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Challenging conventional carbon wisdom: a climate driven vegetation shift from spruce to beech may not change the size of the carbon pool in southeast Norwegian forest soils

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Management of Natural Resources

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Abstract

Forest soils represents the largest terrestrial carbon (C) pool and thus play a critical role in the global C cycle. Predicted climate change is expected to change the size of forest soil C pools by driving a shift in vegetation that will fundamentally transform the ecosystem. It is assumed that the nemoral deciduous tree species European beech (*Fagus sylvatica* L.) will outcompete the boreal coniferous tree species Norway spruce (*Picea abies* (L.) Karst.) in southeastern Norway. The aim of this study was to investigate differences of C storage in the subsoil between a beech forest, a first-generation spruce forest located on a site of former beech forest, and a second-generation spruce forest. Measurements of C and nitrogen (N) concentration, C/N ratio, amount of fungal biomass and pH were conducted along fine-scaled subsoil profiles. Ergosterol was used as a proxy to estimate fungal biomass. The study showed that concentrations of C, N and free ergosterol, C/N ratio and pH varied significantly with soil depth, and the forests caused significant vertical differences in C, N and free ergosterol concentration with soil depth. However, there was no significant difference in total C storage between the three forests. The findings contradict the conventional and well-established assumption that spruce forests soils store more C compared to beech forest soils, and that this may be ascribed to various site conditions.

Sammendrag

Skogsjord utgjør de største karbonlagrene i biosfæren på land og spiller dermed en avgjørende rolle i den globale karbonsyklusen. Forventede klimaendringer antas å føre til forandringer i størrelsen på karbonlagrene i skogsjord ved å forårsake en vegetasjonsendring med påfølgende fundamentale økosystemendringer. Det er forventet at europeisk bøk (*Fagus sylvatica* L.) vil utkonkurrere norsk gran (*Picea abies* (L.) Karst.) i Vestfold, Norge. Hensikten med denne studien var å undersøke forskjeller i karbonlagring mellom en bøkeskog, en førstegenerasjon granskog som er plantet på et område hvor det tidligere var bøkeskog, og en andregenerasjon granskog. Målinger av C- og nitrogen (N)-konsentrasjon, C/N ratio, mengde soppbiomasse og pH-verdier ble utført langs fin-skalerte dybdeprofiler av mineraljorda. Ergosterol ble brukt som en proxy for å måle soppbiomasse. Studien fant at konsentrasjoner av C, N og fri ergosterol, C/N ratio og pH varierte betydelig med jorddybde, og skogene medførte betydelige vertikale forskjeller i C-, N- og fri ergosterolkonsentasjon med jorddybde. Men det var ingen signifikant forskjell i total karbonlagring mellom skogene. Resultatene av dette studiet skiller seg fra den konvensjonelle og veletablerte antakelsen om at granskog lagrer mer karbon i jorda enn bøkeskog.

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1.0 Introduction

Forest soils are the largest terrestrial carbon (C) pool and thus play a critical role in the global C cycle (Lal 2004). The soils represent a large C sink that mitigates the effect of elevated atmospheric CO_2, and the subsequent climatic changes (Schmidt et al. 2011). Of the current total C stock in the world's forests (861 ± 66 Pg C), 44% is stored in the soil (to 1-m depth) (Pan et al. 2011). This is about twice the amount of C in the atmosphere and three times the amount stored in the vegetation (Smith 2004). Geographically, boreal forests store the second most C (272 ± 23 Pg C) after tropical forests, with remarkable 60% in the top meter of the soil compared to 20% in tropical forests (Pan et al. 2011). C storage in the soil is ultimately determined by the balance between labile and recalcitrant C compounds (Ahmed et al. 2016), which in turn varies according to climate, vegetation, soil texture, and historical and current land use (Batjes 1996). Climate change is likely to have its greatest impact on boreal forests because warming is expected to be particularly acute at high latitudes (IPCC 2014). C pools in boreal forests will therefore be more exposed to the impacts of global warming, than C pools in temperate or tropical forests (Vanhala et al. 2011). The warming implies an increased rate in forest growth and decomposition, with varying implications of the soil C pool depending on tree species (Sievanen et al. 2014). Warming is found to increase photosynthesis more than respiration in spring, and the reverse in autumn (Piao et al. 2008). If warming of autumns occurs at a faster rate than that of spring, C sequestration may be diminished. This confirms the importance of respiration in determining the ecosystem C exchange (Valentini et al. 2000). It is also likely that climate change will cause shifts in forest tree species range, with boreal forest giving way to temperate forests at its southern boundaries (Hickler et al. 2012, IPCC 2014). This vegetation shifts may affect the current C pools in the forest soils.

The northern boundaries of temperate forests and hemi-boreal forests in southern Scandinavia are projected to shift 300-500 km northwards, according to the HadCM3 climate scenario, to achieve equilibrium with the new climate (Hickler et al. 2012). In the HadCM3 scenario, boreal trees are replaced by temperate species at considerably higher migration rates than observed in the past, primarily because of longer growing seasons and warmer winters. It is assumed that the nemoral deciduous tree species European beech (*Fagus sylvatica* L.), at its northern range margins, will outcompete the boreal coniferous tree species Norway spruce (*Picea abies* (L.) Karst.) (Bolte et al. 2014). The tolerance of *F. sylvatica* to abiotic and biotic

threats accompanying climate change is predicted to increase, which will boost their competitive ability compared to *P. abies* (Bolte et al. 2010). The present northernmost range of *F. sylvatica* is discontinuous and consists of two distinct and isolated distributions: a single population at Seim in west Norway; and several adjacent populations in Vestfold, southeast Norway (Bjune et al. 2013, Myking et al. 2011) (Fig. 1). *Picea abies* is the predominant tree species on good soils in southeastern Norway (Moen 1999) (Fig. 2), but a rapid northward contraction of the present range due to climate change has been predicted (Bradshaw et al. 2000). The ensuing vegetation shift entails that the forest ecosystems in southeast Norway will be transformed from spruce forests to beech and mixed beech forest ecosystems. Such a transformation will be a truly fundamental ecosystem change that calls for knowledge about potential consequences.

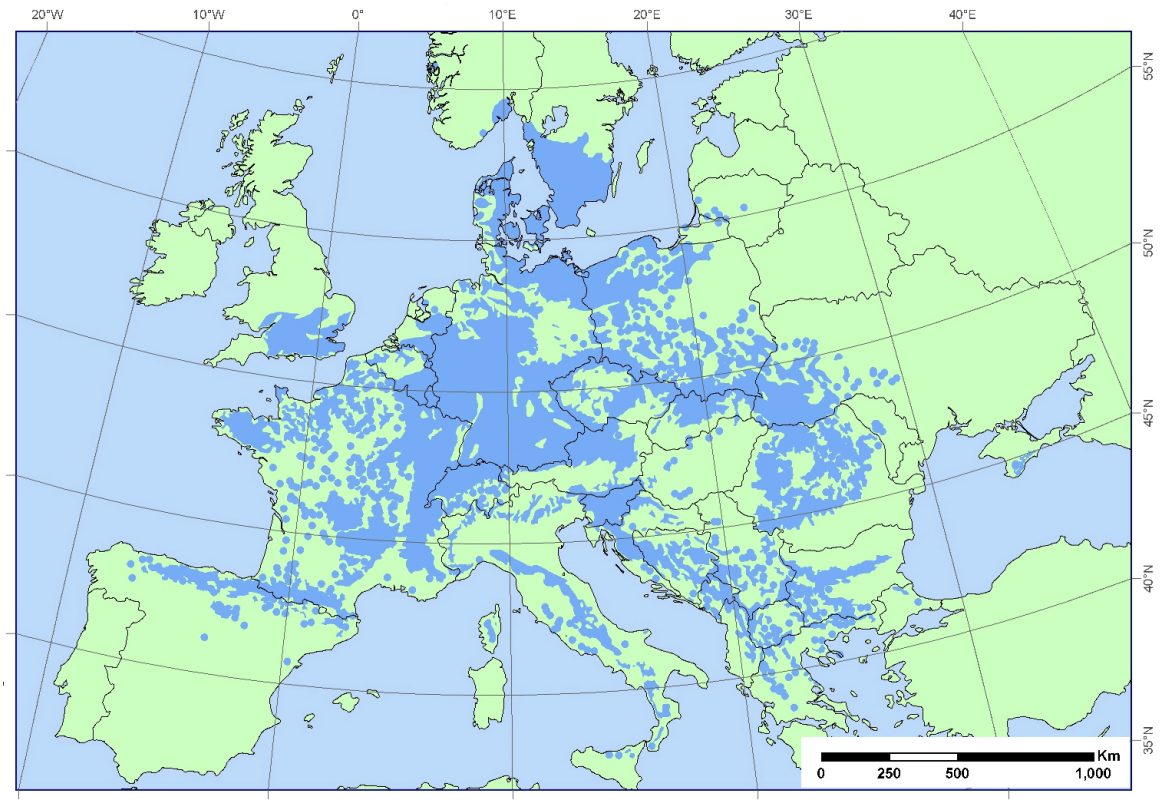

Figure 1: Distribution map of European beech (*Fagus sylvatica*) in Europe (EUFORGEN 2009).

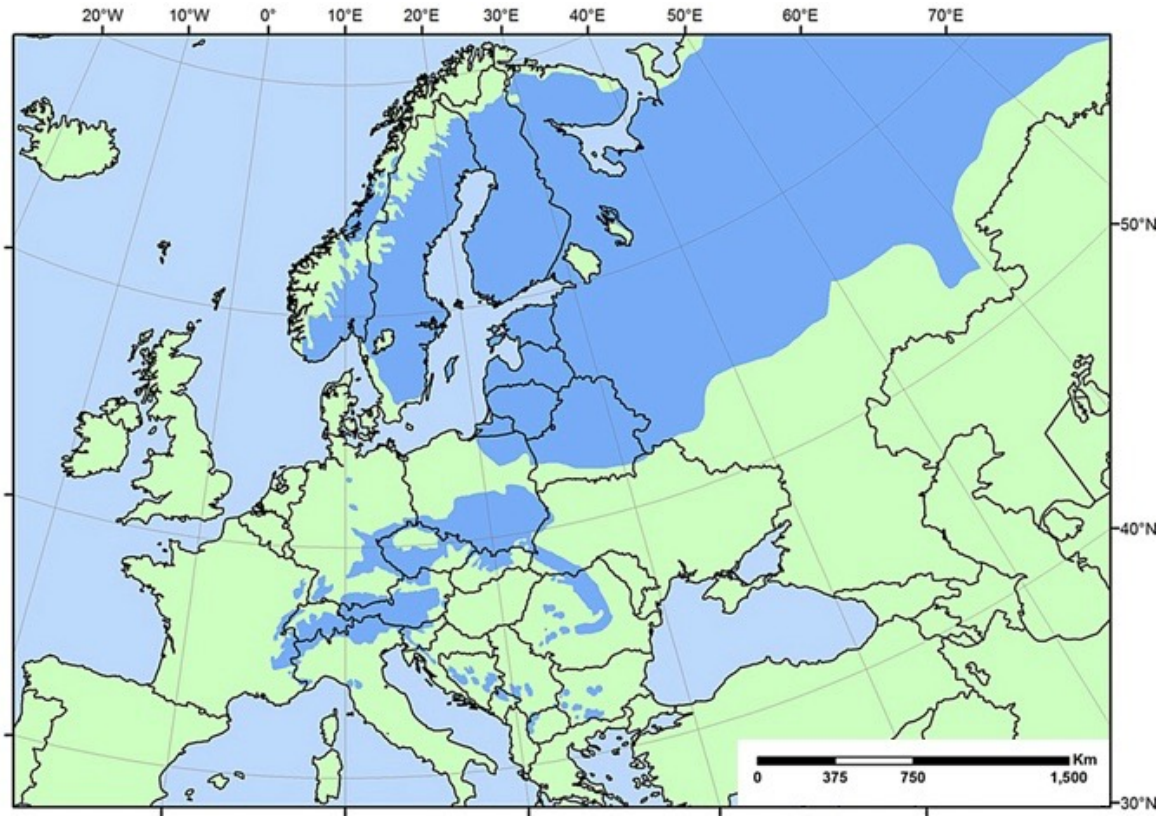

Figure 2: Distribution map of Norway spruce (*Picea abies*) in Europe (EUFORGEN 2009).

The late-successional shade-tolerant tree species *F. sylvatica* is the most abundant and dominating tree species in Central Europe (Gebler et al. 2007). *Fagus sylvatica* forms a dense canopy that produces a large amount of leaf litter and has extensive shallow and intermediate roots (von Wuehlisch 2008). Both *F. sylvatica* and *P. abies* play a key role in their respective forest ecosystems and are economically important tree species in Europe (Bolte et al. 2007, Bjune et al. 2009). The evergreen shade-tolerant tree species *P. abies* forms thick organic layers (humus), has a shallow root system and is known for creating acid soil conditions (Berger & Berger 2012). The pH of soil below spruce forests is thus normally lower than under beech forests (Hojjati et al. 2009), with decreasing acidity in both forests towards the bedrock (Nihlgård 1971). Root system characteristics control plant soil water and nutrient exploitation, and with higher space sequestration efficiency, *F. sylvatica* has a higher competitive ability belowground than *P. abies* (Bolte & Villanueva 2006). Moreover, litter accumulation is higher in spruce forests than in beech forests due to adverse environmental conditions that retard decomposition (Berger & Berger 2012, 2014). Decomposition is an important process for cycling of nutrients in forest ecosystems (Berger et al. 2006), and beech forests are characterized by higher decomposition rates and associated nutrient cycling than

spruce forests (Berger & Berger 2012). In accordance with the respective tree species, more C has been reported in soils under spruce forests than beech forests (Berger et al. 2002, Nihlgård 1971). Galka et al. (2014) found a 25% higher mean soil C stock of spruce forest compared to beech forest in forest litter and the first meter of the mineral soil. Transformation of spruce forests into beech forests may therefore diminish potential C storage.

Along with differences in soil C storage depending on tree species, the soil C also varies by soil depth. Soil C is higher at the surface horizon and decreases with depth in most soils (Adhikari et al. 2014). Several studies indicate that C content in subsoil is less affected by tree species than that in topsoil (Berger et al. 2002, Galka et al. 2014, Vesterdal et al. 2008). Since topsoil C is more vulnerable to decomposition from disturbances than subsoil C (Vesterdal et al. 2008), C sequestered in subsoil is a potentially stable C pool. Radiocarbon measurements, an assessment of the stability of organic matter in the soil, have shown that C stored in the subsoil has an apparent age of several thousand years, and increases with soil depth (Rumpel et al. 2002). The geographical range covered by forest is large globally, so even if there is less C stored in the subsoil compared to the topsoil, it represents substantial amounts of soil C – even at low concentrations (Batjes 1996, Jobbagy & Jackson 2000, Rumpel et al. 2002). Soil nitrogen (N) pools and C/N ratios have been suggested as indicators of C sequestration potential in soils (Vesterdal et al. 2008). Nitrogen is a plant nutrient that affects plant growth and thus C fixation and turnover time in ecosystems (Rumpel et al. 2002). Since the majority of N in is found in organic compounds, the retention of these compounds determines the storage capacity of N. Meanwhile, measurement of the C/N ratio can indicate possible limitation of C or N in the forest soil. Both soil N and C/N ratios vary by soil depth (Vesterdal et al. 2008). The soil properties have for a long time been studied along soil profiles, but the research has primarily focused on bulk soil or relatively large depth intervals (Adhikari et al. 2014, Angst et al. 2016, Kempen et al. 2011, Mishra et al. 2009). Research along a more fine-scaled soil profile, which is rare in the research literature, are essential in detecting smaller variation of soil C belowground.

Aboveground plant litter has been assumed the principal source of soil C, but recent studies have found that belowground roots and root-associated microorganisms contribute respectively to the total C stored in the soil (Clemmensen et al. 2013). Most of this accumulated C originated from fungal mycelium. Fungi are the main decomposers of organic matter in boreal forest ecosystems (Sterkenburg et al. 2015). A common group of fungi, the

mycorrhizal fungi, is a key component of microbial biomass in temperate and boreal forests (Lambers et al. 2008, Wallander et al. 2013). The identity and growth form of mycorrhizal fungi determine C and N sequestration, ultimately leading to either soil C storage or decomposition (Clemmensen et al. 2015). Clemmensen et al. (2015) found that different species of mycorrhizal fungi can play opposing roles in belowground C storage. The mycorrhizal fungi form symbioses with diverse plant roots that allocate a substantial proportion of their C to its symbionts. In return, the mycorrhizal fungi provide water and nutrients, such as N, gathered from its access to greater soil volume reached by the fungi's extensive hyphal mycelium network (up to 25 cm) (Godbold et al. 2006, Lambers et al. 2008). Once the hyphae of the fungi die and decompose, the residues of the fungi form materials containing C that are difficult to convert to CO_2 by decomposers (Phillips et al. 2014, Treseder & Holden 2013, Yuan 2008). This recalcitrant mycelial necromass can be stored in the soil over long time periods, contributing to C storage in the soil (Jastrow et al. 2007). Sterkenburg et al. (2015) has shown that environmental variables can be used to predict the composition and biomass of fungal communities. Analysis of the fungal-specific membrane lipid ergosterol can be used as a means of quantifying mycorrhizal biomass (Sterkenburg et al. 2015). Free ergosterol is a characteristic of newly formed mycelia, while bound ergosterol represents older mycelium with slower biomass turnover (Clemmensen et al. 2013, Wallander et al. 2010, Yuan et al. 2008). Hence, total ergosterol (free plus bound) illustrates the standing fungal biomass (Clemmensen et al. 2013). Soil microbial communities vary between forest types and along vertical scales (Bach et al. 2008). Environmental changes, such as global warming and forest management, are likely to greatly affect soil C sequestration by driving changes in abundance of this dominant component in microbial communities in soils (Clemmensen et al. 2013, 2015, Treseder & Holden 2013).

1.1 Aim of the study

Variations in soil C storage are likely to occur between forests of different dominating tree species, vegetation histories and soil depths. A better understanding of the C sequestration potential of beech forests compared to spruce forests is essential to predict long-term soil C storage and climate feedbacks in northern ecosystems. The aim of this study was to investigate differences of C storage in the subsoil of three distinct forests. They were a beech forest, a first-generation spruce forest located on a site of former beech forest, and a second-generation spruce forest. Three forests were chosen based on comparable climate and site

properties. Concentration of C and N, C/N ratio, amount of fungal biomass and pH were measured along fine-scaled subsoil profiles. Based on the research literature, the following hypotheses were developed: (1) total C storage in the subsoil will be higher for spruce than for beech forests; (2) concentrations of C and N, C/N ratios, amount of fungal biomass, and pH will vary along the soil profile depth, assuming a decrease in C, N and fungal biomass in parallel with an increase in pH with increasing soil depth; (3) the three different forests will display variable patterns that are forest- and soil depth specific.

2.0 Materials and methods

2.1 Study area

The study was carried out in Vestfold county, southeast Norway, where three study sites were located approximately 20 km north of the town Larvik. Two study sites were situated at Brånakollane (59° 11' N, 10° 02' E, 188 m a.s.l.): a beech forest (site A) within the Brånakollane nature reserve; and a first-generation spruce forest (site B) adjacent to the southwest edge of the reserve. The third site was located south of Brånakollane – which is a second-generation spruce forest (site C) next to the small lake Allumtjerna (59° 10' N, 10° 02' E, 68 m a.s.l.) (Table 1, Fig. 3). The present north-western margin of the beech forest range is overlapping the southern margin of boreal spruce forest in this area (Jalas & Suominen 1972). This area belongs to the Oslo Rift geological area, consisting of syenite, granite and monzonite bedrock (Solli & Nordgulen 2007). It is also part of the boreo-nemoral vegetation zone, which forms a transition between boreal coniferous forests and nemoral broad-leaved deciduous forests (Moen 1999). Climate gradients of the region are large within short distances causing the ecotone of boreo-nemoral and southern boreal zones, and associated forest types, to occur over relatively short distances.

The three study sites differ by their vegetation history and by the dominating tree species of the area. The Brånakollane nature reserve (site A) has a very well developed and almost undisturbed *F. sylvatica* forest covering roughly 19 hectare. The nature reserve was established in 1980 to preserve the virtually untouched beech forest and associated wildlife (Norwegian Ministry of Climate and Environment 1980). The nature reserve is surrounded by managed spruce forest, and there has been no significant forestry activity inside the reserve since 1837 (Bjune et al. 2013). A study by Bjune et al. (2013) of the area suggests that the existing beech forest is native, since records of *F. sylvatica* pollen from around 9100 cal B.P were found. They estimated that the establishment and expansion of the local beech forest started between 1300 and 1200 cal B.P., which coincided with a shift to a less diverse landscape mosaic dominated by *F. sylvatica* and *P. abies* trees. Norway spruce is also native to the region and advanced into the area somewhere around 1400 B.P. (Bjune et al. 2013). Their findings also suggest that there previously has been some anthropogenic activity in the area, such as fire and grazing by livestock. There is a clear boundary between *F. sylvatica* inside the reserve, and the *P. abies* on the outside. The *P. abies* forest outside the southwest boundary of the reserve (site B) is a first generation forest that was planted on former mixed

spruce and beech sites in 1956 (Lie, 2016, pers. comm., 5 May). This forest will be referred to as *the first-generation spruce forest*. Soil of both the beech forest and the first-generation spruce forest consist of weathering monzonite/quartz monzonite bedrock (NGU 2016). The study site south of the lake Allumtjerna is also a spruce forest (site C). The forest represent at least a second generation spruce, earlier dominant tree species are not known and therefore, this forest will be referred to as *the second-generation spruce forest*. The spruce forest preceding today's forest was clear-cut and re-planted in 1981 (Lie, 2016, pers. comm., 5 May). A thin layer of humus and peat covers the bedrock of syenite/quart syenite in this forest. The area is also situated below the marine limit; hence the soil is derived from marine sediments (NGU 2016). All three forests have a potential harvestable volume of wood greater than 0.5 m^3 per hectare per year (NIBIO 2016). Climatic data from the Melsom weather station (approximately 18 km from the study area) indicate an annual average precipitation of 1029 mm, with a mean February temperature of -3.8 °C, and a mean July temperature of 16.3 °C for the period 1961 – 1990 (MET 2016).

Table 1. Background information on the 12 sampling plots with corresponding site label with letter prefix and number, geographic coordinates (UTM zone 32 – EU89), elevation (values are interpolated between the two nearest contours), dominant species in the area, depth of topsoil (Thomassen, 2016, pers. comm., 27 April), depth of measured subsoil, and number of samples taken from each hole with total number of samples at the base.

No.	Site	Geographic coordinates		Elevation (m a.s.l)	Dominant species	Topsoil (cm)	Subsoil (cm)	No. of samples
		Latitude N	Longitude E					
	Beech forest							
1	A1	6562173.596	560006.903	188	*F. sylvatica*	6.70	45	10
2	A2	6562213.914	560021.123	192	*F. sylvatica*	7.20	45	10
3	A3	6562189.560	559959.799	186	*F. sylvatica*	4.50	45	10
4	A4	6562266.129	560012.873	199	*F. sylvatica*	6.54	45	10
	First-generation spruce forest							
5	B1	6561987.101	559833.276	168	*P. abies*	6.80	45	10
6	B2	6562154.029	559824.941	179	*P. abies*	3.75	40	9
7	B3	6562146.477	559840.487	176	*P. abies*	7.63	45	10
8	B4	6562096.165	559828.135	175	*P. abies*	5.88	40	9
	Second-generation spruce forest							
9	C1	6560109.158	559678.106	82	*P. abies*	6.25	40	9
10	C2	6560097.152	559693.729	81	*P. abies*	4.75	45	10
11	C3	6560022.685	559703.471	75	*P. abies*	4.25	45	10
12	C4	6560005.966	559702.589	75	*P. abies*	6.25	45	10
								(117)

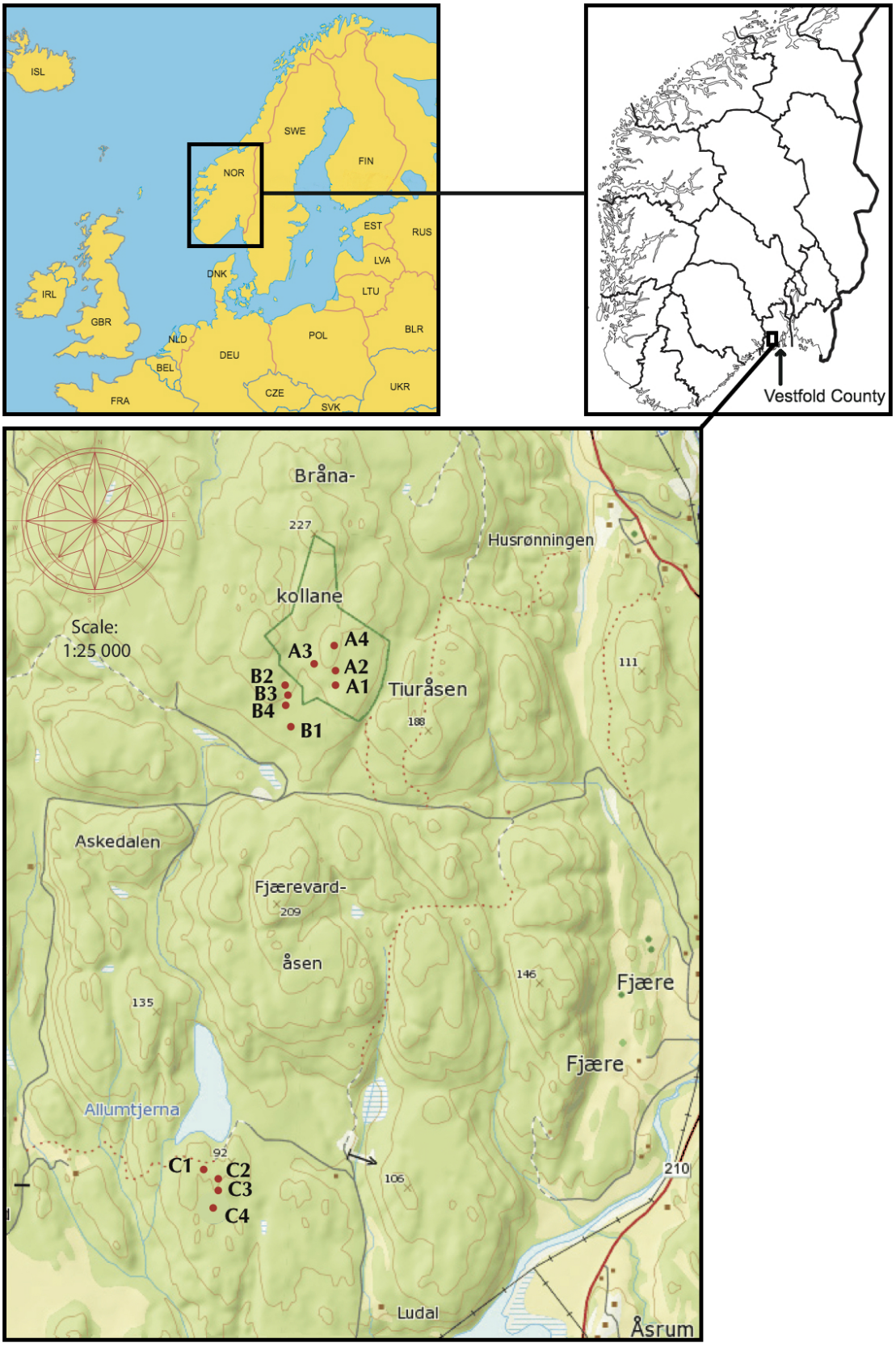

Figure 3: Location of the 12 sampling plots in southeast Norway. Plots labelled with *A* are located in the beech forest, plots labelled with *B* are located in the first-generation spruce forest, and plots labelled with *C* are located in the second-generation spruce forest.

2.2 Sampling design

Soil samples were obtained from 12 different plots during September 2015. A metal rod was used to locate potential plot positions, with soil depths of approximately 50 cm, to assure that desired soil depths could be obtained at a given position. Four plots were randomly selected at each study site, and a circular roughly 50 cm in diameter hole was dug, removing large roots and stones. The thickness of the topsoil layer for beech forest, first-generation spruce forest and second-generation spruce forest were 6.24 ± 0.52 cm, 6.01 ± 0.72 cm, and 5.38 ± 0.45 cm (Mean ± 1 SE, n = 4), respectively (Thomassen, 2016, pers. comm., 27 April). Below the topsoil, samples were taken from the subsoil in a regular pattern with a 2.5 cm diameter steel cylinder horizontally at 5 cm intervals from the upper subsoil level down to the solid bedrock or 45 cm of soil depth (Fig. 4). Depths ranges are been referred to by the smallest depth in the range (i.e. 0 and 45 cm). This imply that the first sample of the depth profile was from 0 - 2.5 cm, and the last from 45 - 47.5 cm. Two rows of samples were collected from the wall of each hole with a minimum of 10 cm horizontal space in-between the two rows, but only one row in each hole were further analysed due to time constraints and limited resources available. Ten samples were taken from 0 cm down to 45 cm depth, but three of the holes had only depths down to 40 cm, leaving 9 samples. 117 soil samples were collected; 40 from site A, 38 from site B and 39 from site C (Table 1). Samples in 20 ml sealed vials were placed in dry ice in a Styrofoam cooler in the field, before stored at a -80°C freezer at the laboratory until analysis.

Figure 4: Illustration photo from two of the sampling plots, one of the beech forest (A3) (left) and the other of the first-generation spruce forest (B3) (right). In the foreground of the photo on the left, from the beech forest, lays the steel cylinder used to collect soil samples.

2.3 Preparation of samples

In the laboratory, all samples were freeze-dried for 48 h, sieved through a 2.5 mm mesh sieve, weighted, milled in a ball mill (MM 400, Retsch, Haag, Germany) to a powder and weighted into smaller subsamples for the various assays. Organic fragments were picked out from the residues after sieving and added to the sample, before the remaining residues were weighted (soil >2.5 mm). All samples were stored at -80°C when not handled to prevent undesirable chemical changes.

2.4 Carbon and nitrogen analysis

Total carbon (C) and nitrogen (N) concentrations were measured with 8 - 22 mg prepared soil sample with an Elementar vario MICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Due to increasing quantities of stone fragments in the soil along the depth gradient, soil samples at deeper soil depths required a larger quantity of soil material and a greater proportion of O_2 to measure the C and N

concentrations. C/N ratios were determined, and C and N concentrations were converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml).

2.5 Analysis of pH

For pH analyses, 3 ml soil from each sample were placed in a 15 ml glass tube with 8 ml purified water, intensively shaken on a Vortex and left overnight. The following day samples were intensively shaken on a Vortex and pH-values were measured with an inoLab 720 precision pH meter (WTW GmbH, Weilheim, Germany).

2.6 Analysis of fungal biomass

Ergosterol was used as a proxy to estimate fungal biomass (Davey et al. 2009). Two approaches of extracting ergosterol were used: free and total ergosterol. Free ergosterol was measured by the method described in detail in Dahlman et al. (2001). Briefly, approximately 200 mg prepared soil sample was mixed with 1 ml EtOH (99.5%), intensively shaken on a Vortex, and incubated on a shaker in darkness for 30 min at room temperature. Afterwards, samples were intensively shaken on a Vortex, centrifuged (c. 16 400 g, 15 min) and the supernatant was analysed for ergosterol content using HPLC (High performance liquid chromatography).

Total ergosterol was measured by the same protocol as used by Davey et al. (2009). Approximately 200 mg prepared soil sample was mixed with 8 ml 3M KOH in EtOH and incubated in a 80°C water bath in darkness for 60 min. The vials were shaken every 10 min. After centrifugation (c. 16 400 g, 15 min), the supernatant was mixed with 2 ml purified water in new tubes. Ergosterol was extracted by adding 5 ml hexane, shaking the vial vigorously on a Vortex, and collecting the hexane phase after the two phases divide. The extraction was done twice. Both extractions were collected in the same vial, evaporated to dryness with N_2, redissolved in 500 µl MeOH and the supernatant was run on an HPLC.

The extractions from the two methods were analysed according to Davey et al. (2009) on an 1100 Series HPLC (Agilent Technologies, Waldbronn, Germany). Ergosterol was separated on a reversed phase ODS ultra sphere column (250 mm × 4.6 mm; particle size 5 µm) using MeOH as the mobile phase at a flow rate of 1.5 ml min^{-1}, and the total analysis time were 12 min. Ergosterol absorption was detected at 280 nm, and the identification of ergosterol was

based on retention time, online UV-spectra and co-chromatography of commercial standard of ergosterol (Davey et al. 2009). Free and total ergosterol was calculated and converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml).

2.7 Estimation of total carbon storage

Moderate estimates of total C storage in the subsoil were calculated, without including larger roots and litter. Due to lack of data for three plots at 45 cm depth, estimates were calculated for 0 to 40 cm depth. Missing values along the soil profile were entered as means of the two adjacent values. Estimates of total C storage of the subsoil from 0 to 40 cm depth in the three forests are referred to as *total C storage*.

2.8 Statistical analyses

All statistical analyses were performed in R Studio, version 3.2.4 (R Development Core Team, 2012). To perform a linear mixed effects analysis of the relationship between each response variable and the fixed and random effects, the package *lme4* (Bates et al. 2011) was used. The entered response variables were C, N, C/N ratio, free ergosterol and pH. Total ergosterol was omitted from the statistical analyses due to data inconsistency. Sites and depth were entered as fixed effects into the model, while holes were set as random effects. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality after log-transforming all variables in order to stabilise their variances. *F* and *P* values were obtained by two-way analyses of variance, using ANOVA from the package *car* (Fox & Weisberg 2011). In order to detect significant interactions between sites, a Tukey's post hoc test from the package *multcomp* (Hothorn et al. 2008) was used. The analyses were done separately for each variable. A Kendall's tau correlation test was done to test the relationship between the response variables, by using a covariance matrix from the package *Deducer* (Fellows 2012). Statistical analyses were regarded as being significant when $P < 0.05$. Means and standard error (SE) were calculated using Microsoft Excel, version 14.3.9 (Microsoft Corporation, 2010), together with calculation of estimates of total C. Of the latter, a one-way ANOVA was used to determine whether there were any significant differences between the sites. The graphic illustrations were generated by using the scientific plotting package Veusz, version 1.23.2 (www.home.gna.org/veusz/).

3.0 Results

3.1 Carbon

All three sites generally decreased in C concentration along the soil profile from 0 to 45 cm, as mean C varied significantly between with soil depth. The C concentration was also significantly different between the forests (Table 2). Overall, C in the first-generation spruce forest was significantly greater compared to the second-generation spruce forest, and significantly lesser compared to the beech forest (Tukey, $P < 0.05$). In the beech forest and the second-generation spruce forest C more or less constantly declined with depth. Meanwhile, C in the first-generation spruce forest declined quickly in the first 10 cm and then slightly increased throughout the rest of the soil profile (Fig. 5a). This resulted in a significant site × depth interaction (Table 2). There was a remarkable increase from 40 to 45 cm depth in the first-generation spruce forest (Fig. 5a).

3.2 Nitrogen

There was a strong correlation between C and N ($r > 0.826$; $P < 0.001$) and this was driven by a similar pattern across all soil profiles (Fig. 5a-b). Mean N varied significantly between the forests (Table 2), with N being significantly greater overall in the beech forest compared to the first-generation spruce forest (Tukey, $P < 0.05$). Concentration of N mainly declined along the soil profile (Fig. 5b), resulting in N varying significantly with soil depth (Table 2). The significant site × depth interaction term (Table 2) was mainly driven by that N in the first-generation spruce forest quickly declined in the first 10 cm and then slightly increased throughout the rest of the soil profile. Concurrently, N more or less constantly declined with depth in the beech forest and second-generation spruce forest (Fig. 5b). As for C, N concentration increased remarkably from 40 to 45 cm depth (Fig. 5b). This rapid increase corresponds with the percentage of C and N in the subsoil at the same level of depth (Appendix, Fig. 1a-b). There was a slight decrease in the soil < 2.5 mm percentage in the subsoil at the same level of depth (Appendix, Fig. 1c).

3.3 C/N ratio

Mean C/N ratio varied significantly between the forests and with soil depth (Table 2). The C/N ratio responded in contrasting directions to depth for the different forests in the upper 10 cm, resulting in a significant site × depth interaction (Table 2, Fig. 5c). At deeper depths, the

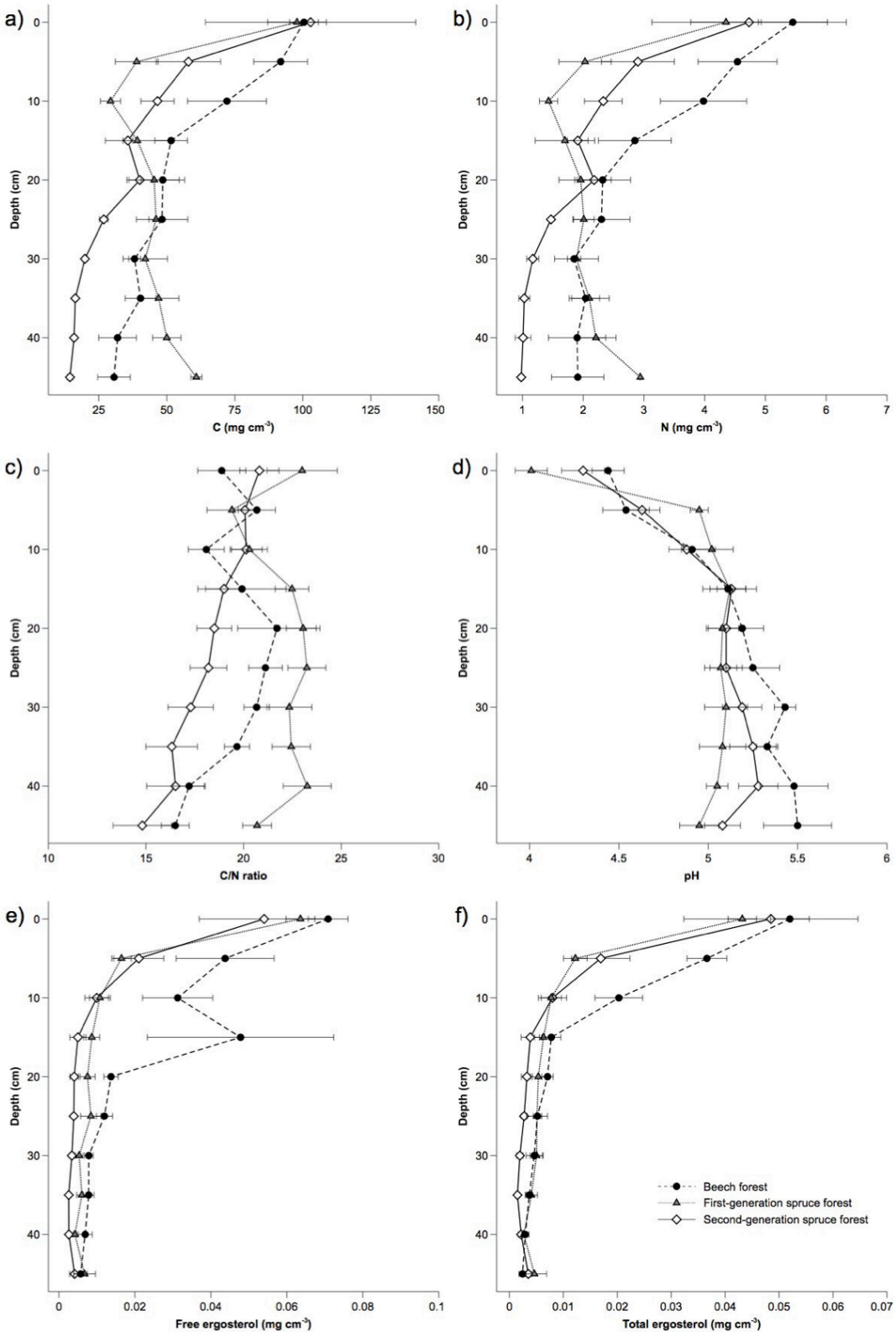

Figure 5. Mean (± 1 SE) for (a) carbon (C) concentration, (b) nitrogen (N) concentration, (c) C/N ratio, (d) pH-values, (e) free and (f) total ergosterol concentration displayed at different soil depths (0 - 45 cm) at beech forest, first generation spruce forest and second generation spruce forest. $n = 4$, except for first-generation spruce forest for which $n = 2$ and second-generation spruce forest for which $n = 3$ at the deepest soil depth (45 cm).

forests displayed a more stable pattern with the second-generation spruce forest constantly declining while the beech forest and the first-generation spruce forest slightly increasing to 20 cm depth before it more or less declined throughout the rest of the soil profile (Fig. 5c). In general, C/N ratios slightly decreased from 0 to 45 cm soil depth. The C/N ratios displayed at all three sites are consistent with the strong correlation between C and N. In the first-generation spruce forest, a rapid decline from 40 to 45 cm depth was present.

3.4 Soil pH

The subsoil pH was unresponsive regarding forests, but varied significantly with soil depth (Table 2). The pH had increasing values with increasing depth before it more or less stabilized from 15 cm depth and throughout the rest of the soil profile (Fig. 5d). A relatively identical pattern in pH-values with increasing soil depth for all forests resulted in no interaction (Table 2). There was a negative correlation between pH and C, N and free ergosterol.

3.5 Fungal biomass

Free ergosterol concentration varied significantly between the forests (Table 2, Fig. 5e). Overall, free ergosterol was significantly greater in the beech forest compared to the second-generation spruce forest (Tukey, $P < 0.05$). The concentration of free ergosterol also varied significantly with soil depth, by generally declining with increasing depth (Table 2, Fig. 5e). The more or less equivalent decline with increasing soil depth among all three forests resulted in no interaction (Table 2). Free ergosterol was positively correlated to C and N. The results from the total ergosterol extractions presented lower values than results returned from the free ergosterol extraction, which is contradictory (Fig. 5e-f). Total ergosterol was therefore omitted from statistical analysis.

Table 2. Two-way split-plot ANOVAs (*F* and *P* values) testing for the effect of site (beech forest, first-generation spruce forest and second-generation spruce forest) and soil depth (0 – 45 cm) as the main plot factors, and holes (1-12) as the random plot factor, on concentration of carbon (C) and nitrogen (N), C/N ratio, concentration of free ergosterol and pH-values.

	Site (S)	Depth (D)	S × D
C	**12.65 (0.002)**	**96.46 (<0.001)**	**44.27 (<0.001)**
N	**7.46 (0.024)**	**73.70 (<0.001)**	**26.52 (<0.001)**
C/N ratio	**8.00 (0.018)**	**12.09 (<0.001)**	**24.08 (<0.001)**
Free ergosterol	**10.12 (0.006)**	**156.51 (<0.001)**	1.06 (0.590)
pH	1.41 (0.493)	**101.46 (<0.001)**	3.65 (0.161)

Degrees of freedom: S = 2, D = 1, S × D = 2.
Bold values indicate significant effects at $P = 0.05$.

3.6 Total C storage

Total C storage of the subsoil from 0 to 40 cm depth of all sites was in the range 16.6 - 24.5 kg m^{-2}, with beech forest displaying a tendency of highest total C storage (Fig. 6). However, there was no significant variation between the forests.

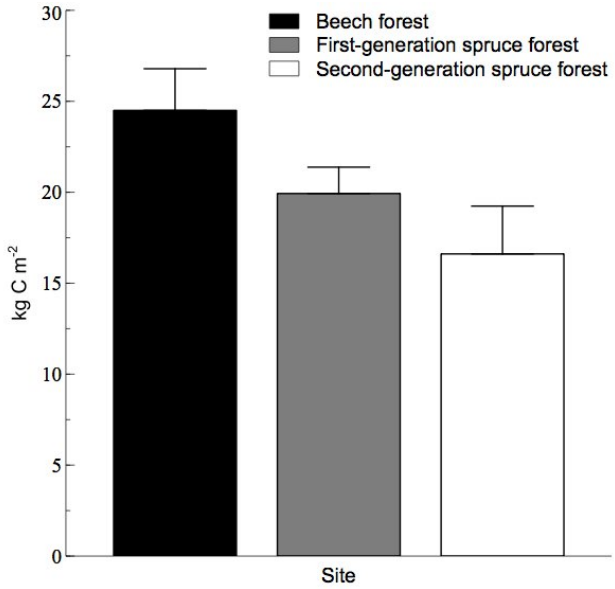

Figure 6. Mean (± 1 SE) for estimates of total C content of subsoil (0 – 40 cm depth) in beech forest, first-generation spruce forest and second-generation spruce forest. $n = 4$.

4.0 Discussion

In contrast to the first hypothesis, which predicted a higher total C storage in spruce compared to beech forest, the results showed no significant difference in total C storage between the forests. Further, in accordance with the second hypothesis, concentrations of C, N and free ergosterol, C/N ratio and pH varied significantly with soil depth. As predicted, C, N and free ergosterol concentrations declined with increasing soil depth, whereas pH increased. Mixed support was found for the third hypothesis considering a predicted varying pattern of the three forests in C, N and free ergosterol concentration, C/N ratio and pH according to soil depth. Concentrations of C, N and free ergosterol, and C/N ratio all varied significantly between the forests, whereas pH was unresponsive. With samples taken at a precise and fine scale, patterns of variation within small soil profile intervals were possible to detect. The small amounts of soil subjected to analysis in this study contributed undoubtedly much to stochastic variation, which makes the significant results more noteworthy. Implications of these results are discussed below.

4.1 Carbon and nitrogen concentration

Concentration of C and N declined with increasing soil depth at all three forests indicating a lower accumulation of C and N at the deeper soil levels. The lower concentrations of C and N at deeper depths likely emerged because as soil depth increases, decomposition of organic matter decreases and thus nutrient cycling decline and turnover time increases. The corresponding C/N ratio slightly decreased throughout the soil profile in all three forests with no significant overall difference in C/N ratio between the forests. This suggests similar accumulation rates of C and N in the beech forest, the first-generation spruce forest and the second-generation spruce forest: which is consistent with previous findings (Zhong & Makeschin 2004). However, beech forest accumulated more C and N throughout the whole soil profile compared to the first-generation spruce forest. The assumption of higher C and N concentration in spruce forests compared to beech forests is due to higher decomposition rates found in beech forests. Conversely, this could be countered by higher soil C and N sequestration under beech forests due to its more extensive and deeper rooting system (Berger & Berger 2012, Schmid & Kazda, 2001). The larger variation between the beech and the first-generation spruce forest in C concentration, than with the beech and the second-generation spruce forest is surprising. This is unanticipated since the beech forest and the first-generation spruce forest are located only about 100 m apart and the first-generation spruce forest until

1956 was a part of the then larger beech forest. Concurrently, the first-generation spruce forest accumulated more C than the second-generation spruce forest. The longer presence of *P. abies* as the dominating species in the second-generation spruce forest would suggest a higher C and N sequestration than the first-generation spruce forest. This is because the highly protected long-living foliage of *P. abies* is causing slow decomposition of its litter and build-up of forest floor over time (Berger et al. 2002). However, additional C input to the subsoil of the first-generation spruce forest by decaying old deep root system of former beech forest could explain the higher accumulation of C in the first-generation spruce forest than that of the second-generation spruce forest.

The first-generation forest had a distinct increase in the C and N concentrations from 40 to 45 cm depth, and a rapid decrease in C/N ratio. This coincided with an increase in percentage concentration of C and N and a slightly decline of soil < 2.5 mm at the same depth level. Lower percentage of soil < 2.5 mm and the relatively shallow soil depth at this forest could suggest that the samples were taken close to the bedrock where higher accumulation of C and N are caused by build-up of organic material. Another possible explanation is that the bulk density of the subsoil in spruce forests is higher, especially right above the bedrock, due to compaction below the flat root system, which is retarding decomposition by reduced soil aeration (Berger et al. 2002). However, it should be noted that the results are based on only two samples, but the low SE could imply a general pattern in the first-generation spruce forest. Sampling the whole soil profile down to the bedrock at each plot in all three forests could have clarified the above circumstances. Another essential notion is that of the high SE of the C, N and free ergosterol concentration in the beech forest at 0 cm. This might be due to imprecise determination of the beginning of the subsoil horizon in the beech forests. Including content from the topsoil to the subsoil samples has the potential to affect the values of the variables. Imprecise determination of the beginning of the subsoil could also be present at the two other forests.

4.2 Soil acidity

Soil pH increased from 0 to 45 cm of soil depth in all three forests, which supports the assumption that the soil will be less acid towards the bedrock (Nihlgård 1971). The relatively low pH displayed at all three forests is likely to be seen as pH > 5 is rare in boreal forest in Scandinavia (Sterkenburg et al. 2015). Acidic soil conditions in boreal forest soils

consequently reduce soil microbial activity, and favour accumulation of organic C as decomposition is retarded (Berger et al. 2002). However, the lack of an effect of forest type on pH is surprising, as forest floor and mineral soil of *F. sylvatica* is commonly described as less acidic than that of *P. abies* (Berger et al. 2002, Hojjati et al. 2009, Nihlgård 1971). Meanwhile, the acidic soil found in the beech forest along with the anticipated acidic soil in both spruce forests, indicates that other factors than dominating tree species determine the pH in this area. On another note, differences in the pH between the forests may be present in the topsoil of the forests.

Although pH correlated negatively with C, N and free ergosterol concentrations, this is unlikely to involve causation because higher pH generally provides more favourable soil conditions stimulating higher rates of plant productivity and decomposition and thus nutrient cycling (Berger et al. 2002). Soil acidity may pose additional stress on the mycorrhizal fungi and constrain their diversity with low levels of available nutrients (Sterkenburg et al. 2015). However, Sterkenburg et al. (2015) suggest that the optimum pH for fungal biomass is around 5. Fertile soils with higher pH generally enhance the environment for soil fauna, which prevent the establishment of large and long-lived mycelial networks (Sterkenburg et al. 2015).

4.3 Fungal biomass

Concentration of free ergosterol declined with increasing soil depth in all three forests, indicating a larger proportion of freshly produced mycelium and thus greater mycelial production in the upper levels of the soil profile. Free ergosterol was positively correlated with both C and N concentrations, suggesting that availability of soil nutrients maintain production of new mycelia (Sterkenburg et al. 2015). In general, the beech forest accumulated more free ergosterol throughout the whole soil profile than the second-generation spruce forest. Higher concentrations of free ergosterol in the beech forest suggest higher C allocation to mycorrhizal symbionts with increased return of N (Sterkenburg et al. 2015). As noted in the above, different mycorrhizal fungi can play opposing roles in C sequestration, and identification of the mycorrhizal diversity in the forest soils may therefore clarify the fungi's contribution to C storage.

Deeper rooting systems in beech forests that allow fungi symbioses at deeper soil levels may explain why free ergosterol was more abundant throughout the soil profile of beech forest

than that of the second-generation spruce forest (Clemmensen et al. 2013). However, this difference may be explained by higher turnover rates in beech forest that affect the production rate of mycorrhizal fungi. Fast decomposition of mycelial networks could cause greater mycelial production to compensate for the loss (Clemmensen et al. 2013). Concentrations of total ergosterol could have helped explained the dynamics of mycorrhizal fungi by quantifying older mycelium in the subsoil. Total ergosterol can be more stable than free ergosterol as dead mycorrhizal fungi can be the driver of high C content with strong C connections that are hard to degrade (Yuan et al. 2008). Results of lower concentrations of total ergosterol than that of free ergosterol in this study suggests that the extraction method of total ergosterol was not preformed properly, or the method was not adequate to preform the analysis of these soil samples. The measurement of extraction of total ergosterol is widely discussed, and improvements of extraction methods are regularly published (Wallander et al. 2013, Yuan et al. 2008, Zhao et al. 2005, Zhou et al. 2015).

4.5 Carbon storage in beech and spruce forests

The estimated total C storage of the subsoil (0 - 40 cm) indicated no significant difference between forests of *F. sylvatica* and *P. abies*, even though significant vertical differences were present in the C concentration along the soil profile between the beech forest and the first-generation spruce forest. The similar total C storage may justify the analogous potential harvestable volume of wood of the forests. Previous studies have claimed that the affect of tree species only can be attributed to the topsoil and the upper subsoil (Ahmed et al. 2016, Vesterdal et al. 2008). Ahmed et al. (2016) found that total C storage to a depth of 1 m was not affected by tree species identity; it only affected the C concentration of soil in the top 20 cm. There were larger differences in C and N concentrations of the upper 15 cm of the subsoil between the beech forest and the first-generation spruce forest, than at deeper depths. Since the mean topsoil of both forests were < 6.2 cm of depth, the highest variation is roughly present in the upper 20 cm of the soil profile. This coincides with a study by Schmid and Kazda (2001) where they found that roots of *P. abies* were more strongly concentrated in the upper soil layers (with maximum root density in top 10 cm) than roots of *F. sylvatica* (with maximum root density in 10-20 cm soil depth). However, they found no differences in total rooting depth down to 1 m of depth. Differences in rooting systems of spruce and beech forest may explain the differences found, but root density and depths were not scrutinised in this study. As pointed out above, the subsoil of the first-generation spruce forest can contain

decaying old deep root systems of former beech forest. If this is the case, the concentration of C in the first-generation spruce forest can change as transformations in the subsoil may emerge after some time (Vesterdal et al. 2008).

The results of this study do not support the conventional and well-established assumption of higher C storage in forests of *P. abies* compared to forests of *F. sylvatica*. It is necessary to stress that the estimates of total C are rather conservative estimates since larger roots and litter were excluded. Measured levels of total C storage are nonetheless relatively high compared to similar studies on *F. sylvatica* in temperate regions at same depths (Ahmed et al. 2016, Vesterdal et al. 2008). Warmer climate in temperate regions implies a higher rate of decomposition (Vanhala et al. 2011), and thus a richer microbial community, than in boreal regions. A detailed mapping of the microbial communities in the forest soils could provide a better understanding of the mechanisms of decomposition. The composition and density of earthworm activity can affect the C storage in forest soils. Earthworms can either contribute to C sequestration by translocation of forest floor materials into deeper soil depths, or obstruct C sequestration by increasing the decomposition rate leading to heterotrophic respiration (Cameron et al. 2015, Melvin & Goodale 2013, Reich et al. 2005, Vesterdal et al. 2012, 2013). Low pH and climate-related limitations at the three forests in this study could reduce the earthworm abundance in the area. However, it is challenging to determine the effect of earthworms on decomposition and C storage, and further research is required to understand the relationship between earthworm activity and C sequestration (Cameron et al. 2015, Vesterdal et al. 2013). Soil moisture can also affect the rate of decomposition, as wet soil may reduce the decomposition rate (Von Haden et al. 2014). However, soil moisture was not assessed in this study, leaving no presumption of the effect of soil moisture on C storage.

In addition, the higher C storage in boreal forest soils could be explained by nutrient poor soils in the north having 5 times more fine roots per basal area than fertile soils in the south (Lehtonen et al. 2016), leaving a potentially higher belowground input of roots and root-associated microorganisms contributing to C sequestration. By recycling of nutrients from deeper soil depths through its deeper root system, beech maintain the activity of soil macro fauna at deeper soil horizons and cause higher turnover rates than in spruce forests (Berger et al. 2006). Storage of long-term C is therefore more likely to occur in spruce forests with lower decomposition rates. So even if both beech and spruce forests accumulate more or less the same amount of C in the subsoil, C in spruce forest may prove to contain more stable C

content, as decomposition is present in beech forests at deeper soil levels. Radiocarbon measurements could have determined the stability of C in the soil, and identified the forest containing most stodgy C in the subsoil.

Even though most C is confined to the first meter of soil, large amounts are also stored further down in fairly stable forms (Batjes 1996). The complexity of C compounds increases with soil depth, and thus C in deeper soils will contribute to a stable C storage (Rumpel et al. 2002). Since most C-budgets studies are confined to the standard 1-m soil depth, global soil C pools and fluxes may be underestimated since they exclude deep soil C (Angst et al. 2016, Callesen et al. 2016, Pan et al. 2011). Depths below 47.5 cm soil depth were not examined in this study. However, the soil depth of the chosen study sites was quite shallow, especially in the first-generation spruce forest.

Higher turnover rates of root growth due to spruce forests' higher sensitivity to waterlogging compared to beech forest can cause higher C sequestrated in the subsoil (Berger et al. 2002). If the forests chosen in this study are not exposed to substantial increases in the water table of the groundwater, this root production might not be as prominent. Also, different bedrock materials have a strong influence on soil-forming processes and have therefore been found to have an effect on C and N stores in forest floor and mineral soil (Berger et al. 2002, Rumpel et al. 2002). The bedrocks underneath all three forests are known to be acid rocks that can provide nutrient-poor soils (Ibrahim et al. 2006). In this case, similar conditions provided by bedrock materials at the chosen sites of this study could explain why the pH of the forests did not differ, and why there was no significant difference in total C storage between the forests. Since the second-generation spruce forest is situated below the marine limit, a finer soil texture is likely to be found towards the deepest soil depths compared to the other two forests. This is partly supported by the second-generation spruce forest tending to contain the highest percentage of soil < 2.5 mm towards the deepest soil depths of the three forests (Appendix, Fig. 1c). A finer soil texture can imply lower levels of soil respiration and input of organic matter. In contrast, the beech forest tend to have the highest percentage of soil > 2.5 mm, which can indicate a higher level of respiration and thus a higher rate of decomposition. Along with this, coarser soil texture can cause leaching of soil organic matter into deeper soil depths. However, it is important to note that statistical analyses were not conducted on the percentage of soil < 2.5 mm of the forests. Although the affect of tending differences in soil texture between the forests were not evident in the results of total C storage, it could explain

the significant overall difference in C concentration throughout the soil profile between the first-generation and second-generation spruce forests. Consistently to findings by Vesterdal et al. (2008), total C storage in the subsoil may be more affected by the site conditions than by tree species.

5.0 Conclusion

The findings of this study show that beech forests do not necessarily store less carbon than spruce forest in the subsoil as postulated. The identity of tree species may only affect the C sequestration in the upper 20 cm of the soil profile, which could explain the significant vertical differences in C concentration between the beech forest, the first-generation spruce forest and the second-generation spruce forest. The findings contradict the conventional and well-established assumption that spruce forests soils store more C compared to beech forest soils, and that this may be ascribed to various site conditions. Further studies are required to reveal the dynamics of soil C storage in beech and spruce forests of southeast Norway. This short-term study is noteworthy in the fine-scaled gradient of soil depth, detecting variation within small intervals along the soil profile. Moreover, climate change will affect the forest soil C pools differently at different locations, as biomass production and decomposition are dependent on physical and chemical soil properties (Callesen et al. 2003). Thus suggesting that some differences are to be expected. Contradictory findings in previous studies on the effect of climate change on soil C pools globally can suggest that changes in forest soil C pools can be unpredictable in response to climate change.

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Appendix

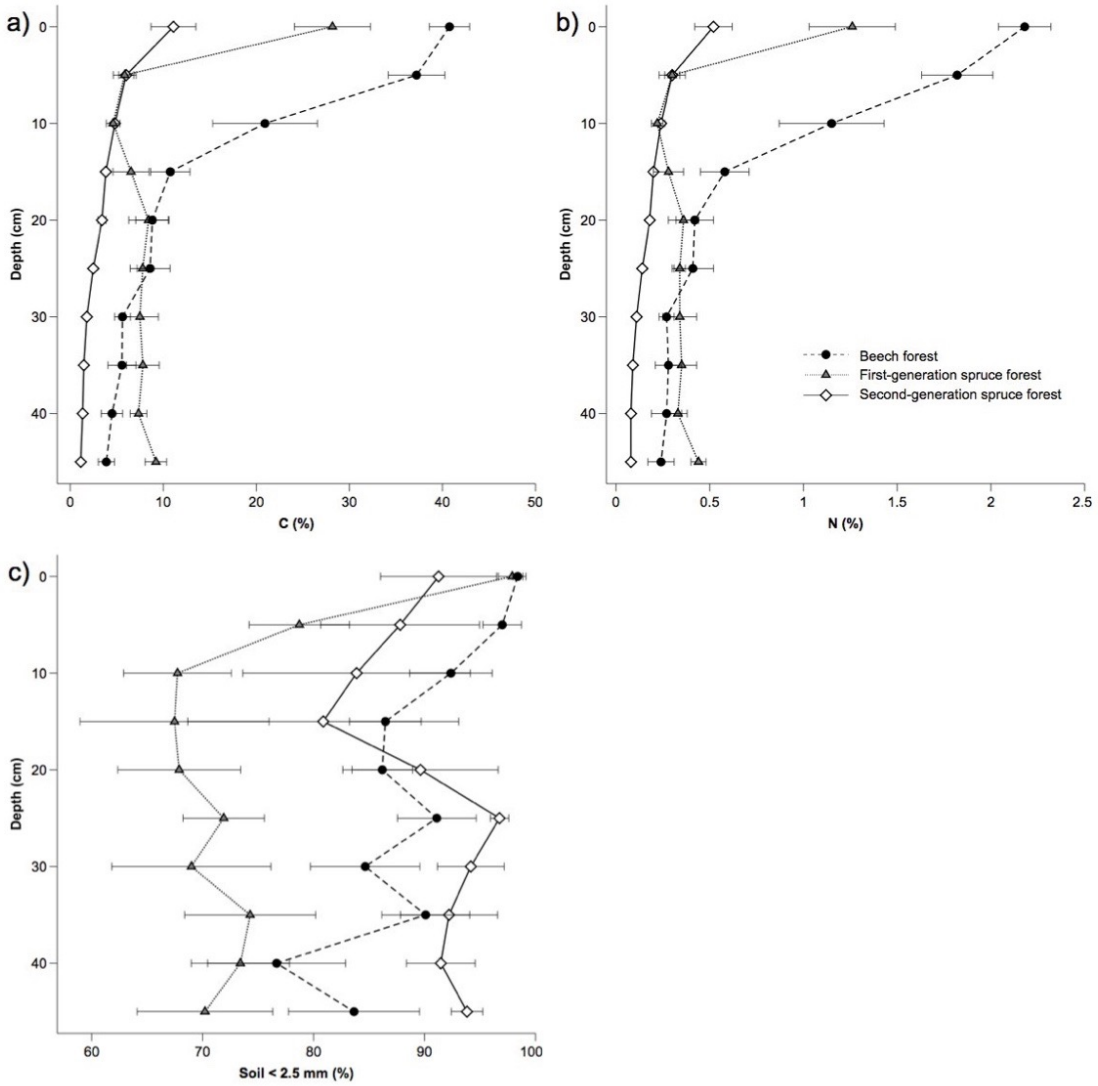
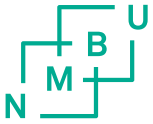


Figure 1. Mean (± 1 SE) for (a) carbon (C) concentration (%), (b) nitrogen (N) concentration (%) and soil < 2.5 mm (%) displayed at different soil depths (0 – 45 cm) at beech forest, first-generation spruce forest and second-generation spruce forest. n = 4, except for first-generation spruce forest for which n = 2 and second-generation spruce forest for which n = 3 at the deepest soil depth (45 cm).



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