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SOIL CARBON AND NITROGEN STOCKS IN SCOTS PINE (*Pinus sylvestris* L.) AFFORESTATION AREAS OF THE ALPINE BELTS OF TURKEY

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ABSTRACT

Forest ecosystems play an important role in reducing the negative effects of global climate change. The soil is considered a significant factor in carbon pools in forest ecosystems. Many studies have been carried out to evaluate carbon and nitrogen stocks in forest ecosystems, but the number of studies examining the change depending on altitude is limited. The study aims to determine the differences in soil organic carbon (SOC) and nitrogen (N) stocks in Scots pine (*Pinus sylvestris* L.) afforestation at different altitudes of the same age in Erzurum province, based on the assumption that SOC and N stocks may vary depending on altitude. In this regard, 22 soil samples were taken from each afforestation from soil depths at 0-10, 10-20, 20-30 by a random method to represent two afforestation. Sampling and evaluation of results were made considering the International Panel on Climatic Change (IPCC) guideline recommendations. Estimated results indicated that soil depth carbon stocks at 0-30 cm are 128.5 Mg C ha^{-1} at high altitude (P1) afforestation and 109.3 Mg C ha^{-1} at low altitude (P2) afforestation. Nitrogen stocks are 10.83 Mg ha^{-1} (P1) and 12.86 Mg ha^{-1} (P2), respectively. Altitude has affected C and N stocks. Hence, this effect should be included for research on stock levels (especially estimation equations). Considering the upper layer's soil organic carbon stock, particularly in silvicultural initiatives in afforestation areas, is critical. Furthermore, the amount of carbon and nitrogen stored in the soil should not be overlooked in greenhouse gas inventories, and ecology-specific management plans should be established in this sense.

Keywords: Scots pine, afforestation, soil organic carbon stock, nitrogen stock, stratification ratio

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INTRODUCTION

Forest ecosystems, covering nearly half of all terrestrial land, are the most important terrestrial carbon (C) cycle components. More than 86% of the carbon pool in vegetation and more than 73% of the carbon pool in the soil are stored in forest ecosystems. The key characteristic of sustainable forest ecosystems and an important component of the global carbon cycle is carbon preservation in soil and biomass. Simultaneously, SOC and N are critical indicators in assessing soil quality and maintaining sustainable land use. As a result, determining C and N stocks in forest soils is essential for understanding how the biogeochemical cycle interacts with climate (Ramesh *et al.,* 2019).

On a global scale, forest vegetation and soil contain about 1146 petagrams of carbon. Soil and peat deposits hold more than two-thirds of the biomass in forest habitats (Dixon *et al.,* 1994). There are five carbon pools in forest ecosystems: trees and ground cover, underground and aboveground biomass, forest floor and dead wood, and soil (Güner and Güner 2020., Weatherall *et al.,* 2022). The amount of carbon and nitrogen retained in mountainous areas varies depending on vegetation, soil type, and land use. Besides, species selection in afforestation is influenced by topography and climatic conditions. The combination of all these variable factors adds carbon retention. C and N stocks in the soil differ depending on soil type, vegetation, land use, slope, slope exposure, precipitation, temperature, and radiation, particularly in mountain terrain (Hoffmann *et al.,* 2014; Lenka *et al.,* 2013; Ping *et al.,* 2015., Çomaklı and Turgut 2021). Changes in C-N stock metrics affect the amount of carbon retained. For example, a low C / N ratio is not suitable for carbon retention as it accelerates the microbial decomposition process (Ferreira *et al.,*2012). The decrease in soil and air temperature with the increase in altitude causes change positively in organic carbon and nitrogen contents (Bangroo *et al.,* 2017; García *et al.,* 2016). The stratification rate (SR) of soil organic carbon (SOC) and N is assessed for soil quality because it reduces soil erosion and enhances water quality. It is used in ecosystem studies, and a high SR indicates improved soil quality (Tfaily *et al.,* 2014). Monitoring and enhancing C and N stocks, one of the most relevant quality measures for soil improvement, is crucial for long-term land management (Ramírez *et al.,* 2021).

Forest ecosystems have the potential to accumulate carbon throughout their life cycle. As a result, among sustainable forest management policy and strategy goals at reducing the impact of climate change, we must assess carbon (C) stocks in natural forests and plantations. (Uddin *et al.,*2019). Afforestation of marginal farmland has positive effects on carbon retention. So that, C retention occurs in both soil and tree biomass with the growth of forests. Changes in the amount of biomass in forests and carbon retained in the soil as well as CO_2 in the atmosphere, are closely related to changes in the amount. However, although the increase in biomass is rapid, especially in the first years of afforestation, the amount of carbon capture in the soil slowly increases. (Ruiz-Peinado *et al.,* 2016).

Forests are identified as essential instruments to minimize climate change impacts by the Intergovernmental Panel on Climate Change (IPCC) and the Kyoto Protocol (KP). The international community pays attention to national and regional monitoring and evaluating forest carbon. Soils constitute a significant part of all carbon pools. The interactions between C and N have become an important research topic in recent years due to their role on important issues such as global climate change, agricultural productivity, and biodiversity. Therefore, it is imperative to estimate the amount of organic carbon and nitrogen stock in the soil.

Several researchers have studied the emergence of carbon and nitrogen stocks in afforestation. However, studies evaluating altitude differences are very few. Under this hypothesis, some soil variables, such as C and N, may vary considerably on a regional and global scale. This study aims to determine carbon and nitrogen stocks of *Pinus sylvestris* L. afforestation at the same age but at different altitudes located in the Alpine belt of Eastern Turkey.

MATERIALS AND METHODS

Description of the study area: The study was carried out in two afforestation areas, Erzurum Palandöken Mountain (P1) (39° 86′N - 41 ° 28′E) and city center (P2) (39 ° 90′N - 41 ° 25′E) (Figure 1). The research area is covered with *Pinus sylvestris* L. trees. In the past, both research areas were used as rangeland. *Pinus sylvestris* L. is the primary species used in afforestation activities in the Erzurum and reaches an altitude of approximately 2500. P1 site is located at 2250 m altitude while P2 is at 1850 m altitude. While the P2 site has a slope of approximately 3%, the average slope of the P1 site is 35%. Each afforestation covers approximately 50 ha.

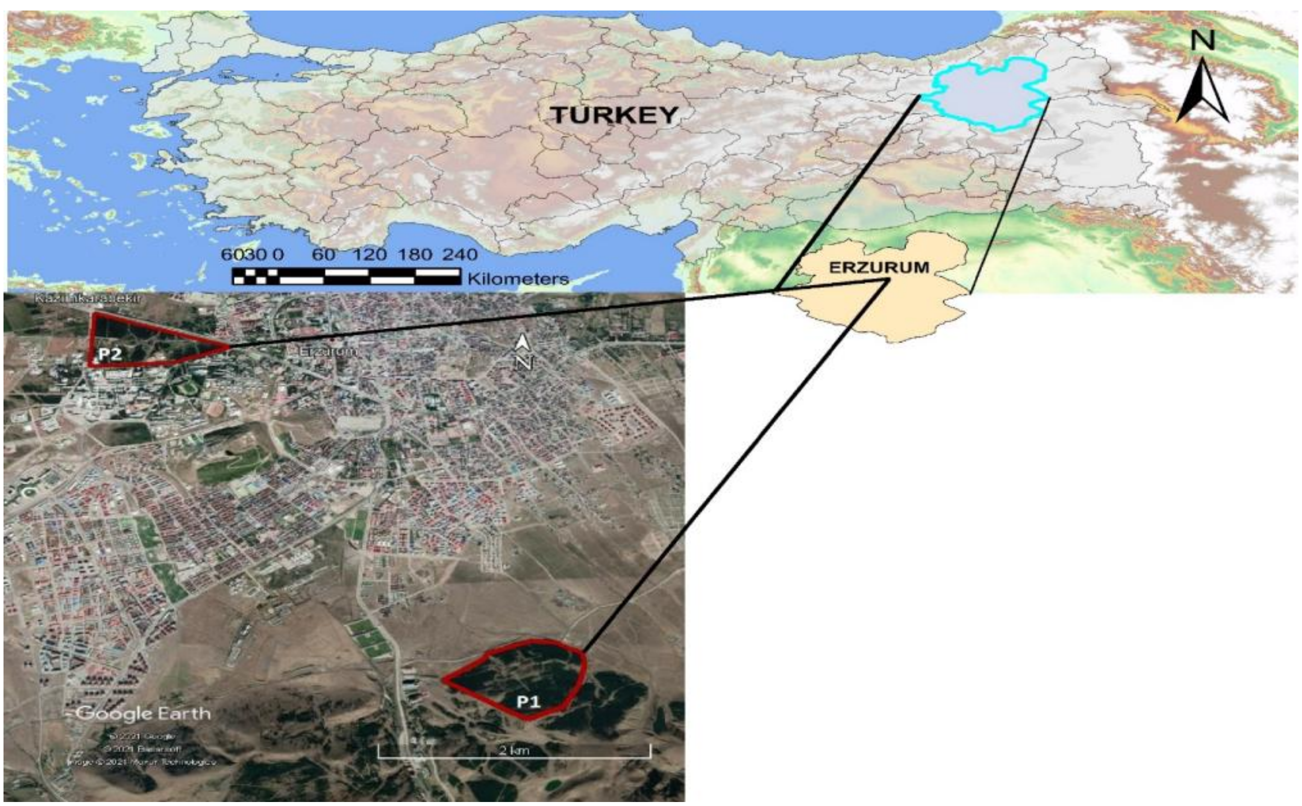

Figure 1. The location of the research area.

Meteorological data were taken from the station data located close to the city center. Long-term mean annual temperature and total annual precipitation are 5.7 ºC and 431.7 mm, respectively. According to Thornthwaite climate classification, the area is half dry, low humidity, and second-degree micro thermal with abundant water supply in winter land climate, coded as C1, C'2, s, b'2. The research areas are in the Iran-Turan flora zone and classified as xeric in humidity regimes. Research areas' soil falls into categories of inceptisol (ordo), ustept (sub ordo), and haplustep (great group) according to the US soil taxonomy (Soil Survey Staff, 1999; Çomaklı and Bingöl 2021).

Data collection and analysis: The stand age was determined with the help of the increasing borer. Field studies were conducted in the fall of 2020, at the age of 35 years of the stand. A total of twenty-two sample points were selected systematically (grid sampling) and 20 m from every sample where they were sampled. Surface soils were 0–10 cm, 10–20 cm, and 20-30 cm in the sites sampled. The sampling points were selected based on the similarity of aspects.

Soil samples were taken from the upper 30 cm considering three depth ranges (0-10, 10-20, and 20-30 cm). Also, soil samples were taken as 3 replicates per soil depth and then evaluated on average values. For this purpose, soil samples were taken from the excavated pits from each depth level, and they were oven-dried at 105 ºC in the laboratory. Bulk density was estimated using the cylinder method. Undisturbed soil samples were taken using a steel cylinder 5 cm high and 5 cm diameter (98.125 cm3). And then, the weight of all soils was noted.

The pH, soil texture, C and N properties were analyzed: pH was determined in soil samples passed through a 2 mm sieve; soil texture (sand, silt, and clay ratio) was determined using hydrometer method (Carter and Gregorich 2008), and C and N concentrations were determined using a LECO TruSpec 2000 analyzer.

C and N stocks were determined by the following equation (IPCC 2003):

SOC (Mg C ha^{-1}) = [SOC] * SBD * SD * SR * 10

SOC is the organic carbon content (g kg^{-1} C/soil), SBD is the soil bulk density in mg m^{-3}, SD is the soil depth in m, and SR is the proportion of soil mass <2 mm in the sample (1 -% skeleton rate), and the conversion constant to adjust for the area was 10.

C-N stoichiometries were determined by the C and N concentrations at the same depth levels. The stratification ratio (SR) was calculated by proportioning the C and N concentrations in the surface layer to those in a deeper layer separately.

Statistical analysis: Data analysis was done with SPSS 26.0 program (IBM SPSS Statistics for Windows). Analysis of all data was done in triplicate. Analysis of variance (Two way ANOVA test) was implemented to evaluate the data. Tukey's Honestly Significant Difference (HSD) multiple comparison tests were used to measure specific differences between pairs of the mean. For differences, $p \leq 0.05$ was accepted statistically significant.

RESULTS AND DISCUSSION

The SOC and N values are presented in Table 1. The mean P1 SOC value was 42,83 Mg ha^{-1}, with values ranging from 21,62 to 64,01 Mg ha^{-1} and P2 SOC value was 36,47 Mg ha^{-1}, with values ranging from 18,31 to 54,65 Mg ha^{-1}. The mean P1 N value was 4,29 Mg ha^{-1}, with values ranging from 2,78 to 5,98 Mg ha^{-1} and P2 N value was 3,61 Mg ha^{-1}, with values ranging from 2,62 to 4,49 Mg ha^{-1}. SOC and N content tended to decrease with increasing soil depth in P1 site. In the P2 site, although the SOC tended to decrease with increasing soil depth, N first increased (10-20 cm) and then decreased (20-30 cm).

Table 1. Descriptive statistical summary of SOC and N (n=22).

Altitude	Soil depth	C (Mg ha^{-1})					N (Mg ha^{-1})				
		Min.	Max.	Mean	SD	CV	Min.	Max.	Mean	SD	CV
P1	0-10	54,37	64,01	58.2	2,65	4,56	4,58	5,98	5.1	0,37	7,36
	10-20	36,88	48,62	43.2	4,04	9,36	4,02	5,02	4.6	0,31	6,72
	20-30	21,62	31,22	27.1	2,88	10,61	2,78	3,54	3.2	0,27	8,44
P2	0-10	42,25	54,65	49.9	3,28	6,56	2,62	3,62	3.1	0,31	9,64
	10-20	34,64	42,44	38.3	2,53	6,60	3,87	4,49	4.2	0,22	5,24
	20-30	18,31	24,51	21.1	2,23	10,57	3,13	3,97	3.5	0,24	7,15

SD: standard deviation. P1 (2250 m.), P2 (1850 m.). CV: Coefficient of variation.

The soil texture was determined as Sandy Clay Loam in the P1 site and Clay Loam in the P2 site. A clay loam texture was found in the sample, with a mean sand content of 40.12%, clay content of 32.74%, and the silt content of 27.21% in the fine soil. A sandy clay loam texture was found in the sample, with a mean sand content of 52.03%, clay content of 24.47%, and silt content of 28.21% in the fine soil. In the P1 and P2 sites, the pH increases as per the depth and varies between 7.64 and 7.86 and between 7.48 and 7.62, respectively (Table 2).

Table 2. Some physical and chemical properties of the soils in the research area (mean ± SD, n = 22).

Altitude	Soil depth (cm)	Sand (%)	Silt (%)	Clay (%)	Ph	Pb ($g\ cm^{-3}$)	C ($Mg\ ha^{-1}$)	N ($Mg\ ha^{-1}$)	C/N
P1	0 - 10	52.0 ± 8.7^{a}	28.2 ± 4.5^{a}	24.5 ± 6.6^{a}	7.64 ± 0.6^{a}	1.39 ± 0.3^{a}	58.2 ± 2.6^{a}	5.1 ± 0.4^{a}	11.5 ± 1.1
	10-20	30.3 ± 4.9^{b}	49.0 ± 4.2^{b}	21.5 ± 1.4^{a}	7.75 ± 0.4^{a}	1.33 ± 0.2^{b}	43.2 ± 4.0^{b}	4.6 ± 0.3^{b}	9.5 ± 0.8
	20-30	30.8 ± 5.3^{b}	45.3 ± 2.3^{b}	22.8 ± 6.3^{a}	7.86 ± 0.2^{b}	1.27 ± 0.3^{b}	27.1 ± 2.9^{c}	3.2 ± 0.3^{c}	8.6 ± 1.3
P2	0 - 10	40.1 ± 7.3^{a}	27.2 ± 5.2^{a}	32.7 ± 4.7^{a}	7.48 ± 0.5^{a}	1.44 ± 0.2^{a}	49.9 ± 3.3^{a}	3.1 ± 0.3^{a}	16.0 ± 2.2
	10-20	21.2 ± 6.6^{b}	56.7 ± 5.1^{b}	22.2 ± 2.7^{b}	7.57 ± 0.2^{a}	1.30 ± 0.3^{b}	38.3 ± 2.5^{b}	4.2 ± 0.2^{b}	9.2 ± 0.7
	20-30	19.1 ± 7.8^{c}	55.1 ± 6.6^{b}	25.7 ± 4.4^{c}	7.62 ± 0.4^{a}	1.39 ± 0.3^{a}	21.1 ± 2.2^{c}	3.5 ± 0.2^{c}	6.0 ± 0.9

SD: standard deviation. P1 (2250 m.), P2 (1850 m.). Different letters show statistically significant differences between depth levels (Tukey p≤0.05).

In both sites, 44% of the average soil organic carbon stock is in the 0-10 cm depth level. Depending on the soil depth (0-10, 10-20, and 20-30 cm), carbon stocks at P1 are 58.2, 43.2, and 27.1 Mg C $ha^{-1,}$ respectively. While in P2, values changed as 49.9, 38.3, and 21.1 Mg C ha^{-1}. The average C stocks of the topsoil (0-30) in the P1 and P2 sites are determined as 128.5 and 109.3 Mg C $ha^{-1,}$ respectively. One research reported 98 Mg C ha^{-1} (to a depth of 20-cm) in afforestation of Scots pine in Veluwe, Netherlands (Ruiz-Peinado *et al.,* 2016). Díaz-Pinés *et al.,* (2011) reported C stocks between 95 and 140 Mg C ha^{-1} (forest floor plus 50 cm mineral soil) in Scots pine stand soils. Charro *et al.,* (2010) reported a higher value than 166 Mg C ha^{-1} (Ah horizon 0-20 cm) in Scots pine reforested area.

Contrary to this study, it was stated in some studies that C stocks decreased due to the increase in altitude. Lower mineralization and net nitrification rates at higher altitudes reduced species diversity, and lower tree density have all been related to the decreasing trend of C with increasing altitude (Bangroo *et al.,* 2017). However, precipitation, air temperature, light intensity, land use, and slope also affect C and N stocks. (Gruba and Socha, 2019; Assefa *et al.,* 2017; Bangroo *et al.,* 2017). Typically, it is understood that C stocks decrease with temperature increase; but increase with precipitation and altitude. However, this study determined that C stocks increase with altitude.

Differences between C and N stocks at the 0-10 cm depth and other depth ranges (10-20 cm and 20-30 cm) were statistically significant. The results obtained in this study are consistent with the results of other studies of this species (Schulp *et al*. 2008., Charro *et al.,* 2010 Mayes *et al.,* 2014). The main source of soil organic matter is organic matter, which mixes with soil due to litterfall decomposition. Since the soil organic matter source is generally litterfall, the top layer of the soil is rich in organic matter, and organic carbon decreases as depth increases (Díaz-Pinés *et al.,* 2011; Måren *et al.,* 2015).

Bulk density has a declining trend depending on soil depth. Bulk density values in the P2 research site were higher than the P1 site. This difference can be elucidated by the scarcity of SOC, less aggregation, and low root penetration in the sub-surface layers. Simultaneously, it is likely that the P1 site is receiving more sunlight and rainfall, which also creates the mentioned outcome. Bulk density values decreased due to the increase in soil depth. The deeper in the forest floor, the stronger the mixture of minerals was present in the profile, and therefore there was a higher bulk density. The main factor explaining the high bulk density on the forest floor is the high organic matter content (Jandl *et al.,* 2007; Lawrence *et al.*, 2015).

The stock of N in the P1 site also decreased with depth, and in the P2 site, it has increased a little and then decreased (Figure 2).

Total stock N at P1 (12.86 Mg ha^{-1}) was higher than P2 (10.83 Mg ha^{-1}). According to the soil depth levels (0-10, 10-20, 20-30 cm), the N stock ratio was 40%, 35%, and 25% in P1. The percentages were 29%, 38%, and 33% in P2, respectively. In the P1 research area, the N stock was found in the soil's 0-10 cm part. On the other hand, in P2, it was detected in 10-20 cm of the soil. Again, the C-N stoichiometries in the soil depth levels tended to decrease with the depth, and it was determined that they were significantly lower in P1 than P2 (Table 1).

The C-N ratio in the soil is among the important indicators concerning soil fertility and soil organic matter. C: N ratios between 12 and 16 show that the organic matter decomposes well. C: N ratios below 10 generally were observed in the subsoil. (Bui and Henderson 2013; Paul 2015). Besides, at values below 15, the litterfall is decomposed by oxidation. Therefore, carbon is emitted into the air as CO_2. The C: N ratios in the soil in the research sample are greater than 10 in the 0-10 cm part of the plain soil in both research sites, but when looking at the mean score, it is less than 10. Decreases in this ratio can indicate increased soil degradation (Sarıyıldız *et al.,* 2020). It may also be attributable to the season in which the sampling was carried out. Indeed, the C: N ratio may be lower in forest areas in the dry season than in the rain season (García *et al.,* 2016).

Stratification rate is examined in two depth levels as SR1 (0-10 cm / 10-20 cm) and SR2 (0-10 cm / 20-30 cm). For both C and N, the SR index increased with depth in both sampling sites (P1-P2), with C decreasing with soil depth. The SR1 index increased with altitude (P1-P2) for both C and N, and the SR2 index increased for N but decreased for C. (Table 3).

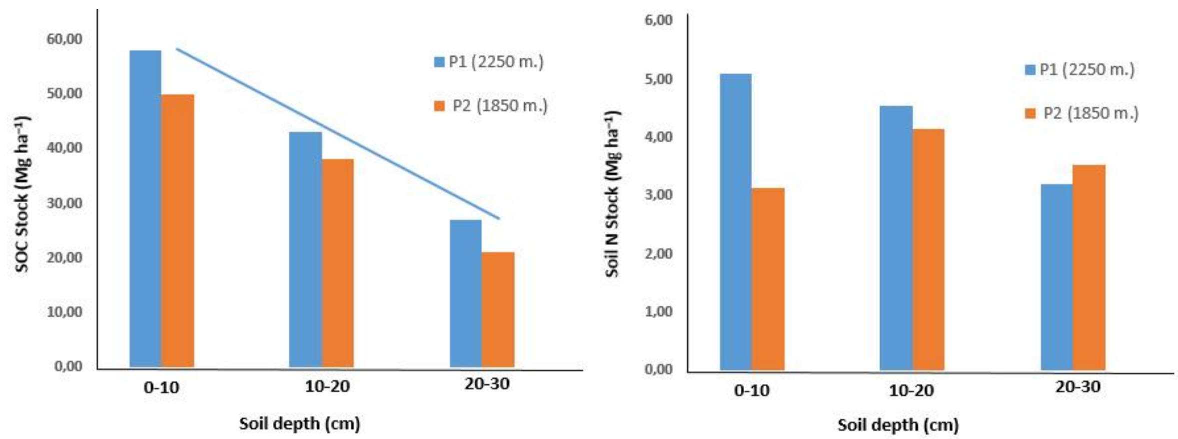

Figure 2. Distribution of soil organic carbon (SOC) and nitrogen stocks at depth levels in research sites.

Table 3. Stratification rates (SR) of C and N concentrations (mean ± SD).

SR	SRCarbon		SRNitrogen	
	P1	P2	P1	P2
SR1	1.36 ± 0.13	1.31 ± 0.12	1.13 ± 0.13	0.75 ± 0.07
SR2	2.17 ± 0.24	2.39 ± 0.29	1.61 ± 0.16	0.90 ± 0.10

SD: standard deviation. P1 (2250 m), P2 (1850 m)

SR value is important in determining soil degradation or quality. In the areas where this value is lower than 2, soil degradation can be mentioned. It is thought that the increase of SR in C and N depending on the depth of the soil depends on the amount of C decreasing with the increase of the depth. However, in certain studies, SR has increased depending on the depth of the soil (Zhao *et al.,* 2021; Bangroo *et al.,* 2017; García *et al.,* 2016).

When the C and N interaction in the research sites were evaluated separately according to soil depth levels, statistically significant differences were detected. Two-way ANOVA of the data proved that different altitudes have a significant effect on carbon and nitrogen at soil depth. Furthermore, it was noticed that there was an interaction between both effect variables. Moreover, soil carbon and nitrogen content were influenced by both altitude x soil depth interaction. (Table 4).

Table 4. Evaluation of C-N amounts according to soil depth and altitude (ANOVA).

		Carbon		Nitrogen		
Source	**df**	**MS**	**F**	**MS**	**F**	**P**
Corrected model	5	4243.4	473.1	13.8	163.0	<0.005
Intercept	1	207483.6	23136.7	2058.5	24193.8	<0.005
Altitude	1	1333.8	148.7	15.1	176.7	<0.005
Soil depth	2	9909.1	1104.9	12.0	141.6	<0.005
Altitude*S.depth	2	32.5	3.6	15.1	177.6	<0.005
Error	126	8.9		0.08		
Total	132					

Df: degrees of freedom, MS: mean square, F: F value, and P: value

A high positive correlation was found between the C concentrations and N concentrations depending on the depth in the P1 research site, r = 0.880, $p < 0.01$. While in the P2 research site, a low negative correlation was observed between the amount of C and N's amount, but the differences were not significant, r = - 0.242, $p < 0.05$. It was observed that the amount of carbon decreased in all soil depths with decreasing altitude. The highest decrease depending on the altitude was observed in the topsoil depth (0-10 cm). The interaction of soil organic carbon depending on depth and altitude is in the form of ordinal interaction (Figure 2).

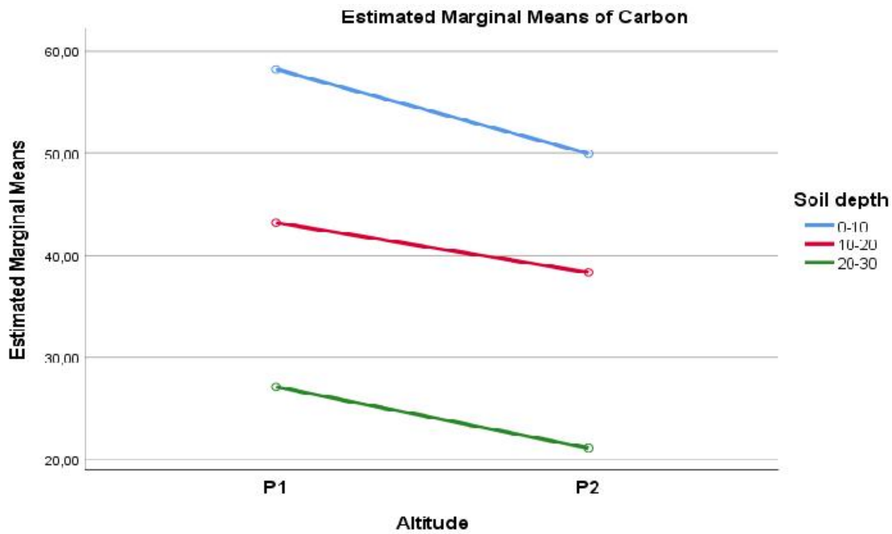

Figure 2. Two-way ANOVA with interaction graph to SOC-Altitude-Soil depth

A significant difference was found in the interaction of nitrogen between altitude and soil depth ($p<0.05$). The interaction of Nitrogen depending on depth and altitude is in the form of disordinal interaction (Figure 3).

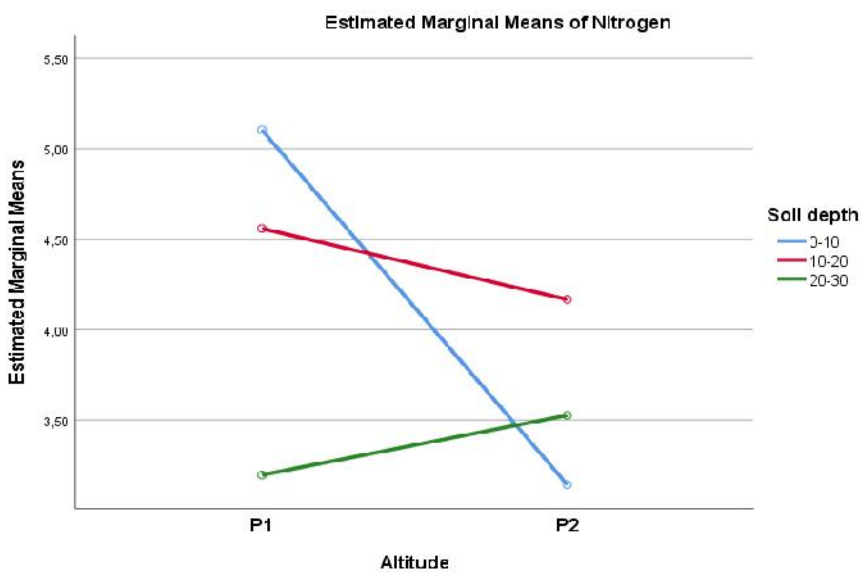

Figure 3. Two-way ANOVA with interaction graph to Nitrogen-Altitude-Soil depth

Conclusions: Soil organic carbon stocks decreased with increasing depth in both research sites. Most of the C stock was found in the soil 0-10 cm deep. More C stock has been found in the high-altitude region, where altitude affects C stock than in the low altitude area. N stocks decreased linearly with increasing depth in the high-altitude area (P1). In the low altitude area (P2), N stocks firstly increased and then decreased.

Stratification rates (SR) showed an increasing trend with soil depth increase. In addition, the SR index increases with altitude for both C and N, especially in the 0-20 cm section. However, high soil quality stratification rates were observed only in C stocks; and the highest was observed in the high-altitude area. The more time spent under snow at high altitudes, the greater the stabilization of C might be. This study emphasized that Scots pine afforestation can be a climate change mitigation approach, particularly in the medium and long term.

Based on results, policies need to be established that recognize forest carbon accumulation capacity. To bond more carbon to forest soils, it is essential to improve the conditions, particularly in disturbed forest areas, increase effective afforestation and deter soil erosion.

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