

No effect on soil C stock, but increased tree C stock 17 years after site preparation: Results from two hemiboreal field experiments

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ABSTRACT

Forest management practices can influence carbon and nitrogen dynamics, which are critical for climate change mitigation. We investigated the effects of disc trenching during stand establishment, in combination with repeated nitrogen fertilisation of the previous stand, on carbon and nitrogen stocks in soil, tree biomass, ground vegetation, and fungal community composition in two 17-year-old *Pinus sylvestris* stands in Sweden (Hagfors and Nissafors). The study applied a split-plot design with factorial combinations of fertilisation (0–1800 kg nitrogen ha⁻¹ in the previous rotation) and disc trenching as sub-plots. Seventeen years after disc trenching, the total soil carbon stocks were not significantly different between disc-trenched and non-disc-trenched treatments, but organic matter was redistributed. At both sites, disc trenching increased tree biomass carbon and nitrogen by ~50% compared to the non-disc-trenched treatment, resulting in significantly higher ecosystem carbon stock at Nissafors; and an increasing trend at Hagfors (albeit non-significant). In contrast, fertilisation of the previous stand had no persistent effects on soil, biomass, or ecosystem carbon and nitrogen stocks in year 17 of the subsequent rotation. Disc trenching and previous fertilisation had limited effects on fungal communities in the soil organic layer. At Hagfors, however, ectomycorrhizal richness was higher in unfertilised non-disc-trenched sub-plots. Overall, disc trenching enhanced ecosystem carbon stock through increased tree growth without causing long-term soil carbon or nitrogen losses, while previous nitrogen fertilisation showed no legacy effects. These results suggest disc trenching can support forest regeneration without causing long-term soil carbon loss.

1. Introduction

Forest management and biomass extraction affect forest carbon (C) stocks and storage (Achat et al., 2015; Mayer et al., 2020; Clarke et al., 2021; Mäkipää et al., 2023). In the temperate and boreal regions, the C stock in the soil far exceeds the C stored in biomass (Scharlemann et al., 2014). In the context of climate change, it is vital to manage forest ecosystems in a way that does not jeopardise their importance as a C sink (Pan et al., 2011, 2024; Mäkipää et al., 2023). In addition, Deluca, Boisvenue, (2012) highlight the role of soil C for improving chemical and physical properties of the soil.

There are multiple forest management strategies, spanning a wide range of intensities and practices, with varying potential to impact C stocks and storage (Jandl et al., 2007; Mayer et al., 2020; Mäkipää et al., 2023). In Fennoscandia, conifers are predominantly managed as even-aged stands, involving various silvicultural practices such as

mechanical site preparation, planting, thinning, and final felling. Fertilisation with nitrogen (N) is restricted but can be used to increase tree growth in mineral soil sites (eg. Jetsonen et al., 2025). In commercial forestry in the northern two thirds of Sweden, current national guidelines allow up to three applications of 150 kg ha⁻¹ of N during a rotation period (Swedish Forest Agency, 2025a, b, Jämtgård et al., 2026). Applications in the southern part of the country are more restricted.

Forest N fertilisation not only affects the biomass C stock but also increases the soil C stocks in boreal forests (eg. Nohrstedt, 2001; Hyvönen et al., 2008; Jörgensen et al., 2021; Mäkipää et al., 2023; Jämtgård et al., 2026). Mayer et al. (2020) conclude that addition of N has an overall effect of increasing soil C stocks in many types of forests, due to increased litter input and reduced decomposition, among other potential causative factors. However, other studies have found no effect, or unclear effects, on soil C (Högbom et al., 2006; Ring et al., 2011). In addition, the long-term persistence of fertiliser-induced soil C is poorly

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understood. A recent study of 24 pairs of conventionally fertilised and unfertilised stands in Sweden observed no significant differences in soil C stocks 4–13 years after clearcutting and 11–49 years after one or two fertilisations (Boeraeve et al., 2025).

Long-term impacts of previous forest fertilisation with N have been investigated, including potential carry-over effects in the next tree generation on, for instance, initial tree growth. Studies in two field experiments (the same sites as considered in the present study) revealed no significant effects on seedling survival and growth up to 17 years after final felling (Johansson et al., 2013; Hjelm et al., 2025). These findings are consistent with results from a recent survey of 18 conventionally fertilised and 18 unfertilised forest stands in Sweden: considering the effect of previous fertilisation on tree ring development 4–13 years after final felling, no significant impact was detected (Boeraeve et al., 2025). However, results are not entirely consistent across studies. In one study of 10-year-old conifer-dominated stands, increased tree height was reported after two fertiliser applications (150 kg N ha⁻¹ each), while a single application had no significant effect (From et al., 2015).

Mechanical site preparation (MSP) is a physical disturbance of the upper soil that is performed to improve the conditions for regeneration. MSP typically involves exposure of the bare mineral soil by removing the superficial organic soil layer and/or mixing the mineral soil with organic matter (Örlander et al., 1990; Sikström et al., 2020). The most common MSP practices used today in Fennoscandia include disc trenching, mounding, and patch scarification (Ring and Sikström, 2024), with the choice of practice depending on site and soil conditions (Örlander et al., 1990; Saksa et al., 2018; Sikström et al., 2020). MSP can substantially increase both seedling survival rates and tree height growth (Sikström et al., 2020).

The disturbance of the upper soil by MSP affects the distribution and flows of C (Mallik and Hu, 1997; Ring and Sikström, 2024). However, concerns have been raised that MSP could lead to a depletion of the soil C stock, thereby impeding climate change mitigation (Mayer et al., 2020). The degree of disturbance is an important factor, with more intensive methods generally associated with greater C losses (Johnson, 1992; Jandl et al., 2007; Mayer et al., 2020). These concerns, however, are based on a limited number of studies conducted in diverse forest ecosystems worldwide, often involving site preparation practices that differ from those currently used in Fennoscandian forestry. On the other hand, a recent study from Canada suggests that MSP may have a smaller impact on soil C than previously assumed (Marty et al., 2024), and results from about 15 experimental sites using the two most common MSP practices in Fennoscandia did not show any consistent soil C loss during the first 2 years after MSP (Mjöfors et al., 2015; Strömgren et al., 2017).

Long-term studies are still scarce, but available evidence indicates no substantial soil C loss even decades after treatment. Mjöfors et al. (2017) found no significant change in soil C stocks 25 years after MSP using methods still applied in Fennoscandia. Similarly, Swedish studies of more intensive practices such as deep ploughing and stump harvest – where more severe soil disturbance would be expected, along with, in some cases, increased biomass removal – did not show substantial soil C losses after 10–40 years (Nordborg et al., 2006; Strömgren et al., 2013; Jurevics et al., 2016; Mjöfors et al., 2017).

Nitrogen addition, through fertilisation or atmospheric deposition, is known to alter ectomycorrhizal fungal communities (Lilleskov et al., 2019; Jørgensen et al., 2022), with *Cortinarius*, *Suillus* and *Piloderma* reacting negatively; *Thelephora* and *Laccaria* reacting positively; and species within *Russula* and *Lactarius* showing diverging responses. Nitrogen addition is furthermore known to shift the abundance and activity of saprotrophic fungi (Morrison et al., 2016; Moore et al., 2021). However, the persistence of the effects of N addition after final felling and MSP has received far less consideration in the research. Boeraeve et al. (2025) found a higher number of fungal ITS2 copy numbers (as a measure of abundance) in the soil organic layer of clearcuts of previously fertilised forests compared to clearcuts of unfertilised forests, mainly comprised of a higher abundance of saprotrophic fungi. MSP

treatments can reduce root colonisation and diversity of ectomycorrhizal fungi (Bzdyk et al., 2022), but the effect of MSP varies between different practices (Harvey et al., 1996). How MSP affects saprotrophic fungi has been studied less, but as the displacement of the litter has been found to affect decomposition rates (Mjöfors et al., 2015), it can be expected to affect saprotrophic fungi.

Although several studies have examined forest fertilisation and site preparation, their long-term effects on soil carbon dynamics remain unclear. MSP is a common practice performed on various types of soils and sites. The practices, for example disc trenching, may be executed at different intensity (Ring and Sikström, 2024). Together, this creates a range of possible outcomes. Long-term studies of disc trenching practiced in Nordic forestry today are scarce, and the persistence of fertilisation-induced changes in soil C stocks following final felling is poorly understood. In addition, the long-term responses of soil fungal communities to mechanical site preparation remain unexplored. Moreover, studies examining the combined effects of fertilisation of the previous stand and site preparation during stand establishment have rarely been examined.

