

## *Soil carbon and nitrogen stocks under anthropogenic influence: A case study in European beech forests of Central Balkan Mountains (Stara Planina), Bulgaria*

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**Abstract.** Understanding how forest management practices influence soil carbon and nitrogen dynamics is crucial for sustainable ecosystem functioning and climate mitigation. This study investigates the effects of thinning on soil organic carbon (SOC) and total nitrogen (TN) stocks in temperate European beech (*Fagus sylvatica* L.) forests in the Central Balkan Mountains of Bulgaria. Fieldwork was conducted in six forest districts using a replicated plot design with three thinning intensities: unmanaged (0%), moderate (10–15%), and higher (20–25%). Soil samples were collected from three depth intervals (0–10, 10–20, and 20–30 cm) and analyzed for SOC, TN, and related properties following standardized laboratory protocols. Results showed that SOC and TN concentrations decreased consistently with depth across all treatments. While higher thinning intensity was associated with modest reductions in subsoil SOC and TN, the overall stocks to 30 cm depth did not differ significantly between managed and control plots. The data suggest that brown forest soils (Cambisols) exhibit strong resilience to moderate thinning, likely due to conservative harvesting practices, stable canopy structure, and the retention of organic inputs. These findings highlight that moderate-intensity thinning, as applied under Bulgarian forestry regulations, can be compatible with the preservation of soil carbon and nitrogen pools. The study emphasizes the importance of site-specific management and long-term monitoring to ensure soil sustainability under changing climatic and land-use conditions.

**Key words:** temperate Cambisols, carbon stock, anthropogenic influence, beech forests.

### Introduction

Forests cover about 4–5 billion hectares, which is roughly 30% of the Earth's terrestrial area (Bastin et al., 2017) and their soils are widely recognized as important reservoirs of stable carbon (C) in the biosphere, storing significant amounts of soil organic carbon (SOC) that play a crucial role in the global carbon cycle (Lal, 2005; Pan et al., 2011). The balance of organic inputs through litterfall and root deposition and the process of decomposition of organic matter, which is influenced by organic matter chemistry, soil properties, climate and site conditions, determines the development of soil carbon stocks. Changes in soil organic matter (SOM) inputs and carbon losses

influence the soil carbon balance. These changes may result from natural factors, such as wildfires and pest activity, or human activities, including land use and management practices (Smith, 2008).

Forest thinning is a management practice that selectively removes trees to increase the availability of resources to the remaining trees improving their growth and productivity (Nazari et al., 2023). Thinning enhances tree growth and alters forest structure (Shen et al., 2019; Loverin et al., 2024). By reducing competition for nutrients, water and light among the remaining trees, thinning leads to increased tree size and timber quality, which has positive economic effects (Horner et al., 2010). Thinning practices also affect the soil

properties. According to some studies, thinning activities can reduce the soil organic carbon (SOC) by decreasing litterfall inputs and through microclimate-driven warming and acceleration of decomposition rates (Jandl et al., 2007; James & Harrison, 2016), with a moderate intensity thinning (on average 12% of total volume) showing higher dissolved organic carbon and biological activity (Romeo et al., 2020). At the same time other studies reported no significant effects on mineral soil C stocks after thinning (Zhou et al., 2013; Noormets et al., 2015; Strukelj et al., 2015; Kim et al., 2018). However, controversy and debate persist, particularly over whether undisturbed forests store more SOC than managed forests. These uncertainties arise from variations in study findings, differences in methodology, and the complex interactions between forest management practices, vegetation, soil types, and environmental conditions (Clarke et al., 2015; Dean et al., 2017). In addition, the effects of thinning on SOC are influenced by factors such as intensity, recovery time, and forest type.

European beech (*Fagus sylvatica* L.) is a dominant and ecologically significant tree species in Europe (Černý et al., 2024), occupying mountain areas in Bulgaria (Tzonev et al., 2006; Grigorov et al., 2023). The undisturbed beech forests are found to store approximately 15% more SOC than managed forests, with significant differences in the subsoil (Leuschner et al., 2022). Depending on the occurrence of natural disturbances or the harvesting system used, the amount, composition and distribution of harvest debris, including branches, tops, rooted snags and pre-existing woody debris, can vary considerably (Udali et al., 2024). For example, the conventional stem-only harvesting system results in the retention of woody debris, whereas whole-tree harvesting is usually associated with higher soil disturbance and limited amounts of debris left on site (Mäkipää et al., 2023). The residues and the woody debris are a significant reservoir of carbon, nitrogen and various nutrients suggesting that its removal could negatively impact soil processes and carbon sequestration (González-Polo et al., 2013; Stutz & Lang, 2017). This indicates that forest management practices and natural processes are important in preserving and enhancing soil carbon pools in forests.

Furthermore, research focused on specific regions is refining our understanding of the effects of management and land use on SOC in soils and providing important information regarding region-specific findings. For example, recent studies in Bulgaria have highlighted the effect of the land use changes in SOC stock (Zhiyanski et al., 2016) and assess the implications of natural disturbance and felling intensity on soil parameters (Kirova & Zhiyanski, 2021; Kirova, 2024). However, these studies have been conducted on a very small scale with small sample sizes, which limits the ability to draw region-specific conclusions.

To gain a more comprehensive understanding of how thinning affects the accumulation of soil organic carbon in broadleaf forests, we analyzed key soil parameters under different thinning intensities in beech stands (*Fagus sylvatica* L.) compared to unthinned controls at six forest districts in the Central Balkan Mountains in Bulgaria. We hypothesized that thinning would alter some soil properties leading to increased carbon and nitrogen storage, with moderate thinning and extended recovery time generally favoring SOC accumulation.

## Materials and methods

### Site description

This study was conducted in 2022 in six State Forestry Enterprises, located in the middle part of Stara Planina/Balkan Mountains in Bulgaria, on the north slopes of the mountain – Etropole, Teteven, Troian, Apriltsi, Rositsa and Gabrovo (Fig. 1). The research area lies at an altitude between 950 – 1150 m above sea level. The soils in the studied fields are brown forest soils (Cambisols, WRB 2014). The silvicultural treatment applied in the study area was thinning at varying intensities – 0%, 10–15%, and 20–25% – based on data from the forest management plans for the territories of the studied State Forest Enterprises.

### Experimental design

In each State Forestry, 3 sampling plots have been set in natural beech forests (*Fagus sylvatica* L.): 1) M0 - controls – unmanaged plots, without any activities for more than 20 years; 2) M10 - thinned with 10-15% intensity in the past 10 years; and 3) M20 - thinned with 20-25% intensity in the past 10 years.

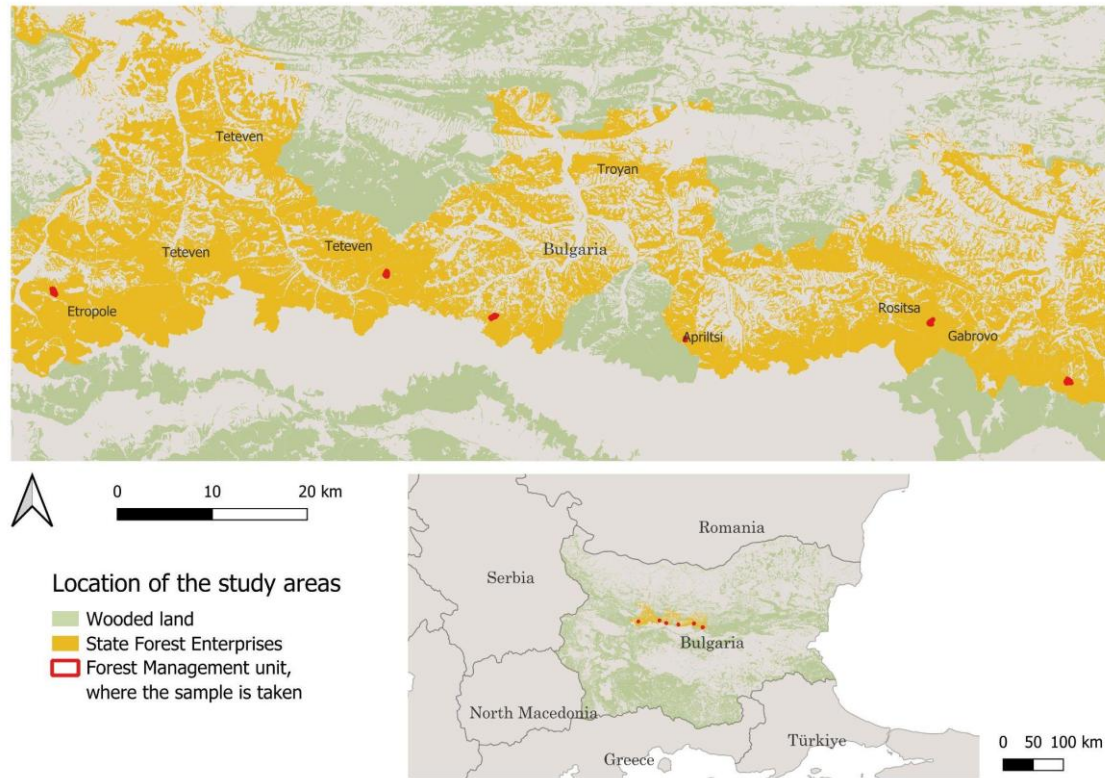


Fig. 1. Study area in the central part of Balkan Mountains, Bulgaria.

Table 1. Characteristics of the sampling plots

Each sampling plot is with a size of 0.1 ha. Soil samples were collected with a stainless-steel auger at depths of 0–10, 10–20, and 20–30 cm (3 replicates per depth), following stratified random sampling to account for microrelief variations (FAO, 2006). Samples were stored in labeled plastic bags, and wet weight was measured within 2 hours of collection (Blake & Hartge, 1986).

#### *Measurements and Laboratory analyses*

In the laboratory, the soil samples were dried at 105°C for 24 h to calculate the soil moisture content and dry bulk density (Donov et al., 1974).

The content of the coarse fragments was determined by weight method (Donov et al., 1974), which includes the following procedures: 1) a mean sample close to 100 g was taken from the dried soil sample; 2) it was weighted; 3) all the organics were washed; 4) the residue was dried and sieved through 2 mm sieve; 5) all coarse fragments > 2 mm were measured and their content was calculated.

The soil texture was determined by Kaczynski's pipette method, in which the sample was treated with hydrochloric acid (Donov et al., 1974).

In order to measure the soil's chemical properties, soil particles <2 mm were used for the experiments. The Tjurin method (Donov et al., 1974) was used to determine the organic carbon (OC) content as percentage in the soil - oxidation with a bichrome mixture  $K_2Cr_2O_7/H_2SO_4$  at a temperature of 160°C for 20 min with a pumice catalyst and silver sulfate  $Ag_2SO_4$ , followed by a titration with a 0.2N solution of Morr's salt  $(NH_4)_2SO_4.FeSO_4.6H_2O$ , with phenylanthranilic acid as an indicator. The Kjeldahl method (Donov et al., 1974) was used to measure total N, for the soil and forest floor samples - ISO 11261.

#### *Data processing and statistical analysis*

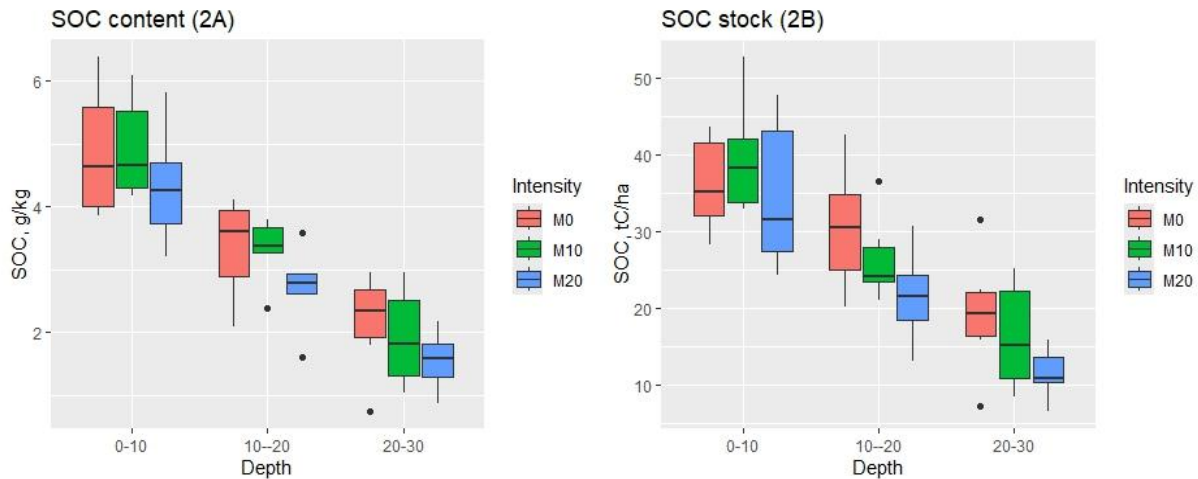
The results of the analyses of the samples were statistically processed. An average value together with the standard error for each individual sample plot was estimated from the 3 replicates of the sample. Carbon and nitrogen stocks were derived using the IPCC GPG-LULUCF formula (IPCC, 2003), adapted for nitrogen stocks by Ellert & Bettany (1995). The data were then

grouped and analyzed considering the different layers and management regimes. The data distribution of the individual groups (i.e by layers and by treatment, 9 groups), were tested for normality with the Shapiro-Wilk test. A two-way ANOVA analysis was conducted to assess the impact of thinning and soil depth, and their interaction on the C and N stock as well as the C:N ratio. In addition, the same analyses on C stock, N stock and the C:N ratio were also conducted for the whole studied depth (0–30 cm). The C and N stocks for 0–30 cm were derived as a sum of the stocks in the analyzed layers. The CN ratio was estimated as an average value. The processing of the data and the statistical analysis were done in R programming.

#### **Results**

All examined soils belong to textural class sandy-loamy - light to heavy, with clay content between 38 and 42%, and 54–62% sand. Bulk density (BD) in all plots increased with depth, varying between 1.05 and 1.19 g/cm<sup>3</sup> for the controls (M0), 1.08–1.16 for the managed with 10–15% intensity plots (M10), and from 1.03 to 1.16 for M20 plots. Coarse fraction content follows the same trend of increasing in depth as BD in the managed plots with values between 25–29% for M10, and between - 22–36% in M20 plots. The CF is decreasing in depth in the not managed plots varying between 27% and 24% (data not presented).

The SOC content is highest in the topsoil layer for all the treatments, and it is characterized with higher dispersion. There is a clear trend for decrease of the SOC content in depth in the three studied intensities (Fig.2A). In the low intensity managed plots, the soil organic carbon content is similar with the controls for 0–10 and 10–20 cm layers, and a trend for decreasing is found in the 20–30 cm layer. For the plots with higher intensity of the management (M20) SOC content is lower in all studied depths compared to the controls (Fig. 2A). SOC stock follows the trend of SOC content for the topsoil layers, and it decreases in all other depths compared to the controls for both intensities (Fig. 2B). For SOC and SOC stock the distribution of the data is normal, although the observed outliers.

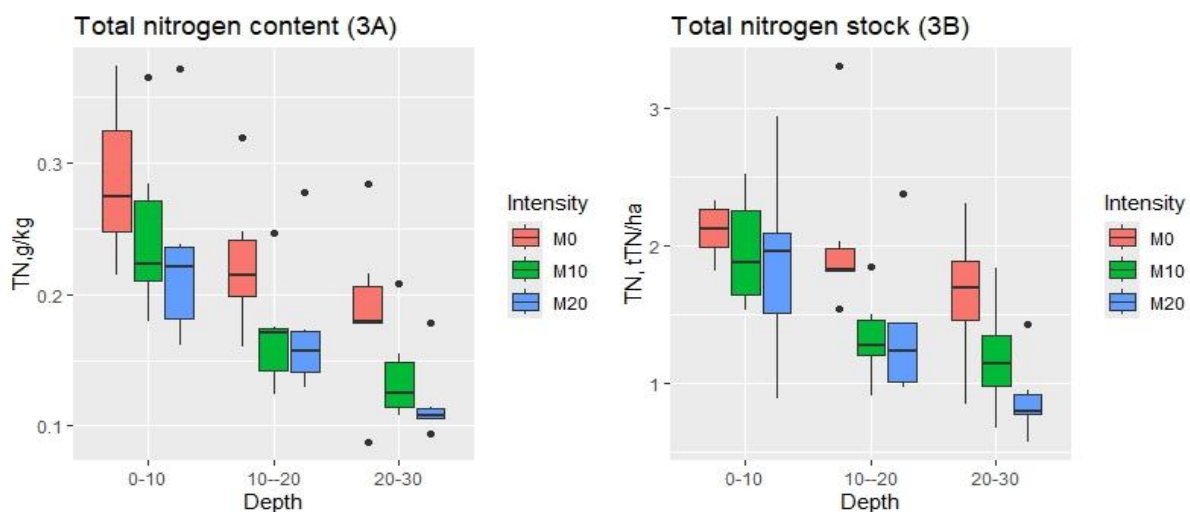


**Fig. 2.** SOC content (2A) and SOC stock (2B) for the controls and two thinning intensities, grouped by soil layers.

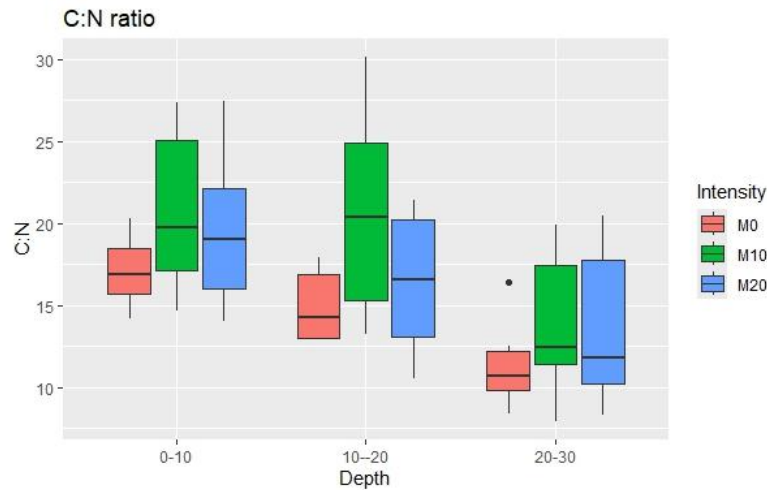
The nitrogen content follows the same tendency as the carbon content - with highest values in the topsoil layer and decreasing in depth for all the treatments. The dispersion of the TN content, however, is again higher in the 0-10 cm layer, compared to the other two depths, where most of the outliers are detected. It is also noticeable that the TN content in depth has lower variability in all the plots. For all the treatments the total nitrogen content (TN) and the TN stock in the managed plots (M10 and M20) are similar in between, and lower compared to the controls for the three studied soil layers. The differences are greater in the 10-20 and 20-30 cm layers (Fig. 3A and 3B). The normality test shows that all the distributions of

TN content and TN stock are normal, except the TN content in 10-20 cm layer under M10, 20-30 cm under M10 and M20 and in the N stock of 20-30 cm under M20. However, these distributions are close to normality, which allows further statistical analyses.

CN ratio varies in depth and between the management activities. There is a noticeable trend of gradual decrease in depth of the ratio in the not managed plots as well as in the plots with 20% thinning intensity. The largest variability is observed in the data from M10 plots and the lowest in control plots (Fig.4). Data distribution of CN ratio is normal in all the analyzed groups according to the Shapiro Wilk test.



**Fig. 3.** Total nitrogen content (3A) and total nitrogen stock (3B) for the controls and two thinning intensities, grouped by soil layers.



**Fig. 4.** Carbon-nitrogen ratio (C:N) for the controls and two thinning intensities, grouped by soil layers.

The analysis of variance shows that there are significant differences in means of the studied parameters in respect to the different depth and intensity treatment. Depth is the most significant factor variable in all the parameters with p-values < 0.001. The thinning intensity is also a significant factor considering the analyzed parameters with p-values ranging between 0.01-0.05. The analysis of the variance was then followed by a Tukey test to

find the means that are significantly different from each other. The results of the test are presented in Table 2.

Despite the observed differences between the studied soil layers, the ANOVA test for the whole studied depth - 0-30 cm, does not show that the plots differ significantly, considering the management (Table 3).

**Table 2.** Mean values and standard deviations of soil parameters referred to three layers of soil (0-10; 10-20; 20-30 cm).

| Depth     | 0-10                 |                      |                    | 10-20             |                    |                    | 20-30             |                    |                    |
|-----------|----------------------|----------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|--------------------|
| Intensity | M0                   | M10                  | M20                | M0                | M10                | M20                | M0                | M10                | M20                |
| SOC       | 4.86±1.04<br>aa      | 4.93±0.82<br>aa      | 4.32±0.92<br>aa    | 3.35±0.80<br>ba   | 3.32±0.50<br>ba    | 2.72±0.65<br>ba    | 2.15±0.80<br>ca   | 1.92±0.78<br>ca    | 1.55±0.47<br>ca    |
| TN        | 0.29±0.06<br>aa      | 0.25±0.07<br>ab      | 0.23±0.08<br>ab    | 0.23±0.05<br>4 ba | 0.17±0.04<br>3 bb  | 0.17±0.05<br>bb    | 0.19±0.06<br>ba   | 0.14±0.04<br>bb    | 0.12±0.03<br>bb    |
| CN        | 17.10±2.2<br>2<br>ab | 20.79±5.2<br>1<br>aa | 19.62±5.01<br>aab  | 14.96±2.3<br>1 ab | 20.70±6.6<br>5 aa  | 16.41±4.4<br>8 aab | 11.41±2.8<br>4 bb | 13.81±4.6<br>9 ba  | 13.64±5.1<br>1 bab |
| SOC stock | 36.15±6.2<br>6<br>aa | 39.61±7.6<br>7 aab   | 34.69±10.0<br>7 ab | 30.49±8.2<br>0 ba | 26.33±5.6<br>5 bab | 21.51±6.1<br>3 bb  | 19.20±8.0<br>5 ca | 16.26±7.1<br>7 cab | 11.4±3.31<br>cb    |
| TN stock  | 2.11±0.20<br>aa      | 1.96±0.41<br>ab      | 1.87±0.70<br>ab    | 2.06±0.63<br>ba   | 1.34±0.32<br>bb    | 1.38±0.54<br>bb    | 1.65±0.50<br>ba   | 1.19±0.41<br>bb    | 0.89±0.30<br>bb    |

*Legend:* soil organic carbon (SOC, %); total nitrogen (TN, %); C/N ratio (CN); carbon stock (Cstock, t/ha); total nitrogen stock (Nstock).

For each parameter the sample size was n=6 per layer for treatment.

Means by depth followed by the same letter combination are not statistically different at  $p \leq 0.05$  (two-way ANOVA followed by Tukey test).

**Table 3.** Soil organic carbon stock (SOC stock, t/ha), total nitrogen stock (TN stock, t/ha) and carbon-nitrogen ratio (CN ratio) for the 0-30 cm depth, for the three intensities.

| Intensity | SOC stock   | TN stock  | CN ratio   |
|-----------|-------------|-----------|------------|
| M0        | 85.83±17.93 | 5.82±0.99 | 14.49±2.35 |
| M10       | 82.21±16.38 | 4.49±1.08 | 18.43±5.06 |
| M20       | 67.60±18.67 | 4.14±1.46 | 16.56±4.40 |

## Discussion

Our study reflects the need for more extensive research on forest soils at a regional scale in Bulgaria to better understand the effects of thinning on soil carbon and nitrogen stocks. By increasing the number of plots and expanding the spatial scope across six forest districts, this study improves upon earlier local-scale investigations and enhances the reliability of observed trends. It aims to build on existing findings from other small-scale studies by expanding the investigation to a larger scale.

Earlier local studies conducted in the central part of the Balkan Mountains found no significant differences in carbon content after thinning in beech forests but showed a slight increase in soil organic carbon (SOC) stocks in deeper soil layers (Kirova, 2018). Similarly, a later study observed comparable changes in SOC stocks across the entire depth studied in the western part of the Balkan Mountains (Kirova & Zhiyanski, 2021), and the latest presented no significant differences in the SOC and TN stocks (Kirova, 2024). In contrast, the present study observed slight decreases in SOC and TN content and stocks in thinned plots, especially in the subsoil layers, particularly under higher thinning intensity. However, these changes were not statistically significant at the profile level (0–30 cm), supporting the notion that thinning under current forest management practices in Bulgaria does not lead to substantial soil degradation.

This divergence from previous findings may be attributed to several factors, including differences in sampling design, landscape variability, and recovery time since thinning. The inclusion of multiple forest districts in this study allowed for capturing greater heterogeneity in site conditions, which strengthens the robustness of our conclusions.

As discussed in the introduction, there are global studies reporting contrasting results on the change in soil parameters after thinning, emphasizing that the outcome depends on many factors such as harvesting system, intensity, forest type, climatic conditions, etc. The lack of effect on stands could be due to the thinning intensities studied in this experiment, which are limited to 30% by national regulations.

In addition, the harvesting system used in Bulgaria is cut-to-length harvesting, and the timber export processes do not involve the use of heavy mechanization, which contributes to soil disturbance. The cut-to-length harvesting system is associated with more biomass left in place compared to the conventional whole-tree harvesting system (Huber et al., 2017).

Another aspect is the type of thinning conducted during stand cultivation. In Bulgaria, forest management practices are dominated by thinning from below. This is usually associated with the preservation of the canopy cover, which limits the amount of light that can penetrate. This influences microclimatic conditions (e.g., temperature, moisture). Although in our study this is not an object of discussion, it is worth noting that opening the canopy cover allows more light and rainfall to reach the forest floor. This stimulates microbial activity, leading, on one side, to faster decomposition of litter and increased nitrogen mineralization. The decreased number of trees, on the other side, leads to reduced leaf, branch, and root litterfall. This reduction in organic material limits the inputs of N and C to the soil, slowing nutrient cycling.

The balance between these opposing forces – enhanced decomposition due to a warmer, more open microclimate, and reduced organic matter inputs from thinning, may explain the slight

decreases observed in SOC and TN under higher thinning intensity.

In brown forest soils, SOC levels often recover within several years after thinning due to organic matter inputs from decomposition of logging residues and regeneration of vegetation (Tejedor et al., 2017). This resilience depends on the thinning intensity and post-thinning management practices, which means that the observed soils have a good retention to the observed thinning intensities.

The observed variation in C:N ratio among treatments, particularly the greater fluctuations under moderate thinning (M10), may reflect altered organic matter quality and microbial community dynamics. Stable C:N ratios in unmanaged plots suggest that undisturbed soils maintain more balanced nutrient cycling processes.

Additionally, while this study did not assess microbial activity, enzyme dynamics, or below-ground biomass directly, these are known to influence SOC stabilization. Thinning could reduce root biomass and rhizodeposition, especially in deeper layers, thus contributing to the slightly lower subsoil carbon and nitrogen stocks in managed plots. Conversely, increased understorey vegetation following canopy opening may offset these effects by enhancing organic inputs in surface layers.

After all, the expectations, after prevailing the first years after cutting – characterized with rapid changes – might be for reaching an equilibrium in the SOC and TN stocks and results close to the non-managed plots, as found in the present study.

Overall, the findings suggest that moderate-intensity thinning, as practiced in Bulgaria, maintains soil carbon and nitrogen pools within a stable range and does not compromise soil health. This highlights the ecological sustainability of these silvicultural interventions, particularly in montane forest ecosystems with relatively undisturbed soil profiles.

## Conclusions

This study provides regionally grounded evidence that moderate-intensity thinning (10–25%) in temperate European beech (*Fagus sylvatica* L.) forests does not significantly alter soil organic carbon (SOC) and total nitrogen (TN) stocks within the upper 30 cm of soil. Despite slight reductions in SOC and TN – particularly in the

subsoil under higher thinning intensity, no statistically significant differences were found between managed and unmanaged plots at the profile scale, indicating the resilience of these forest soils to management interventions.

The results underscore the role of site-specific conditions and sustainable forest management practices in maintaining soil function. The dominance of brown forest soils (Cambisols), combined with conservative thinning practices (cut-to-length harvesting and thinning from below), likely contributes to the buffering capacity observed across the study sites. Such practices preserve canopy cover, minimize soil disturbance, and maintain organic inputs through logging residues and natural regeneration.

Our findings align with broader ecological literature suggesting that the effects of thinning on SOC and TN dynamics are context-dependent and mediated by interactions among harvesting intensity, forest structure, recovery time, and microclimatic feedbacks. The observed variability in C:N ratios and the relative stability of SOC in the topsoil layer further point to a dynamic balance between organic matter inputs and decomposition processes following thinning.

These insights are particularly relevant for forest management in montane regions, where maintaining soil carbon pools is crucial for long-term ecosystem productivity, biodiversity, and climate mitigation goals. The study supports the continued use of moderate, ecologically sensitive thinning as a tool for forest management that can balance timber production with the conservation of belowground carbon and nutrient stocks.

To strengthen these findings, future research should incorporate longer-term monitoring, microbial and enzymatic indicators, and assessments of belowground biomass contributions. Expanding the spatial scope to include additional soil types and climatic gradients would also enhance the applicability of the results for national and regional forest policy planning.

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