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Ecosystem carbon stocks in Danish deciduous forests: impact of land-use history and management on major carbon pools

Master's thesis in Biology Supervisor: Bente Jessen Graae Co-supervisor: Richard Strimbeck & James D. M. Speed May 2023



Photo: Tonje Rostad Solhus



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Abstract

Forests are important carbon sinks and can help mitigate climate change. Sustainable management of forests is needed to maximize the carbon storage potential. Land-use legacy and management may affect the forests' current carbon stocks and future storage, currently this is little known. Therefore, knowledge about how factors like previous land-use and management affect the ecosystems carbon stocks is important to understand how to best manage the forests in a climate mitigation perspective. This thesis investigates differences in the major carbon stocks, i.e., soil, deadwood, litter, and vegetation in Danish deciduous forests, and if these potential differences could be explained by different land-use history or management. All the forest stands in this thesis were given either a land-use history category (i.e., defined by land-use the forest stand had at the 17th century, i.e., forest, fields, or plains, and dominating tree species, beech (Fagus sylvatica) or oak (Quercus robur)) or a management category (i.e., defined by harvest and other management measures). The results showed no statistically significant differences between forest stands with different land-use history or management in any of the major carbon pools, with an exception for some of the land-use history categories in the shrub layer (i.e., ancient oak forest vs. beech forest on previous plains, and ancient oak forest vs. oak forest on previous fields, where the ancient oak stands were smallest) and the tree layer and below-ground vegetation (i.e., beech forest on previous fields vs. ancient beech forest, and beech forest on previous fields vs. beech forest on previous plains, where the beech stands on previous fields were smallest). For both the wood litter, some of the land-use history categories were close to be statistically significant different from each other. The above-ground carbon pools were statistically significantly larger than below-ground carbon pools. As expected, the three different soil depths showed a significant difference, with the soil carbon content decreasing with depth, but this trend was only observed in the forest stands with different land-use history and not management. No clear difference was found between the forest stands with different land-use legacy and management. This could be linked to the small sample size in this project making any small differences untenable, or because for the management comparison among the forest stands no differences were found, this could be because managed forests were treated similarly to the unmanaged during the last 25 years, or maybe no difference were found because there is no difference between the stands. Overall, in conclusion, there are not any clear differences in the major carbon pools between the forests with different land-use history or management.

Sammendrag

Skog er viktig karbonsluk, og bidrar med å hjelpe å dempe klimaendringene. Bærekraftig forvaltning av skogen er nødvendig for å kunne maksimere effekten av karbonslukene. Effekten av tidligere arealbruk, og nåværende forvaltning på skogens karbonlagre er lite kjent. For å kunne forvalte skogene gunstig i et karbon- og klimaperspektiv er det nødvendig med kunnskap om hvordan arealbruksendring og forvaltning påvirke skogen. I denne avhandlingen undersøker jeg om det er noe forskjell i de viktigste karbonlagrene (dvs. jord, død ved, strø, og vegetasjon) i dansk løvskog, og om denne forskjellen kan knyttes til arealbruksendringshistorien og forvaltning. Alle skogbestand i denne oppgaven befinner seg i Sjælland, Danmark, og er gitt en arealbruks kategorien eller forvaltning kategori. Arealbruks kategorien er definert av hvilken tresort som dominere, bøk (Fagus sylvatica) eller eik (Quercus robur), og arealbruk i skogbestand på 1600-tallet (dvs. skog, dyrka mark, eller sletter/beitemark). Ved forvaltning kategorien er skogene definert som enten forvaltet (dvs. hogst) eller ikke-forvaltet (dvs. urørt, ingen hogst). Resultatet viste ingen statistisk signifikant forskjell i karbonlagrene mellom skogbestander med ulik arealbrukshistorie eller forvaltning, med unntak av noen av arealbrukskategoriene i busk-sjiktet, tre-sjiktet, og underjordisk vegetasjon. For vedaktig strø tyder det på at det kunne vært en forskjell mellom noen av arealbrukshistorie kategorien. Som forventet avtok karbon innholdet med dybden i jorden innad en arealbruksendring kategorien, men ingen tydelig forskjell mellom skogbestander med ulik arealbruksendring kategori. Ingen klar forskjell ble funnet i økosystem karbonlagrene i bestander med ulik arealbruksendring og forvaltning, dette kan skyldes begrenset datagrunnlag som er for lite til å oppdage mulige små forskjeller. En annen mulig forklaring er at de forvaltet skogene har de siste 25 årene blir behandlet som ikke forvaltet, og har bygget opp karbon lagrene på lik linje som det ikke-forvaltete. Eller rett og slett fordi det ikke er noen forskjell mellom skogbestandene. Avslutningsvis, denne avhandling kunne ikke bekrefte noen av hypotesene om at det er en forskjell i karbonlagrene i disse danske løvskogene som kan forklares av ulik arealbruksendring eller forvaltning.

Keywords/nøkkelord: Carbon stocks; ecosystem carbon; carbon storage; land-use change; management; temperate forest; deciduous forests; Denmark; soil; litter; deadwood; vegetation; shrub layer; tree layer; above-ground; below-ground vegetation; climate mitigation

Acknowledgements

I want to thank my supervisors Bente Jessen Graae (NTNU), Richard Strimbeck (NTNU), and James Speed (NTNU University Museum) for always being patient with me and being super helpful with all your knowledge. I am thankful for all the help and support I have received throughout the whole process of doing a Master's project. With all the feedback I have gotten from my supervisors and others in Plant Ecology team I have found motivation and support for all the work I have been through. I have really enjoyed the process, and have learned much, both about this field of science and about myself.

I want to thank Thomas Nord-Larsen (KU), Charles Davidson (KU), and Naia Morueta-Holme (KU) for the help of getting LiDAR-data and obtaining biomass-data for the forests stands in this project. Also, I want to thank Siv Anina Etter (NTNU) and Mari-Ann Østensen (NTNU) for the help with doing the C/N-analysis and helping me through that process. Thanks to Per Gundersen (KU) and Lars Vesterdal (KU) for borrowing the soil corer, and other field equipment.

During the fieldwork in Denmark, I had a great time and will be looking back at all the things I learned and all the fun memories. The fieldwork would not be possible without the muchneeded help from Hanne Alise Heien, Birte Maaseide, Lukas Seifert, Håkon Nilsen, Martijn L. Vandegehuchte (NTNU), Sigrid Lindmo (NTNU), Hans Henrik Bruun (KU), and of course Richard and Bente.

I am very grateful of all the support and motivation I have gotten from classmates, friends, and family. Thanks for always being there for me and being available for me when a muchneeded break from the work was necessary, everything from lunch breaks with something sweet from café SITO, hikes in Trondheim, and fun evenings together.

In the end I am really grateful for everything I have had the opportunity to experience and learn during my time as a student at NTNU and Trondheim, it has been a great time I will be looking back at as I now go into the next chapter of my life, with many new experiences.