The aim of this study is to examine the individual and combined effects of (1) disc trenching performed at the time of stand establishment and (2) repeated N fertilisation of the former stand on C and N stocks in the soil, the biomass, and the entire ecosystem 17 years after clearcutting in two field experiments with 17-year-old Scots pine. Additionally, we investigate how these treatments affect soil fungal communities in the organic soil layer.

We expected that disc trenching would not significantly affect the total soil C stock, but would lead to a decrease in C stock in the organic layer and a translocation of organic matter into the mineral soil, mainly because the organic layer of the furrows becomes part of the mineral soil layer of the tilts. The C stock in biomass is expected to increase because improved seedling establishment and growth after disc trenching have been reported for the two studied sites (Johansson et al., 2013; Hjelm et al., 2025). As a result of the unchanged total soil C stock, a small ground vegetation C stock, and an increased tree C stock, we expected that the total ecosystem C stock will show a significant increase. We also expected that the effects of disc trenching on N stocks will mirror those observed for C stocks.

Regarding carry-over effects of N fertilisation, we expected that fertilisation during the previous rotation period will lead to a higher N stock in the entire soil profile, while its effect on soil C stock will be minor.

Lastly, we expected that disc trenching substantially alters the composition of soil fungal communities in the organic soil layer with the strongest effect for saprotrophic fungi and fungi associated with ground vegetation roots. Because clearcutting has a strong impact on ectomycorrhizal fungal communities (Kyaschenko et al., 2017), we further expected subsequent disc trenching to have only a limited effect on this functional group. While N fertilisation is known to alter soil fungal communities in mature forests (Marupakula et al., 2021; Jørgensen et al., 2022), we expected to find limited carry-over effects of fertilisation due to the intense disturbance during clearcutting.

2. Material and methods

2.1. Site description

The study was conducted at two field experiments situated in Sweden, 165 Hagfors (60°00'N, 13°42'E) and 132 Nissafors (57°24'N, 13°37'E), hereafter referred to by the location name alone. The sites are situated on a gentle slope composed of sandy-silty till (Hagfors) and a flat sandy sediment (Nissafors). The soils are classified as Haplic Podzols according to the FAO-UNESCO soil classification system and are characterised by mor-type humus.

Both the previous and current tree stands were dominated by *Pinus sylvestris* L. The mean site productivity was 5.9 m³ ha⁻¹ year⁻¹ at

Hagfors and $5.5 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ at Nissafors. This is close to the mean site productivity of $5.4 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ for productive forest land outside formally protected areas in Sweden (Table 3.11b, National Forest Survey, <https://skogsstatistik.slu.se/pxweb/en/OffStat/>, accessed 15 January 2026). Over the last 30-year climate reference period (1991–2020), the average annual precipitation was 750 mm in the Hagfors area (gauging station Forshult) and 865 mm in the Nissafors area (gauging station Hestra D) (Swedish Meteorological and Hydrological institute, <https://www.smhi.se/data>, accessed 26 April 2024). The mean annual air temperatures for the same period were 5.3 and 6.5 °C, respectively. In the Hagfors and Nissafors areas, the inorganic nitrogen deposition amounted to approximately 4–6 and 6–8 kg N ha^{-1} , respectively, during the hydrological year 2022/2023 (The Swedish Throughfall Monitoring Network, <http://www.krondroppsnatet.ivl.se>, accessed 10 October 2025).

The study sites are typical for sites exposed to fertilisation in Swedish forestry, although the national guidelines recommend no commercial fertilisation in the Nissafors area. They represent forest land where tree growth is limited by the availability of N, as in most forests growing on mineral soils in the boreal and hemiboreal region (Tamm, 1991). Previous investigations report significant increases in tree growth after N fertilisation at both Hagfors and Nissafors (Jacobson and Pettersson, 2001; 2010). Furthermore, in practical forestry, regeneration of this type of sites would generally involve some kind of mechanical site preparation (Sikström et al., 2020). Thus, the sites were found suitable for the present investigation.

2.2. Experimental design

The original aim of the experiments was to explore the growth-response of middle-aged *Pinus sylvestris* L. stands to varying intensities of fertilisation (Jacobson and Pettersson, 2010; Johansson et al., 2013). Fertilisation in the former stand constituted the main-plot treatment, while disc trenching served as the sub-plot treatment. Both experiments utilised a randomised split-plot design, replicated in three blocks (Fig. 1). The main plots measured $30 \times 30 \text{ m}$, except in one block at Nissafors, where they were $25 \times 25 \text{ m}$. Beginning in 1981 (Hagfors) and 1977 (Nissafors), nitrogen (N) was added as NH_4NO_3 to study plots at a rate of $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. From 1991 onwards, dolomite and boron were applied with the NH_4NO_3 . At Hagfors, the application interval was 8, 4 and 2 years, reaching total levels of 450 kg, 900 kg, and 1800 N ha^{-1} (450 N, 900 N and 1800 N). The 1800 N treatment also included magnesium and micronutrients (Ring et al., 2011). At Nissafors, a total of

450 kg ha^{-1} of N was added at 5- to 7-year intervals. In 1977 and 1978, the plots in blocks 1 and 2 received an annual N application of 75 kg ha^{-1} , while the plot in block 3 received six applications of 25 kg ha^{-1} in 1977. In 1984 and 1989, a single application of $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was made to each plot, with the addition of 20 kg ha^{-1} of P in 1989 (Ring et al., 2011). Unfertilised control plots (0 N) were part of the experimental design at both sites. There were also additional treatments at both sites that were not included in the present study.

Following final felling and removing of logging residues from the study plots in 2006, site preparation by disc trenching was performed. Each study plot was divided into two sub-plots, with one undergoing disc trenching (DT) and the other remaining without disc trenching (no DT). The sub-plots measured $30 \times 15 \text{ m}$, except in one block at Nissafors, where they were $25 \times 12.5 \text{ m}$. Subsequently, the study plots were planted with *Pinus sylvestris* L. seedlings at a density of 2500 seedlings per hectare. For further descriptions of the experimental treatments and experimental design, see Jacobson and Pettersson (2010), Ring et al. (2011), (2013), Johansson et al. (2013) and Hjelm et al. (2025).

2.3. Tree C and N stocks

The tree inventory was conducted in September 2022 after annual growth had ceased. Height and diameter at breast height (1.3 m) were measured for all trees, including both planted and naturally regenerated trees, within each sub-plot utilising a buffer strip of approximately 2 m. For additional details regarding the tree inventory, see Hjelm et al. (2025).

The dry weight of tree biomass was calculated using biomass functions for *Pinus sylvestris* from Marklund (1988): for stems (function T-2), living branches (function T-14), dead branches (function T-22), and needles (function T-18). The dry weight of roots with a diameter greater than 2 mm was determined using functions derived by Petersson and Ståhl (2006). To convert dry weight of tree biomass into C and N stocks, published C and N concentrations specific to different tree compartments were applied (Table 1).

2.4. Ground vegetation C and N stocks

Sampling of ground vegetation was conducted between 15 and 21 August 2022 at Hagfors and Nissafors. From each sub-plot without site preparation (no DT treatment), six $30 \times 30 \text{ cm}$ quadrats were randomly positioned along pre-determined lines located between two tree rows, 7, 14 and 21 m from the short side of the sub-plot. For the disc-trenched

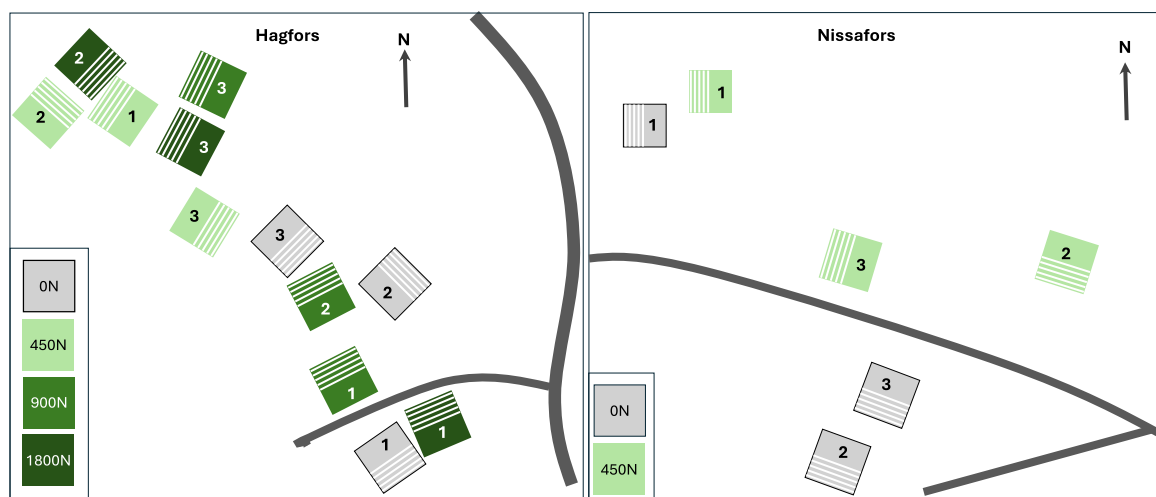


Fig. 1. Experimental design in Hagfors and Nissafors sites. Main plot treatments: Unfertilised control (0 N) and fertilised treatments corresponding to a total dose of 450, 900, and 1800 kg N ha^{-1} (450 N, 900 N and 1800 N). Sub-plot treatments: Disc trenching (stripes) and without disc trenching (no stripes). Numbers on the plots denotes the block identity.

Table 1
Concentrations of C and N used for calculating the tree biomass stock at Hagfors and Nissafors.

Compartment	C (%) ^a	N (%) ^b
Stem with bark	50.30	0.27
Living branches	52.55	0.35
Dead branches	50.67	0.27
Needles	52.56	0.80
Stumps and roots	50.79	0.27

^a Sourced from Mensah and Petersson (2024). The C concentration for dead branches was assumed to be equivalent to the C concentration given for the whole tree in Mensah and Petersson (2024).

^b Assumed to be the same as those measured during the fifth growing season after planting at Hagfors (Johansson et al. (2013)). The N concentration in dead branches, stumps, and roots, not measured at the sites, were assumed to equal the concentration in the stem.

sub-plots, the sampling design was similar, but six quadrats were established in each of the three types of soil disturbance: tilt, furrow, and area between furrows (Hjelm et al., 2025).

The ground vegetation within each quadrat was clipped at the ground surface, then combined to create a composite sample for each sub-plot and soil-disturbance type, and finally dried at 70 °C until a constant weight was reached.

The C stock of the ground vegetation was calculated by multiplying its dry weight with a C concentration of 50%. The N stock was calculated using the N concentrations measured in the fifth growing season after final felling at Hagfors, namely 1.09%, 1.28%, 1.20% and 1.27% for the 0 N, 450 N, 900 N and 1800N treatments, respectively (Johansson et al., 2013).

The C and N stocks in disc-trenched sub-plots were scaled up to hectare values based on the coverage of the three soil-disturbance types tilt, furrow, and area between furrows. At Hagfors, tilts, furrows and area between furrows were estimated to cover 37%, 41% and 21% of the ground surface, respectively, based on the mean widths of the different soil disturbance types provided by Ring et al. (2013). At Nissafors, the corresponding estimates were 32%, 35% and 33%, respectively.

2.5. Soil C and N stocks

2.5.1. Soil sampling

Soil sampling was conducted from September to November 2023 to assess soil C and N stocks. Within each sub-plot, 18 sampling locations were systematically positioned in a regular grid pattern (see Fig. 2). At each sampling location of the disc-trenched sub-plots, three samples were collected from each type of soil disturbance (i.e., tilt, furrow, and

area between furrows). Cores were taken from the organic horizon (O), encompassing litter, fermentation and humus layers, using a sharp-edged steel cylinder with a diameter of 50 mm. Additionally, cores of the mineral soil down to a depth of 20 cm from the original soil surface were obtained using a steel corer with 20 mm diameter. Any organic matter present within the mineral soil, such as in the tilts, was integrated into the mineral soil sample (see Fig. S1, Supplement). In the furrows at Hagfors, samples were taken to a depth of 5 cm from the current surface of the mineral soil given the practical challenges to delineating the original soil surface for the furrows. At Nissafors, samples in the furrows were taken to a depth of 17 cm from the mineral soil surface, assuming an estimated furrow depth of 3 cm from the original mineral soil surface.

The large boulder and stone content in the soil at Hagfors posed challenges to reaching a depth of 20 cm for the mineral soil samples. Consequently, the following adjustments were made to the soil sampling protocol for Hagfors:

- For sub-plots without site preparation and the areas between furrows within disc-trenched sub-plots, soil samples were collected to a depth of 10 cm from the original mineral soil surface. Where feasible, an additional soil sample was obtained at depths of 15–20 cm.
- For the tilts, mineral soil samples were taken down to the original surface of the mineral soil (as indicated by the upper red line in Fig. 2). Additionally, a separate sample was collected from the original surface of mineral soil to a depth of 10 cm where feasible. This criterion was fulfilled in 7 out of the 12 sub-plots.
- No alterations were made to the sampling protocol for furrows.

Before further analysis, the soil samples of each horizon and soil disturbance type (no DT, area between furrows, furrow, and tilt) were merged into one composite sample for each plot.

2.5.2. Analysis for C and N in soil

Pre-treatment of the composite soil samples and analysis for C and N followed the procedure used by the Swedish Forest Soil Inventory (Karlton et al., 2022) and were conducted in the same laboratory. First, the soil samples were air-dried at 35–40 °C until they reached a constant weight. Then, roots exceeding 2 mm in diameter were removed from the sample and weighed separately. Thereafter, the samples were sieved through a 2 mm mesh using a soil mill (Ånö) for humus samples and a vibration sieve shaker (AS 200 control, Retsch GMB Haan, Germany) for mineral soil samples. The samples were then weighed individually.

The sieved compartments underwent analysis for C and N concentrations by dry-oxidation utilising a LECO TruMac CN instrument (Leco Corporation, St Joseph, MI, USA). To adjust for any remaining water

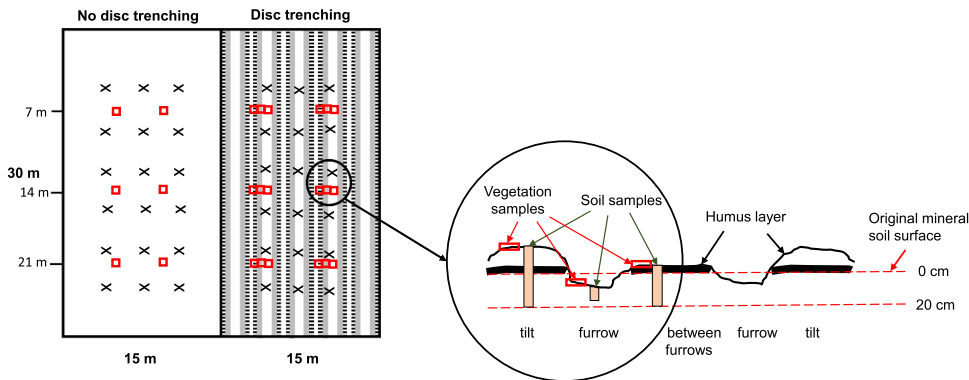


Fig. 2. Schematic outline of the study plots with location for sampling of soil (x) and ground vegetation (□). Sampling in the disc-trenched sub-plots: at every sampling location, one sample was collected from each of the three soil disturbance types for both ground vegetation and soil. The mineral soil sample, except for the furrows, was taken to a depth of 20 cm from the original mineral soil surface (indicated by lower broken line). In the furrows, mineral soil samples were obtained to a depth of 5 cm at Hagfors and 17 cm at Nissafors from the current mineral soil surface, as the original surface position could not be determined. Adjustments to the soil sampling protocol in response to the high levels of stoniness at Hagfors are described in the main text.

content in the samples, dry weight was determined based on a selection of five humus samples, five samples of mineral soil from intact soil profile (sub-plot without disc trenching and area between furrows), and two samples from the mineral soil of the tilts. The dry matter content was 96.9% (SD 2.0%) for the humus samples. The averages of the mineral soil samples were 99.4% (SD 0.1%) and 98.8% (SD 0.2%) for the intact and furrow strata, respectively. The mean dry matter content of the seven mineral soil samples was 99.2% (SD 0.3%), and this value was employed in the subsequent calculations.

2.5.3. Estimations of soil C and N contents

The total stock for a stratum was obtained by summing the stocks of the humus layer and the mineral soil. The following assumptions were also made:

1. The coarse compartment of the humus layer (> 2 mm): This compartment consisted of hard woody debris, and the C concentration were therefore assumed to be 50% (see Table 1). The N concentration, however, was assumed to be the same as in the fine compartment (< 2 mm). The coarse compartment accounted for a minor part of the organic layer (17% and 6% at Hagfors and Nissafors, respectively).
2. Stoniness: The C and N content in the mineral soil was adjusted for the presence of stones and boulders at site-level, estimated using the Viro method adapted by Stendahl et al. (2009). This content was estimated to be 36% at Hagfors and 0% at Nissafors.
For Hagfors, the soil sampling protocol was adjusted due to significant stoniness. Additional assumptions were made for calculations of soil C and N stocks, namely:
3. Intact soil profiles: Where the soil profile remained intact (i.e., in sub-plots without site preparation and areas between furrows in disc-trenched sub-plots), the C and N concentrations, as well as weight of at 10–20 cm depth from the original mineral soil surface, were estimated using the mean of the soil samples taken at 15–20 cm depth.
4. Furrows: The depth of this mineral soil profile were assumed to be 7 cm based on earlier measurement of depth by Ring et al. (2013) and thickness of organic layer by Ring et al. (2011). The C and N concentration, as well as the bulk density, of the mineral soil samples from furrows were considered representative for the mineral soil to a depth of 20 cm from the original position of the mineral soil surface in this stratum. Therefore, for calculating the C and N stocks in this mineral soil profile, the C and N content in the sampled mineral soil samples at a depth of 5 cm needed to be multiplied by a factor of 1.4 (7/5).
5. Tilts:
 - a. The compartment of the mineral soil placed on top of the original mineral soil surface: The amount of mineral soil was assumed to equal the amount moved from furrows during disc trenching. This quantity was estimated by assuming (1) rectangular cross sections of furrows and tilts using the mean depths and widths of furrows and tilts presented in Ring et al. (2013); and 2) a bulk density according to the 0–10 cm depth profiles of the intact soil in the same plot.
 - b. At 0–10 cm depth from the original soil surface: The C and N concentration in the mineral soil was either measured (7 plots) or assumed to be the same as in the intact soil profiles in the same plot (5 plots). This assumption seems reasonable given the insignificant differences for these concentrations across plots for this horizon (Table 2). Bulk density was assumed to be the same as measured at 0–10 cm depth in the intact soil profiles in the same plot.
 - c. For 10–20 cm depth from the original surface of mineral soil, see assumption 4.

Soil C and N stocks in disc-trenched sub-plots were scaled up to

Table 2

Concentrations of C and N in 0–10 cm depth of mineral soil for different soil disturbance types at Hagfors. Mean $\pm$ 1 standard deviation.

Disc trenching	Soil disturbance type	C (%)	N (%)
Yes	Tilt	1.46 $\pm$ 0.72	0.041 $\pm$ 0.014
Yes	Between furrows	1.53 $\pm$ 0.31	0.049 $\pm$ 0.008
No	Intact	1.72 $\pm$ 0.78	0.051 $\pm$ 0.018

hectare values in the same way as for ground vegetation.

2.6. Soil fungal communities

2.6.1. Soil sampling

A separate soil sampling was conducted for determination of the soil fungal communities. During September to October in 2023, samples of the humus layer were taken from the areas with intact soil profiles in the same locations as the sampling for soil C and N stocks (see Fig. 2). Thus, 18 humus samples were taken from each sub-plot. The samples were taken using a steel corer with a diameter of 20 mm. Any abundance of fresh needle litter in the samples was carefully removed, before all samples were merged to one composite sample per sub-plot. The samples were stored frozen until they were freeze-dried and homogenised according to the same procedure for soil samples for DNA-analysis used by the Swedish Forest Soil Inventory (Karlton et al., 2022).

2.6.2. Molecular analyses

DNA was extracted from the freeze-dried soil with the Nucleospin Soil kit (Machery-Nagel), following the manufacturer's instructions. From diluted DNA extracts, the ITS2 region and part of the LSU region were amplified with the gITS7/TW13 primer pair (Jhrmark et al., 2012). PCR products were equimolarly pooled, cleaned, and sequenced on the PacBio Sequel II platform (Pacific Biosciences).

The sequencing dataset was processed using the SCATA bioinformatics pipeline (<https://scata.mykopat.slu.se>). After quality-filtering, sequences were clustered into operational taxonomic units (OTUs) using single-linkage clustering at 98.5% similarity threshold. OTUs were assigned a taxonomy based on the UNITE database, using the Species Hypothesis (SH) matching service incorporated in the PlutoF platform (Abarenkov et al., 2010; Nilsson et al., 2019). These identifications were subsequently double-checked by BLASTing representative sequences of the OTUs against NCBI Genbank. OTUs were assigned to an ecological group using the FungalTraits database (Pölmé et al., 2020), with some modifications (see extended methods in the Supplementary Material). ITS2 copy numbers were determined on a CFX Connect Real-Time System (Bio-Rad) with primer pair fITS7-ITS4/ITS4arch, in duplicate. The ITS2 copy numbers ng^{-1} DNA were converted to ITS2 copy numbers mg^{-1} dry soil, which were then corrected to fungal ITS2 copy numbers mg^{-1} dry soil using the proportion of fungal sequences in the metabarcoding dataset. The ITS2 copy number mg^{-1} dry soil was then also used to correct the OTU table (see extended methods in the Supplementary Material). From this copy number-corrected OTU table, fungal OTUs were selected and used in further analyses.

2.7. Data analysis

To assess treatment effects on C and N stocks across different compartments in the ecosystem, a mixed model analysis of variance was performed using SAS Statistical software (Proc mixed, v9.4, SAS Institute, Cary, NC, USA). The analysis also included the entire soil column studied (i.e., the sum of the stocks of the humus layer and mineral soil strata/compartments) and the whole ecosystem stock (i.e., the sum of all soil and biomass compartments). These split-plot models incorporated fertilisation treatment (main plots), site preparation (subplots), and their interaction as fixed factors, employing the ddfm method according to Kenward Roger. Additionally, block and block $\times$ fertilisation

treatment were included as random factors. Separate analyses were conducted for Hagfors and Nissafors.

The effects of different soil-disturbance types on C and N stocks were also investigated with a similar method, but with a split-plot model incorporating fertilisation treatment (main plots) and soil disturbance type (subplots), and their interaction as fixed factors.

If a fixed effect was significant, paired *t*-tests adjusted for multiple comparisons were used to identify differences between the treatments, following Tukey–Kramer’s method. A difference was considered significant if $p < 0.05$.

To assess treatment effects on the abundance (fungal ITS2 copy numbers), diversity, and community composition of the soil fungal communities, linear mixed models (LMMs), non-metric multidimensional scaling (NMDS) and permutation analyses of variance (PERMANOVAs) were used in R. Separate analyses were carried out for Hagfors and Nissafors. The diversity metrics OTU richness, Shannon diversity, and Pielou’s evenness were calculated using the vegan R package (Oksanen et al., 2022), for all soil fungi and for the three most abundant ecological groups (root-associated ascomycetes, saprotrophic ascomycetes, and ectomycorrhizal fungi) and used as response variables in LMMs. Explanatory variables were fertilisation treatment and site preparation treatment, and their interaction, and block as a random variable. Copy number-corrected OTU tables were used as input for the NMDS; and the Bray–Curtis dissimilarity matrix from the OTU tables as input for the PERMANOVAs. The PERMANOVAs included fertilisation treatment and site preparation treatment as independent variables, and were repeated with the interaction between fertilisation treatment and site preparation treatment. PERMANOVAs were executed with the *adonis2* function from the *vegan* R package (Oksanen et al., 2022) and were carried out for the soil fungal community as a whole, and for the three most abundant ecological groups (root-associated ascomycetes, saprotrophic ascomycetes, and ectomycorrhizal fungi). In case of

significant effects of the fertilisation treatment, post hoc pairwise comparisons were carried out with pairwise Adonis (Martinez Arbizu, 2020).

3. Results

3.1. Ecosystem C and N stocks

Seventeen years after disc trenching, total ecosystem C stocks, including the entire soil profile down to 20 cm, ground vegetation, and tree biomass, ranged from 70 to 90 Mg ha⁻¹ at Hagfors and from 104 to 130 Mg ha⁻¹ at Nissafors (Fig. 3, Table S1). Soil C stocks constituted the largest ecosystem C compartment, accounting for approximately 70% and 80% of the total ecosystem C stock at Hagfors and Nissafors, respectively. Tree biomass also contributed substantially, whereas the contribution from ground vegetation was minor, especially at the Nissafors site.

Ecosystem C stocks were significantly higher in the disc-trenched treatment (DT) compared to the non-disc-trenched treatment (no DT) at Nissafors, with an increase of approximately 15% (Table 3, Table S1). A similar trend was observed at Hagfors, but the difference was not statistically significant. Earlier N fertilisation of the former stand had no significant effect on ecosystem C stocks in either site.

Depending on treatment, total ecosystem N stock ranged from 1.54 to 2.06 Mg ha⁻¹ at Hagfors and from 2.85 to 3.45 Mg ha⁻¹ at Nissafors (Fig. 3, Table S2). Similar to C, the majority of the N stock was stored in the soil compartment. Tree biomass contributed substantially to the ecosystem N stock, whereas ground vegetation N stocks were small in comparison to the other compartments.

Statistical analyses did not show any significant effect on ecosystem N stock of either disc trenching or fertilisation alone, or in their interaction (Table 3).

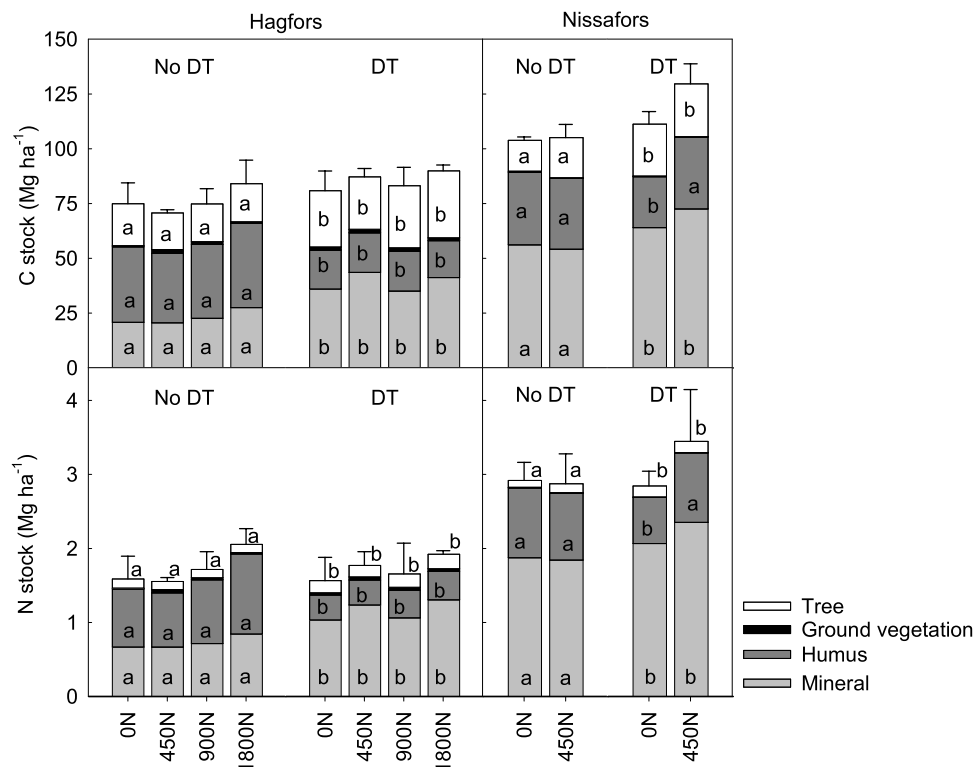


Fig. 3. C and N stocks in ecosystem, tree biomass, ground vegetation, humus layer, and mineral soil down to a depth of 20 cm for non-disc-trenched treatment (No DT) and disc-trenched treatment (DT) with various nitrogen additions at Hagfors and Nissafors. Error bars denote one standard error for the ecosystem C and N stock. Different letters denote a significant difference ($p < 0.05$) between treatments (fertilisation or disc trenching (DT)) within a specific site for the tree, humus and mineral soil compartments. For further details regarding significant differences in C and N stock in ground vegetation and ecosystem, mean and standard deviation for different compartments see Table S1 and S2.

Table 3

p-values of the ANOVAs for Hagfors and Nissafors, respectively, referring to the effect of fertilisation (Fert), disc trenching (DT) and their interaction on C and N stocks in the entire ecosystem (trees, ground vegetation, and soil) and soil (humus layer and mineral soil down to 20 cm depth). Full Type 3 tests of fixed effects, including degrees of freedom and F values, are provided in Supplement (Table S5-S6).

Variable	Site	Fixed effects	Ecosystem	Tree	Veg	Soil	Humus	0–20 cm
C stock	Hagfors	Fert	0.62	0.50	**	0.65	0.95	0.34
		DT	0.10	***	**	0.85	***	***
		Fert x DT	0.84	0.47	*	0.53	0.55	0.44
		Fert	0.29	0.29	*	0.46	0.15	0.69
	Nissafors	DT	*	**	***	0.12	0.06	*
N stock	Hagfors	Fert x DT	0.06	0.37	0.18	0.07	*	0.17
		Fert	0.12	0.51	**	0.09	0.22	0.09
		DT	0.97	***	**	0.49	***	***
		Fert x DT	0.61	0.45	0.06	0.52	0.15	0.53
	Nissafors	Fert	0.45	0.31	0.47	0.49	0.05	0.70
		DT	0.12	**	***	0.20	*	*
		Fert x DT	0.06	0.35	0.35	0.07	*	0.13

Note: Organic matter embedded in the mineral soil was considered part of the mineral soil. * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$.

3.2. Soil C and N stocks

Seventeen years after disc trenching, the buried layer of soil organic matter within the tilts remained clearly visible at both Hagfors and Nissafors sites. As this layer was included in the mineral soil compartment, it resulted in a significant increase in C stock within this compartment in the DT treatment compared to the no DT treatment at both sites (Fig. 3, Table 3, Table S1). At Hagfors, this increase was accompanied by a significant reduction in C within the top organic layer. At Nissafors, however, a reduction in C in the top organic layer was only observed in the unfertilised DT treatment, whereas the fertilised DT treatment maintained a C stock in the organic layer comparable to that of the no DT treatment.

When considering the entire soil profile, including both the top organic layer and the mineral soil down to a depth of 20 cm, no significant difference in total soil C stock was observed (Fig. 3, Table 3, Table S1). Thus, the observed decrease in C within the top organic layer was offset by an increase in the mineral soil.

Soil C stocks varied across different soil-disturbance types in the DT treatments, with the lowest content in the furrows, the highest in the tilts, and intermediate levels in the undisturbed soil between the furrows (Fig. 4, Table 4). This pattern was consistent for both the entire soil profile and the mineral soil compartment. The C content in the organic layer, however, was significantly lower in both the furrows and the tilts, with the exception of the tilts at Nissafors, which exhibited values equivalent to those of the organic layer in the undisturbed soil between the furrows. At this site, tilts did not always contain mineral soil layer that originated from the furrows, and the sampled organic layer of the tilts therefore likely included both the original organic layer and translocated organic material from the furrows (see Fig. S2). No significant difference in soil C stocks could be observed when comparing the soil in the no DT treatment and the soil between the furrows (which can be assumed to have been undisturbed), for either the entire soil profile or its individual compartments.

Over the 17 years since disc trenching, the organic layer in the furrows has gone from being absent to reaching a C stock of approximately 12.2 Mg ha^{-1} at Hagfors and 7.3 Mg ha^{-1} at Nissafors (Fig. 4). This corresponds to approximately one-third and one-fourth, respectively, of the organic layer C stock in the no DT treatment at each site.

The N stock in the entire soil profile and its compartments followed a similar pattern to that of the C stock at both locations (Fig. 4, Table 4, Table S2).

C and N concentrations in humus layer and mineral soil are presented in Supplement (Table S3-S4).

3.3. Ground vegetation C and N stocks

The C stock in the ground vegetation was minor compared to the soil

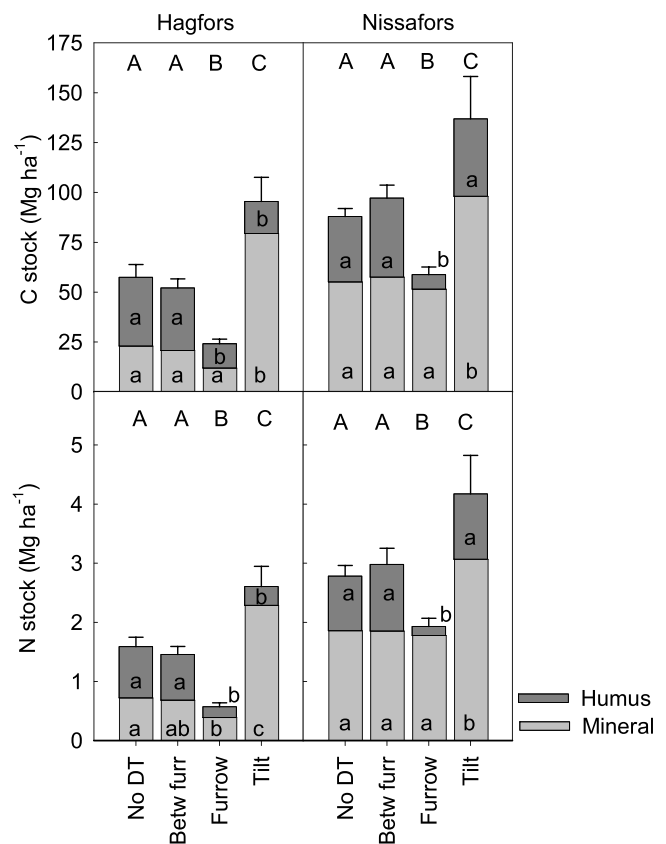


Fig. 4. C and N stocks in whole soil profile, humus layer, and mineral soil to a depth of 20 cm from the original mineral soil surface for different soil disturbance types (no DT, area between furrows, furrow, and tilt) with all N treatments combined at Hagfors and Nissafors. Different lower-case letters denote significant differences between soil disturbance types within each nutrient stock, compartment, and site. Difference in upper-case letters denote significant differences in C or N stock in the whole soil profile between soil disturbance types within each site. Note that organic matter embedded in the mineral soil of tilts was accounted as mineral soil.

and biomass stocks (Fig. 3, Table 3). It ranged from 0.2 to 1.7 Mg C ha^{-1} depending on site and treatment. Despite the low absolute values, statistical analysis revealed significant effects of both fertilisation and DT at both sites (Table 4). At Hagfors, but not at Nissafors, the interaction between these two fixed effects was also significant. At Hagfors, the no DT treatments that were either unfertilised (0 N) or received the highest N addition (1800N) had approximately half as much the vegetation C

Table 4

p-values from the ANOVAs for Hagfors and Nissafors, referring to effects of fertilisation, soil disturbance type (no DT, area between furrows, furrow, and tilt) and their interaction in C and N stocks in whole soil, humus layer, and mineral soil to a reference depth of 20 cm from original soil surface. Full Type 3 tests of fixed effects, including degrees of freedom and F values, are provided in Supplement (Table S7–S8).

Site	Fixed effects	C stock			N stock		
		Soil	Humus	0–20 cm	Soil	Humus	0–20 cm
Hagfors	Fert	0.68	0.99	0.42	0.19	0.39	0.22
	Soil dist. type	***	***	***	***	***	***
	Fert x soil dist. type	0.92	0.50	0.82	0.94	0.14	0.81
Nissafors	Fert	0.35	0.07	0.57	0.33	0.05	0.56
	Soil dist. type	***	***	***	***	***	***
	Fert x soil dist. type	0.21	**	0.76	0.22	**	0.81

Note: Organic matter embedded in the mineral soil of tilts was treated as mineral soil. * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$.

stock as the other treatments. At Nissafors, both fertilisation of the previous stand and disc trenching resulted in lower ground vegetation C stocks.

As the C stock in ground vegetation was minor, the N stocks were also low, ranging from 0.005 to 0.042 Mg N ha⁻¹ depending on treatment and site. The N stock followed a similar pattern across treatments as the C stock.

3.4. Tree C and N stocks

Tree C stocks ranged from 14 to 31 Mg ha⁻¹ depending on site and treatment (Fig. 3, Table S1). DT had a clear effect (Table 4), with the tree C stock in DT treatments being approximately 50% higher than in no DT treatments (cf. Fig. 3). However, earlier fertilisation of the previous stand did not show a significant effect in the ANOVA at either Hagfors or Nissafors (Table 4).

Tree N stock ranged from 0.09 to 0.20 Mg ha⁻¹, with the DT treatment storing, on average, 52% and 44% more N than the no DT treatments at Hagfors and Nissafors, respectively (Table 5).

3.5. Soil fungal communities

In both Hagfors and Nissafors, there were no significant differences in total fungal abundance (i.e., fungal ITS2 copy numbers mg⁻¹ dry soil) or in the abundance of any of the main ecological groups (Fig. S3, Table S10). Likewise, OTU richness of all soil fungi and root-associated ascomycetes did not significantly differ between DT and no DT plots and among the different N fertilisation treatments (Table 5). For ectomycorrhizal fungi, OTU richness was significantly affected by previous fertilisation and the interaction between previous fertilisation and site preparation at Hagfors, but not at Nissafors (Table 5, Fig. 5). In the no DT plots, ectomycorrhizal fungal OTU richness was significantly higher in the unfertilised plots than in the plots that received 450 or 900 kg N ha⁻¹, and tended to be higher than in the plots that received 1800 kg N ha⁻¹ ($p = 0.055$). In the DT plots, on the other hand, there was no significant difference in ectomycorrhizal fungal OTU richness among the fertilisation treatments. For saprotrophic ascomycetes, OTU richness

was significantly higher in the DT plots than in the no DT plots, but did not differ significantly between fertilisation treatments at Nissafors (Fig. S4). At Hagfors, no significant effect of disc trenching or fertilisation was found for OTU richness of saprotrophic ascomycetes, but there was a significant effect of fertilisation on OTU richness of saprotrophic agaricomycetes (Table 5, Table S9). OTU richness of saprotrophic agaricomycetes at Hagfors was higher in the plots that received 1800 kg N ha⁻¹ compared to those that received 900 kg N ha⁻¹, but did not differ significantly from the other fertilisation treatments (Fig. S5, Table S9).

Soil fungal communities differed significantly between Nissafors and Hagfors ($F_{1,28} = 8.3$, $p < 0.001$), and ectomycorrhizal fungal communities tended to differ between fertilised and unfertilised plots at Nissafors ($F_{1,9} = 1.9$, $p = 0.08$, Table S11). At both sites, no significant differences in total fungal communities (Fig. S6), root-associated ascomycete, saprotrophic agaricomycete, saprotrophic ascomycete, or ectomycorrhizal fungal communities were found between DT and no DT treatments and among the various N fertilisation treatments (Table S11).

4. Discussion

Understanding how forest management practices affect long-term carbon (C) and nitrogen (N) dynamics is essential in the context of climate change mitigation. Practices such as mechanical site preparation (MSP) and fertilisation may influence the distribution and storage of C and N in forest ecosystems, but long-term field-based evaluations remain limited. Results from this study show that disc trenching, performed 17 years before this study, led to no change in soil C and N stocks, and increased tree biomass C and N, resulting in statistically significant increase in total ecosystem C stock in one of the two study sites. In contrast, N fertilisation applied to the previous stand did not have any clear effect on C or N stocks in the subsequent stand, in either the soil or in the various compartments of the biomass. The contribution of ground vegetation to the total ecosystem C and N stocks was minor. These results are in line with the expectations for the disc trenching treatment. However, the expected persistent effect of N fertilisation on soil N stocks was not observed.

Table 5

p-values from the linear mixed models for Hagfors and Nissafors, referring to effects of fertilisation (Fert), disc trenching (DT), and their interaction on OTU richness of all fungi, root-associated ascomycetes, saprotrophic ascomycetes, saprotrophic agaricomycetes and ectomycorrhizal fungi. Full Type 3 tests of fixed effects, including degrees of freedom and F values, are provided in Supplement (Table S9).

Site	Fixed effects	All fungi	Root-associated ascomycetes	Saprotrophic ascomycetes	Saprotrophic agaricomycetes	Ectomycorrhizal fungi
Hagfors	Fert	0.46	0.88	0.64	*	*
	DT	0.47	0.80	0.19	0.54	0.10
	Fert x DT	0.52	0.67	0.96	0.21	**
Nissafors	Fert	0.70	0.80	0.65	0.91	0.26
	DT	0.18	0.22	*	0.44	0.07
	Fert x DT	0.46	0.32	0.60	0.91	0.61

* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$

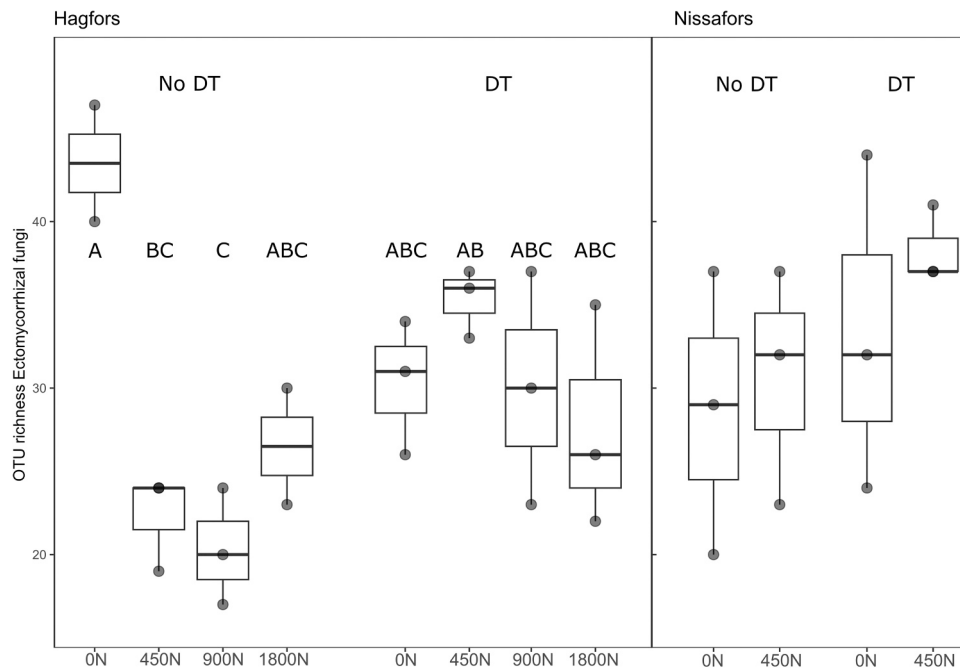


Fig. 5. OTU richness of ectomycorrhizal fungi for non-disc-trenched treatment (No DT) and disc-trenched treatment (DT) with various nitrogen additions at Hagfors and Nissafors. The boxplots indicate the median, first and third quartiles (indicated by the hinges), and largest values up to 1.5 times the inter-quartile range from the first and third quartiles (indicated by the whiskers). Individual data points are overlaid on the boxplots. Different letters denote significant difference between treatments.

4.1. No significant effect of disc trenching on soil C

The results confirm that disc trenching did not significantly affect the total soil C stock, which aligns with our expectations. Instead, a redistribution of organic matter was observed, with lower C stocks in the humus layer and a corresponding increase in the mineral soil. This suggests that the main impact of disc trenching consists in physical relocation rather than net gain or loss of soil carbon. This pattern agrees with earlier studies in boreal and hemiboreal forests, where disc trenching has been shown to change the vertical distribution of soil organic matter without altering the total C pool (e.g. Mjöfors et al., 2017; Marty et al., 2024).

It is also in congruence with *in situ* measurements on soil CO₂ fluxes for two years following site preparation, showing no general increase in soil C loss after either MSP (Strömgren et al., 2017), experimental soil disturbance (Pumpanen et al., 2004; Mjöfors et al., 2015), or stump extraction (Strömgren et al., 2012, 2017). Like MSP, stump extraction disturbs the soil physically, but the proportion of the soil surface area disturbed is greater than for the MSP practices normally used in Fennoscandian forests (cf. Strömgren et al., 2017; Sikström et al., 2020). Furthermore, the removal of a biomass compartment such as the stumps may in turn contribute to a decrease in the buildup of soil C. However, two series of Swedish experimental studies on the long-term consequences of stump harvest for soil C did not observe any significant soil C loss after 25 years (Strömgren et al., 2013) and 32–39 years (Jurevics et al., 2016; see also review by Persson and Egnell, 2018). Taken together, these results suggest no general effect of disc trenching on soil C, which is also supported by the conclusions presented in the reviews by Mäkipää et al. (2023) and Ring and Sikström (2024).

Several earlier reviews raised concerns regarding soil C loss as a result of MSP (e.g., Johnson, 1992; Jandl et al., 2007; Mayer et al., 2020), in contrast to the results of this study. Since field studies on site preparation effects on soil C are scarce, earlier reviews included a variety of methods with different intensities, such as litter raking, prescribed burning, and deep ploughing, applied in a range of forest ecosystems around the world. This may explain the contrasting findings

in our current study, and suggest that the concerns raised may not be valid for disc trenching in boreal and hemiboreal forest ecosystems.

Örlander et al. (1996) measured soil C stocks down to either 25 cm or 100 cm soil depth 29–63 years after site preparation. Each experiment had one control plot without site preparation and one or two plots subjected to either spade inversion or ploughing, disturbing almost the entire soil surface down to a depth of 20 cm. In these experiments, representing high intensity site preparation, soil C losses of 6–41% were observed. Egnell et al. (2015) found that deep ploughing to 50–60 cm combined with stump harvest (a so called “worst case” treatment) reduced the soil C stock by 10–25% compared with manual patch scarification at two experimental sites in Sweden, although the effect was not significant when both sites were analysed together. However, Nordborg et al. (2006), who investigated the same two sites (along with one additional site) 10 years earlier, found no significant overall effect of deep ploughing on soil C stocks. Likewise, Mjöfors et al. (2017), in a study of two other experimental sites subjected to deep ploughing, but no stump removal, did not observe any soil C loss. In contrast to the more intensive methods discussed above, disc trenching involves a more limited disturbance of the soil surface, and our results support the interpretation that this type of site preparation does not lead to measurable losses in total soil C stocks 17 years after treatment. Nevertheless, it is important to recognise the methodological challenges associated with assessing soil C dynamics: small relative changes must be detected against the background of a large and spatially heterogeneous pool, and differences in system boundaries, such as sampling depth or whether disturbed layers are included, can strongly affect estimates and complicate comparisons across studies.

4.2. Disc trenching increased tree C stock

Our findings also supported that disc trenching would enhance tree biomass C stock. This was likely due to improved seedling establishment and growth, facilitated by reduced competition and enhanced soil conditions during the establishment phase, as reported by Johansson et al. (2013) and Hjelm et al. (2025) for the sites used in this study. Similar

effects have been reported in other studies on MSP in boreal forests, with increased tree productivity being a well-documented response (e.g., see reviews by Örlander et al., 1990; Sikström et al., 2020).

Disc trenching affected the ground vegetation C stock in different ways at Hagfors and Nissafors, suggesting that the effect can be site specific. This C stock was however small in comparison to the other stocks measured, and makes a minor contribution to total ecosystem C stock.

The net effect at the ecosystem level was an increase in total C stock, primarily driven by tree biomass accumulation rather than changes in soil C pools. This was however only significant at Nissafors, even if Hagfors showed the same tendency (see Table 4). As the stand continues to develop the proportion of total ecosystem C stock comprised by tree C stock will increase. Because disc trenching resulted in higher tree C stocks as a response to reduced tree mortality and increased growth (Hjelm et al., 2025, cf. Hjelm et al., 2019), it is unlikely that the non-disc-trenched stands will fully compensate for this difference in the near term. The difference in ecosystem C and N stocks may therefore increase during the coming decades, when stand growth rates are still relatively high.

4.3. Disc trenching effects on N stocks mirrored those on C stocks

To our knowledge, only few studies have specifically quantified the long-term effects of MSP on N stocks in boreal forest soils (e.g. Örlander et al., 1996). Most existing research has focused on mineralisation rates, or losses through leaching, rather than total N pool sizes. Our findings therefore contribute novel insights by showing that disc trenching, while significantly altering the spatial distribution of soil N, does not appear to cause long-term losses or gains in total N stock. This supports the interpretation that the observed shifts are primarily due to physical relocation of organic material, rather than altered N cycling or accumulation.

A previous modelling study at the Hagfors site estimated that disc trenching increased N leaching by about 1.5–3.7 kg N ha⁻¹ yr⁻¹ compared to non-disc-trenched treatments during the first 6 years after site preparation (Rappe George et al., 2017). Although this represents a substantial increase in N loss, it corresponds to only a few per mill of the soil N stock, and is therefore difficult to detect by studies of N stock changes.

The effects of disc trenching on N stocks largely mirrored those observed for C stocks. The increase in tree N stock following disc trenching was most likely a direct consequence of enhanced tree growth. However, these estimates should be interpreted with caution, as the calculations were based on generic N concentrations for different tree compartments derived from the literature (see Table 1). Thus, potential changes in tissue N concentration because of treatment were not accounted for.

For ground vegetation, N concentrations were taken from a previous study conducted at the Hagfors site (Johansson et al., 2013). While concentrations differed among fertilisation treatments, no effect of disc trenching on ground vegetation N concentration was observed. Nevertheless, this assumption represents a simplification, and changes in N concentration over time cannot be ruled out. This introduces a degree of uncertainty, particularly at the Nissafors site where N concentration values from Hagfors were used. Despite these limitations, the ground vegetation N stock was small in absolute terms and is therefore unlikely to substantially influence the conclusions regarding total ecosystem N stocks.

4.4. Minor effects of N fertilisation on C and N stocks

Nitrogen fertilisation applied to the previous stand had no significant effect on either soil C or N stocks across the entire soil profile 17 years after final felling. This outcome is consistent with the observations of no significant fertilisation effects on total C and N stocks in soil just prior to

final felling 17 years earlier in the two sites (Ring et al., 2011). Similar results were reported by Larsson et al. (2024), who found no differences in 23-year-old stands, and by Högbom et al. (2001). In their study, repeated fertilisation in the previous rotation at the lower doses (three applications of 120–240 kg N ha⁻¹) did not significantly affect soil C or N stocks in 11-year-old stands. However, Högbom et al. (2001) reported a positive trend in soil C content with increasing N dose when including the entire range of N doses (three applications of 0–600 kg N ha⁻¹).

In contrast to the results from our study, increases in soil C stocks are frequently observed following N fertilisation, particularly in the organic layer (eg. Hyvönen et al., 2007; Mäkipää et al., 2023). Recent evidence supports a transient impact of fertilisation on soil C. Boeraeve et al. (2025) found that while the organic layer C stock was significantly higher one year after final felling in previously fertilised stands, this effect could not be detected in independently sampled stands aged 4–13 years. This indicates that fertilisation effects on soil C stocks may diminish over time. At our study sites, however, fertilisation had no significant effect on soil C stocks either prior to final felling (Ring et al., 2011) or 17 years after final felling.

Just as no effect was observed of previous fertilisation on soil C and N stocks, no significant effect was observed on either the C and N stocks in trees. This suggests that any growth-promoting effects of past N addition on the next rotation is limited (Pettersson and Högbom, 2004).

4.5. Methodological considerations and limitations

In boreal forests, investigations of long-term effects of forest management require study sites, like Hagfors and Nissafors, where treatments have been performed and documented over several decades. Maintaining this type of sites is challenging for several reasons, which is why the number of long-term sites is limited. We regard the forest stands, soils, and site preparation practice used at Hagfors and Nissafors as representative of many productive forests in these regions. In a Swedish context, the investigated N fertilisation treatments all exceed the maximum total application rates according to current national guidelines (Swedish Forest Agency, 2025a, b; Jämtgård et al., 2026). However, the wide span in N application (at Hagfors) helps identify and understand the impact.

One common challenge in *in situ* studies of soil C dynamics and soil fungal communities is that potential changes can be hidden by natural variability. Soil C stocks are large, while the expected changes are relatively small: possibly substantial in ecological terms, yet difficult to detect statistically. Similarly, due to the patchy distribution of fungal mycelia, between-sample variation can be high. Addressing this issue requires a high number of true replicates to increase statistical power; however, this is rarely feasible, as each plot must be large enough to allow for reliable measurements of tree growth and associated changes in tree C stocks. In our study, this limitation is particularly relevant for the fertilisation treatments, where the number of replicates is constrained. In contrast, the disc trenching treatment benefits from a high number of replicates, as plots from the original fertilisation experiment were divided into two sub-plots. However, it also imposes a limitation: the smaller plot size may reduce the duration over which tree biomass can be accurately monitored, particularly as trees grow and edge effects become more pronounced.

Soil C stocks in the present study were similar to or higher than those reported for Hagfors and Nissafors prior to clear-felling by Ring et al. (2011), after correcting for stoniness, which was not accounted for in the earlier study. Organic layer C stocks at Hagfors were higher in our study. The underlying causes of these differences in soil C between occasion remain unclear but may reflect differences for instance in sampling procedures and methodology. We therefore believe that the changes in soil C stocks at the Hagfors and Nissafors sites do not indicate true differences, while the relative patterns within each sampling occasion remain quite robust.

At the Hagfors site, the high abundance of rocks and boulders made

soil sampling particularly challenging, especially in deeper layers. As a result, certain assumptions had to be made to estimate the C and N content in the 10–20 cm depth interval from the original soil surface, as sampling could not be conducted as originally planned. These estimates were based on a limited number of samples for both the tilts and the no DT treatments (Table S1). For the tilts, assumptions were also required for the 0–10 cm mineral soil layer. However, given that both C and N concentrations typically decline with soil depth, and no significant differences were observed in the 0–10 cm layer between treatments (as described in the Materials and Methods section), we consider the impact of these assumptions on the interpretation of the results to be minor.

4.6. Organic layer C declines after final felling may reflect effects of mechanical site preparation

Harvesting is known to reduce soil C stocks (Mäkipää et al., 2023). A recent study based on national forest inventory data from Nordic countries and Canada reported a long-lasting decline in C stocks in the organic layer following final felling (Johannesson et al., 2025, cf. Piirainen et al., 2015). Similar results were earlier reported in a meta-analysis for temperate forests (Nave et al., 2010). However, final felling may be followed by for example MSP, which is common in Sweden and Finland, countries from which a large share of the data in Johannesson et al. (2025) originates. It is therefore difficult to disentangle the effect of harvesting from MSP in these studies. The low C stocks found in the organic layer after MSP in our study suggest that the decline in organic layer C stocks after final felling reported by Johannesson et al. (2025) may partly be attributed to mechanical site preparation.

While the mineral soil C stocks (0–20 cm) were not significantly affected in the studies by Johannesson et al. (2025) and Piirainen et al. (2015), the decrease in the organic layer C stock in our study was followed by an increase in mineral soil C stock. One explanation for this discrepancy may lie in methodological differences. Estimates of mineral soil C stocks are sensitive to sampling design. Many inventories and studies measure to a fixed mineral soil depth without accounting for physical disturbance from MSP, which can introduce bias. In contrast, our study explicitly considered the effects of such disturbance, which may explain the divergent results.

4.7. Limited soil fungal community responses

Overall, the differences in soil fungal communities in the organic soil layer among the different treatments were relatively limited, and varied between the two study sites and among the different ecological groups. Interestingly, OTU richness of ectomycorrhizal fungi at Hagfors was significantly affected by the interaction between fertilisation and disc trenching. More specifically, in non-disc-trenched plots, ectomycorrhizal fungal OTU richness was significantly higher in the unfertilised plots compared to all three fertilisation treatments. However, in the disc-trenched plots, there was no difference between the fertilisation treatments, suggesting that the disc trenching annihilates the fertilisation effect on ectomycorrhizal fungi. This is in accordance with the results of Bzdyk et al. (2022), who found negative effects of MSP on ectomycorrhizal root colonization levels and of Boeraeve et al. (2025), who also found no differences in ectomycorrhizal fungal communities between clearcuts of fertilised and unfertilised forests, all of which had been subjected to MSP. Despite significant effects of fertilisation and disc trenching on the (mostly ericoid) ground vegetation, no significant effect was found of either treatment on the root-associated ascomycetes (which include ericoid mycorrhizal fungi). Contrary to our expectations, disc trenching had limited effects on saprotrophic fungi, with only an increase in OTU richness of saprotrophic ascomycetes at Nissafors, and no effect at Hagfors or on community composition.

4.8. Concluding remarks and implications

By examining the long-term effects of disc trenching and previous stand fertilisation on ecosystem C and N stocks and soil fungal communities in two field experiments, this study provides new insights into how these common forestry practices influence ecosystem C and N stock dynamics and soil fungal communities during the first decades after stand establishment. Taken together, the results from this study suggest that, under current forestry practices, disc trenching can promote tree growth and increase ecosystem C stocks without causing substantial long-term reductions in soil C or N stocks. This supports its role as an effective regeneration practice in boreal and hemiboreal forestry as currently practised. However, potential side effects must also be considered, such as impacts on N leaching, ground vegetation cover, and erosion. From a climate perspective, these findings are encouraging, although minimising soil disturbance remains an important principle to avoid other undesirable effects of MSP.

Furthermore, the study found no evidence that N fertilisation of the previous stand resulted in persistent carry-over effects on soil or biomass C and N stocks in the subsequent rotation. This indicates that the climate mitigation potential of stand-level N fertilisation is limited in the long term, at least under the conditions studied.

The analyses of soil fungal communities in the soil organic layer 17 years after MSP revealed limited treatment effects and no lasting legacy of either disc trenching or fertilisation in the previous stand. However, we did find some indication that fertilisation and disc trenching can negatively affect ectomycorrhizal fungi, but the effect was site-specific. Overall, the main long-term consequences of disc trenching and fertilisation seem to be more pronounced in tree growth and ecosystem level C stock, rather than in fungal communities of the soil organic layer.

Finally, the study highlights the need for long-term experiments extending beyond the first two decades following mechanical site preparation, particularly to capture stand-level biomass development. Accurate assessment of soil C stocks requires systematic and appropriate sampling strategies that account for the physical changes introduced by MSP.

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CRedit authorship contribution statement

Karin Hjelm: Writing – review & editing, Resources. **Eva Ring:** Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Margaux Boeraeve:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Monika Strömgren:** Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lars Högbom:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (OpenAI) in order to improve clarity and quality of language and to suggest structure of the discussion. After using this tool, the authors

reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2026.123745](https://doi.org/10.1016/j.foreco.2026.123745).

Data availability

Data will be made available on request.

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