, J. F., Bey, I., Eickhout, B., Fiore, A. M., Hauglustaine, D., Horowitz, L. W., Krol, M., Kulshrestha, U. C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., Van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Müller, J. F., Pitari, G., Rodriguez, J., Sanderson, M., Solmon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S. & Wild, O. (2006). Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Global Biogeochemical Cycles,* 20**,** n/a-n/a.

Driebe, E. & Whitham, T. G. (2000). Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. *Oecologia,* 123**,** 99-107.

Endara, M. J. & Coley, P. D. (2011). The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology,* 25**,** 389-398.

Evans, J. R. (1983). Nitrogen and photosynthesis in the flag leaf of wheat (Triticum aestivum L.). *Plant physiology,* 72**,** 297-302.

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology,* 51**,** 565-581.

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Sardans, J., Luyssaert, S., Campioli, M., Chapin Iii, F. S., Ciais, P., Malhi, Y. & Obersteiner, M. (2014). Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate Change,* 4**,** 471-476.

Fierer, N., Schimel, J. P., Cates, R. G. & Zou, J. (2001). Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology and Biochemistry,* 33**,** 1827-1839.

Foulkes, M. J., Hawkesford, M. J., Barraclough, P., Holdsworth, M., Kerr, S., Kightley, S. & Shewry, P. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research,* 114**,** 329-342.

Galloway, J. N. & Cowling, E. B. (2002). Reactive Nitrogen and The World: 200 Years of Change. *AMBIO: A Journal of the Human Environment,* 31**,** 64-71, 8.

Ganthaler, A., Stöggl, W., Kranner, I. & Mayr, S. (2017). Foliar phenolic compounds in Norway spruce with varying susceptibility to Chrysomyxa rhododendri: analyses of seasonal and infection-induced accumulation patterns. *Frontiers in plant science,* 8**,** 1173.

Gauslaa, Y., Lie, M. & Ohlson, M. (2008). Epiphytic lichen biomass in a boreal Norway spruce forest. *The Lichenologist,* 40**,** 257-266.

Gonçalves, J. D. M. & Carlyle, J. (1994). Modelling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. *Soil Biology and Biochemistry,* 26**,** 1557-1564.

Gonzalez-Hernandez, M., Karchesy, J. & Starkey, E. E. (2003). Research observation: hydrolyzable and condensed tannins in plants of northwest Spain forests. *Rangeland Ecology & Management/Journal of Range Management Archives,* 56**,** 461-465.

Gregersen, P. L., Holm, P. B. & Krupinska, K. (2008). Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biology,* 10**,** 37-49.

Gundale, M. J., Deluca, T. H. & Nordin, A. (2011). Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Global Change Biology,* 17**,** 2743-2753.

Gundale, M. J., From, F., Bach, L. H. & Nordin, A. (2014). Anthropogenic nitrogen deposition in boreal forests has a minor impact on the global carbon cycle. *Global Change Biology,* 20**,** 276-286.

Hagerman, A. 2002. The tannin handbook. Miami University Oxford, OH 45056.

Hättenschwiler, S., Hagerman, A. & Vitousek, P. (2003). Polyphenols in litter from tropical montane forests across a wide range in soil fertility. *Biogeochemistry,* 64**,** 129-148.

Haynes, B. E. & Gower, S. T. (1995). Below-ground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree physiology,* 15**,** 317-325.

Hedwall, P.-O., Gong, P., Ingerslev, M. & Bergh, J. (2014). Fertilization in northern forests – biological, economic and environmental constraints and possibilities. *Scandinavian Journal of Forest Research,* 29**,** 301-311.

Herms, D. A. & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The quarterly review of biology,* 67**,** 283-335.

Hernes, P. J. & Hedges, J. I. (2000). Determination of condensed tannin monomers in environmental samples by capillary gas chromatography of acid depolymerization extracts. *Analytical Chemistry,* 72**,** 5115-5124.

Hofland-Zijlstra, J. D. & Berendse, F. 2009. The effect of nutrient supply and light intensity on tannins and mycorrhizal colonisation in Dutch heathland ecosystems. *In:* Van Der Valk, A. G. (ed.) *Herbaceous Plant Ecology: Recent Advances in Plant Ecology.* Dordrecht: Springer Netherlands.

Iason, G., Hartley, S. & Duncan, A. (1993). Chemical composition of Calluna vulgaris (Ericaceae): do responses to fertilizer vary with phenological stage? *Biochemical Systematics and Ecology,* 21**,** 315-321.

Iason, G. R. & Hester, A. J. (1993). The response of heather (Calluna Vulgaris) to shade and nutrients--predictions of the carbon-nutrient balance hypothesis. *Journal of Ecology***,** 75-80.