Table of content

1		Intro	duction1
2		Meth	nods7
	2.	1	Study sites7
	2.	2	Data collection and carbon analysis10
		2.2.1	Transect10
		2.2.2	Soil carbon pool11
		2.2.3	Deadwood carbon pool12
		2.2.4	Litter carbon pools13
		2.2.5	Vegetation carbon pool14
		2.2.6	CN analysis15
	2.	3	Statistical analysis16
3		Resu	lts17
	3.	1	Impact of management on carbon pools18
		3.1.1	Soil
		3.1.2	Deadwood
		3.1.3	Leaf litter
		3.1.4	Wood litter
		3.1.5	Shrub layer19
		3.1.6	Tree layer19
		3.1.7	Below-ground vegetation19
	3.	2	Impact of land-use history on carbon pools20
		3.2.1	Soil
		3.2.2	Deadwood
		3.2.3	Leaf litter
		3.2.4	Wood litter
		3.2.5	Shrub layer21

	3.2.6	5 Tree layer2	1
	3.2.7	7 Below-ground vegetation	2
4	Disc	zussion2	3
	4.1	The impact of forest management strategies on ecosystem carbon pools	4
	4.2	Ancient forests and legacy effects of former land-use on ecosystem carbon pools 2	6
	4.3	The impact of afforestation on ecosystem carbon pools and the role of previous	
	land-us	se2'	7
	4.4	Limitations of the study2	9
5	Con	clusion	0
6	Refe	erences	1
7	App	endix	7

1 Introduction

The global climate on Earth is undeniably changing, and human society has played a significant role in causing this change. The impact of climate change on natural and human systems has already been severe, and the damage may be irreversible if nothing is done. Burning fossil fuels and the resulting emission of greenhouse gases are the primary contributors to the current state of global warming (IPCC, 2022). It is crucial to address the impact of climate change and prevent the many consequences that will occur if it continues unabated. Climate mitigation is therefore essential, and many countries have signed agreements and treaties to reduce carbon emissions and combat climate change through agreements such as the Kyoto Protocol and the Paris Agreement. However, to meet the goals set forth in the Paris Agreement (United Nations, 2015), we must not only reduce greenhouse gas emissions but also increase the removal of carbon from the atmosphere. This is where forests come in as a potential climate mitigation, as they have the ability to sequester large amounts of carbon (IPCC, 2022). The role of forests in climate mitigation cannot be understated, and understanding their potential is crucial for finding a sustainable solution to climate change.

Carbon sequestration is an important process that could help mitigate climate change, and forests play a crucial part in this by absorbing and storing carbon. Forests can act as either carbon sinks (i.e., taking up more carbon than it releases) or as carbon source (i.e., taking up less carbon than it releases) (Keenan & Williams, 2018). Initially trees take up the carbon and storing it in the plant biomass. Then the carbon is transferred to the soil, either directly through roots as exudates or indirectly from decomposition of plant biomass such as litter or deadwood. The carbon stays in the soil until it is released through respiration or washed away (Chapin et al., 2011a, 2011b, 2011c; Lorenz & Lal, 2010). Being a carbon sink, an ecosystem has the potential to reduce the atmospheric CO₂-concentration and contribute to cooling the climate.

Sustainable forest management has been pointed out as a cost-effective method of climate warming mitigation (Favero et al., 2017; Lal, 2013). Examples of sustainable forest management is to increase forest area and current carbon stocks through reforestation and afforestation, and reduce emission from forest degradation and deforestation (Canadell & Raupach, 2008).

Changes and disturbance to the forest ecosystem will change the flow of carbon (Lal, 2005). For example, deforestation will reduce the carbon concentration in the ecosystem, were the type of harvest determines how much carbon is lost (James et al., 2021). Removal of biomass (i.e., harvest) will move carbon out of the ecosystem and decrease the input of new carbon into the ecosystem (i.e., through photosynthesis), and disturbance of soil will cause emission of stored carbon (Durigan et al., 2017; Zummo & Friedland, 2011). Land-use change is an example of a type of change that may alter the carbon dynamic of an area. Conversion from grassland and forest to cropland cause emission of soil organic carbon (Andrés et al., 2022), while the carbon stocks stored in forests may be quickly lost when areas are converted into crop fields or grassland (Ordóñez et al., 2008).

Denmark's native forests consists of temperate deciduous trees like beech (Fagus sylvatica) and oak (Quercus robur). Beech forests is often considered to be the natural forest type in Denmark, even though most of this forest area today is semi-natural or planted. Similar with beech, oak forests in Denmark today is mainly a result of planting (Lawesson, 2000). Despite Denmark in early times being almost completely covered by forests and mires, by the beginning of the 19th century Denmark had a forest cover of only 2-4% due to increasing human population and exploitation of the land by the industrialization of agriculture (Fritzbøger, 1992; Heilmann-Clausen et al., 2020; Mather et al., 1998; The Danish Environmental Protection Agency, 2022). To protect the forest and increase the forest cover the Danish Forest Act of 1805 (Fredskovordningen) was established (Fritzbøger, 1992). Following this act, most of the remaining forest areas were protected, and harvest was limited unless immediately replaced with new stands and protected from grazing by livestock, and new forest were planted. The majority of the forests planted consisted of conifers like Norway spruce (*Picea abies*) since this tree species grows fast and can thrive in harsher environments, making it profitable for the forest owners (The Danish Environmental Protection Agency, 2022). Therefore, the native deciduous forests in Denmark no longer dominate the forest cover, with 50/50 distribution of the conifers and the deciduous forests.

Temperate forests cover 25% of global forest area (Martin et al., 2001), and it is estimated that 14% of all carbon in the world's forests are in temperate forests (Pan et al., 2011). Studies of ecosystem carbon stocks in Danish forests have shown that the distribution of the ecosystem carbon between the above-ground and below-ground carbon stocks are around 50/50 (Nord-Larsen et al., 2019; Vesterdal & Christensen, 2007). Nord-Larsen et al. (2019) found that the above-ground carbon pool is the largest with 58% of the whole ecosystem carbon stock with 47% of the ecosystem carbon stock in the above-ground live standing biomass, 9% in the deadwood, and 2% in the forest floor, and the below-ground carbon pool being 42% of the whole ecosystem carbon stock with 31% in the top 75 cm of soil and 11% in below-ground biomass, while Vesterdal and Christensen (2007) found that the above- and below-ground biomass made up 59% of the ecosystem carbon stock, while the deadwood made up 6% of the ecosystem carbon stock, 1% in the forest floor, and 34% in top 100 cm of the soil.

Carbon sequestration by trees is thought to decrease with age to become carbon neutral after the trees turn approximately 200 years (Gundersen et al., 2021). Other studies though suggests that old-growth forests are an overlooked carbon sink and will sequester carbon longer than first believed (Luyssaert, 2008; Luyssaert et al., 2021). A literature review by Luyssaert (2008) suggests that forest up to 800 years old still acts as carbon sinks. The potential of old-growth forests to be carbon sinks have caused debate as the interpretation of the data differ. Old-growth forests have larger carbon stocks than younger forests, while younger forests have a higher rate of carbon sequestration (Nord-Larsen et al., 2019; Pregitzer & Euskirchen, 2004; Pugh et al., 2019; Wang & Huang, 2020; Zhu et al., 2019). Hence, while younger forests take up more carbon day-to-day, the old-growth forests have over long time accumulated much carbon making the carbon stocks large. How much and for how long old-growth forests will sequester carbon depend on disturbance of the forests. Little or non-disturbed forests will fix and store carbon longer than more disturbed forests (Martin-Benito et al., 2021).

Harvest of wood lead to a decrease of the carbon stocks, were forest with high frequency and intensity management had the least carbon. Sustainable management is thought be used to increase the carbon sequestration, sustainable management is defined by the frequency, intensity, and the retention of woody debris. Forest management with low frequency and high structural retention (allow deadwood build up) can sequestrate up to 50% more carbon than the opposite management (i.e., high frequency and low structural retention) (Nunery &

Keeton, 2010). What type of harvest affects the ecosystems carbon stocks effect the carbon stocks. A study of Swedish forests shown that harvest of stems only or stems and stubs gave larger ecosystems carbon stocks, compared to harvest of stems, stubs, and slash (Strömgren et al., 2013). A study that investigated forests 15 years following two different management strategies (i.e.,1) thinning and planting of oak or 2) clear cut and plating of oak) of a Scots pine (*Pinus sylvestris*) stand in Belgium did not find any difference in the ecosystem carbon stock. Only difference found were variation in allocation of carbon between the carbon pools (Van Damme et al., 2022).

Management of soil carbon pools can be done through management of litter production and decomposition. Litter raking is a type of management (i.e., harvest for animal food), and the effects of this could still be seen long after, both on soil carbon (Gimmi et al., 2013) and diversity patterns (Vild et al., 2018). Investigation of Swiss forests showed that still 130 years after litter raking were abandoned as a management strategy, the forest is still recovering, with the soil carbon pools being reduced compared to similar forest without litter raking (Gimmi et al., 2013). Therefore, by not removing the litter from the forest as positive effect on the soil carbon pool could be observed.

