Norwegian University
of Life Sciences

Master's Thesis 2021 60 ECTS
Faculty of Environmental Science and Natural Resource Management

Effect of Nitrogen Fertilization on Decomposition in Boreal Forest

Puspa Subedi
Ecology (M.Sc)

Acknowledgement

This thesis is a part of my Master's degree in Ecology in Norwegian University of Life Sciences, under the supervision of Professor Line Nybakken. The major objective of the project is to advance the knowledge on the fertilization effect on decomposition on boreal forest soil.

This thesis is not my sole effort. It would have been impossible to have written without help and support from several people. Firstly, I am grateful to my supervisor Professor Line Nybakken and co-supervisor Professor Johan Asplund, Faculty of Environmental Science and Nature Management, Norwegian University of Life Sciences (NMBU). I would like to thank you for letting be a part of the project and for all your help and constructive feedback throughout the project be it from preliminary planning, design, field and laboratory work, to statistical analysis and your valuable input during the whole writing process. Secondly, I would like to thank Annie Aasen and Claus D. Kreibich for the guidance, instructions and help with the lab work.

Further, I also have a sincere gratitude to my fellow friend Mrs. Zannatul Ferdous Runa for working together with me during the process and assistance in lab and review on my writing. I would also like to thank my dear friend Ashesh Raj Gnawali for his help in data analysis and document preparation in LaTeX.

Finally, I am grateful for my parents and family for their encouragement, love and support without which this would never have been possible.

Puspa Subedi

Ås, 9th March 2021

Abstract

Anthropogenic activities have resulted in escalating deposition of nitrogen in many forms that impact several aspects of ecosystem especially lying on boreal region where N is a limiting factor for tree growth. Although N is used as forest fertilizer to some extent to enhance growth and carbon storage, it alters the ecosystem by altering microbial community, soil properties, aboveground species abundance and diversity and nutrient cycling through decomposition. To better understand the effects of N addition on decomposition, an experiment was carried out in a long-term N forest-fertilization set-up in south east Norway. The experiment followed the Tea Bag Index (TBI) protocol, where green and rooibos tea were used as standard litters and buried in fertilized and control plots for three months. The tea bags were sampled afterwards, and mass loss was measured to calculate the decomposition rate constant (k) and proportion of stabilized material (S). Mineral soil samples were also analyzed in the lab to measure pH, concentration of condensed tannins, and moisture. Bilberry and spruce litters were collected for a lab incubation experiment where they were kept on mesh on petri-discs filled with soil samples. This was done to analyze how litter quality affects the mass loss and nitrogen release during the decomposition process.

N fertilization significantly affected k and S values. Interestingly, k was higher in fertilized plots S was higher in control plots indicating higher decomposition under N fertilization. However, N treatment did not show any significant impact soil pH, C, N and CT, neither did the interaction between them significantly affected k and S. The litter incubation experiment showed that treatment and initial N concentration significantly affected N release from the litter, whereas no significant effect was found on mass loss percentage. My results indicate that increased N concentration in litter as a result of fertilization does not necessarily indicate faster decomposition.

Keywords: decomposition rate, Tea Bag Index, N fertilization, boreal forest

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Abbreviations

C Carbon

CT Condensed Tannin

HCL Hydrochloric acid

k Decomposition Rate Constant

MeOH Methanol

N Nitrogen

S Proportion of Stabilized Material

SE Standard Error

TBI Tea Bag Index

1 Introduction

Human activities have altered the environment in different ways and scales (Mahmood et al., 2007). Production and use of fertilizers, burning of fossil fuels and land use intensification and associated ammonia (NH_3) emissions have led to an accelerated rate of nitrogen (N) deposition into the atmosphere (Gundale et al., 2014; Maaroufi et al., 2015). As a result, N concentration has increased three to five folds in the past century mainly in the forms of NH_x and NO_y (Reay et al., 2008; Galloway et al., 2008). In cold ecosystems, such as boreal forests in far northern latitudes, N is a limiting factor for tree growth. This is because of the lower rate of N fixation and slower soil mineralization process in those ecosystems (Maaroufi et al., 2016; Gundale et al., 2014; Vitousek and Howarth, 1991). Therefore, addition of sufficient amount of human derived N has been proposed as an effective way to enhance forest growth and productivity. In recent years, N addition is used to some extent in forest fertilization in the Nordic countries to increase timber production (Pukkala, 2017) and carbon sequestration.

Change in the availability of N also affects other parts of the ecosystem such as microbial community shifts (Pan et al., 2014), biomass alternation (Treseder, 2008), effect on microbial metabolic and enzymatic activities (Ramirez et al., 2010) and alternation of soil properties such as pH, soil porosity and proportion of organic materials (Marinari et al., 2000). In boreal ecosystems, where nutrients are insufficient, N fertilization can enhance soil mineralization through enhanced microbial enzymatic activity. These varied effect of N on soil and microbes can have a considerable effect on the nutrient cycling through decomposition and alternation of the food web structure and function (Meunier et al., 2016). Similarly, increased N concentration results in decreased aboveground plant species richness and a significant change in species composition (He et al., 2016) through acidifying effects (Stevens et al., 2010). Nitrophilic plants outcompete species growing in N limited conditions (Fang et al., 2012) especially forbs, grasses and bryophytes (Stevens

et al., 2010).

Decomposition is the process in which complex organic compounds are broken down into simpler forms. The process starts with detritivores breaking down the litter pieces which is followed by chemical reduction and mineralization into basic inorganic molecules such as ammonium, phosphate, carbon-dioxide, and water by microorganisms (bacteria and fungi). These molecules are readily taken up by plants and micro-organisms and released back to the atmosphere via respiration (Swift et al., 1979). The decomposition process is affected by several factors in which litter quality, the physio-chemical environment, climate (temperature and moisture) and the composition of decomposer community play a major role (Swift et al., 1979; van Zuijlen et al., 2020). Climate and litter chemistry, especially N and C:N ratio are the primary determinants of decomposition at a global scale (Keiser et al., 2013; Aerts, 1997; Gholz et al., 2000). However, at a local scale, the properties of the soil and soil microbial community play a greater role (Strickland et al., 2009; Wall et al., 2008). Although, earlier decomposition studies concentrated on climate and litter quality, recent studies have successfully demonstrated that soil with different characteristics such as differences in microbial communities have different effects on the decomposition process (Delgado-Baquerizo et al., 2015). Soil properties such as pH and nutrient content are very important for the biological composition of the soil biota, making these properties crucial aspects of decomposition studies (Delgado-Baquerizo et al., 2015).

Mineral elements, and especially N, plays a significant role in the decomposition of organic matter (Ågren et al., 2001). N fertilization can be beneficial to the fungal dominated micro-organisms in the soil and leads to more efficient mineralization (Austin et al., 2004). Similarly, increased N concentration and lower C:N ratios in the microbial substrate are the indication of higher mineralization (Schimel and Bennett, 2004). Soils with higher microbial abundance and microbial functional diversity tend to have a greater decomposition rate (Delgado-Baquerizo et al., 2015). For example, litter decompose faster in their

home soil where local microbes are adapted to the litter than in a foreign soil (Ayres et al., 2009a). In general, sites with higher initial N and lower C:N ratio have higher decomposition rate (Cornwell et al., 2008; Zhou et al., 2018). Therefore, artificial addition of N in a N-limited ecosystem accelerates the decomposition rate through increased soil N supply and retarded C:N ratio (Norris et al., 2013; Hobbie, 2005). However, the case is reverse for the nitrogen- sufficient ecosystems (Song et al., 2015). Studies have found a varied effect of N addition on decomposition rate which can be neutral (Hobbie and Vitousek, 2000; Van Vuuren and Van der Eerden, 1992), positive (Conn and Day, 1996; Hunt et al., 1988), or negative (Magill and Aber, 1998; Prescott, 1995).

In boreal ecosystems, where nutrient availability is a limiting factor for microbial activity, addition of N should promote organic matter decomposition. In fact, carbon release as a result of increased decomposition due to N fertilization have been observed in some ecosystems (Khan et al., 2007; Mack et al., 2004). However, there is also a substantial scientific evidence proving that in a long-term, N deposition has a negative relation with decomposition although it starts with a positive or a neutral relation in the early stages of fertilization (Franklin et al., 2003). This similar pattern was also observed in an experiment (Hobbie, 2008) in which decomposition was negatively correlated to N fertilization only after 5 years of fertilization reducing the decomposition rate by 20% which was previously neutral or slightly positive. Both lab and field experiments have now successfully demonstrated that N addition often decelerates the litter decomposition rate as well as microbial activity (Buchkowski et al., 2015; Ramirez et al., 2010). Several factors that contribute to a decreased decomposition rate upon fertilization include an increased ratio of microbial biomass and assimilation, and retarded growth of the underlying decomposer community (Olsson et al., 2005). A meta-analysis found that the addition of N in temperate forests reduced microbial respiration by nearly 15% (Janssens et al., 2010). Similarly, another meta-analysis showed a 6%-15% decrease in microbial biomass under fertilization (Treseder, 2008).

Soil N can move to litter through processes such as diffusion, leaching and fungal mycelia network (Frey et al., 2000; Lummer et al., 2012). Several studies have demonstrated such N transfer from soil to the nitrogen poor organic substrate (Manzoni et al., 2008) or from nitrogen-rich to nitrogen-deficient litter during decomposition processes (Bonanomi et al., 2010; Schimel and Hättenschwiler, 2007). According to Zheng et al. (2021), N lost during the decomposition of the litter was recovered in soils in both laboratory and field studies. The study further concludes that litters with a lower decomposability rate can contribute to a significantly higher efficiency of C and N transfer into the soil. Through this transfer, microbes can take an advantage to balance their nitrogen requirement and increase the decay rate (Berglund and Ågren, 2012). Based on this fact, the stoichiometric decomposition theory predicts that the phenomenon of N transfer occurring in N rich soil, enhances the decay rate for high C:N ratio plant residues in a significant manner (Bonanomi et al., 2017).

Physical and chemical properties of the litter are also major controlling factors in decomposition (Melillo et al., 1982; Keiser et al., 2013). Litter properties varies according to the plant species and so does the decay rates. For example, the decay rate of deciduous leaves in general is higher than the conifers (Gosz et al., 1973; Mikola, 1960). Broad leaves with larger Specific Leaf Area (SLA) have a higher concentration of potassium and phosphorus while less lignin. The decay rate of leaves is also higher in comparison to twigs and branches (Krishna and Mohan, 2017). A review on decomposition rate constant (k) values of different litter types from varied ecosystems globally concluded that total nutrient concentration and C:N ratio comprises of 70.2% variation in litter decomposition rate making the litter quality a crucial element in litter decomposition (Zhang et al., 2008). High and low quality litters show different decomposition rates within the forest, and the rate differ greatly in boreal forest ecosystems (Gholz et al., 2000). After fertilization, low quality litters with high lignin and C:N ratio can show a retarded decomposition rate, whereas high quality litter with low lignin concentration and low C:N ratio show increased

decomposition rate (Knorr et al., 2005). In boreal ecosystems, fertilization with N can cause increased N concentrations in the litter (Maaroufi et al., 2016) (Maaroufi et al., 2016b) through N uptake and thereby alter litter quality (Maaroufi et al., 2017), eventually affecting litter C:N ratio; another most important determinant of litter decomposition (Aerts, 1997). There is evidence that N concentration in Scots pine needle litter and Norway spruce increased as a N fertilization effect (Berg, 2000). This results to lower C:N ratio in litter (Xu et al., 2020) and thus promoting the decomposition of senescent plant tissue (Xu et al., 2020). The margin of the C:N ratio for N to be mobilized is 20:1 (Swangjang et al., 2015). When litters have C:N ratio less than 20, the microbes can actively mineralize the N and make it readily available for plants (Brust, 2019). However, C:N ratio more than 35 indicates microbial immobilization (Brust, 2019; Swangjang et al., 2015). The C:N ratio in soil microbes is approximately 8, and they require C and N from the soil to keep the ratio in balanced condition. The microbes perform best when they are successful to compensate the required C:N, which is supported when litter or their diet has a C:N ratio of 24 (Brust, 2019).

Plant Secondary Metabolites (PSMs) such as lignin, tannin, and other polyphenols, which are essential as defensive compounds against herbivores, are themselves resistant to decomposition. They can also limit the decomposition process through various mechanisms such as forming protein-tannin complexes, altering N availability to the soil organism, causing direct toxicity to the microbial species, and inhibiting the microbial enzymatic activities (Kraus et al., 2003). Previous studies suggest that a high concentration of Condensed Tannins (CT) in litters is associated with slower decomposition rates (Zhang et al., 2013) and fertilization has a negative relation with litter CT concentration (Kraus et al., 2004). The protein complexes formed by the tannins inhibit the microbes from mobilizing N and converting it into the forms that plants can take up quickly, resulting in the reduced microbial ability to decompose plant litter (Gundale et al., 2010).

Scientific knowledge on litter decomposition and how external fertilization affects the

process has extensively increased after the development of litter bag technique in 1960s which made it possible to measure the mass loss and decomposition rate. Later during 1980s and 1990s, the recognition of its importance as a critical ecological process for nutrient cycling, carbon storage and mitigation of climate change further increased the scientific concern into the topic and has remained high since then (Prescott, 2010). In 2013, an easy, convenient and cost-effective approach was introduced to measure decomposition which uses green and rooibos tea as representation of dead plant materials (Keuskamp et al., 2013). This method creates a global database with participants all over the world and compute a Tea Bag Index (TBI) that provides information on soil functions at a local, regional, and global scale. This involves a simple process of burial of rooibos and green tea and measuring their mass loss after a period of time.

Despite extensive research and results, the effect of N fertilization on soil decomposition ability is still unclear. There have been several studies on N effect on litter quality and soil decomposition rate on boreal forest. However, to my knowledge, this is the first study to use tea bag approach under natural conditions. Similarly, no other studies before this have addressed soil CT concentration with respect to N addition.

This experiment was carried out in a spruce (*Picea abies*) forest in Southeast Norway which is being fertilized annually by Nitrogen since 2003. I carried out tea bag experiment as per the Tea Bag Index (TBI) protocol given by (Keuskamp et al., 2013) where mass loss is measured to calculate decomposition rate using Lipton red and Lipton rooibos tea. Similarly, litter incubation experiment was performed to understand the effect of fertilization on litter quality and how litters with different quality decompose.

1.1 Aim of the study

The broad objective of this study was to increase the understanding of how nitrogen addition effects the decomposition rate of boreal forest soil. This study adds knowledge on understanding of effect of human-added nitrogen on decomposition and also the litter quality as a determinant of decomposition. The following hypothesis were tested:

1. The decomposition rate constant (k) will decrease upon fertilization and proportion of Stabilized material (S) for the tea material will be higher in fertilized plots.
2. The concentration of soil CT will decrease under fertilized condition.
3. Mass loss percentage and N release during decomposition will be higher in litter samples from fertilized plots.

2 Materials and Methods

2.1 Study Area and Experimental Set-up

The study forest is an old boreal forest dominated by Norway Spruce *(Picea abies)* in Kittilbu in South East Norway at an altitude of 800 meters above sea level and latitude 61° 10' N, 09° 09' E. The forest is situated only 100 meters below the tree line and has a great variation in tree age and size. The tree composition varied from mature plants that stand 20 meters tall and 220 years old (Gauslaa et al., 2008), with smaller trees and seedlings in gaps. *Vaccinium myrtillus* and bryophytes such as *Pleurozium schreberi*, *Hylocomium splendens*, *Polytrichum commune* and *Sphagnum girgensohni* dominate the ground vegetation in the experiment site together with herbs such as *Vaccinum vitis-idaea* and *Avenella flexuosa* (Gauslaa et al., 2008). There is no record of intensive logging carried out in the forest since past 50 years, however, some slight selective logging was observed. The mean annual temperature and annual precipitation of the site are -0.1°C and 810 mm (Gauslaa et al., 2008) respectively.

Twenty experimental plots with an area 225 m^2 ($15m \times 15m$) were set up in 2003. Ten of these are untreated control plots, while ten have been supplemented with Nitrogen granules at a rate of 150 kg per hectare per year containing 24.6% N, 2% P and 6% K (YaraMilaTM Fullgjødsel, by Yara, Norway) (Davey et al., 2017). The fertilization has been done manually every year in spring with an exception in 2018. The plots are 50 to 350 meters apart from each other and have five assigned sub-plots.

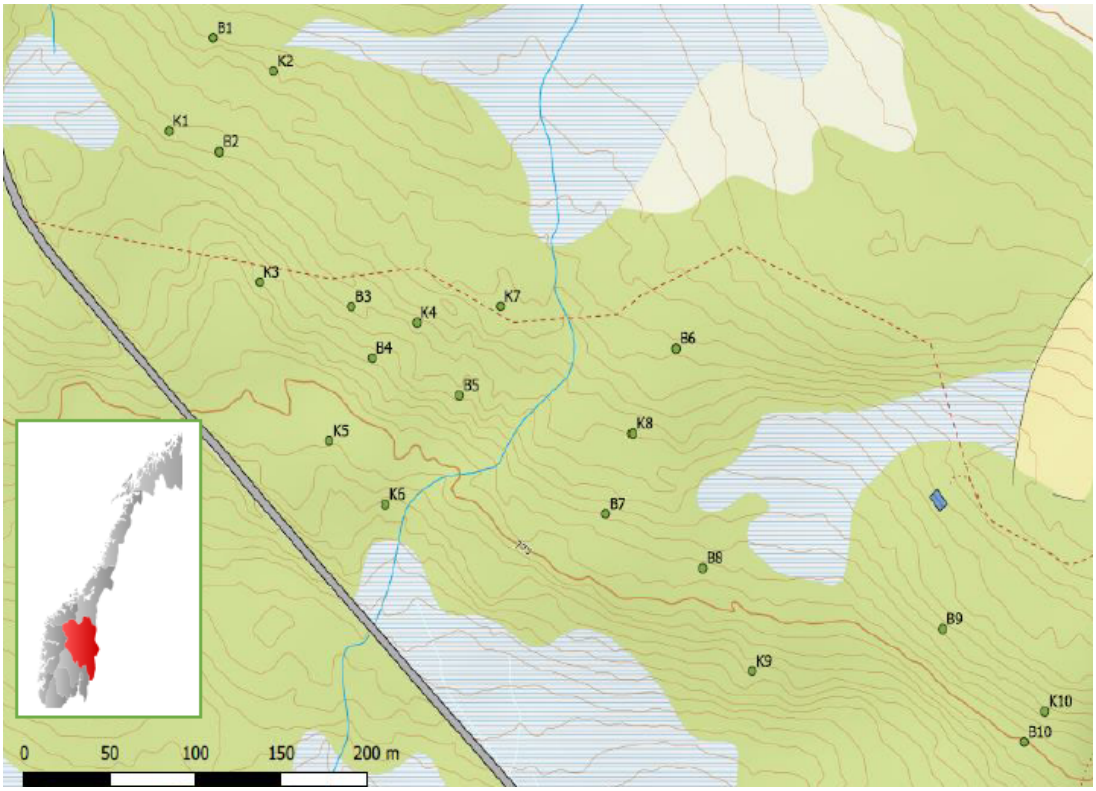


Figure 2.1: Map of the study area: K refers control plots and B refers fertilized plots

2.2 Tea Bag Experiment

I sat up a tea bag decomposition experiment following the Tea Bag Index (TBI) protocol (Keuskamp et al., 2013). The Tea Bag Index (TBI) approach uses tea bags as standardized plant litter to estimate the decomposition rate and stabilization through burial of green and rooibos tea bags followed by measurement of mass loss over a period of time (Keuskamp et al., 2013).

I used Lipton green tea and Lipton rooibos tea of tetrahedron shape (fig. 2). The mesh size of 0.25 mm was large enough for the microorganism and mesofauna to enter the bags and enhance the decomposition, however, the macrofauna were excluded (Keuskamp et al., 2013; Setälä et al., 1996).

The Tea Bag Index primarily consists of two major components, explaining the de-

composition rate (k) and stabilization factor (S). The decomposition rate is higher in the initial phase leaving the recalcitrant fractions to decompose slower. The rooibos tea has a relatively slow decomposition rate and will be in its initial phase of decomposition even after three months. This gives the estimation of decomposition rate constant (k). On the other hand, green tea decomposes faster. All the easily decomposable materials are gone by the end of the experiment period, leaving only the resistant material that gives the value for stabilization factor (S). The use of two different types of organic litter for the decomposition experiment also allows understanding the decomposition on the initial and final stages of the experiment. This further eliminates the requirement of doing a time series experiment for k and S calculation (Keuskamp et al., 2013). After measuring the actual tea content inside the bag after three months, the Stabilization factor S and decomposition rate k were calculated.

2.3 Preparation and Field Work

100 tea bags of each Lipton green and Lipton red tea were numbered from 1 to 200 with a permanent marker on the white side of the label prior to going in the field. One marked tea bag of each type was deployed in the forest soil in all five sub plots of all 20 plots on June 16, 2020. The tea bags were buried below eight cm in forest soil using a shovel and were separated about 20 cm apart. The labels were kept above the soil and every sub-plot were marked by red sticks which were also labeled with the plot ID. Each tea bag was also marked by white sticks to make it easier to identify their location (fig 3). After three months, on September 16, the tea bags were collected, soil particles attached to the bags were removed, and dried the tea bags in an oven at 70°C for 48 hours, before the dry weight was measured. Soil samples were also collected using a soil sampler in each plot. The mineral and organic layers were separated and put in separate paper bags. Spreuce and bilberry litters were also collected from the same fertilized and control plots for the lab incubation experiment to measure mass loss and N release.

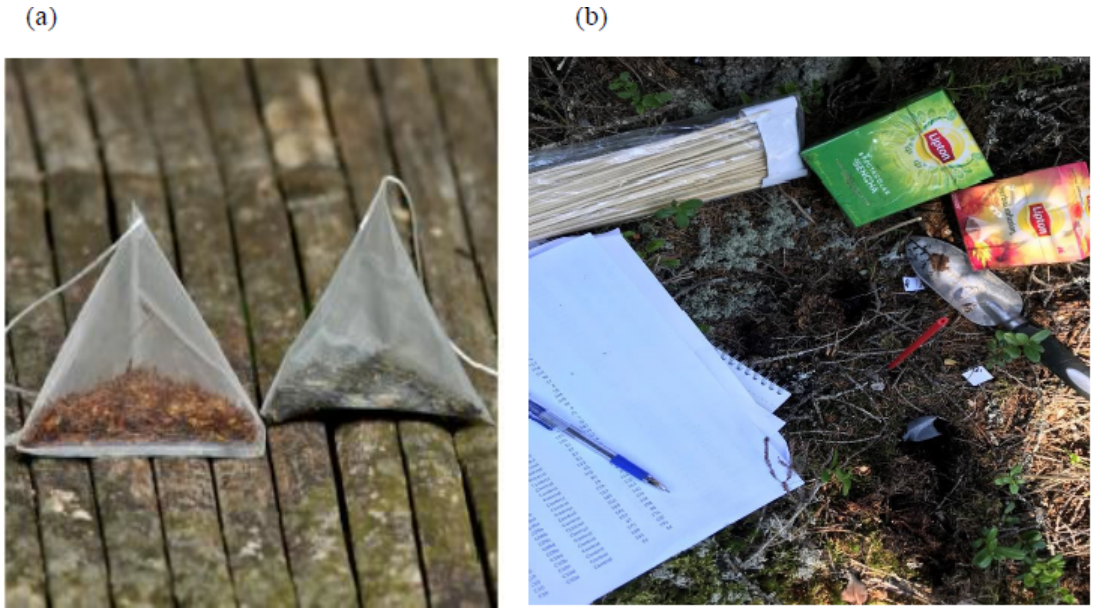


Figure 2.2: a) Tetrahedron teabags (image: Keuskamp et al. (2013)) used in the experiment: Rooibos tea (left) and green tea (right), b) Burial of teabags in the forest soil, Kittilbu

2.4 Decomposition Experiment

Standard procedures for the decomposition experiment was in accordance with (Wardle et al., 1998). Fresh soil was collected from the spruce forest near NMBU, Ås to fulfil the substrate requirement. 40 Petri dishes were filled with the soil up to 2/3rd portion and covered on the top by 1mm nylon mesh cut in a circular shape. 1 gram of each litter type (bilberry and spruce) from fertilized and control plots was kept on the top of the mesh and tightly sealed using a isolation tape to avoid water loss. The samples were stored in room temperature under dark condition (inside a box) for three months. Afterwards, the litters were taken out very carefully in paper bags and oven-dried in 47°C for 48 hours before weighing. Decomposition was represented as a percentage mass loss during the 3 months incubation time. Nitrogen release during the decomposition time was calculated as proportion between total biomass * N concentration before incubation minus biomass N concentration after decomposition and total biomass *N concentration before incubation

(Asplund and Wardle, 2013).

2.5 Lab Work and Chemical Analysis

2.5.1 Preparation of Soil Samples

The fresh soil samples were weighed before they were dried in 70°C for 48 hours. The mineral soil was then sieved through a 2 mm sieve, grinded into powder using a Retsch MM400 (RETSCH, Germany) ball mill with a frequency 20-25 revolutions per second for 25-40 seconds. These powdered soil samples were later used to estimate the concentration of condensed tannins, total C and N, as well as pH of the soil.

2.5.2 Analysis of Concentration of Condensed Tannins

I followed the acid butanol assay protocol as instructed by The Tannin Handbook (Hagerman, 2002). 0.2 gram of each soil sample was mixed with 4 ml 70% acetone and shaked on a planar shaker (KS 501 digital, IKA-WERKE, Germany) at frequency 200 rpm for one hour.

This was followed by vortexing and centrifuging (c. 16,400 rpm, 10 minutes), before the supernatant was collected in a 15 ml tube. This process was replicated three times, and all the accumulated extractions were evaporated on Eppendorf Concentrator Plus 5301 (Eppendorf, Hamburg, Germany). I followed the acid butanol-HCL-iron assay for the analysis of condensed tannins (CT) as given by (Hagerman, 2002). After evaporation, dried extractions were mixed with 0.5 ml MeOH and shaked in a vortex. 3 ml acid butanol (95% butanol, 5% HCL) and 0.1 ml iron reagent (2% ferric ammonium sulphate in 2N HCL) were added and the samples were kept in boiling for one hour and cooled. Absorbance (550 nm) of the samples was determined by a UV spectrometer (Shimadzu, Kyoto, Japan). Soil CT was converted into mg cm^{-3} by using volumetric mass sample of each sample (20 ml) and CT/C ratios was determined.

2.5.3 Analysis of pH

For pH measurement, a 5 ml soil sample was mixed with 12.5 ml deionized water, well shaken and left overnight. The next day, the samples were well-shaked, and pH values were measured using an intoLab 720 precision pH meter (WTW GmbH, Weilheim, Germany).

2.5.4 Analysis of Carbon and Nitrogen

For estimation of C and N content, 10 milligram of oven-dried soil samples were packed in a tin foil and then run into a vario MICRO cube elemental analyzer (Elementaar, Hanau, Germany) to determine C:N ratios. Concentration of C and N were converted into mg cm-3 by using volumetric mass of each sample (20 ml).

Similar method was followed for the C and N analysis of the bilberry and spruce litter samples. C and N concentration (%) and C:N ratio of both litters from fertilized and control plots were measured before and after the incubation experiment to measure the N release during the decomposition process.

Table 1: Mean initial values of C and N concentration (%) and C:N ratio in bilberry and spruce litter.

Litter type	**Treatment**	**%C**	**%N**	**C:** N
Bilberry	Control	47.3 ±0.34	1.07 ±0.36	44.8 ±1.54
	Fertilized	47.3 ±0.42	1.74 ±0.05	27.4±0.7
Spruce	Control	48.8 ±0.73	0.54 ±0.01	90.3 ±2.15
	Fertilized	48.6 ±0.19	0.95 ±0.07	53.4 ±3.56

2.5.5 Statistical Analysis

All data processing and primarily calculation was performed in Excel (Office 365), whereas all statistical analysis was done using RStudio, R version 4.0.3 (R Studio Team 2020). Two treatments were taken into consideration (fertilized and control) to analyze the relation-

ship between these treatments and responses i.e. degradation constant (k) and proportion of stabilizing material (S).

Linear Mixed Effect analysis between each of the response variable and the fixed and random effects was performed using R package *lme4* in library *lmer*. The response variables entered were decomposition rate constant (k) and proportion of stabilizing material (S). The mixed effect was tested by modelling k and S as a function of treatment and interaction between treatment and other predictive variables such as C, N, C:N ratio, CT, Moisture and pH. The residual variation i.e. unexplained variation of the response variable associated with the experimental plots were also modelled through variance. F and P values were obtained from the ANOVA table obtained from the same test. Only analysis with P values less than 0.05 were regarded as significant.

3 Results

3.1 Soil Properties

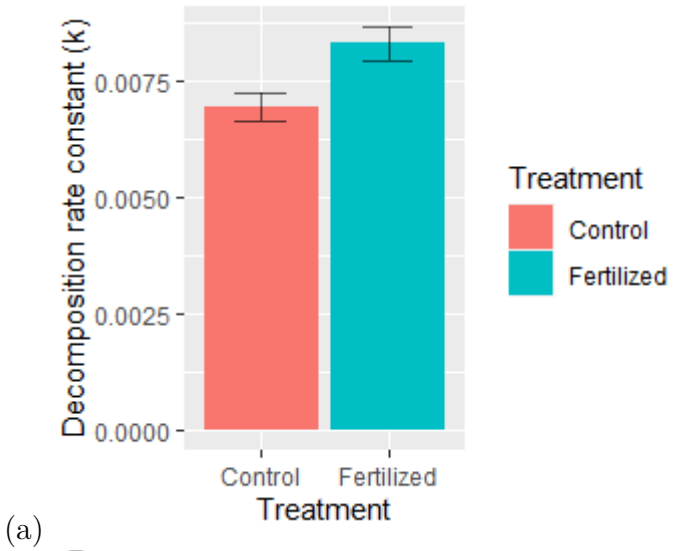
None of the measured soil parameters varied significantly between treatments (Table.2.

3.2 Tea bag experiment: Decomposition rate constant (k) and proportion of Stabilized material (S)

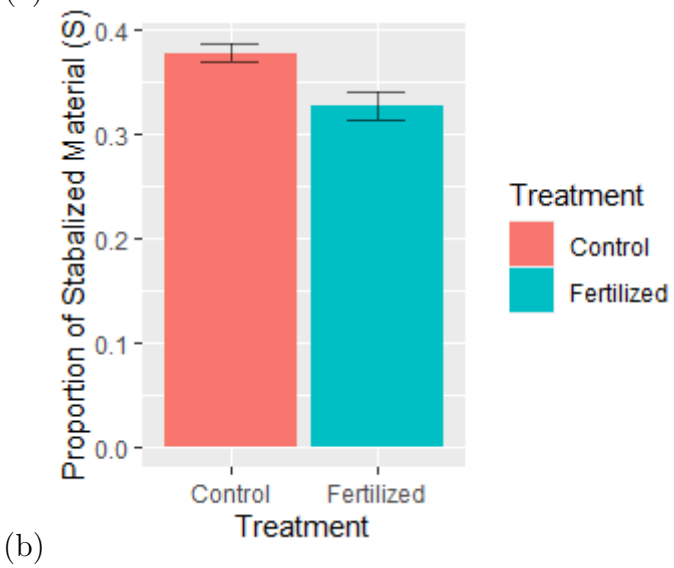
The rate of decomposition was significantly higher in fertilized plots (Table 2, Fig. 3.1 and the proportion of stabilization material was significantly higher in control plots (Table 2, Fig. 3.1). Other predictor variables and their interactions with treatment have no significant impact on k and S. The average decomposition rate was 9.0% higher in fertilized plots (Fig. 3.1) whereas the stabilization factor was 7.2% higher in control plots (Fig. 3.1)

Table 2: Summary of ANOVA from basic linear mixed model of different response variables. Significant P-values at 95% confidence interval obtained from Satterthwaite's test are presented in bold letters.

Response	F	P
Decomposition rate constant (k)	6.01	**0.027**
Stabalization constant (S)	5.1	**0.038**
Condensed Tannin (CT)	0.03	0.869
Carbon (C)	0.03	0.868
Nitrogen (N)	0.29	0.598
C:N ratio	2.63	0.124
pH	0.28	0.601



(a)



(b)

Figure 3.1: (a) Mean ($\pm SE$) decomposition rate constant (k) and (b) Mean ($\pm SE$) proportion of stabilized material (S) in control and fertilized plots.

Table 3: ANOVA output, F and (P) values for mass loss and N release for bilberry and spruce litter, treatment, initial Nitrogen, and interaction effects. The bold values are significant represented by *p<0.05, **p<0.01.

	Bilberry			Spruce		
	T	N1	T*N1	T	N1	T*N1
Mass loss	3.26 (0.088)	3.43 (0.081)	0.11 (0.752)	0.46 (0.505)	0.02 (0.883)	0.29 (0.597)
Nitrogen release	6.58 **(0.0197)**	8.45 **(0.009)**	1.35 (0.270)	4.66 **(0.005)**	9.44 **(0.007)**	0.059 (0.081)

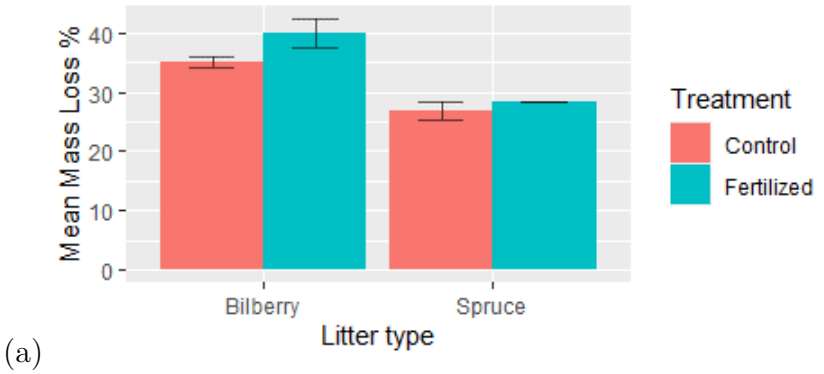
3.3 Incubation Experiment

3.3.1 Litter Mass Loss

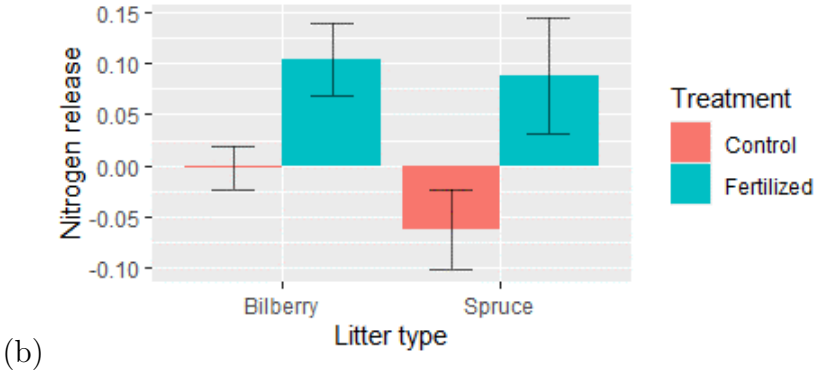
There was no significant effect of fertilization or initial N on mass loss of any of the two studied species (Table 3, Fig. 3.2). Bilberry lost 40% in fertilized and 35% in control plots while spruce lost 28% in fertilized and 26% in control plots.

3.3.2 Nitrogen Release

The treatment and concentration of initial N had significant effect on N release during decomposition (Table 3). However, no interaction effect was found. Positive and negative values indicate that both bilberry and spruce litter released N under fertilized condition while the N remained immobilized under control conditions respectively (Fig. 3.2). Released N was higher in the bilberry litter while spruce litter have a significantly higher proportion of immobilized N.



(a)



(b)

Figure 3.2: Mean $(\pm SE)$ percentage mass loss (a) and N release (b) of the bilberry and spruce litter during the 3 months incubation period for decomposition experiment. The colors (orange and blue) indicate the type of treatment (control and fertilized).

4 Discussion

4.1 Degradation rate constant (k)

The decomposition rate constant (k) in the tea bag experiment was higher in N fertilized plots than in the control plots, which contradicted my hypothesis. This might have occurred as a result of difference of fungal dominance in different treatment sites. Although, fungal biomass study was beyond the scope of this research, there are studies which support my results. Less fertile soils (high C:N) are more likely to be dominated by saprotrophic fungi than the mycorrhizal fungi which leads to higher nutrient immobilization or slower decomposition in nutrient poor soils (Fernandez and Kennedy, 2016). This stands in accordance with my result that decomposition rate is higher in fertilized plots than on control plots. Another study on fertilization effect on soil carbon and ectomycorrhizal (EM) fungi also showed a suppressed EM biomass and increased total fungal biomass indicating faster decomposition (Hauken, 2020) under fertilized condition. An experiment on effect of N fertilization on decomposition using the TBI method (Koceja, 2019) showed a greater decomposition rate (k) of rooibos tea litter (high C:N) in fertilized plots than in control plots while similar values of k were observed for green tea litter (low C:N) in both treatments. This indicates the decomposition of low-quality litter is enhanced under fertilization through increased microbial activity. This is also supported by several other researches that microbial community shift as a result of N enrichment can result to a higher decomposition rates (Khan et al., 2007; Mack et al., 2004).

A similar result was found in a meta-analysis (Knorr et al., 2005) where data from 900 incubation and field fertilization experiment concluded that decomposition is inhibited if the fertilization rate is 2-20 times than anthropogenic N deposition but decomposition is enhanced if fertilization is higher than 20 times in an ambient N deposition of 5-10kgNha-1kg-1. Therefore, excess N deposition over an extended period leading to exceed the

ambient N concentration of the site can be an explanation for the increased decomposition rate in N fertilized plots. However, significant amount of researches showed a decelerated decomposition of litter (Chen et al., 2013) resulting to an increased C storage capacity of fertilized soils (Maaroufi et al., 2015; de Vries et al., 2009) through suppressed microbial biomass (Li et al., 2015) and respiration (Yan et al., 2016). Increased decomposition rate in fertilized plots as obtained in the result may have occurred as a result of a very strong N fertilization over so many years.

Another explanation for this can be an increased microbial functioning under fertilized condition. Scientific evidence supports that soil moisture content also plays a role in decomposition capacity of soil. Generally, a higher moisture content in soil is a promotor of decomposition. Mass loss for both high and low quality litters were declined in the order wet > moist>dry soil conditions with a more pronounced effect in high quality litter (Petraglia et al., 2019). My results showed a higher moisture content in the unfertilized soils than the fertilized ones. However, the difference was not statistically significant to explain the result.

N addition is expected to have a negative effect on decomposition of recalcitrant material through supression of oxidative enzyme production that degrade recalcitrant materials (Fog, 1998). However, the result showed a lower proportion of stabalized (recalcitrant) material in fertilized plots thus suggesting a lower carbon storage.

N fertilization did not show any significant effect on soil CT concentration which is contradicting to my second hypothesis. To my knowledge, no scientific studies have investigated the concentration of the phenolics like tannins in N fertilized soil directly. However, there are several studies investigating their concentration in leaf litter under fertilized condition (De Long et al., 2016; Sundqvist et al., 2012; Nybakken et al., 2018). The concentrations of several phenolics were lower in young spruce needles taken from N fertilized plots than in control plots where CT in fertilized needles was half as much as in the control plot's needles (Nybakken et al., 2018).

The source of phenolics like tannins, into the soil is primarily the litter deposition from aboveground. After the leaf senescence or plant death, tannin reaches the soil surface as a component in the litter for decomposition. Since, litter deposition is a major pathway for CT to enter the soil, decrease concentration in foliage due to N fertilization was the basis to assume soil CT concentration will decrease under fertilization. However, the result in this study was not significant to support the assumption. One reason for this might be the analysis of the mineral layer of the soil instead of organic layer where the concentration is relatively low. Another study on organic layer, as a part of this project (unpublished), done in the same site at the same time following the same method showed a significant decrease in organic layer CT concentration in fertilized condition. CT studies in soil is still in initial phase and therefore further research on how soil phenolics on organic and mineral layer of soil are affected by long term N fertilization are required to draw a stronger conclusion.

4.2 Litter Mass Loss and Nitrogen release during incubation experiment

Results from the litter incubation experiment partly supports my hypothesis that mass loss and N release will be higher in fertilized litters. Mass loss of bilberry and spruce litter in the incubation experiment did not differ between treatments which was unexpected. However, I still found fertilization effect on N release. More interestingly, bilberry with twice as much as initial N decomposed faster than spruce. Mass loss percentage for bilberry and spruce litter in the incubation experiment did not have a significant difference in two treatments which implies that the litter mass loss was not affected by the treatment which was unexpected. The reason for this might be the use of foreign soil in incubation experiment. There are indications that litter decomposition is accelerated in home soil than in foreign soil due to specialized soil biotic community and adapted microclimate Ayres et al. (2009b).

Several scientific works on decomposition showed a strong association between decomposition rate and litter N concentration and C:N ratio (Krishna and Mohan, 2017). A large proportion of literature supports the fact that high quality (nutrient-enriched) litters show an accelerated decomposition rate than low quality (nutrient-deficient) litters (Pichon et al., 2020; Liu and Sun, 2013). N concentration in boreal forest litter increases as a result of N fertilization which reduces the C:N ratio in them (Maaroufi et al., 2017). This increased N and decreased C:N has increased decomposition of litter under fertilized conditions (Knorr et al., 2005). Similarly, increased decomposition rate as a result of high N concentration under fertilized condition was observed in Scots pine and Norway spruce needles (Xu et al., 2020). However, results from my study showed no effect of added N on the decomposition of the litter. Although, N fertilization was able to change the litter quality in terms of decreased C:N ratio (Table 1), the difference was not significant enough to affect the decomposition of the litter indicating that higher N content in the litter does not necessarily mean a rapid decomposition rate of those litters.

In a study with herbaceous species, to test whether species growing in nutrient rich soils produced fast decomposing litters, it was found that N fertilization changed some leaf and litter traits, but those changes were not significant to alter the decomposition (Kazakou et al., 2009) which was exactly the similar result as in this study. Another study on effect of litter quality and climate on decomposition showed that litters from fertilized plots with five times higher N content decomposed at the same rate as litters from control plots. This decomposition was more derived by lignin content than initial nitrogen suggesting that litter decomposition is more dependent on carbon substrates (Murphy et al., 1998).

Both spruce and bilberry litters taken from fertilized plots released nitrogen whereas litters from control plots gained N (Fig. 3.2) by the end of the 3 months incubation experiment. This can be explained by higher initial N concentration in both litter types from fertilized plots than in control plots (Table 1). Treatment and initial litter N content

showed a significant effect on N release (Table 3) for both litter types. Increase in N during the early stages of decomposition process was first described by (Tenney and Waksman, 1929) and have been reported repeatedly (Melillo et al., 1982). When organic material with a lower N concentration enter the soil, the decomposers in the soil need to overcome the lacking N from the surrounding. This occurs through either one or more of these mechanisms: immobilization, fixation, absorption of atmospheric ammonia, insect frass, green litter, fungal translocation through fall and/or dust (Melillo et al., 1982).

My results showed increased N concentration in fertilized litters (Table 1) which is consistent with several other studies (van Diepen et al., 2015; Nybakken et al., 2018; Sjöberg et al., 2004) and therefore, release N during decomposition. Significant effect of fertilization on N release despite of unresponsive mass loss is an indication of increased N mineralization which in turn could accelerate fertilization effect.

4.3 Factors that might have influenced my results

A substantial amount of literature support that climatic factors play a determining role when it comes to litter decomposition rate on a larger spatial scale. However, on a smaller spatial scale or a similar site, like the one in this study for tea bag decomposition, the climatic conditions are similar and therefore do not significantly influence litter decomposition rate. Also, the soil for the incubation experiment was collected for another spruce forest so that the sol fauna do not vary significantly.

Another important factor in decomposition experiment is the mesh size of the litter bags. Tea bags with mesh size 0.25 mm do not allow the macrofauna to access the litter. Soil organisms can control the litter decomposition rate to a large extent and smaller mesh size allows only microfauna, fungi and microbes to decompose the litter. A larger mesh size will allow other decomposing organisms, but also can cause loss of litter resulting to an overestimation of the degraded material. However, exclusion of soil meso and macro fauna does not have a significant effect on decomposition when it comes to high altitude

systems Wall et al. (2008) and therefore may not have affected my results.

5 Conclusion

Since boreal forest is a large sink for global carbon sequestration, small changes in decomposition rate could have a significant impact on global carbon budget. The purpose of this study was to investigate the effect of long-term N deposition on litter decomposition on boreal forest soil. Based on the results, N fertilization increased litter decomposition which can release more carbon in the atmosphere. Moreover, litters with different qualities did not show any variation in decomposition rate. My results, although contrasting to majority of the existing literature, is an indication that decomposition study is a complex phenomenon and therefore several factors should be embedded in a study to draw a clear conclusion. Since many studies are concentrated to C:N ratio as a major determinant for litter decomposition, future research may want to focus on other factors in litter quality. Furthermore, none of the studies accounted for the changes in PSMs such as tannin concentrations on soil especially mineral layer, which can affect decomposition process through effect on soil microorganisms.

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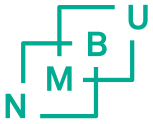

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway