Norwegian University
of Life Sciences

Master's Thesis 2017 60 ECTS
Faculty of Environmental Science and Natural Resource Management

Impact of fertilization on field vegetation and litter layer in a boreal forest

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Preface

This thesis is a part of my Master's degree in Biology at the Norwegian University of Life Sciences. I have been interested in plants since I was little and was glad when I got the opportunity from my supervisor, researcher Marit H. Lie, to do research on the field vegetation in a boreal forest.

First, I would like to thank Marit for wonderful help throughout, every step of the way. I would also like to thank Annie Aasen and Claus D. Kreibich for help with the lab work, and Marit's parents for borrowing their cabin during my field work. Thank you, Gausdal state-owned common (Gausdal statsallmenning), for using your land for the fertilization experiments.

Finally, a great thanks to family and friends for the support, comfort and help these last months.

Norwegian University of Life Sciences

Ås, May 12th 2017

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Abstract

Fertilization of Norwegian boreal forests to increase carbon uptake is suggested as a measure to reduce CO_2 emissions. However, studies of the effects of fertilization on field vegetation and litter layer in these forests in Norway are few.

This study was based on a fertilization experiment in a Norwegian boreal forest where fertilization has been added yearly since 2003. The aim was to examine the effects of fertilization on field vegetation and litter layer in Norwegian boreal forests. To do this, field vegetation, nitrogen concentrations and litter layer were analysed in both control and fertilized plots.

Results indicated significant effects on the field vegetation when fertilized. There was observed a shift in species composition along with a decreased total cover. Fertilization seemed to favour grass, while heather species and large bryophytes were more associated with no fertilization. Analysis of litter biomass samples indicated that species composition reflected field vegetation. There was no difference in biomass litter weight, indicating that even though species composition changed from fertilization, the amount of litter produced was about the same. Overall, there was an increase in nitrogen concentration in the analysed plants when fertilized, which led to decreased CN ratios.

On this basis, fertilization of Norwegian boreal forests seems to cause a shift in species composition which is reflected by the litter layer. Further studies on the effects over time should be conducted to achieve better understanding of what goes on.

Sammendrag

Gjødsling av de norske boreale skogene for å øke karbonopptaket er foreslått som et tiltak for å redusere CO_2 utslipp. Studier av gjødslingseffekter på bunnvegetasjonen og strølaget i disse skogene er få.

Denne studien er basert på et gjødslingseksperiment i en norsk boreal skog der det har vært gjødslet årlig siden 2003. Målet var å undersøke effektene av gjødsling på bunnvegetasjonen og strølaget i norske boreale skoger. For å gjøre dette ble bunnvegetasjon, nitrogen konsentrasjoner og strølaget analysert i både kontroll og gjødslede områder.

Resultatene indikerte signifikante forskjeller på bunnvegetasjonen som ble gjødslet. Det ble observert et skift i artssammensetning i tillegg til en nedgang i vegetasjonsdekning. Gjødsling så ut til å favorisere gress, mens lyngarter og store moser var mer assosiert med fravær av gjødsling. Analyse av biomasseprøvene fra strølaget indikerte at artssammensetningen reflekterte bunnvegetasjonen. Det var ingen forskjell i vekt på biomasseprøvene, noe som indikerte at selv om artssammensetningen endret seg med gjødsling, var mengden av strø som ble produsert omtrent den samme. Det var en økning i nitrogenkonsentrasjonen i de analyserte plantene som var gjødslet, noe som ledet til lavere CN ratio.

Basert på dette ser gjødsling av norske boreale skoger ut til å gi et skift i artssammensetningen som igjen blir reflektert av strølaget. Videre studier på effekter over tid bør bli utført for å få bedre forståelse av hva som foregår.

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1. Introduction

Norway is working towards reducing greenhouse gases, especially CO_2, to prevent global warming. The expert group - Climate cure 2020 – presents various options in different sectors for achieving a set reduction in CO_2-emission by 2020. One of the sectors mentioned is forestry (Klima- og forurensningsdirektoratet 2010). The Norwegian boreal forests are known to store large amounts of carbon (Haugland et al. 2014) which contributes to reducing CO_2-emissions (Klima- og forurensningsdirektoratet 2010).

To reduce CO_2 emissions, The Ministry of the Environment has suggested different measures to increase carbon-uptake in the forest. One of these is fertilization (Meld. St. 21 (2011-2012)). Boreal forests are important carbon stocks (Haugland & Rosland 2010), and compared to the living biomass, the soil in these forests store much more carbon (Meld. St. 21 (2011-2012)). Studies show that fertilization in boreal forests might increase the carbon content in the soil (De Wit & Kvindesland 1999; Johnson & Curtis 2001; Mäkipää 1995a). Since most boreal forests are known to be nitrogen limited (Bobbink et al. 2010), increasing plant production caused by fertilization might increase the carbon concentration in the forest floor (Haugland & Rosland 2010; Olsson et al. 2005).

Few studies have looked at the effects of fertilization on the forest floor in boreal forests in Norway. Studies in other countries indicate that when fertilized, heather and bryophytes tend to decrease, while grasses increase in abundance (e.g Strengbom & Nordin 2008). According to Hjeljord (2008), the heather species *Vaccinium myrtillus* is a key species in boreal forests and an important food source for Norwegian wildlife. A decrease could affect multiple species depending on this plant. A potential change in species composition in the living biomass might, in turn, affect the litter composition. Fertilization might also influence the nitrogen concentration in the plants which in could influence plants susceptibility to diseases (Strengbom et al. 2002) and changes in herbivory patterns (Gurevitch et al. 2006). If nitrogen concentration in plants changes due to fertilization, this might influence the amount of nitrogen that enters the soil through litter.

This study is based on a subalpine boreal forest in south-east Norway containing control plots and plots that have been fertilized since 2003. The aim is to investigate the effects of fertilization on both field vegetation and litter layer. Based on this, the following research questions have been formulated: i) How will field vegetation change when fertilized over time?, ii) How would fertilization affect the litter layer?, iii) What would fertilization over time do to nitrogen and carbon concentration in the field vegetation?

I hypothesize that, when fertilized, there will be fewer species and higher productivity, leading to a higher vegetation cover. There should be a change in vegetation, from less heather species to more grass in fertilized plots. I also predict lower CN ratios in plants due to higher nitrogen concentrations. I hypothesize that there will be a change in litter when fertilized, both in higher litter biomass due to increased productivity, but also a litter composition reflecting species composition of the field vegetation.

2. Materials and methods

2.1. Study area and study species

The study area is located along east side of Dokkfløyvatnet in Gausdal, SE Norway, 61°10'N, 09°90'E, 800 m a.s.l (Gauslaa et al. 2008). The annual mean temperature is -0.1 °C while the annual mean precipitation is 810 mm. The bedrock consists of sedimentary rocks of Cambro-Ordovician age with moraine deposits (Gauslaa et al. 2008). This area is a subalpine boreal forest dominated by Norway spruce (*Picea abies*), *V. myrtillus* and has a dense bryophyte layer (Figure 1). This is typical for the bilberry forest vegetation type which is common in Norway (Bratli 2016). The age of the trees varies between 68-213 years with a mean age of 131 years. Selective logging was done in the past, but stopped at least 50 years ago (Gauslaa et al. 2008).

Figure 1: Study area in Gausdal, SE Norway (Photo: Mathilde Norby Lorentzen).

Study area

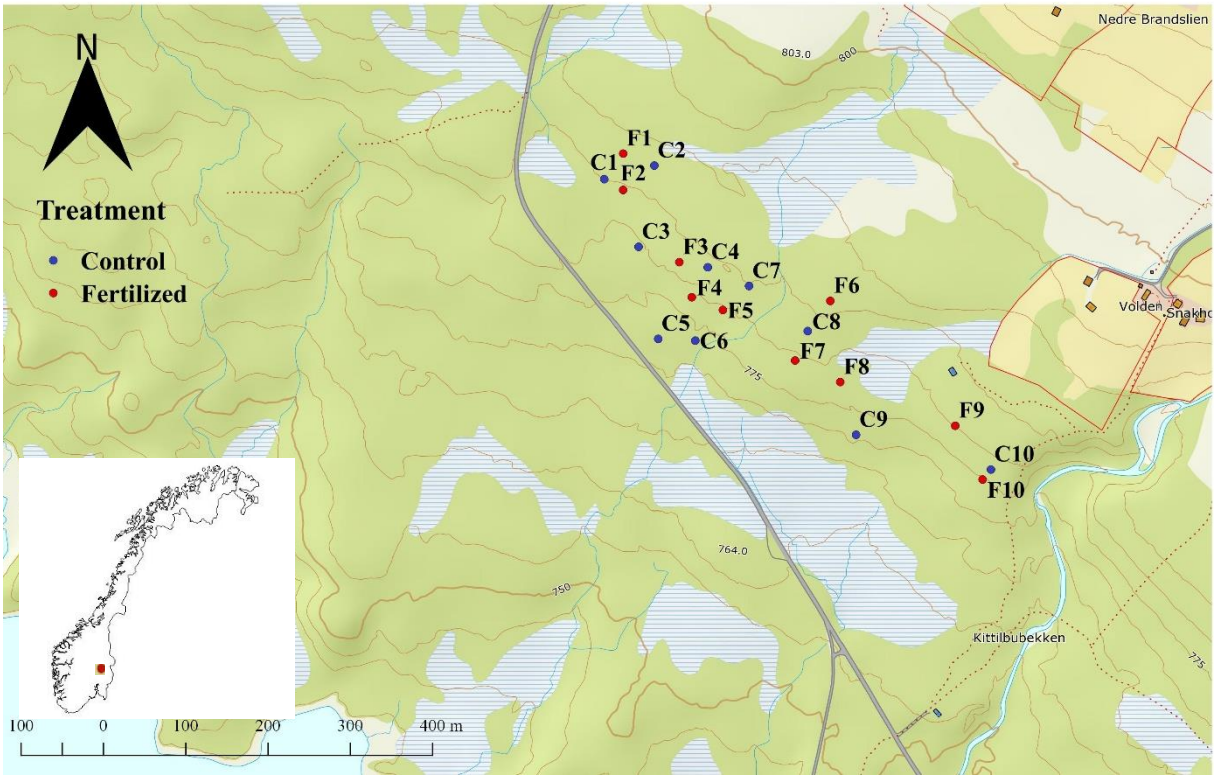

Figure 2: Map of the study area in Gausdal, SE Norway (Quantum GIS Development Team 2017).

In 2003, there were established 20 plots, each 15 m^2 (Figure 2). They were marked with red bands, red sticks as well as metal tubes in the corners. Each plot was situated between 50 and 350 m from the next plot. Since 2003, 10 plots were fertilized annually by hand with 150 kg N per ha in the form of granulated pellets containing 24,6 % N, 1,6 % P, 6 % K and other nutrients such as Ca and Mg (YaraMila™ Fullgjødsel ® by Yara, Norway) (Davey et al. 2016). The nitrogen contained 41-48 % NO_3- and the rest as NH_4+ (YaraMila™ Fullgjødsel ® by Yara, Norway). The other 10 were control plots with no fertilization.

2.2. Data collection and data processing

The study was conducted in July 2016. To randomize the location of vegetation and litter sampling in the treatment and control plots, each plot was divided into 25 subplots, á 3 m^2, with subplot number one in the north-west corner and subplot number 25 in the south-east corner (Figure 3). A total of 15 random subplot numbers in each plot was calculated with no replacement. The first four random subplots were used in the field vegetation analysis, the next eight were used in the litter analysis and the last three were used in the species carbon and nitrogen analysis.

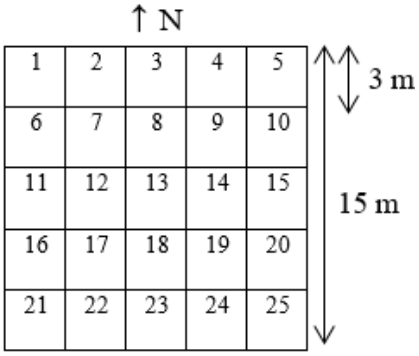

Figure 3: Plot layout.

2.2.1. Field vegetation analysis

Vegetation analyses were done in four 1 m^2 sample plots (squares) in each control and fertilized plot (Figure 4). Two different methods were used for quantifying species abundances based on Økland's paper (1988): (1) Species frequency was calculated by dividing the square into 16 parts and calculate how many of them contained the species. (2) Species percent cover (hereafter referred to as species cover) was measured by the total coverage of each species in the sample plots. The square was positioned in the north-west corner of the random subplot. If this location was unsuitable, i.e. containing trees or large bushes (>1 m), the square was moved to the next corner, clockwise. Total cover in each subplot was measured by summing percent cover for all species in each square.


Figure 4: Vegetation analysis in a control plot (Photo: Mathilde Norby Lorentzen).

2.2.2. Species carbon and nitrogen analysis

Young parts of the dominating plants *V. myrtillus, Avenella flexuosa* and *Pleurozium schreberi* were collected in three random subplots inside every plot. This resulted in a total of 60 samples of each species. Shoots from *P. abies* were sampled on the north side of three trees in all plots. The trees were in the middle of the plots. Three new shoots from 2016 and three older from 2015 were collected at each tree. There were in total 120 samples.

For all the samples in the carbon and nitrogen analysis, silica was added to collect moisture. The samples were dried in a drying cabinet on 35 °C for approx. 48 hours. After drying they were ground in a Retsch MM400 ball mill apparatus at 30 Hz until homogenized (Retsch GmbH, Haan, Germany). They were then packed in 5 mg samples and analysed for nitrogen concentration and carbon concentration using an Elementar Vario MICRO cube analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). Silica was removed prior to the analyses.

2.2.3. Litter sampling

Samples of 18 cm x 18 cm, cut out with the living biomass and litter layer were gathered from eight random subplots in each plot resulting in a total of 160 samples. I started in the north-west corner of each subplot and rejected this and moved clockwise if the sample area had a rocky surface or big roots, keeping the samples homogenous.

Silica was added to the samples to collect moisture and the biomass samples were dried in a drying cabinet on 35 °C for approx. 48 hours. Then they were weighed and checked for amount of grass, heather, bryophytes and spruce needles (none, small amount, medium amount and large amount). This was done by spreading the samples on a white sheet and scoring the amounts subjectively by looking over the sample. Silica was removed prior to the analysis. Samples dominated by *Sphagnum* spp. were removed before analysis because they were too different from the focused forest type and might have disturbed the results.

2.3. Statistical analysis

Treatment (control or fertilized) was used as the explanatory variable in all analyses. A significance level of $\alpha=0.05$ was used on all statistical analyses.

For the numerical data, a Shapiro-Wilk normality test was conducted on the response variables to see if they were normally distributed. The response variables were species richness, total vegetation cover, species frequency, species cover, litter biomass weight, nitrogen concentration, carbon concentration and CN ratio. To see if there was a difference between the control and the fertilized plots, a t-test was applied if the data were normally distributed ($p>0.05$) or a two-tailed Wilcoxon test if not ($p<0.05$) (Verzani 2014). Boxplots were used to visualize differences between fertilized and control plots. The 15 most frequently observed species were shown in a table with species cover.

Non-metric multidimensional scaling (hereafter referred to as NMDS) ordination using Bray-Curtis distance was used to analyse species composition (Kruskal 1964). The NMDS ordination was based on the species cover data for all the species. To bring down the stress level, the number of dimensions used was four ($k=4$). An ordination plot showing species and treatment plots, using NMDS, was constructed to visualize if there were patterns in species distribution in relation to treatment.

To see if there was a difference in species composition in the litter layer between fertilized and control plots for the categorical data (grass, heather, bryophytes and spruce needles), the data were organized in contingency tables before the Fisher's exact test for count data was used. The Fisher's exact test was used because some of the counted data were small (Freeman Jr 1987).

The species frequency data and the species cover data were both tested on treatment and used in the NMDS ordination analysis. However, the species frequency data showed the same trends as the species cover data when the statistical analysis was done. The species cover data gave slightly clearer results, and to minimize the clutter of repeating the same trends I choose not to include the frequency data from now on.

To perform the statistical analysis and make the figures, the softwares R version 3.2.3 (R Core Team 2015) and Rstudio version 1.0.44 (Rstudio 2016) were used, along with the packages vegan (Oksanen et al. 2016), ggplot2 (Wickham & Chang 2016) and gridextra (Auguie & Antonov 2016). The map of study area was made with Qgis version 2.18.4 (Quantum GIS Development Team 2017).

3. Results

3.1. Vegetation

There were a total of 44 species found in the plots. The mean number of species found in control plots were 11 (min. 6, max. 19), and in fertilized plots 10 (min. 7, max. 14). There was no significant difference in species richness ($p=0.236$), but a significantly lower total vegetation cover in the fertilized plots compared to the control plots ($p<0.001$).

Non-metric multidimensional scaling (NMDS) ordination plot showed that the control plots were clearly different from the fertilized plots (Figure 5). The NMDS1 axis seemed to represent a fertilization gradient, however, it was less apparent what NMDS2 axis represent. The species composition seemed to reflect the treatment. Species that had their main distribution on the lower side of the fertilization gradient seemed to be associated with no fertilization while species on the higher side of the fertilization gradient seemed to be associated with fertilization. Species that were observed only a few times were mostly located outside of the plots. For more details on each species see Appendix 1. From Figure 5, it seems like heather, large bryophytes and some other plants are associated with no fertilization. Smaller bryophytes, lichens, grass and some herbs seem to be associated with fertilization.

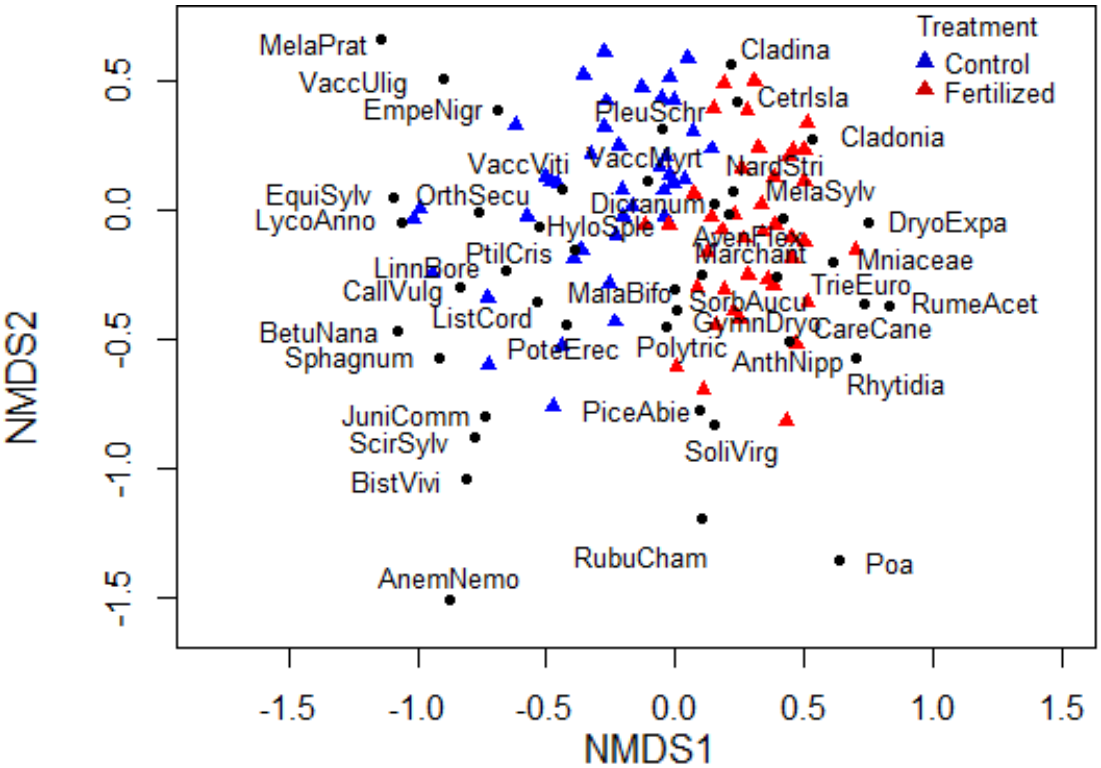

Figure 5: Non-metric multidimensional scaling (NMDS) ordination plot (Bray-Curtis distance, k=4, stress=0,119) based on species coverage data from all 44 species and 80 subplots. The subplots consist of 40 control (▲) and 40 fertilized yearly since 2003 (▲). Displaying species composition along the fertilization gradient (NMDS1). Species are written with the first four letters in the genus and species name, or only the genus.

Several frequently observed species showed a difference when fertilized (Table 1). The species *A. flexuosa*, *Marchantiophyta* spp. and *Trientalis europaea* showed a significant increase in cover in the fertilized plots (Table 1). In contrast, *V. myrtillus, P. schreberi, Hylocomium splendes, Vaccinium vitis idaea, Empetrum nigrum* and *Linnaea borealis* decreased in the fertilized plots. There was no significant difference in cover between control and fertilized for *Dicranum* spp., *Polytrichaceae* spp., *Cladina* spp., *Cetraria islandica, Maianthemum bifolium* and *Cladonia* spp. (Table 1).

Table 1: Overview of the 15 most frequently observed species with the total number of subplots they occurred in and number of subplots in control/fertilized (C/F) for each species. The mean species coverage (%) and standard deviation (SD) for control and fertilized are also included. Significant P-values from Wilcoxon tests are represented by the symbols: *p < 0.05; **p < 0.01; ***p < 0.001.

	Number of plots	Number of plots	Species cover			
Species	Total	C/F	Control	SD	Fertilized	SD
*V. myrtillus****	80	40/40	71.88	±18.27	31.70	±16.51
*A. flexuosa****	78	38/40	20.93	±16.10	51.09	±24.42
Marchantiophyta spp.**	75	36/39	8.82	±9.66	15.07	±11.92
*P. schreberi****	74	36/38	42.41	±33.51	14.44	±15.20
Dicranum spp.	67	32/35	5.38	±5.09	6.79	±6.95
*T. europaea****	55	21/34	3.67	±4.93	25.77	±18.18
*H. splendes****	46	33/13	31.17	±31.33	1.87	±3.75
*V. vitis idaea****	44	31/13	5.61	±7.40	1.01	±1.45
Polytrichaceae spp.	40	16/24	2.96	±6.66	2.65	±2.93
Cladina spp.	36	18/18	2.18	±3.07	1.40	±1.54
C. islandica	31	15/16	1.32	±1.96	1.87	±2.87
*E. nigrum****	28	23/5	8.12	±17.73	0.39	±1.03
M. bifolium	26	12/14	4.69	±11.75	1.79	±3.55
Cladonia spp.	19	6/13	0.47	±1.11	1.32	±2.30
*L. borealis****	17	15/2	4.53	±8.78	0.31	±1.53

3.2. Nitrogen and carbon

The three species, *A. flexuosa*, *P. schreberi* and *V. myrtillus*, did all show a significant increase in nitrogen concentration when fertilized ($p<0.001$, Figure 6). However, the carbon concentration was not significantly different between the treatments ($p>0.05$). This is consistent with the CN ratio decreasing significantly for all three species when fertilized ($p<0.05$, Figure 6). *A. flexuosa* had the lowest CN ratio while *P. schreberi* had the highest in fertilized plots.

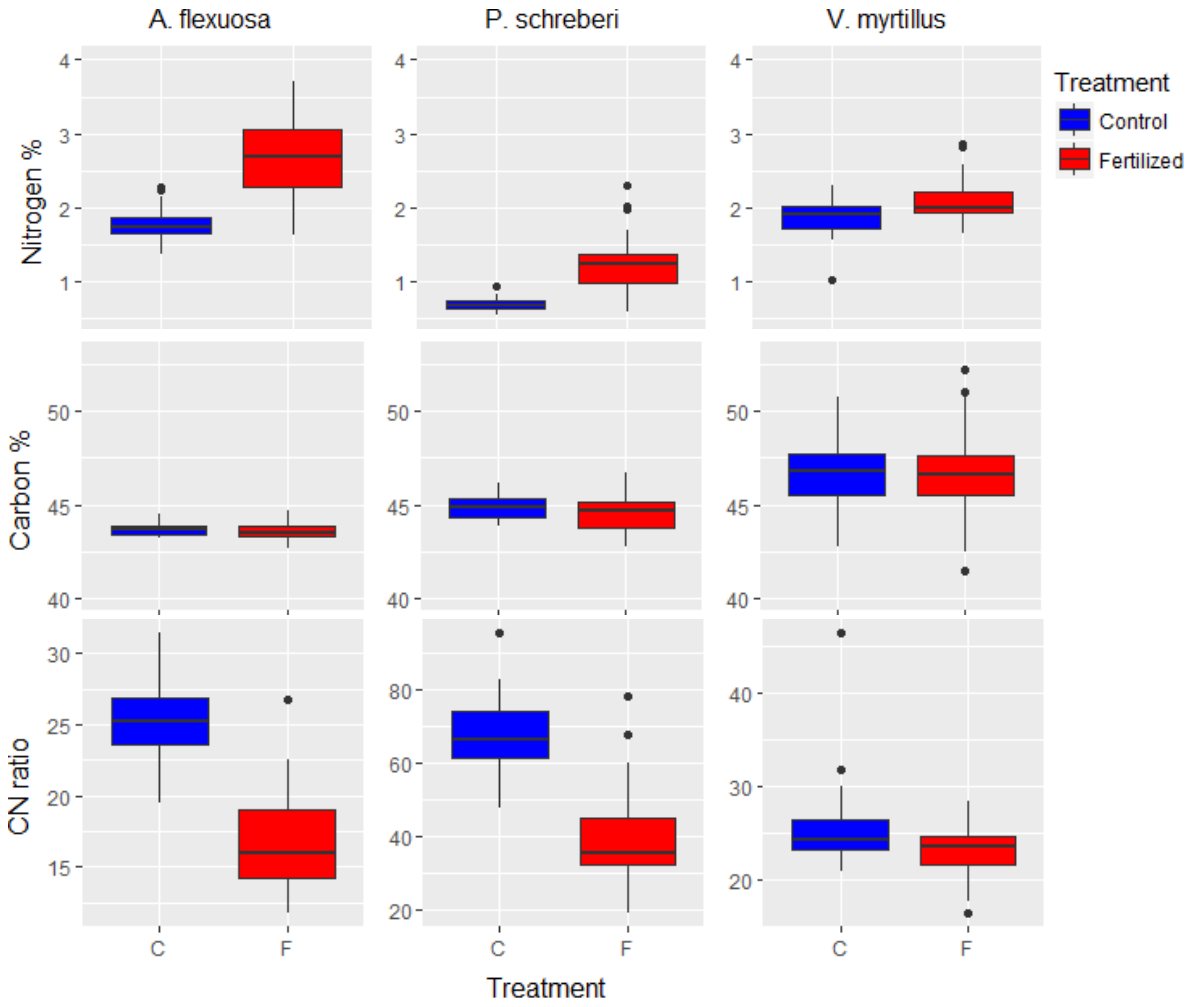

Figure 6: Nitrogen concentration (%), carbon concentration (%) and CN ratio of *V. myrtillus*, *P. schreberi* and *A. flexuosa* from control (C, blue) and fertilized (F, red) plots.

According to the results represented in Figure 7, the nitrogen concentrations for *P. abies* shoots were significantly higher in fertilized plots compared to control ($p<0.001$). There were higher concentrations in younger (2016) compared to older shoots (2015) in both control and fertilized plots ($p<0.001$).

The carbon concentration in the shoots did, however, not differ between control and fertilized plots ($p>0.05$, Figure 7). On the other hand, it decreased significantly in the younger (2016) compared to the older shoots ($p<0.001$).

Results from nitrogen and carbon concentrations in *P. abies* showed a decrease in CN ratio for all analyses ($p<0.001$, Figure 7).

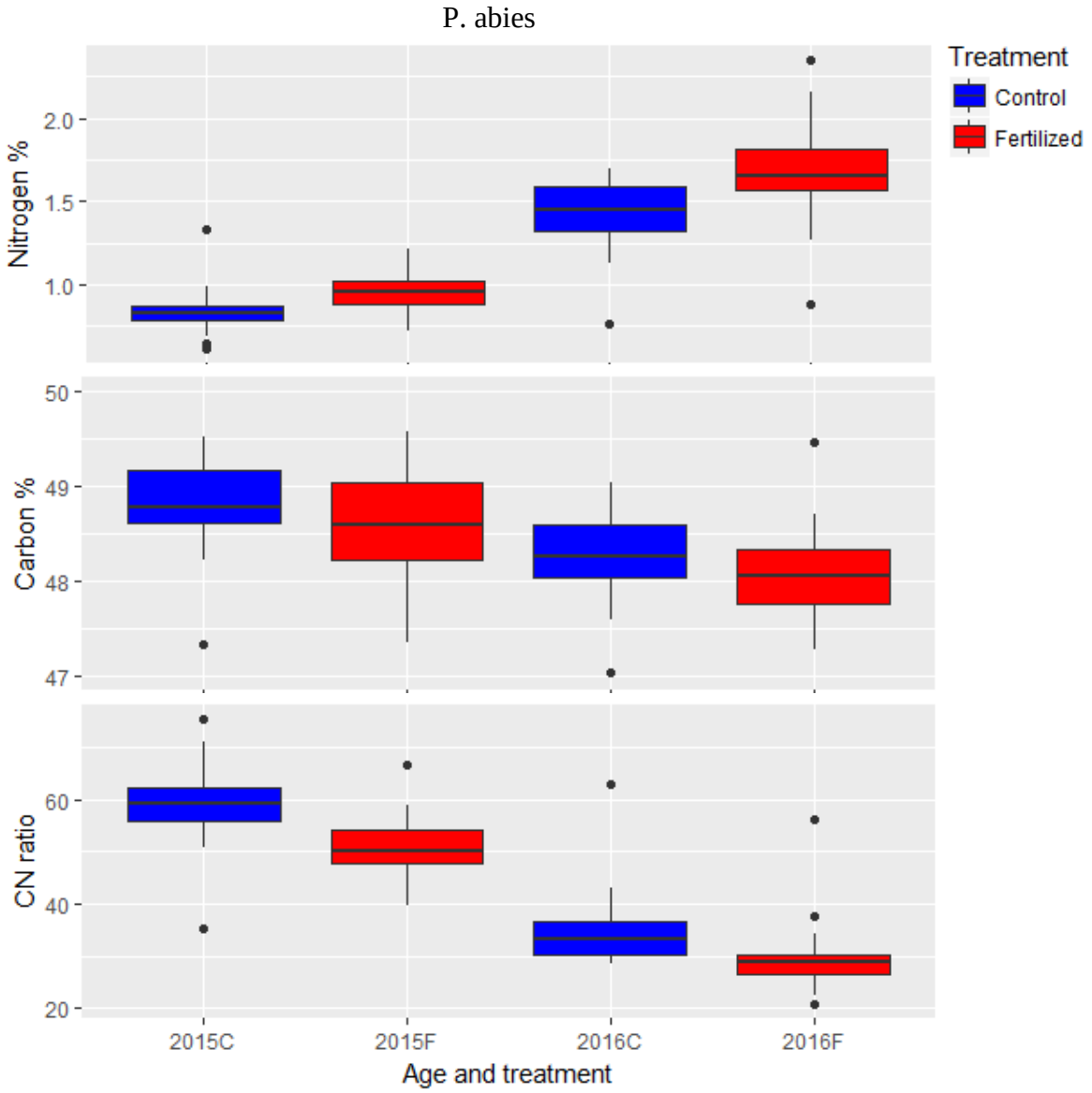

Figure 7: Nitrogen concentration (%), carbon concentration (%) and CN ratio for *P. abies* shoots in 2015 and 2016, for control (C, blue) and fertilized (F, red) plots.

3.3. Litter

The results showed that average litter biomass weight in control (46 g ± 17) was slightly lighter than fertilized plots (51 g ± 24). However, there was no significant difference (p=0.601).

The results from the litter biomass samples showed that when fertilized, the amount of grass and spruce needles increased significantly, while heather and bryophytes decreased significantly (For all; p<0.001, Figure 8).

Figure 8: Litter sample from control plot and fertilized plot (Photo: Mathilde Norby Lorentzen).

4. Discussion

4.1. Vegetation

4.1.1. Species richness, abundance and composition

The results showed that fertilization can clearly change the field vegetation in a boreal forest. There were a slightly lower mean and maximum number of species in the fertilized plots, which could indicate a decreasing trend. However, there was no significant difference in species richness. In accordance to the study, Hedwall et al. (2013) and Bobbink (2004) found no significant difference in species richness. Other studies have concluded with a decrease in species richness (Hedwall et al. 2011; Strengbom & Nordin 2008). The results indicated that there where a shift in species composition rather than a decrease in species richness. However, this could also be a part of a gradual decrease in species richness. If so, this could result in reduced biodiversity (Gilliam 2006).

The results also showed a significant decrease in total vegetation cover when fertilized. In contrary of the results, Strengbom and Nordin (2008) found an increase. A possible explanation for the decrease in total cover found in this study could be because fertilization might lead to larger tree crowns and therefore decreased light availability for the field vegetation. This could possibly contribute to a decrease in cover (Haugland et al. 2014; Hedwall et al. 2010). On the other hand, observed vegetation cover during field study indicated that normally large and dominant species decreased when fertilized. There might be other explanations for the decrease in cover than decrease in light because the observed light availability did not seem to change between control and fertilized plots. The estimation of total cover might have been done differently in other studies. A better estimation might be the total vegetation cover in percent in each subplot. Even though, the indication of decrease in cover was also clearly observed when gathering data.

The non-metric multidimensional scaling (NMDS) ordination plot showed that there were differences between the control and fertilized plots. The species composition changed with the fertilization gradient (NMDS1). From the ordination plot, it seemed like heather and large bryophytes were associated with no fertilization, while grasses, some herbs, smaller bryophytes and lichens were associated with fertilization. This indicates that fertilization leads to a shift in species composition, which is consistent with other studies (Bobbink 2004; Hedwall et al. 2013). In the analysis, there was used four dimensions, which complicated the interpretation of the axes.

Results from the most frequently observed species found were consistent with the NMDS ordination plot, showing that heather species such as *V. myrtillus, V. vitis idaea* and *E. nigrum* decreased when fertilized. This is in accordance with similar studies (Gundale et al. 2014; Nordin et al. 2005; Strengbom et al. 2003; Strengbom & Nordin 2008). There are several possible explanations for the decreases. The keystone and dominating plant, *V. myrtillus,* has been shown to be more susceptible to an attacking fungal pathogen when nitrogen concentrations are elevated, possibly contributing to a decrease (Nordin et al. 1998; Nordin et al. 2006; Strengbom et al. 2002; Strengbom et al. 2003). This is also in accordance with other studies indicating a higher abundance of some attacking fungal pathogens when fertilized (Davey et al. 2016; Strengbom et al. 2003; Wiedermann et al. 2007). Another explanation for the decrease in *V. myrtillus* could be an increase in herbivorous *Operophtera* spp. larvae species feeding on the leaves when nitrogen concentrations increases (Nordin et al. 1998; Nordin et al. 2009). On the other hand, during the field study, there were no observed signs of either fungal pathogens or herbivory on *V. myrtillus,* indicating that these might not be likely explanations. A third explanation could be a decreased advantage when fertilized. Heather species, such as *V. myrtillus,* are generally more adapted to nitrogen limited environments, suggesting that when exposed to elevated nitrogen concentrations, more nitrogen efficient species get favoured and replace heather (Mäkipää 1999; Nilsson et al. 2002; Nordin et al. 2006).

The ordination plot indicated that the grass *A. flexuosa* was associated with fertilization. This is consistent with the significant increase in species cover, and several others found the same increase (Gundale et al. 2014; Nordin et al. 2005; Strengbom & Nordin 2008). *A. flexuosa* is known to be nitrogen efficient (Nordin et al. 2006) and the increase in species cover is a likely contributor for the decrease in heather species (Nilsson et al. 2002). As heather and large bryophytes decreased, the light availability increased and possibly gave even better growing conditions for *A. flexuosa* (Strengbom et al. 2002; Strengbom et al. 2004). The decreased vegetation cover could eventually be covered with nitrogen efficient species such as *A. flexuosa* in the future, but this requires further research.

Results did also indicate that the dwarf shrub *L. borealis* decreased, the herb *M. bifolium* showed no difference and *T. europaea* increased when fertilized. *L. borealis* is, as the heather species, adapted to nitrogen limited environments and might therefore also be replaced by more nitrogen efficient species (Mäkipää 1999). Strengbom and Nordin (2012) found that *T. europaea* increased in fertilized plots, which is consistent with the results.

A possible explanation for the increase could be that, when fertilized, the reproduction below ground increases due to higher nutrient availability (Piqueras et al. 1999). Factors such as decreased competition from large aboveground species might also contribute, but few studies have looked at this species response to fertilization.

The NMDS ordination plot indicated that there was a shift in bryophytes when fertilized. The most frequent species analyses showed that large bryophytes, such as *P. schreberi* and *H. splendes*, were clearly associated with no fertilization. This is in accordance with other studies (Gundale et al. 2014; Nordin et al. 2005; Strengbom & Nordin 2008). Davey et al. (2016) did a research in the same study area. They found that, when exposed to elevated nitrogen concentrations, pathogenic fungi attacking bryophytes increased in both *H. splendes* and *P. schreberi.* On the other hand, bryophytes are known to lack a effective cuticle resulting in absorption of nitrogen through all surface area (Oishi 2016). This makes bryophytes sensitive to elevated nitrogen concentrations. Paulissen et al. (2004) suggested that bryophytes take up toxic NH_4^+ when fertilized, which potentially would lead to a decline. This could be another explanation for the decrease in *H. splendes* and *P. schreberi.* The smaller bryophytes *Marchantiophyta* spp. showed an increase when fertilized while *Dicranum* spp. and *Polytrichaceae* spp. showed no difference. The results, showing that *Marchantiophyta* spp. increased, indicates that other factors might be more important than increased fertilization. However, there are few studies on the effects on *Marchantiophyta* spp. A possible reason could be eased competition since the total cover decreased, but this needs further research.

From the NMDS ordination plot it looked like lichens preferred fertilized plots, however, none of the frequently found lichens showed any significant difference. This contrasts with other studies who found a decrease in abundance (Hedwall et al. 2010; Mäkipää 1995b; Olsson & Kellner 2006; Strengbom & Nordin 2008). Possible reasons for this could be that some lichens utilize increased nitrogen concentrations, which could arise from fertilization, for growth (Nybakken et al. 2009) or that other factors might be more important (Fremstad et al. 2005). As it was difficult to interpret the NMDS axes, other factors could be more important than fertilization. The observed decrease in total vegetation cover could possibly have eased the competition for lichens, but they have a slow growth indicating that significant changes could take time.

When the fertilization experiments started in 2003, the placement of plots, fertilization type and amount, were originally based on an epiphytic lichen study (Gauslaa et al. 2008). There was mostly nitrogen in the fertilizer, but it also contained P, K, Ca and Mg (YaraMila™ Fullgjødsel ® by Yara, Norway). This paper mainly considers the effects of nitrogen, but the other elements should be taken under consideration as they could have contributed to the results. Worth noting is that, in this study, the amount of fertilization is larger and repeated more often than other studies, which might give more extreme results (e.g Hedwall et al. 2013; Strengbom et al. 2002; Strengbom et al. 2004; Strengbom & Nordin 2008).

4.1.2. Species nitrogen content

When fertilized, *A. flexuosa*, *P. schreberi* and *V. myrtillus* increased in nitrogen concentration. However, none showed any significant difference in carbon concentration, which is consistent with the CN ratio decreasing for all three species when fertilized. This is in accordance with Gundale et al. (2014) and Strengbom and Nordin (2008) who found that the nitrogen concentration increased for *A. flexuosa* and *V. myrtillus*. Gundale et al. (2014) did also found that *P. schreberi* increased in nitrogen concentration, while Strengbom and Nordin (2008) detected no difference. In contrast, Strengbom and Nordin (2008) found that only one species, *A. flexuosa,* showed a significant difference in carbon concentration between control and fertilized plots. It increased in carbon concentration when fertilized. The increase in nitrogen is most likely due to the nitrogen-limited boreal forest being exposed to fertilizers containing nitrogen (Bobbink et al. 2010). The results indicated that fertilization did not have any significant effects on carbon concentrations in dominating plants.

The analysis of *P. abies* shoots indicated that there was a significant increase in nitrogen concentration when fertilized for both 2015 and 2016 shoots. This is in accordance with other studies concluding that fertilization leads to higher nitrogen concentrations (From et al. 2015; Gundale et al. 2014; Strengbom & Nordin 2008). There was a higher nitrogen concentration in younger shoots (2016). A possible reason could be that younger shoots require high nitrogen concentrations to support growth (Mattson Jr 1980). Results indicated lower carbon concentrations in the younger shoots (2016). This is in accordance with Mattson Jr (1980) who found that young and growing shoots have lower carbon concentrations than older shoots. Lower CN ratios in *P. abies* shoots due to higher nitrogen is in accordance with Strengbom and Nordin (2008) and From et al. (2015).

4.2. Litter

There was no significant difference between the litter biomass weight in control and fertilized plots. Results showed that, when fertilized, the amount of grass and spruce needles increased, while heather and bryophytes decreased. No difference in litter biomass weight could be because grass and spruce needles in fertilized plots produce approximately the same amount of litter as heather and bryophytes in control plots. Based on this, it seemed like it was no difference in plant production. The increase in grass is consistent with the increase in *A. flexuosa* cover found in the field vegetation analysis, suggesting that grass is favoured when exposed to elevated nitrogen concentrations. Increased amounts of spruce needles in the fertilized litter could indicate a higher production of spruce needles, although it could also indicate a slower long-term decomposition (Berg 2014; Bobbink et al. 2010; Franklin et al. 2003; Perakis et al. 2012). The decrease in heather and bryophytes is consistent with the decreased cover of *Vaccinium* species and large bryophytes such as *P. schreberi* and *H. splendes* found previously. Although *Marchantiophyta* spp. increased in cover when fertilized, the species are small and so the amount did not make up for the loss of the larger bryophytes. Based on this, the litter biomass seemed to clearly reflect field vegetation in both control and fertilized plots.

The carbon storage in the soil is known to potentially increase in fertilized plots when litter biomass increase due to higher plant production and slower long-term decomposition (Franklin et al. 2003). However, litter biomass weight did not increase, which indicates no difference in carbon storage in this study. On the other hand, the litter biomass composition changed and this could potentially alter the amount of carbon stored in the soil depending on species carbon concentrations. The analyses of living biomass indicated that carbon concentration was at the lowest in *A. flexuosa* and highest in *P. abies.* This suggests that each species found in the litter could alter the potential carbon storage in the soil. However, to understand more of the potential carbon storage, it requires a more thorough analysis of both litter biomass and carbon concentrations.

5. Conclusion

Fertilization in Norwegian boreal forests can clearly affect both field vegetation and litter layer. Although there was no significant difference in species richness when fertilized, a decrease in total vegetation cover was clear. Fertilization seemed to alter the species composition, from domination of heather species and large bryophytes, to more grass, herbs and smaller bryophytes. There were increased nitrogen concentrations in the species tested, but fertilization had little impact on carbon concentrations. This resulted in decreased CN ratios for all species. Although there was no difference in litter biomass weight, the litter layer changed from heather and bryophytes, to grass and spruce needles when fertilized. While this indicates no clear change in plant production, litter layer did reflect field vegetation.

Fertilization of Norwegian boreal forests seem to have similar effects as studies done in other countries. However, to achieve a better understanding of what goes on, further studies on the effects of fertilization in Norway over time would need to be conducted.

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7. Appendix

Appendix 1: Overview of species found in the plots with number of plots they were found in, along with mean and standard deviation (SD) for both frequency data (x/16) and species cover data (%) in control (C) and fertilized (F) plots. Significant P-values for species coverage are represented by these symbols: *p < 0.05; **p < 0.01; ***p < 0.001.

	Plots	Frequency				Species cover			
Species		C	SD	F	SD	C	SD	F	SD
Anemone nemorosa	1	0.00	±0.02	0.00	±0	0.08	±0.48	0.00	±0
Anthoxanthum nipponicum	2	0.00	±0	0.00	±0.02	0.00	±0	0.16	±0.67
*Avenella flexuosa****	78	0.63	±0.33	0.84	±0.17	20.93	±16.1	51.09	±24.42
Betula nana	3	0.02	±0.06	0.00	±0	0.23	±0.81	0.00	±0
Bistorta vivipara	1	0.00	±0	0.00	±0	0.08	±0.48	0.00	±0
Calluna vulgaris	3	0.02	±0.05	0.00	±0	0.23	±0.81	0.00	±0
*Carex canescens**	5	0.00	±0	0.03	±0.07	0.00	±0	1.17	±4.1
Cetraria islandica	31	0.09	±0.14	0.11	±0.17	1.32	±1.95	1.87	±2.86
Cladina spp.	36	0.17	±0.24	0.09	±0.13	2.18	±3.06	1.40	±1.54
Cladonia spp.	19	0.01	±0.04	0.05	±0.08	0.47	±1.1	1.32	±2.3
Dicranum spp.	67	0.30	±0.25	0.45	±0.26	5.38	±5.08	6.79	±6.95
*Dryopteris expansa***	7	0.00	±0	0.02	±0.03	0.00	±0	0.54	±1.17
*Empetrum nigrum****	28	0.21	±0.29	0.01	±0.04	8.12	±17.73	0.39	±1.02
Equisetum sylvaticum	2	0.01	±0.05	0.00	±0	0.31	±1.53	0.00	±0
Gymnocarpium dryopteris	13	0.05	±0.11	0.03	±0.1	1.25	±2.86	1.09	±3.52
*Hylocomium splendes****	46	0.53	±0.39	0.07	±0.13	31.17	±31.32	1.87	±3.74
Juniperus communis	4	0.01	±0.04	0.00	±0	0.23	±0.81	0.08	±0.48
*Linnaea borealis****	17	0.24	±0.36	0.03	±0.12	4.53	±8.78	0.31	±1.53
Listera cordata	6	0.03	±0.12	0.00	±0	0.55	±1.69	0.08	±0.48
Lycopodium annotinum	1	0.01	±0.05	0.00	±0	0.24	±1.46	0.00	±0
Maianthemum bifolium	26	0.16	±0.3	0.09	±0.17	4.69	±11.75	1.79	±3.54
Marchantiophyta spp.**	75	0.48	±0.28	0.69	±0.31	8.82	±9.65	15.07	±11.91
Melampyrum pratense	1	0.00	±0.02	0.00	±0	0.08	±0.48	0.00	±0
Melampyrum sylvaticum	1	0.00	±0	0.00	±0.02	0.00	±0	0.08	±0.48
Mniaceae spp.**	11	0.00	±0	0.06	±0.15	0.08	±0.48	1.32	±3.33
Nardus stricta	14	0.02	±0.07	0.05	±0.1	0.78	±2.5	2.57	±7.04
Orthilia secunda	3	0.04	±0.12	0.00	±0	0.55	±2.08	0.00	±0
Picea abies	4	0.00	±0.01	0.00	±0.01	0.16	±0.67	0.16	±0.67
*Pleurozium schreberi****	74	0.70	±0.38	0.49	±0.32	42.41	±33.51	14.44	±15.2
Poa sp.	3	0.00	±0	0.03	±0.13	0.00	±0	1.88	±9.82
Polytrichaceae spp.	40	0.12	±0.22	0.13	±0.14	2.96	±6.66	2.65	±2.93
Potentilla erecta	10	0.03	±0.08	0.01	±0.03	0.70	±1.77	0.23	±0.81
Ptilium crista-castrensis	2	0.01	±0.06	0.00	±0.02	0.08	±0.48	0.08	±0.48
Rhytidiadelphus spp.	1	0.00	±0	0.01	±0.06	0.00	±0	0.24	±1.46
Rubus chamaemorus	6	0.03	±0.14	0.06	±0.2	0.55	±2.94	3.67	±12.51
Rumex acetosa	1	0.00	±0	0.00	±0.02	0.00	±0	0.08	±0.48
Scirpus sylvaticus	2	0.00	±0.02	0.00	±0.02	0.08	±0.48	0.08	±0.48
Solidago virgaurea	2	0.00	±0	0.00	±0	0.08	±0.48	0.08	±0.48
Sorbus aucuparia	3	0.00	±0	0.00	±0.01	0.08	±0.48	0.16	±0.67
Sphagnum spp.	13	0.14	±0.31	0.02	±0.08	10.24	±25.87	0.78	±3.01
*Trientalis europaea****	55	0.23	±0.28	0.76	±0.35	3.67	±4.93	25.77	±18.18
*Vaccinium myrtillus****	80	1.00	±0.01	0.87	±0.21	71.88	±18.27	31.70	±16.51
*Vaccinium uliginosum****	10	0.08	±0.16	0.00	±0	1.64	±3.75	0.00	±0
*Vaccinium vitis-idaea****	44	0.37	±0.3	0.04	±0.08	5.61	±7.39	1.01	±1.45

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