Afforestation of agricultural soils will in general result in constant or increasing carbon sequestration, and thus also carbon stocks (Bárcena et al., 2014a; Mayer et al., 2020). Ecosystem carbon stocks increases after afforestation on former agricultural land by increasing the above-ground carbon pools (i.e., vegetation biomass, litter, and deadwood), when the trees are mature the accumulation of carbon levels out and a new equilibrium between carbon input and output is found (Jandl et al., 2007b). Afforestation on cropland and grasslands will change the vegetation structure from annuals to perennials, this will increase the biomass in the ecosystem and thus increase the accumulation of dead biomass (i.e., deadwood and litter), and in the end increase the carbon stocks (Vesterdal et al., 2007). Soil nutrient-richness before afforestation and previous agricultural practices determine the outcome of afforestation, for example, agricultural land that has been used for more than 50 years ago may give another outcome after afforestation compared to more recently used agricultural land due to current agricultural practices of tilling and fertilization/liming (Jandl et al., 2007b). Nutrient-poor and sandy soil will sequester more carbon after afforestation, compared to more nutrient-rich and clayey soil (Vesterdal et al., 2007). Nitrogen rich soil will enhance soil microbes, and this causes higher levels of respiration that releases carbon back to the atmosphere (Begum et al., 2021). The quality of leaf litter is important for the rates of decomposition, leaf litter with high nitrogen content decomposes faster than low nitrogen content leaf litter (Chapin et al., 2011b; Janssens et al., 2010). Nitrogen fertilization of forests as a management treatment has been shown to increase soil carbon pools, mainly due to reduced respiration as the microbial communities changes due to the changed nitrogen deposition (Janssens et al., 2010).

In the EU, 31.7% of all soil organic carbon (SOC) is found in agricultural soils, with 9.3% found in grassland and 21.4% in cropland. While most of the original forests in Europe have been felled during the Roman age to make way for agricultural land, the most important recent land-use change in Europe have been abandonment of cropland, and this causes an increase of forest cover, and this have a general trend by resulting in increased carbon stocks in Europe (Andrés et al., 2022).

After a disturbance, no matter how small or large, the ecosystem must restabilise. The disturbance may cause a loss of carbon. The carbon stocks must be rebuilt, which takes some time. Right after a disturbance like a fire, the carbon stocks will decline, apart from the deadwood that will increase after a fire before declining. After around 35 years stocks will begin to rise again. Up to 230 years may pass before the forest's carbon stocks is fully recovered (Kashian et al., 2006; McKinley et al., 2011). It takes 80-100 years after agricultural abandonment for the soil carbon stocks to show an increase as the natural forest succession starts (Foote & Grogan, 2010).

Old agricultural soils can lose up to half to two-thirds of their carbon stocks after having turned into agricultural lands (Lal, 2004). How big of a positive effect afforestation has on carbon stocks depends on several factors, like species of tree planted, soil properties, and climate. Berthrong et al. (2009) found that when *Pinus* were planted there were a loss of carbon, and Vesterdal et al. (2013) found that the tree species did have a significant influence on the soil carbon, with deciduous forests having more soil carbon than conifers. For deciduous tree species, Vesterdal et al. (2013) showed that beech and oak forests had higher soil carbon stocks compared to other deciduous species. The tree species is mostly important when looking at the stability of the forests, a resilient forest will handle disturbance better and emit less carbon during these disturbances (Jandl et al., 2007b).

In this thesis I will investigate the previous land-use effects on current carbon stocks, and how management strategies impact the carbon stocks and the distribution of the carbon pools. I have three hypothesise for this project, looking at the effect of management, the effect of age, and the effect of land-use change on forest ecosystem carbon stocks.

First, I hypothesize that non-intervention forests with old trees will have had time to accumulate more carbon, thus having larger carbon stocks compared to managed forests of the same age due to not being harvested. Therefore, I expect to find a larger soil carbon stock in these unmanaged forests, and because of the absent of harvest in the unmanaged forest, the deadwood and litter carbon pool will be larger in the unmanaged compared to the managed.

Second, I hypothesize that ancient forests will have had the time to build up larger carbon stocks, compared to younger forests with previously another land-use e.g., plains and fields. Previous land-use of grazing or agricultural activity may have drained the ecosystem carbon and the depletion is still visible in the soils.

Third, I hypothesize that due to high soil disturbance (e.g., tilling of soil) in the previous fields, the soil carbon pools in forests on previous fields will have been lower than in forests on previous plains. This difference may be seen even after centuries of forest occupation when comparing forests on previous fields and forests on precious plains.

2 Methods

2.1 Study sites

In this project 34 forests stands at Zealand, Denmark (55.355, 11.748) were visited, all the forest stands included in this project have been studied before (Graae, 2000; Graae & Sunde, 2000; Graae et al., 2003; Jessen & Andersen, 1994).

Six of the 34 forests stands are in three locations in north-eastern Zealand (Fig. 1). Each location consist of a pair of ancient forest stands, that is dominated by the same tree species for more than 200 years, where one of the stands in the pair is managed (i.e., even-aged monocultures and selective harvesting), and the other is unmanaged forest (i.e., without significant management for at least 35 years to more than 130 years) (Graae & Heskjær, 1997; Jessen & Andersen, 1994). These forests pairs are Farum Lillevang, Nørreskoven, Bredvig Mose (Fig. 1).



Figure 1: Management forests, 2) Bredvig Mose, 3) Farum Lillevang, and 4) Nørreskoven. Shows the forests stands with their assigned management strategy and transect line.

The forests with different land-use history are all located in the Bidstrup forests, here 28 of the 34 forests stands in this project. The Bidstrup forest is a large forest complex with an area of 1900 ha, where 900 ha is state owned (Miljøministeriet Naturstyrelsen, 2022). Currently, large parts (500 ha) of the Bidstrup forest is in the process of becoming a national park, as a way of protecting important habitats and species (Miljøministeriet Naturstyrelsen, 2023b; Nationalpark Skjoldungernes Land, 2021). Bidstrup forests play an important role in Denmark, both because Bidstrup houses rare and threatened species, like the hazel dormouse (*Muscardinus avellanarius*), and species that have some of their last known Danish occurrence in Bidstrup, like the butterfly high brown fritillary (*Fabriciana adippe*) (Skorski et al., 2014). But is also important because the Bidstrup forests are used much for recreational use, with many roads and hiking trials in the forests, and lakes and camping sites that people use. The aim of making parts of Bidstrup a national park is protecting and creating a variation of habitat that is important for many species (Miljøministeriet Naturstyrelsen, 2022, 2023b).

In the Bidstrup forest there are many forests stands of different size. These forests stand have different tree species as the dominant species, most of the forest stands have a deciduous tree species as the dominated species. The two most common tree species in Bidstrup are beech (Fagus sylvatica) and oak (Quercus robur), which respectively cover 46.5 % and 16.8% of the forest in Bidstrup (Miljøministeriet Naturstyrelsen, 2023a). These forest stands in Bidstrup have different forest history (Skorski et al., 2014), and the 28 sites in this project are classified into one of five categories (Fig. 2) determined by the previous land-use and the currently dominating tree species in the forest stand (i.e., beech or oak). The three land-use categories are: ancient forests (i.e., established sites where there have been forests since the late 17th century), *plains* (i.e., treeless landscape during 18th and 19th century), and *fields* (i.e., ploughed fields in the 18th century) (Graae et al., 2003). The forest stands with other previous land-use were planted during late 1800s or early 1900s, while the ancient forest is assumed to already have been a mature and established forest in the 17th century. The 28 stands are hence categorized into these categories, six ancient beech forest (BA), five beech forest on previous fields (BF), six beech forest on previous plains (BP), six ancient oak forests (OA), and five oak forests on previous fields (OF).



Figure 2: Overview of the forest stands in Bidstrup, with their assigned forest history category and transect lines.

2.2 Data collection and carbon analysis

The data collection was done in two rounds of fieldwork, May, and July 2022. All measurements of deadwood were done in May, while in July, all sampling of the four carbon pools in the forest (i.e., *soil, deadwood, litter, and vegetation*) was done. After sampling, all samples were prepared for CN-analysis.

2.2.1 Transect

All the sampling and measurements were done along a 100 m long and 10 m wide transect in the longest diagonal of the forest stand, with transect points at every 10 m (Fig 3a). Compass bearing of the transect were noted to use as a reference when setting up the transect for the next visit to the forest.



Figure 3: a) Whole transect $(10 \times 100 \text{ m})$, with transect point at every 10 m. The two sampling units b) big plots $(10 \times 5 \text{ m})$, and c) microplot $(0.25 \times 0.25 \text{ m})$.

Within the transect there were two different sampling units: *big plots, and microplots*. The big plots were 10 x 5 m (Fig. 3b) and placed at the first, the middle, and the last transect point (i.e., transect point 1, transect point 5, and transect point 10). The big plots were placed on the left side of the transect and were marked up using measuring tape and levelling rods. The microplots were 0.25 x 0.25 m and marked up using a wooden frame (Fig. 3c), and as the big plots they were places in the first, middle, and the last transect point.

2.2.2 Soil carbon pool

In the middle of the microplot (Fig. 3c) a soil sample was taken using a soil corer (split corer, Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands) with a diameter of 87 mm and length of 40 cm. Each soil sample was divided into tree soil depth; 0-10 cm, 10-25 cm, and 25-40 cm (Sampling method modified from Bárcena et al. (2014b)), and placed into separate paper bags and later a plastic bag to prevent any spillage if the paper bag rupture due to the soil moisture. While in Denmark, the bags were opened to allow the soil to air-dry before returning to Trondheim.

With the results from the CN-analysis (see chapter 2.3.5 for more details) and the full weight of each soil sample after sieving, the carbon content (kg) in each soil sample was determined (Eq. 1).

$$C(kg) = \frac{weight(g) * C(\%)}{100} * 0.001(kg)$$

Equation 1

The soil sample is shaped as a cylinder, the volume (cm³) of the soil sample was calculated (Eq. 2).

volume (cm³) =
$$\pi * \left(\frac{\text{diameter (cm)}}{2}\right)^2 * \text{ lenght (cm)}$$

Equation 2

The volume (cm^3) of the soil sample was so converted to area (m^2) (Eq. 3).

$$area(cm^2) = rac{volume(cm^3)}{length(cm)}$$

Equation 3

Then the amount of carbon in kilogram pr. square meter was calculated (Eq. 4).

C pr. area
$$(kg/m^2) = \frac{C(kg)}{area(m^2)}$$

Equation 4

For each transect the carbon content (kg) were calculated per m^2 for each plot, and then pooled together to find the ecosystem carbon (kg/m²) on transect-level.

2.2.3 Deadwood carbon pool

1

Deadwood, both laying and standing woody debris, were measured in the big plots (Fig. 3b). Length and diameter were measured, and decay class (1-5 - see (Pfeifer et al., 2015)) subjectively determined (Table 1). Only deadwood with a diameter > 4 cm on the middle of the log was included (Methods modified from Awuah et al. (2022)). For determining the carbon content in the different decay classes, 10 samples consisting of smaller sticks from each decay class were collected from all forests stands and brought to the lab for further analysis (see chapter 2.3.5 for more details).

Table 1: Description of how to classify deadwood into different decay classes.

Decay class	Description (Modified from Pfeifer et al. (2015))
1	No decay. Bark, leaves, and twigs are intact
2	Log still intact. Some bark is lost, all leaves and twigs are lost
3	The log is starting to fall apart, with a soft outer and hard inner wood, the log still has some bark
4	The log is completely soft and is falling apart, all bark is lost
5	The log is falling apart, hard to identify what is deadwood and soil

The volume of the deadwood sample (Eq. 2) and the weight-to-volume ratio (Eq. 5) were calculated.

$$ratio (g/cm^3) = \frac{weight (g)}{volume (cm^3)}$$

Equation 5

The volume of the deadwood logs measured in the field were calculated (Eq. 2). Using the mean weight-to-volume ratio of each decay class calculated from the deadwood samples, the weight of the deadwood measured in field were found (Eq. 6).

weight deadwood
$$(g)$$
 = volume deadwood (cm^3) * mean ratio (g/cm^3)

Equation 6

Two samples of each decay class were analysed using CN-analysis. This gave the carbon content (%) for each sample, and the mean carbon-content (%) in each decay class were calculated from these two samples. With this I was able to find the amount of carbon in kilograms in each deadwood piece measured in field (Eq. 1). The sampling plots for deadwood were 10 x 5 m, giving the plot an area of 50 m². Then the amount of carbon in

Page 12 of 51

kilogram pr. square meter was calculated (Eq. 5). For each transect the carbon content (kg) were calculated per m^2 for each plot, and then pooled together to find the ecosystem carbon (kg/m²) on transect-level.

2.2.4 Litter carbon pools

A wooden frame with inside measurements of 0.25×0.25 m marked up the microplots (Fig. 3c), and all litter (including small pieces of deadwood < 4 cm) within the frame were collected and placed in bags.

Carbon content in leaf litter and wood litter was treated as distinct carbon pools and calculated separately (see chapter 2.3.5 for more details). The carbon content in kilograms for each leaf litter sample was determined by utilizing the average carbon percentage in leaf litter and the weight of leaf litter (Eq. 1). The litter samples were collected from an area of 0.0625 m^2 . Subsequently, the amount of carbon per square meter was calculated (Eq. 4). For each transect the carbon content (kg) were calculated per m^2 for each plot, and then pooled together to find the ecosystem carbon (kg/m²) on transect-level. The same procedure was repeated for wood litter, but with the average wood carbon content being used instead, and the same surface area of 0.0625 m^2 .

2.2.5 Vegetation carbon pool

A wooden frame with inside measurements of 0.25 x 0.25 m marked up the microplots (Fig. 3c), and all herbaceous vegetation (<0.5 m) within the frame were collected and placed in bags.

Using the carbon content (%) from the CN-analysis and the weight of the samples collected (see chapter 2.3.5 for more details), the amount of carbon in kilograms in all shrub layer (i.e., herbaceous vegetation (< 0.5 m)) samples were calculated (Eq. 1). With the area of the plot, 0.0625 m², the amount of carbon pr. square meter was calculated (Eq. 4). For each transect the carbon content (kg) were calculated per m² for each plot, and then pooled together to find the ecosystem carbon (kg/m²) on transect-level.

The rest of the tree layer (i.e., standing biomass (> 0.5 m)) in the forest is estimated using Lidar-data (i.e., *light detection and raging data*, remote sensing data that uses lasers to measure objects (Wandinger, 2005; Zalevsky et al., 2021)). With the help from Thomas Nord-Larsen (University of Copenhagen) biomass maps of all forest stands were made (Nord-Larsen et al., 2023). Using ArcGIS Pro, the above-ground biomass and below-ground biomass for each forest stand were found by taking the mean of all biomass points (i.e., the biomass points show the mean biomass within an area of 25 x 25 m) within a forest stand. As 50% of the biomass is carbon, the carbon content pr. square meter was calculated using (Eq. 7) with the biomass in the forest stand and the conversion factor of 0.5 (Joosten et al., 2004).

 $C(kg/m^2) = biomass(kg/m^2) * 0.5$

Equation 7

2.2.6 CN analysis

All samples were placed in paper bags in a furnace (Termkas TS 9135) and dried at 60°C (Bárcena et al., 2014b; Haukenes et al., 2019). The soil samples were dried for 48 hours, while the vegetation, litter, and deadwood samples were dried for 24 hours.

The samples were weighed (Sartorius BP 4100), and using a 2 mm sieve the soil samples were sieved (Bárcena et al., 2014b; Haukenes et al., 2019). Roots and stones left behind were weighed separately, and the sieved soil were weighed again (Haukenes et al., 2019). For the litter samples, woody debris of a considerable size were sorted from the litter and weighed separately, this gave two litter fractions: leaf litter and wood litter. After drying the deadwood samples, the length, diameter, and weight of the samples were taken.

Vegetation, litter, and deadwood samples were grinded by hand to a powder before C/Nanalysis. Leaves and plants were grounded using a mortar and pestle, while woody material was "grounded" using a knife.

Around 20 mg of the sieved soil and 10 mg of grounded plant material were weighted (Sartorius BP 221 S) and placed in tin capsules (Santis analytical tin capsules for solids, 5 x 9 mm) for C/N-analysis. The tin capsules were tightly to closed avoid air being trapped in the cups. The tin capsules were placed in the wells of the plastic plates (Thermo scientific Nuclon Delta surface, 96-Well MicroWell Plate). The plates were taped shut and stored in a glass desiccator with silica before all plates were prepared and the C/N-analysis could start. For all 298 soil, 10 vegetation, 10 leaf litter and 10 deadwood samples CN-analysis was performed with standardised methods with a Vario EL cube from Elementar AS

2.3 Statistical analysis

To test for differences in the ecosystem carbon stocks for the major carbon pools between forest stands with different land-use history and management, the forest stands within the same category were compared to other categories. Two datasets were made for each carbon pools, one for *Bidstrup forests* and one for the *management forests* (i.e., managed, and unmanaged forests).

For the Bidstrup forests, a *linear model* was made for each carbon pool, with amount of carbon (kg/m^2) as the response variable, and forest history group as predictor variable. For the model for the soil carbon pool, a second variable were added, soil depth (i.e., the depth of the soil sample) to check for difference for the three soil layers. Since this model has two predictor variables, interaction between them was investigated. Using AIC, it was tested if the model were a good fit for the data and ANOVA-test that the interaction between forest history and depth was non-significant.

For the carbon pools in the management forests, a *paired t-test* were performed to test for any differences in means between these two management strategies. For the soil carbon pool, the overall mean between the two management strategies were tested against each other, and the means of each depth were tested against each other between and within management strategies, by doing a paired t-test for all combinations of comparisons of soil depth and management.

All statistical analysis were performed in R version 4.2.2. A significance level of p-value < 0.05 was chosen. All values are reported as the mean \pm SE, unless stated otherwise.

3 Results

The above-ground carbon stocks (i.e., deadwood, litter, and above-ground vegetation) were much larger $(12.34 \pm 0.91 \text{ kg/m}^2)$ (72.46% of the whole ecosystem carbon) than the below-ground carbon stocks (i.e., soil and below-ground vegetation) (4.64 ± 0.23 kg/m²) (27.54% of the whole ecosystem carbon) (p-value = <0.001, 95% confidence interval (CI) [5.98, 9.42]) within a land-use history or management group. There were not found any statistically significant differences in the ecosystem carbon stock between the land-use history or management groups (Fig. 4).



Figure 4: Ecosystem carbon (kg/m^2) in a) Bidstrup forest and b) management forests. The black line shows the separation of above-ground carbon stock and below-ground carbon stock. The letters show the significance between the groups, the same letter show that they are not different, but different letter show that they are different.

Soil b Leaf litter Wood litter а С n = 3 0.4 n = 3 0.40 n = 3 n = 3 NS N S Carbon (kg/m2) 0.20 0.25 Carbon (kg/m2) 03 0.2 0.0 Managed Unmanaged 1.2 Managed Unmanaged Management Management d Shrub layer Tree layer е Carbon (kg/m2) 0.03 n = 3 n = 3 Carbon (kg/m2) (kg/m2) (kg/m2 0.01 • 10 0.00 Managed Managed Unmanaged Unmanaged . Management Management f Below ground vegetation Deadwood g 0.4 0.6 Carbon (kg/m2) Carbon (kg/m2) 0-10 10-25 25-40 Soil depth Management 🔸 Managed 🔸 Unmanaged Managed on... Management Managed Unmanaged . Unmanaged Sample size: Managed = 3, Unmanaged = 3 Management

3.1 Impact of management on carbon pools

Figure 5: Carbon pool (kg/m2) (mean \pm SE) for the management forests, there are three forest stands pr. management strategy, a) soil, b) leaf litter, e) wood litter, d) shrub layer, e) tree layer, e) below-ground vegetation, g) deadwood. Significant differences are shown with asterisk or letters. The letters show the significance between the groups, the same letter show that they are not different, but different letter show that they are significantly different from each other.

3.1.1 Soil

The unmanaged forests tend to have a similar sizes overall soil carbon pool as the managed (Appendix, Table A 1, Fig 6a). There were not found any difference between the managed and unmanaged forests in the soil carbon pool for any of the soil depths, apart from the second depth (10-25 cm) (p-value = 0.03, 95% CI [0.03, 0.27]) (Appendix Table A 2).

3.1.2 Deadwood

The unmanaged forests $(3.57 \pm 1.81 \text{ kg/m}^2)$ showed a higher estimated deadwood carbon stock than the managed $(1.71 \pm 0.77 \text{ kg/m}^2)$ (Fig. 6g), but this difference were found to not be statistically significant (p-value = 0.30, 95% CI [-7.59, 3.87]).

3.1.3 Leaf litter

The leaf litter carbon stock was found to be the same in the managed forest $(0.30 \pm 0.07 \text{ kg/m}^2)$ as for the unmanaged forest $(0.25 \pm 0.04 \text{ kg/m}^2)$ (Fig. 6b), but this difference was found to be not significant (p-value = 0.61, 95% CI [-0.26, 0.35]).

3.1.4 Wood litter

The managed forests and the unmanaged forest had same sized wood litter carbon pool, respectively, $0.19 \pm 0.08 \text{ kg/m}^2$ and $0.17 \pm 0.11 \text{ kg/m}^2$ (Fig. 6c), there was no significant difference between the two management strategies (p-value = 0.74, 95% CI [-0.23, 0.27]).

3.1.5 Shrub layer

It was found that the shrub layer in the managed forests $(0.009 \pm 0.009 \text{ kg/m}^2)$ were slightly larger than the unmanaged forests $(0.006 \pm 0.003 \text{ kg/m}^2)$ (Fig. 6d). It was shown that there was no significant difference between the two management strategies in the shrub layer carbon pool (p-value = 0.69, 95% CI [-0.030, 0.037]).

3.1.6 Tree layer

The managed forests were estimated to be the similar sized as the unmanaged forests in the tree carbon pool, respectively, $12.20 \pm 2.55 \text{ kg/m}^2$ and $12.10 \pm 1.64 \text{ kg/m}^2$ (Fig. 6e). No significant difference in the tree carbon pool between the managed and unmanaged forest were found (p-value = 0.95, 95% CI [-5.72, 5.90]).

3.1.7 Below-ground vegetation

The managed forests $(2.61 \pm 0.52 \text{ kg/m}^2)$ showed a same size estimated below-ground carbon pool as the unmanaged forests $(2.59 \pm 0.33 \text{ kg/m}^2)$ (Fig. 6f). No significant difference in the below-ground vegetation carbon pool between the managed and unmanaged forest were found p-value = 0.95, 95% CI [-1.18, 1.22]).



3.2 Impact of land-use history on carbon pools

Figure 6: Carbon pool (kg/m2) (mean \pm SE) for the forest stands in the Bidstrup forests, a) soil, b) leaf litter, e) wood litter, d) shrub layer, e) tree layer, e) below-ground vegetation, g) deadwood. The sample size is given in the figure, BA = 6, BF = 6, BP = 5, OA = 6, and OF = 5. Significant differences are shown with asterisk or letters. The letters show the significance between the groups, the same letter show that they are not different, but different letter show that they are significantly different from each other.

3.2.1 Soil

There were not found any significant differences in the overall soil carbon stock between the forest stands with different former land-use (Appendix Table A 4). The estimates of mean soil carbon stock were estimated to be largest in the upper soil depth (0-10 cm), and a significant decrease in carbon within a forest group when soil depth increases (Appendix Table A 3, Fig 7a).

3.2.2 Deadwood

I found no statistically significant difference between the forests with different forest history group in the deadwood carbon stock (Appendix, Table A 4). Of the beech dominated forests, ancient beech forest $(0.70 \pm 0.45 \text{ kg/m}^2)$ and beech on previous fields $(0.44 \pm 0.20 \text{ kg/m}^2)$ had a larger deadwood carbon stock than beech on previous plains $(0.34 \pm 0.06 \text{ kg/m}^2)$. While for the oak dominated forests, it was estimated that oak forest on previous fields $(0.55 \pm 0.09 \text{ kg/m}^2)$ had a larger deadwood carbon stock than ancient oak forest $(0.37 \pm 0.09 \text{ kg/m}^2)$ (Fig. 7g).

3.2.3 Leaf litter

Again, I found no statistically significant difference between the forests with different forest history in the leaf litter carbon stock (Appendix Table A 5). Beech forest on previous plains was the beech dominated forest with largest estimated leaf litter carbon stock $(0.41 \pm 0.06 \text{ kg/m}^2)$, followed by beech forest on previous fields $(0.37 \pm 0.07 \text{ kg/m}^2)$ and the smallest leaf litter carbon pool were in ancient beech forest $(0.34 \pm 0.04 \text{ kg/m}^2)$. While for oak dominated forests, ancient oak forests $(0.40 \pm 0.06 \text{ kg/m}^2)$ had the largest leaf litter carbon stock compared to oak forest on previous fields $(0.35 \pm 0.07 \text{ kg/m}^2)$ (Fig. 7b).

3.2.4 Wood litter

No statistically significant difference between the forests with different forest history was observed in the wood litter carbon stock, but ancient oak forest and oak forest on previous fields, are close to having a significantly lower carbon stock than beech on previous fields (p-value of 0.06) (Appendix Table A 6). Beech forest on previous fields $(0.21 \pm 0.05 \text{ kg/m}^2)$ and ancient beech forest $(0.17 \pm 0.03 \text{ kg/m}^2)$ had a higher wood litter carbon pool than beech forest on previous plains $(0.15 \pm 0.05 \text{ kg/m}^2)$. Ancient oak forest $(0.11 \pm 0.03 \text{ kg/m}^2)$ had the same wood litter carbon as oak forest on previous fields $(0.11 \pm 0.02 \text{ kg/m}^2)$ (Fig. 7c).

3.2.5 Shrub layer

Beech forest on previous plains $(0.014 \pm 0.005 \text{ kg/m}^2)$ and oak forest on previous fields $(0.014 \pm 0.005 \text{ kg/m}^2)$ both had significantly higher shrub layer carbon stocks than ancient oak forest $(0.002 \pm 0.005 \text{ kg/m}^2)$, with a p-value of 0.03 for both. The other forest stands were not significantly different from each other (Appendix Table A 7). Beech forest on previous plains $(0.014 \pm 0.005 \text{ kg/m}^2)$ and ancient beech forest $(0.006 \pm 0.004 \text{ kg/m}^2)$ had a larger shrub layer carbon pool than beech forest on previous plains $(0.005 \pm 0.005 \text{ kg/m}^2)$, but none of the beech stands did differ significantly. (Fig. 7d).

3.2.6 Tree layer

There were no observed differences between the beech stands and the oak stands, but beech forest on former plains ($11.88 \pm 1.58 \text{ kg/m}^2$) and ancient beech forest ($11.82 \pm 1.34 \text{ kg/m}^2$) tended to have larger (p-values of 0.06; Appendix Table A 8) tree carbon stock than beech forest on previous fields ($8.28 \pm 1.81 \text{ kg/m}^2$), and ancient oak forest ($9.50 \pm 0.41 \text{ kg/m}^2$) and oak forest on previous fields ($9.34 \pm 0.26 \text{ kg/m}^2$) (Fig. 7e) did not differ significantly.

3.2.7 Below-ground vegetation

Beech stands and oak stands were not found to have a significant difference in the belowground carbon pool. A significant difference was observed within the beech stands, with the below-ground vegetation carbon pool was largest in ancient beech forest $(2.57 \pm 0.27 \text{ kg/m}^2)$ and beech forest on previous plains $(2.57 \pm 0.31 \text{ kg/m}^2)$ than beech forest on previous fields $(1.83 \pm 0.37 \text{ kg/m}^2)$ both with a p-value of 0.05 (Appendix Table A 9). The ancient oak forest $(2.09 \pm 0.09 \text{ kg/m}^2)$ did not differ significantly from oak forest on previous fields $(2.05 \pm 0.05 \text{ kg/m}^2)$ (Fig. 7f).

4 Discussion

This study only demonstrated limited differences in ecosystem carbon when comparing forest stands with different former land-use or different management. The unmanaged forests had larger ecosystem carbon stocks than the managed, even though this difference were not significant (Fig. 6), the managed forest and unmanaged forest had the same-sized estimated means for all carbon pools apart from the deadwood carbon pool where the unmanaged had the highest estimated mean (Fig. 6g). For the forests stands with different forms of former land-use, the only significant difference observed were for the shrub layer, tree layer and below-ground vegetation. For the shrub layer the difference was between ancient oak forests and beech forests on previous plains, and between ancient oak forests and oak forests on previous fields, where the ancient oak forests were considerable smaller (Fig. 7d), while for the tree layer and the below-ground vegetation the difference were between ancient beech forest and beech forest on previous fields, with the beech on previous fields being the smallest (Fig. 7f).

As expected, we found a statistically significant difference in carbon content between the soil depths, but only in the Bidstrup forests. Most carbon is stored in the upper layer of soil, and as one move down in the soil the carbon content decreases (Keen et al., 2011), this is because in the first layers the input of biomass from vegetation is highest, and as the carbon is moving down it is respired away and/or washed away, thus decreasing the carbon content.

I found that the above-ground carbon pool was statistically significantly larger (2.7 times) than the below-ground carbon pool. Studies of the Danish forest support my finding by also having found the above-ground pool tend to be larger than the below-ground pool (Nord-Larsen et al., 2019; Vesterdal & Christensen, 2007). My findings show that the soil carbon pool contributing 13.74% of the ecosystem carbon stock, and the tree carbon pool being almost 5 times larger than the soil carbon pool is also supported by Liski et al. (2002) for finding that in northern European temperate forests, the soil carbon stock were estimated to be 40% smaller than the tree carbon pool (respectively 2.31 kg/m² and 10.73 kg/m²). Others have found that the soil carbon stock should be larger than the tree carbon pool in European temperate deciduous forest (de Vries et al., 2003), contradicting my results. A potential explanation for this difference may be that geographical difference, where in central and south Europe the soil carbon pool has been reported to often be larger than the tree carbon

pool, but in northern Europe the contrary is found (Liski et al., 2002). As Denmark is a part of northern Europe, all these results support my findings of having the tree carbon pools being larger than all other carbon pools. The forests stand in this project are consists of many old trees, these old trees are huge carbon pools. This could explain why I find the tree carbon pool to be larger than the soil carbon pool. Especially in the management forests, the trees are old and have over time accumulated large quantity of carbon in its biomass. In addition of there being a spatial difference in carbon allocation in the ecosystem of European temperate deciduous forests, there is also a temporal difference, with a shift from the soil carbon pool being largest to the tree carbon pool being the largest, this is observed looking at forest inventories from 1950s to 1990s, and simulating the future using this data (Liski et al., 2002).

4.1 The impact of forest management strategies on ecosystem carbon pools As the unmanaged forests has had longer time accumulate carbon without disturbance, it is expected that that would have larger carbon pools than the managed, especially the soil and deadwood carbon pools should be larger. The unmanaged forests ecosystem carbon pool was estimated to be larger than the managed, but the difference between the managed and unmanaged forest were found to not be statistically different from each other for none of the carbon pools. Unmanaged forests have shown to have larger overall carbon stocks compared to managed forests (Nunery & Keeton, 2010). However, this is not what my data showed as the managed forests had a similar sized carbon stocks for all carbon pools apart from the deadwood were the unmanaged forest were estimated to be larger than the managed.

The soil carbon pool is the carbon pool mostly expected to see the differences between these two management strategies because of the accumulation and decomposition of biomass. There was no clear trend in the soil carbon stock between these two strategies, but second depth (10-25 cm) were statistically different between the managed and unmanaged forests, with the unmanaged forest being larger than the managed (respectively 1.23 ± 0.17 kg/m² and 1.08 ± 0.19 kg/m²). Soil type affects soil carbon stock, nutrient-rich mineral soil has been found to have three times less carbon in the forest floor compared to poor and sandy soil types (Vesterdal & Christensen, 2007). There was a noticeable variation in soil type between the managed and unmanaged forests, ranging in many different colours, and differing in being clay soil, or loamy soil (pers. Obs).

Forest sequestrates carbon and remove carbon from the atmosphere, but they also release greenhouse gasses. Soil hydrology play an important role in the balance between uptake of

carbon and greenhouse gas emission. A study of Danish temperate forest showed that poorlydrained forests have significantly larger soil carbon pools, compared to the more well-drained forests. The lack of oxygen in these poorly-drained soils are thought to be the reason for this, as decomposition will go slower (Christiansen et al., 2012). The managed forests in Bredvig Mose has been drained (Jessen & Andersen, 1994) and thus one would expect to find more soil carbon in the unmanaged part of Bredvig Mose than the managed part, the overall soil carbon stock was higher in the unmanaged than the managed, but this difference were not significant.

Unmanaged forests were expected to have larger deadwood carbon pools than managed forests, due to harvest of wood in the managed forests, and this were observed in this project by the managed forests having less deadwood biomass and thus deadwood carbon. In the managed forests only small twigs and sticks are left in the forests, while the large logs are harvested. This causes large variation in deadwood content in forests with different management (Christensen et al., 2005). Generally, in Danish managed forests and semi-natural forests there is little deadwood, due to harvest and clear-cutting (Vesterdal & Christensen, 2007), which is the management of the forests stands in this project (Graae & Heskjær, 1997; Jessen & Andersen, 1994). I found a larger deadwood carbon stock in the unmanaged forests compared to the managed forests, even though the results were not statistically significant.

The lack of larger differences between managed and unmanaged and the large woody biomass pools in both of these forest types ay relate to increased focus on natural forests in Denmark during the last decades. The managed forest stands that in the 90ies were selected as managed references in this project have become more or less unmanaged, with decreased harvest of wood, leaving deadwood behind to accumulate, often with the aim of protecting biodiversity and climate (Heilmann-Clausen et al., 2020). This may also have caused an accumulation of deadwood more similar to unmanaged forest for the forests included in this study. Conservation of previous managed forests will build up the deadwood and soil carbon pool (Krueger et al., 2017), since these managed forests in this project now are not harvests the same as before, the carbon pools will start to reach the levels of the unmanaged. My results show that the unmanaged have larger deadwood carbon pool and overall soil carbon pool, this is most likely because of the time the unmanaged forests have had to build up these carbon stocks before and around the 90ies.

4.2 Ancient forests and legacy effects of former land-use on ecosystem carbon pools

In the Bidstrup forests there are many forests stands with different former land-use. Because of the legacy effects these former land-uses may have on the ecosystem, it was expected to see some difference in the carbon pools, especially the soil carbon pool. Because those ancient forests stands are standing on ground that have been covered by forest longer than the other forests, they were expected to have accumulated more carbon. Overall, the ancient forests were not being significant different from the forest stands on previous fields or plains for neither beech nor oak, apart from the vegetation carbon pools. The ancient oak stands had a smaller shrub layer carbon pool than oak stands on previous fields and beech stands on previous plains, and the beech stands on previous fields were smaller than the ancient beech stands, and beech stands on previous plains for both the tree layer carbon pool and below-ground carbon pool. There though seemed to be a trend of the previous fields to hold smaller carbon stocks than the other forests, which could be explained by the former soil disturbance from tilling or ploughing that they have released considerable amounts of carbon.

For the wood litter carbon pool in this project, the beech forests on previous fields were close to be statistically significant different from both oak stands, with the beech forests on previous fields being larger than both oak forests. The litter biomass input contributes to the soil carbon pool as the litter is decomposed. The effect of afforestation on former agricultural land and pastures have a larger effect on the forest floor than the soil, this comes from the increased biomass production, thus litterfall (Jandl et al., 2007a) with litterfall pointed out to be the source of 70-80% of the carbon input into soils (Liski et al., 2002), this could be a potential explanation for why there seems to potentially be a trend of the previous fields or plains to have more litter than the ancient forests. The non-ancient forests have a younger vegetation and some of them also had smaller trees and hence production of litter is less than the ancient forest stands with older and larger trees. Reduced litter input was shown to reduce the soil carbon pools as there is less carbon input to the soil, but increased litter input did not have a positive effect on soil carbon pool. Even with 20 years with increased litter input, the soil carbon pool did not increase more than it increased under normal litter input. Decomposition of the litter may have been the limited factor, high rates of litter decomposition limited the possibility of storing the carbon in the soil (Bowden et al., 2014), so even if the non-ancient forests do produce more litter by having younger vegetation, the soil carbon does not increase at the same time. This is seen in my results by the non-ancient

forests having higher estimated means in the litter carbon pools than the ancient forests, but the ancient forests have higher soil carbon pools. Oak stands and beech stands have been found to produce similar amounts of leaf litter, but since oak leaf litter decomposed faster than beech leaf litter in the same environment, the forest floor in beech forest accumulated faster (Jonard et al., 2008), my data did not find any difference in leaf litter between the oak stands and beech stands, but the wood litter were found to be lower in both oak stands compared to beech on previous fields.

These temperate deciduous forests that are a part of this project are thought to become carbon neutral when the trees are hitting 200 years, and as they reach this age the productivity will decrease (Gundersen et al., 2021), as the only the ancient forest stands have had a forest cover close to 200 years, the non-ancient forests are still experiencing increased carbon sequestration and the carbon pools are expected to still increase in the future. A study of Hestehave, a beech dominated forest in Jutland, Denmark, showed that over a 50-year long period that the biomass and productivity increased, even when the forest were close to 150 years, and it were concluded that the forest stand were still a carbon sink. This increase in biomass caused an increase in litterfall, where around 50% of all litter were decomposed, causing an accumulation of carbon in the above-ground carbon pools (Andersson, 2015). This illustrates that one could expect that older forests (before hitting carbon neutrality) should experience increased biomass and thus litterfall as they get older, having a positive effect on the above-ground carbon pool, this could support that the forests in this project could still have increasing carbon pools. It has been shown that Danish oak stands tend to have increased litterfall the first 20 years after afforestation before it levels out, as this levels out quickly the forest floor of old oak (200 years) stands are similar to younger oak stands (25-30 years). And therefore, when looking at the forest floor and litter carbon pool in these oak stands one does not really see the effect of age (Vesterdal et al., 2007), and this could support the findings I have of no clear difference between the two oak forests.

4.3 The impact of afforestation on ecosystem carbon pools and the role of previous land-use

The forest stands that were not ancient forests, have experienced difference in disturbance before afforestation. In the previous fields, the soil disturbance would be expected to have been much higher than previous plains. This soil disturbance may have caused considerable amount of carbon loss, therefore at the time of afforestation these previous fields would have smaller soil carbon pools than the previous plains. As these afforested stands move forward in the forest succession, the above-ground carbon pools increase as live biomass, litter, and deadwood accumulate as there is a shift from annuals to perennials in early afforestation phase (Vesterdal et al., 2007). My data did not show any clear differences in any of the carbon pools when comparing the forests stands on previous plains and previous fields in none of the carbon pools apart from above-ground live standing vegetation and below-ground vegetation, were the beech stands on previous plains are larger than the beech stands on previous fields. Studies that have looked at the effect on soil carbon pools after afforestation have found that the effect on the soil carbon pool differs depending on what previous landuse were. Shi et al. (2013) found that the effect of soil organic carbon (SOC) differs between afforestation on cropland and grassland, where afforestation of grassland reduces SOC, and afforestation of cropland increases SOC, this is also supported by Bárcena et al. (2014a) that found a positive effect on SOC after afforestation on cropland, and a negative effect on SOC after afforestation on grasslands. A meta-analysis showed that conversion of pasture and cropland into forest had different results, where pasture to plantation conversion decreased soil carbon and pasture to secondary forest conversion did not have a significant change, while cropland to forest increased soil carbon. For cropland conversions, the effect was largest when converted into secondary forest (i.e., natural process after land abandonment) compared to plantation (i.e., human-made) (Guo & Gifford, 2002). These studies support the trend that my results seem to indicate, with the stands on previous plains have more carbon than stands on previous field in the vegetation carbon pools. But this trend of negative effect following afforestation of grassland is not clear and there are contradicting results found. A study from US showed that there in general is an increase in SOC after afforestation, but previous land-use affects this, shrub encroachment of native grassland had a positive significant effect on SOC, but afforestation on agricultural land did not have a significant effect (Nave et al., 2013). Even after 100 years following afforestation, previous grasslands showed reduced soil carbon in 75% of cases studied (Poeplau et al., 2011). This nonsignificant results of afforestation of previous grasslands, could explain why I did not find any differences in the carbon pools for my stands. In the review of Mayer et al. (2020) it were made clear that afforestation of cropland increases soil carbon pools, and it were discussed the different effect of afforestation of grasslands that are observed (i.e., small increase, stay unchanged or decrease), here it were thought that the soil type played an effect, where the negative changes were found on shallow and deep peat soils. Within the Bidstrup forest, the soil type was more similar (pers. Obs), but still there were differences in soil type depending Page 28 of 51

on moisture and nutrients (input) (pers. Obs), that could potentially explain the findings of no difference between the different land-use, and it is expected that the forests with more moisture will have higher levels of carbon stored (Christiansen et al., 2012). The differences in these forest stands could potentially be because of different soil and forest hydrology, and not land-use directly. In Bidstrup, the different forest stands are in many cases grouped with forest stands of the same history. All the oak stands on previous fields are situated close to each other, and all the beech stands on previous fields are situated close to each other and far away from the oak stands on previous fields (Fig. 2). The places oak stands are situated are in many ways different from where the beech stands are, the difference can be seen in the landscape and the intensity of the management and harvest.

4.4 Limitations of the study

In this project no statistically significant differences were found, other than between the soil depths as one expects. This could be because of the small sample size of the project. There could be a small difference between these forests, but the sample size is too small to be able to detect this difference. For the Bidstrup forest dataset, there are only five to six forest stands in each forest history group, and for the management dataset the sample size is even smaller with only three forests stands in each management group. By increasing the sample size, one could be more certain if there are any difference or not.

For some of the plots the soil corer was not able to go deep enough to collect the three depths fully because of stones and hard soil, this caused that for some plots the last (and sometimes the second depth) were missing and not a part of the analysis and amplifying the small sample size.

5 Conclusion

There were not found any difference in ecosystem carbon between forests with different landuse history or management, expect of the above-ground and below-ground where ancient beech forest and beech forest on previous plains were found to be significantly larger than beech forest on previous fields. In the shrub layer, the ancient oak forest was significant smaller than oak fields and beech plains. Disproving the hypotheses for this thesis were 1) the unmanaged forests would have more carbon than the managed due to harvest and removal of biomass, and not fully supporting the hypotheses 2) ancient forest will have larger carbon stocks than forest with previous land-use other than forest because of the time ancient forests have had to accumulate carbon, and 3) the high levels of soil disturbance in forests on previous fields, will cause smaller carbon stock in these forest in previous fields compared to forests on previous plains. These results also contradict much of the literature that are on this topic, that state that unmanaged forests will be significantly larger than managed, and age of the forest and levels of previous soil disturbance have an impact on the forest carbon pools following afforestation. The small sample size of this project is pointed out to be one of the reasons why a potential difference between the forests stands with different land-use history and management were not detected.

6 References

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7 Appendix

	Mean (kg/m ²)	SE
0-10	1.14	0.12
10-25	1.08	0.19
25-40	0.49	0.12
0-10	1.21	0.14
10-25	1.23	0.17
25-40	0.38	0.14
	0-10 10-25 25-40 0-10 10-25 25-40	Mean (kg/m²) 0-10 1.14 10-25 1.08 25-40 0.49 0-10 1.21 10-25 1.23 25-40 0.38

Table A 1: Soil carbon stock in the management forest (mean and SE) for all management strategies and soil depths.

			Unmanaged	
	r	0-10	10-25	25-40
	0-10	t-value: -0.31009 d.f., = 2 p-value: 0.79	t-value: -1.0047 d.f., = 2 p-value: 0.42 95 % CI [-0.47.	t-value: 3.09 d.f., = 2 p-value: 0.09
		95% CI [-0.92, 0.80]	0 29]	95% CI [-0.30, 1.83]
Managed	10-25		t-value: -5.3825 d.f., = 2 p-value: 0.03 95% CI [-0.27, - 0.03]	t-value: 2.18 d.f., = 2 p-value: 0.16 95% CI [-0.69, 2.11]
				t-value: 2.3388
	25-40			p-value: 0.14
				95% CI [-0.10, 0.33]
	l		Managed	
	_	0-10	10-25	25-40
			t-value: 0.51591	t-value: 2.8028
	0-10		d.f., = 2	d.f., = 2
q	0 10		p-value: 0.66	p-value: 0.11
age			95% CI [-0.43, 0.55]	95% CI [-0.35, 1.65]
an				t-value: 2.8028
Σ	10-25			u.1., -2 p-value: 0.18
				95% CI [-0.68, 1.86]
	25-40			
			Unmanaged	
		0-10	10-25	25-40
			t-value: -0.093729	t-value: 0.16125
	0-10		d.f., = 2	d.f., = 2
			<i>p-value:</i> 0.93	p-value: 0.02
ed			9570 CI [-1.25, 5.16]	55 / 0 CI [0.55, 1.55]
lag	10.5-			$d_{1}f_{1} = 2$
nai	10-25			p-value: 0.11
Um				95% CI [-0.47, 2.18]
-				
	25-40			

Table A 2: Results from t-test for the soil-data in the management forests. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

		Mean (kg/m ²)	SE
	0-10 cm	1.03	0.18
BA	10-25 cm	0.74	0.06
	25-40 cm	0.29	0.02
	0-10 cm	0.84	0.10
BF	10-25 cm	0.71	0.07
	25-40 cm	0.35	0.03
	0-10 cm	0.92	0.07
BP	10-25 cm	0.85	0.12
	25-40 cm	0.47	0.04
	0-10 cm	0.98	0.09
OA	10-25 cm	0.88	0.09
	25-40 cm	0.39	0.08
	0-10 cm	0.85	0.08
OF	10-25 cm	0.93	0.07
	25-40 cm	0.42	0.02

Table A 3: Soil carbon stock in Bidstrup forest (mean and SE) for all land-use history groups and soil depths.

Table A 4: P-value for comparison between the forest history groups for the whole soil carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.65	0.57	0.54	0.67
BF			0.32	0.297	0.4
BP				0.958	0.91
OA					0.87
OF					

Table A 5: P-value for comparison between the forest history groups for the deadwood carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.47	0.28	0.34	0.68
BF			0.76	0.84	0.76
BP				0.91	0.53
OA					0.61
OF					
BP OA OF				0.91	0.53 0.61

Table A 6: P-value for comparison between the forest history groups for the leaf litter carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.66	0.33	0.42	0.97
BF			0.63	0.74	0.69
BP				0.87	0.37
OA					0.46
OF					

Table A 7: P-value for comparison between the forest history groups for the wood litter carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.47	0.71	0.21	0.21
BF			0.28	0.06	0.06
BP				0.37	0.36
OA					0.96
OF					

Table A 8: P-value for comparison between the forest history groups for the shrub layer carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.88	0.15	0.41	0.13
BF			0.13	0.53	0.11
BP				0.03	0.89
OA					0.03
OF					
OA OF					0.03

Table A 9: P-value for comparison between the forest history groups for the tree layer carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.06	0.97	0.19	0.18
BF			0.06	0.50	0.58
BP				0.18	0.17
OA					0.93
OF					

Table A 10: P-value for comparison between the forest history groups for the below-ground vegetation carbon pool. Significant values are in bold, and close to significant (p-value < 0.1) values are in italic.

	BA	BF	BP	OA	OF
BA		0.05	0.99	0.17	0.16
BF			0.05	0.48	0.56
BP				0.18	0.17
OA					0.92
OF					
	1				