Ilek, A., Kucza, J. & Szostek, M. (2015). The effect of stand species composition on water storage capacity of the organic layers of forest soils. *European Journal of Forest Research,* 134**,** 187-197.

IPCC (2014). Synthesis report: summary for policy makers.

Jacobson, S. & Pettersson, F. (2010). An assessment of different fertilization regimes in three boreal coniferous stands. *Silva Fennica,* 44**,** 815-827.

Jones, C. G. & Hartley, S. E. (1999). A Protein Competition Model of Phenolic Allocation. *Oikos,* 86**,** 27-44.

Jung, H.-J. G., Batzli, G. O. & Seigler, D. S. (1979). Patterns in the phytochemistry of arctic plants. *Biochemical Systematics and Ecology,* 7**,** 203-209.

Kraus, T., Zasoski, R. & Dahlgren, R. (2004). Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. *Plant and Soil,* 262**,** 95-109.

Kraus, T. E., Dahlgren, R. A. & Zasoski, R. J. (2003). Tannins in nutrient dynamics of forest ecosystems-a review. *Plant and soil,* 256**,** 41-66.

Kuiters, A. (1990). Role of phenolic substances from decomposing forest litter in plant-soil interactions. *Acta botanica neerlandica,* 39**,** 329-348.

Kuiters, A. & Sarink, H. (1986). Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. *Soil Biology and Biochemistry,* 18**,** 475-480.

Linder, S. & Axelsson, B. (1982). Changes in carbon uptake and allocation patterns as a result of irrigation and fertilization in a young Pinus sylvestris stand. *Carbon uptake and allocation in subalpine ecosystems as a key to management***,** 38-44.

Lorentzen, M. 2017. *Impact of fertilization on field vegetation and litter layer in a boreal forest.* M.Sc. thesis. Ås, Norwegian University of Life Sciences.

Maaroufi, N. I., Nordin, A., Hasselquist, N. J., Bach, L. H., Palmqvist, K. & Gundale, M. J. (2015). Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. *Global change biology,* 21**,** 3169-3180.

Madritch, M. D. & Lindroth, R. L. (2015). Condensed tannins increase nitrogen recovery by trees following insect defoliation. *New Phytologist,* 208**,** 410-420.

Mäkipää, R. (1994). Effects of nitrogen fertilization on the humus layer and ground vegetation under closed canopy in boreal coniferous stands.

Mälkönen, E. (1990). Estimation of nitrogen saturation on the basis of long-term fertilization experiments. *Plant and Soil,* 128**,** 75-82.

Meunier, C. L., Gundale, M. J., Sánchez, I. S. & Liess, A. (2016). Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments. *Global Change Biology,* 22**,** 164-179.

Nabeshima, E., Murakami, M. & Hiura, T. (2001). Effects of Herbivory and Light Conditions on Induced Defense in Quercus crispula. *Journal of Plant Research,* 114**,** 403-409.

Nissinen, A. & Hari, P. 1998. Effects of nitrogen deposition on tree growth and soil nutrients in boreal Scots pine stands. *Nitrogen, the Confer-Ns.* Elsevier.

Nohrstedt, H.-Ö., Jacobson, S. & Sikström, U. (2000). Effects of repeated urea doses on soil chemistry and nutrient pools in a Norway spruce stand. *Forest Ecology and Management,* 130**,** 47-56.

Northup, R. R., Dahlgren, R. A. & Mccoll, J. G. 1998. Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback? *Plant-induced soil changes: Processes and feedbacks.* Springer.

Nybakken, L., Lie, M. H., Julkunen-Tiitto, R., Asplund, J. & Ohlson, M. (2018). Fertilization Changes Chemical Defense in Needles of Mature Norway Spruce (Picea abies). *Front Plant Sci,* 9**,** 770.

Osier, T. L. & Lindroth, R. L. (2001). Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *Journal of chemical ecology,* 27**,** 1289-1313.

Pallant, E. & Riha, S. J. (1990). Surface soil acidification under red pine and Norway spruce. *Soil Science Society of America Journal,* 54**,** 1124-1130.

Pregitzer, K. S., Burton, A. J., Zak, D. R. & Talhelm, A. F. (2008). Simulated chronic nitrogen deposition increases carbon storage in Northern Temperate forests. *Global change biology,* 14**,** 142-153.

Prescott, C., Corbin, J. & Parkinson, D. (1992). Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. *Plant and soil,* 143**,** 1-10.

Price, D. T., Alfaro, R., Brown, K., Flannigan, M., Fleming, R., Hogg, E., Girardin, M., Lakusta, T., Johnston, M. & Mckenney, D. (2013). Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews,* 21**,** 322-365.

Rivero, R. M., Ruiz, J. M., Garcıa, P. C., Lopez-Lefebre, L. R., Sánchez, E. & Romero, L. (2001). Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Science,* 160**,** 315-321.

Saarsalmi, A. & Mälkönen, E. (2001). Forest fertilization research in Finland: a literature review. *Scandinavian Journal of Forest Research,* 16**,** 514-535.

Salminen, J.-P. & Karonen, M. (2011). Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology,* 25**,** 325-338.

Smolander, A., Kanerva, S., Adamczyk, B. & Kitunen, V. (2012). Nitrogen transformations in boreal forest soils—does composition of plant secondary compounds give any explanations? *Plant and Soil,* 350**,** 1-26.

Strengbom, J. & Nordin, A. (2008). Commercial forest fertilization causes long-term residual effects in ground vegetation of boreal forests. *Forest Ecology and Management,* 256**,** 2175-2181.

Strengbom, J., Witzell, J., Nordin, A. & Ericson, L. (2005). Do multitrophic interactions override N fertilization effects on Operophtera larvae? *Oecologia,* 143**,** 241-250.

Tamm, C., Aronsson, A. & Popovic, B. (1995). Nitrogen saturation in a long-term forest experiment with annual additions of nitrogen. *Water, Air, and Soil Pollution,* 85**,** 1683-1688.

Tamm, C. O. 1991. Introduction: geochemical occurrence of nitrogen. Natural nitrogen cycling and anthropogenic nitrogen emissions. *Nitrogen in Terrestrial Ecosystems.* Springer.

Throop, H. L. & Lerdau, M. T. (2004). Effects of Nitrogen Deposition on Insect Herbivory: Implications for Community and Ecosystem Processes. *Ecosystems,* 7**,** 109-133.

Vogt, K., Vogt, D., Gower, S. & Grier, C. 1990. Carbon and nitrogen interactions for forest ecosystems. Air Pollution Research Report Series.

Wallace, Z. P., Lovett, G. M., Hart, J. E. & Machona, B. (2007). Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. *Forest ecology and management,* 243**,** 210-218.

Wam, H. K., Stolter, C. & Nybakken, L. (2017). Compositional changes in foliage phenolics with plant age, a natural experiment in boreal forests. *Journal of chemical ecology,* 43**,** 920-928.

Zucker, W. V. (1983). Tannins: does structure determine function? An ecological perspective. *The American Naturalist,* 121**,** 335-365.

7. Appendix

Table 2: T-values and P values of each of the measured covariates and their interaction effect with treatments for soil organic layer. Analysis was done separately for each of the covariates. Significant P-values from Satterethwaite's t-test are representing by bold and the symbols: *p<0.05; **p<0.01; ***p<0.001.

Response	Covariates	T value	P-Value
CT	WHC	-1.748	0.086
	Treatment x WHC	1.869	0.068
	Depth	-0.112	0.911
	Treatment x Depth	0.630	0.530
	Total Graminoid	0.052	0.959
	Treatment x total Graminoids	0.019	0.985
	V. myrtillus	-0.473	0.643
	Treatment x *V. myrtillus*	**2.163**	**0.046***
pH	WHC	0.818	0.417
	Treatment x WHC	-0.686	0.497
	Depth	-1.041	0.302
	Treatment x Depth	0.564	0.574
	Total Graminoids	-0.621	0.543
	Treatment x total Graminoids	0.826	0.421
	V. myrtillus	-0.438	0.668
	Treatment x *V. myrtillus*	-1.196	0.249
N	WHC	**2.441**	**0.020***
	Treatment x WHC	-1.288	0.206
	Depth	-1.000	0.322
	Treatment x Depth	0.194	0.847
	Total Graminoid	0.150	0.882
	Treatment x total Graminoids	-0.082	0.936
	V. myrtillus	-0.227	0.823
	Treatment x *V. myrtillus*	0.246	0.809
C	WHC	1.614	0.110
	Treatment x WHC	0.150	0.881
	Depth	0.121	0.904
	Treatment x Depth	0.953	0.343
	Total Graminoid	-1.178	0.256
	Treatment x total Graminoids	1.196	0.249
	V. myrtillus	**-2.250**	**0.040***
	Treatment x *V. myitillus*	-0.655	0.522
CN	WHC	**-2.412**	**0.020***
	Treatment x WHC	1.950	0.056
	Depth	1.733	0.087
	Treatment x Depth	-0.200	0.842
	Total Graminoid	-1.401	0.180
	Treatment x total Graminoids	1.106	0.285
	V. myrtillus	-1.286	0.217
	Treatment x *V. myrtillus*	-0.365	0.720

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway