

Atf için: Çomaklı E, Bilgili A, Öztaş T, Çomaklı T, 2022. Determination of Carbon, Nitrogen and Phosphorus Stocks and Stoichiometry in Broadleaf Mixed Forest Soil and Litterfall: A case study in Oltu district, Erzurum. *İğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 12(1): 464-475.

To Cite: Çomaklı E, Bilgili A, Öztaş T, Çomaklı T, 2022. Determination of Carbon, Nitrogen and Phosphorus Stocks and Stoichiometry in Broadleaf Mixed Forest Soil and Litterfall: A case study in Oltu district, Erzurum. *Journal of the Institute of Science and Technology*, 12(1): 464-475.

Determination of Carbon, Nitrogen and Phosphorus Stocks and Stoichiometry in Broadleaf Mixed Forest Soil and Litterfall: A case study in Oltu district, Erzurum

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ABSTRACT: It is necessary to provide plant nutrients in soil at optimal levels for the sustainability of forest ecosystems. The soil stoichiometry of total carbon (C), total nitrogen (N) and total phosphorus (P) allow monitoring and assessment of ecosystem structures and variations in nutrient cycle. Studies on determination of C-N-P stoichiometry in forest ecosystems, however, are somewhat inadequate. This study aims to determine change of C-N-P stoichiometry depending on litterfall condition and soil depth in broadleaf mixed forest (European Hophornbeam - *Sypisrensis* Oak) soil and the C-N-P stocks in soil. In this context, we were determined both C-N-P stoichiometry and C-N-P stock in soil and litterfall by conducting field studied at 10 different points in the Broad Leaf Mixed Forest of Erzurum-Oltu district. The results indicated that as the depth of the soil increased, the C-N ratio decreased, whereas the N-P and the C-P ratios increased. Positive correlations were observed between C-N in all soil depths, but negative correlations between C-P and N-P. The correlation coefficients between C and N ($r_{0-10}= 0.58$, $r_{10-20}= 0.52$ and $r_{20-30}= 0.44$) and between C and P ($r_{0-10}= 0.64$, $r_{10-20}= 0.54$ and $r_{20-30}= 0.42$) and between N and P ($r_{0-10}= 0.52$, $r_{10-20}= 0.35$ and $r_{20-30}= 0.36$) decreased as soil depth increased. The mean scores of the C-N-P stocks were determined as 5.9, 1.3, and 0.2 ton ha⁻¹ in litterfall and 157.68, 24.60, and 2.68 tons ha⁻¹ in soil, respectively. It is important to rehabilitate degraded forests and minimize the negative effects of erosion in order to increase the amount of carbon captured in forest soils. In addition, the variable C: N: P stoichiometry in forest ecosystems; It can be considered as a leading indicator of soil degradation and drought and climate changes.

Keywords: Stoichiometry, soil organic carbon, nitrogen and phosphorus stocks, litterfall, broadleaf forest, climate change

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INTRODUCTION

Most of the ecological processes, including energy balance of ecosystem and the contributions of elements to these mechanisms and the composition of carbon, nitrogen, and phosphorus cycles in soil, are related to stoichiometry. Information regarding nutrient cycle fluctuations and biological processes in the ecosystems is also provided by ecological stoichiometry (Alberti et al., 2015; Qiao et al., 2020; Yang et al., 2017; Zechmeister-Boltenstern et al., 2015). The existence of organisms in terrestrial ecosystems is attributed to the presence of C-N-P and is one of the essential components of research about ecological stoichiometry (Chen et al., 2016; Tan and Wang, 2016). Comprehending C-N-P stoichiometry in the soil is important for the protection of the environment, C retention and soil quality preservation (Alberti et al., 2015; Yang et al., 2017). Commonly, stocks of C, N, and P vary with soil depth. However, these variations also differ. To illustrate, most of the C substrate is distributed in the topsoil and litterfall layer. (Hu et al., 2008; Jobbagy and Jackson, 2001; Lal, 2009; Wang et al., 2019). For regulating the carbon and nutrient cycle, litterfall decomposition plays a vital role. However, the process of litter decomposition depends on different factors, such as climate, carbon input quality, soil, chemical, and physical characteristics of litterfall and also soil biota. Additionally, one of the most significant considerations influencing litterfall decomposition and thus C-balance in the soil is the composition of the plant organisms (Barantal et al., 2014; Magill and Aber, 2000).

Plants uptake nitrogen in forms of NH_4^+ and NO_3^- . The transformation of inorganic nitrogen into a structure beneficial to the plant relies on the decomposition of organic material by microorganisms. Bonding of N and mineralization are carried out microbially in soil system. Particularly in ecosystem conditions where the N amount is high, the management of immobilization processes requires an unstable carbon source. Soil pH also takes a significant role in nitrogen mineralization (Qualls et al., 1991). Whereas the soil C / N ratio is a significant indicator of soil quality and nutritional balance, it also impacts the quality of soil pH, nutrient, aggregation and humic matter (Jiang et al., 2018; Zhang et al., 2011). The solubility of organic matter in the soil, the production of organic carbon, and its mobility depend, especially in sandy soils, on the chemical composition of organic materials and the C/N ratio of them. On top of this fact, mineralization of soil organic matter increases soil carbon stock and regulates CO_2 emissions which have critical significance in terms of climate change (Ostrowska and Porębska, 2015; Silveira et al., 2011). Litterfall decomposition, microbial N fixation and decomposition of parent material also affect N accumulation in soil. This deposition, however, creates an imbalance along with the profile in N distribution that differs as per different soil depths (Andres, 2019; Houlton et al., 2018; Morford et al., 2016).

The main source of P is decomposition process of bedrock. However, in the short term, litterfall decomposition also affects the amount of P (Xia et al., 2015). While the amount of P is generally low in older soils, several studies have also revealed that the P content of soil increased with forest age (Frizano et al., 2002; Zarin and Johnson, 1995). By decreasing both leaf area index and photosynthesis rate of leaves, P deficiency obviously influences photosynthetic performance of plants. It also has an indirect effect on symbiotic and asymbiotic N fixation phase in roots (Augusto et al., 2013; Gough et al., 2010; Ise and Moorcroft, 2010; Pourhassan et al., 2016; Zhong et al., 2020). N accumulation stimulates plant development and facilitates P uptake of plants (Deng et al., 2016).

Determination of stoichiometric variations in litterfall and soil contributes to ecosystem's sustainable management by offering knowledge on ecosystem's working mechanisms. In several studies contradictory findings regarding the change (increase or decrease) of the C, N, and P stocks corresponding to soil depth were reported (Chen et al., 2016; Makineci, 2005; Qiao et al., 2020; Tashi

et al., 2016; Ward et al., 2016). Different results gained in several studies raise the ambiguity concerning soil C-N-P stoichiometry. It is therefore essential to ascertain whether correlations between C-N-P and soil depth in broadleaf forests change and, how if it happens. This research assessed distribution of the soil C-N-P stoichiometry in three soil depths (0–10, 10–20, and 20–30 cm) and litterfall C-N-P stock in deciduous broadleaf mixed forests in the Erzurum region. The soil depth and C, N, and P ratios in soil were assumed to vary in this ecosystem, and the factors causing the spatial variation in soil concentrations of C, N, and P accepted as not differing between levels of soil depth.

MATERIALS AND METHODS

Research Area

This study was carried out in a deciduous forest area with broadleaf trees in the Erzurum - Oltu region (Turkey) (40 ° 37 'N, 41° 57' E). The altitude of the experimental area from sea level varies between 1760 m and 1820 m with a mean of 1790 m. The mean slope gradient in the experimental area is 40% and the mean exposure is northeast. For sampling, an area of approximately 1.5 hectares representing a mixed forest was defined (Figure 1). Height and exposure variation in the basin where the research area is situated generates the development of microclimatic areas. The research area, which can be characterized as the semi-arid, low humidity climate type, and its annual precipitation mean is 390.5 mm. The annual mean temperature is 9.9 °C, and the coldest month is January (-3.6 °C) while the hottest month is august (22.5 °C). The dominant soil type in the study area is Brown Earth soil formed on Oligocene gypsiferous marl sediment. Dominant tree species are European Hophornbeam (*Ostrya carpinifolia* Scop.) and Sypirensis Oak (*Quercus macranthera* subsp. *sypirensis* (K.Koch) Menitsky). According to the stand canopy classification, the area is in the "very dense forest" (>70%) class.

Sampling and laboratory analysis

The pH of the soils was measured in 1: 2.5 soil-water suspension with a glass electrode and the electrical conductivity value was determined as dS m⁻¹ with an electrical conductivity meter (FAO, 2020). Organic matter content was determined by the modified Walkey-Black (Chapman and Pratt, 1962), soil texture by Bouyoucos hydrometer (Gee et al., 1986) and lime content according to the Scheibler calcimeter method (Allison and Moodie, 1965). Bulk density was estimated using the cylinder method. Undisturbed soil samples were taken using a steel cylinder 5 cm high and 5 cm in diameter (98.125 cm³).

The litterfall and soil sampling were taken from 10 locations and three samples were taken from each location. Litterfall samples were collected as mixed from 0.25 m² areas (50cm x 50cm). In addition to the disturbed soil samples collected from a depth of 0-30 cm indicating the areas where litterfall sampling was conducted, steel cylinder were used to assess the bulk density at 3 soil depths (0-10 cm, 10-20 cm, 20-30 cm) from each sample point for undisturbed soil samples. The litterfall and soil samples gathered were delivered to the laboratory and fully prepared for analysis. The ambient temperature and moisture content at sampling was determined via the Hobo MX1101 data logger. Using a computer program called ArcMap 10.5, slope, exposure and height of the sampling area were determined. The litterfall samples were dried for 48 hours in an oven at 70 °C and their moisture contents and oven-dry weights were measured. It was then pulverized (<100 mm) for analysis. The soil samples were washed from roots and stones, dried in the oven at 105 °C for 24 hours, and sifted through a 2 mm sieve. It was also sifted through a 0.25mm sieve for C, N, and P analysis. C and N concentration in litterfall and soil samples were measured in the Leco CHNS-932 elementary chemical analyzer using a combustion element analysis. The soil and litterfall samples were carefully combined for the assessment of the P

concentration, weighing approximately 0.3 grams from each sample for the purpose of analysis. Then, in the Milestone Ethos Up brand microwave machine, the burning process needed for analysis was conducted. 3 ml of nitric acid, 1 ml of hydrogen peroxide, and 6 ml of hydrochloric acid were added to the litterfall samples. Soil samples are left in a microwave for 1 hour after adding 9.9 ml of nitric acid and 0.1 ml of HF (Çomaklı and Bingöl, 2021). The samples were diluted 100 times with distilled water after these phases and the Agilent 7800 brand ICP-MS system was used to estimate their P concentrations. Also we calculated average molar ratios (C: N, C: P and N: P) of both the total soil and the litterfall.

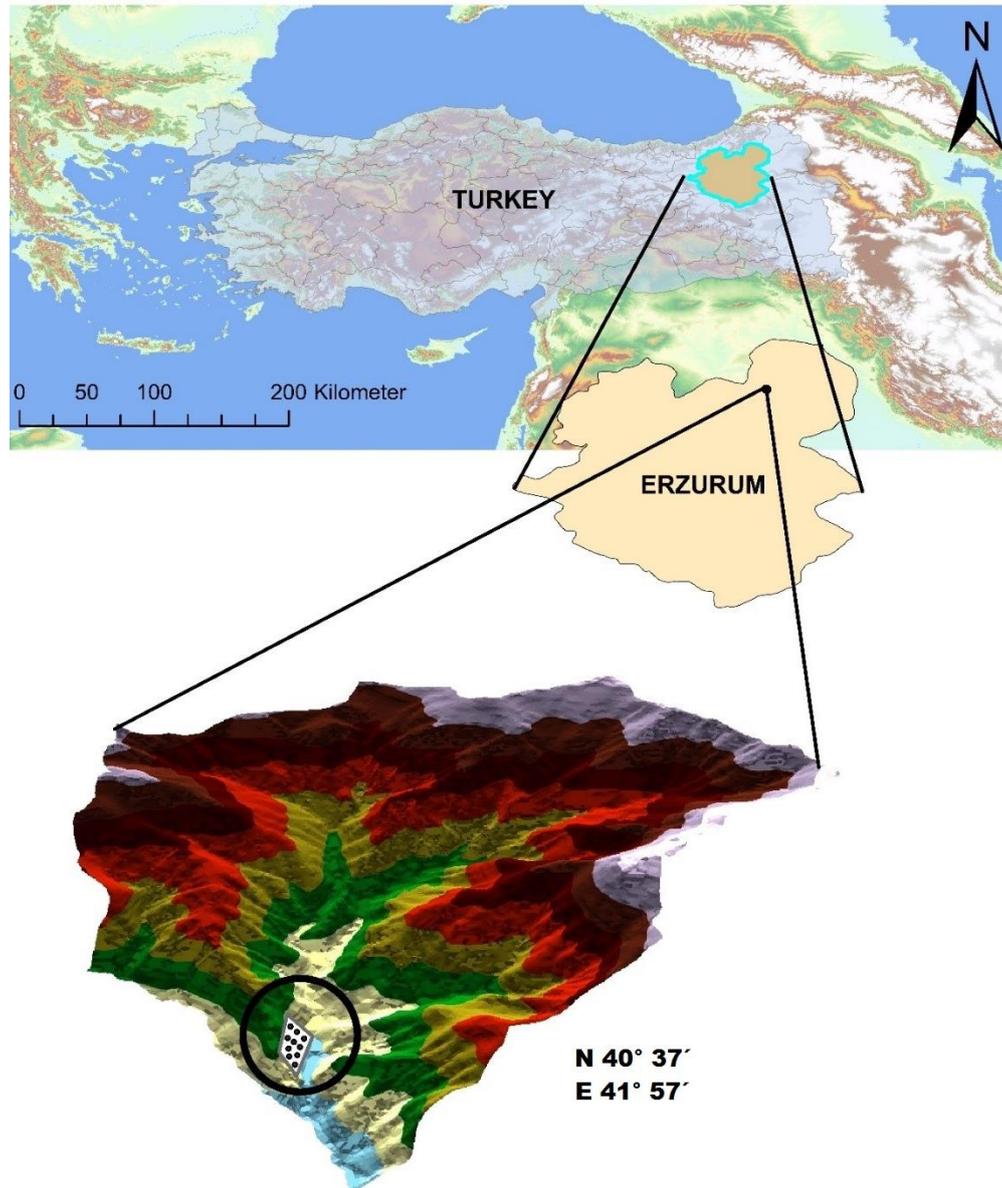


Figure 1. The location of the research area

The following formula was used to calculate the stocks of soil organic carbon (SOC), soil total nitrogen (STN), and soil total phosphorus (STP) per hectare in soil (IPCC GPG 2003): (Example is given only for C)

$$\text{SOC (ton C ha}^{-1}\text{)} = [\text{SOC}] * \text{SBD} * \text{SD} * \text{SR} * 10^4$$

Where, SOC is organic carbon content (g C per kg soil), SBD is soil bulk density in mg m^{-3} , SD is soil depth in m, and SR is proportion of soil mass <2 mm in the sample (1 -% skeleton rate), the conversion factor to adjust for the area was 10^4 ($\text{m}^2 \text{ha}^{-1}$).

Statistical analysis

In order to assess the distribution and concentrations of C, N, and P at various soil depths (0-10 cm, 10-20 cm, 20-30 cm), the SPSS 20.0 software was used to evaluate non-parametric tests. Depth-dependent changes in C, N, and P amounts were determined by regression analysis while the degree of bivariate analysis between C, N, and P was determined with the help of Nonparametric Spearman rank-correlation coefficients analysis.

RESULTS AND DISCUSSION

The soils of the research area are sandy loam (SL) textured and containing 3.79% organic matter, on the average (Table 1). The soils are 'moderately alkaline' and 'slightly calcareous' without salinity problem.

Table 1. Some soil characteristics of the research area

Sand %	Silt %	Clay %	Texture class	pH	Organic matter %	EC dS m^{-1}	CaCO_3 %
72.5	20.8	6.7	SL	8.33	3.79	0.092	1.10

The C: N ratios at 0-10 cm, 10-20 cm, and 20-30 cm depths were determined as 10.12 kg ha^{-1} , 6.55 kg ha^{-1} and 6.02 kg ha^{-1} respectively, while the C: P ratios as 90.89 kg ha^{-1} , $103.62 \text{ kg ha}^{-1}$ and $140.62 \text{ kg ha}^{-1}$, and the N: P ratios as 10.89 kg ha^{-1} , 16.69 kg ha^{-1} and 28.23 kg ha^{-1} respectively (Table 3 and Figure 2). The C, N, and P concentrations were 52.56 kg ha^{-1} , 8.20 kg ha^{-1} , and 0.90 kg ha^{-1} , respectively. C content decreased dramatically at 0-20 cm soil depth but increased again at 20-30 cm soil depth. The mean scores of SOC concentrations were 58.31 kg ha^{-1} at 0-10 cm depth, 40.64 kg ha^{-1} at 10-20 cm depth, and 58.74 kg ha^{-1} at a depth of 20-30 cm. Similar to C concentrations, P concentrations decreased at 0-20 cm and increased again at 20-30 cm of soil depth. While the P concentration mean scores were 0.86 kg ha^{-1} at 0-10 cm depth, 1.10 kg ha^{-1} at 10-20 cm depth, and 0.73 kg ha^{-1} at a depth of 20-30 cm, no increases were observed in N concentrations depending on soil depth. Depending on the depth levels, N concentration mean scores were reported as 6.52 kg ha^{-1} , 7.09 kg ha^{-1} and 10.98 kg ha^{-1} respectively. While the ratios C:N and C:P did not change substantially based on the depth, the ratios N:P rose with the depth of the soil (Figure 3).

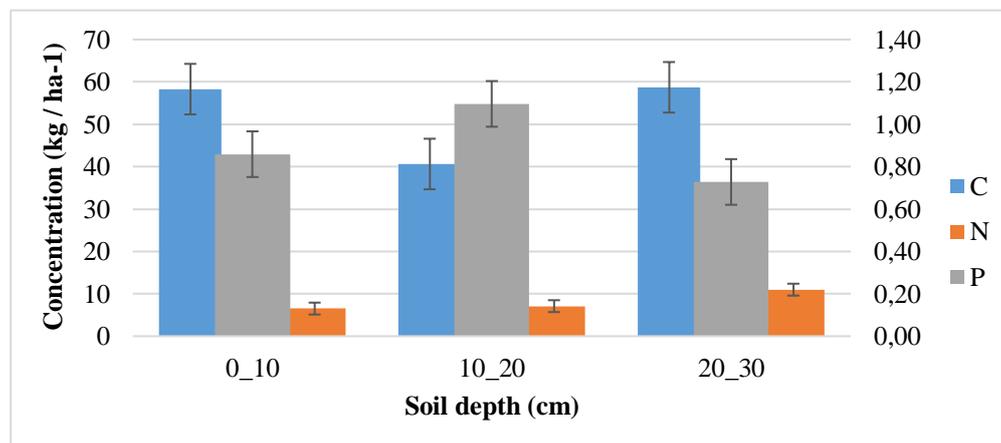


Figure 2. Variation of C-N-P mean concentrations according to soil depth levels

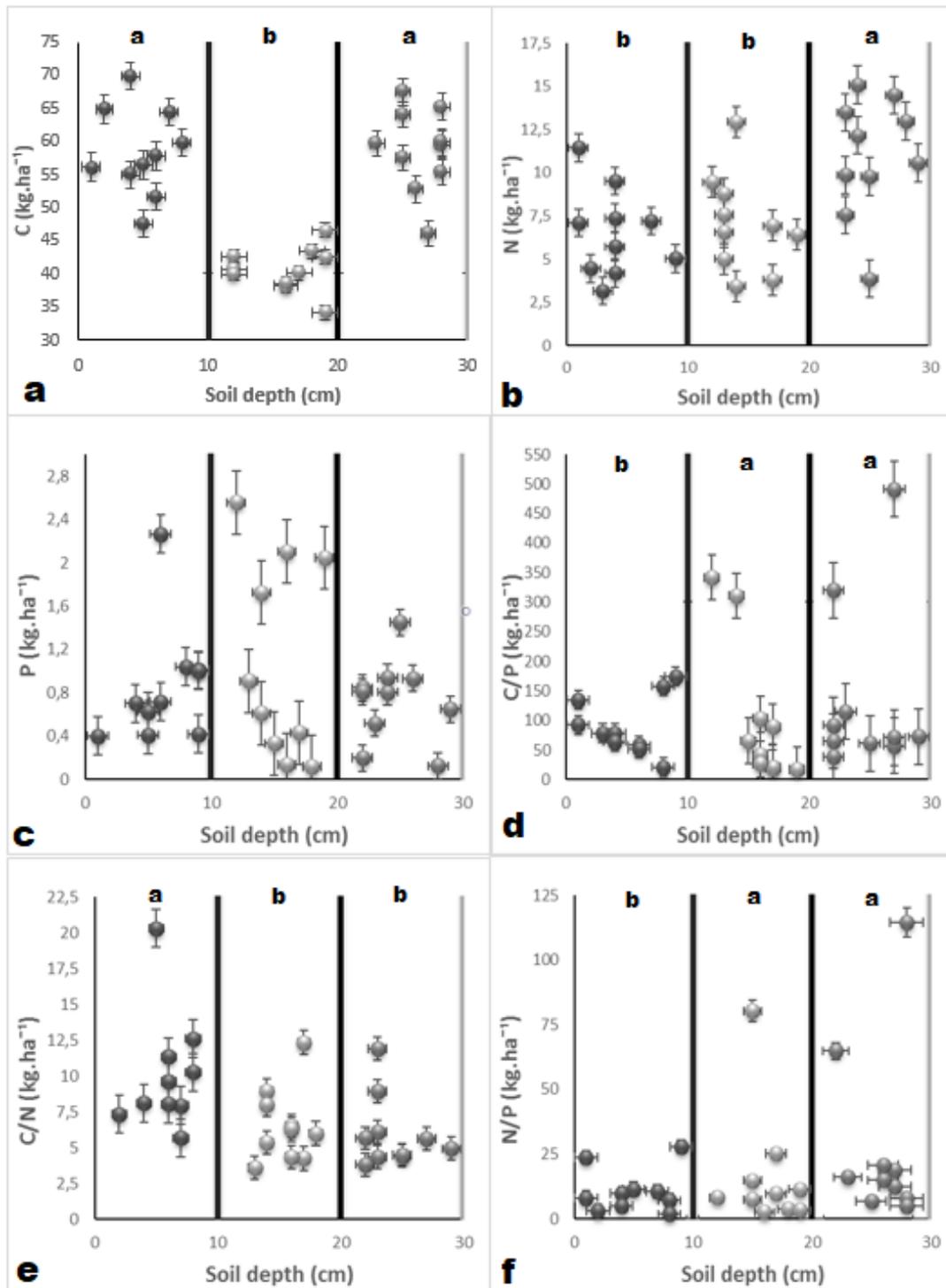


Figure 3. Vertical distributions of C (a), N (b), and P (c) and the ratios of C: P (d), C: N (e), and N: P (f) at different soil depths (0-10 cm, 10–20 cm, and 20–30 cm). Different lower case letters in the boxes suggest differences among different depths ($P < 0.05$).

Considering three soil depth levels, the correlation coefficient (r) between C and N decreased slightly from 0,34 (0–10 cm) and 0,27 (10–20 cm) to 0,20 (20–30 cm). The r between N and P concentrations decreased from 0.27 (0-10 cm) to 0.13 (20-30 cm). Likewise, the r value between the C and P concentrations decreased to 0.18 (20-30 cm) from 0.41 (0-10 cm) (Table 2).

Table 2. Correlation coefficients of C-N-P at different soil depth levels

		C			N			P		
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
C	0-10	1								
	10-20	-0,49	1							
	20-30	0,03	0,04	1						
N	0-10	0,58	-0,47	0,05	1					
	10-20	-0,72	0,52	-0,18	-0,63	1				
	20-30	-0,22	0,55	0,44	0,06	-0,13	1			
P	0-10	-0,64	0,72	0,18	-0,52	0,70	0,33	1		
	10-20	-0,08	0,54	-0,19	-0,25	0,34	0,19	0,51	1	
	20-30	0,62	-0,27	-0,42	0,02	-0,23	-0,36	-0,28	0,45	1

Table 3. C-N-P concentrations and their ratio in different depth levels (kg ha⁻¹)

Variable	0–10 cm	10–20 cm	20–30 cm	Mean
C	58.31 ± 6.62a	40.64 ± 5,95b	58.74 ± 6.29a	52.56± 10.15
N	6.52 ± 2.55b	7.09 ± 2.83b	10.98 ± 3.43a	8.20± 3.50
P	0.86 ± 0.55	1.10 ± 0.92	0.73 ± 0.38	0.90± 0.65
C:N*	10.12 ± 4.11a	6.55 ± 2.62b	6.02 ± 2.52b	7.56± 2.51
C:P*	90.89 ± 49.29b	103.62 ± 121.34a	140.62 ± 154.32a	111.71± 20.65
N:P*	10.89 ± 8.51b	16.69 ± 23.33a	28.23 ± 34.83a	18.60± 13.21

Significant differences between soil depths are shown in different lower case letters (P < 0.05) after mean ± standard deviation (SD). * The values given are the ratios of the averages.

The C, N, and P stocks in the 0-30 cm depth level of soil were determined as 157.68 tons ha⁻¹, 24.60 tons ha⁻¹ and 2.68 tons ha⁻¹, respectively. Concentrations of C, N, and P in litterfall were estimated to be greater than soil concentrations between 1 and 5 times. The amount of litterfall was calculated as 21.7 tons ha⁻¹. The litterfall's mean amount of C was 27%, N was 6%, and P was only 1%. The mean scores of C, N and P amounts in the litterfall were determined as 5.9 tons ha⁻¹, 1.3 tons ha⁻¹, and 0.2 tons ha⁻¹, respectively. As a change in C-N-P stocks in soil (0-30 cm) and litterfall samples were taken from the research area was analyzed, it was observed that the C and N stocks in the soil exhibited less variability than the C and N stocks in the litterfall. So, it was concluded that C and N stocks in the soil are more important. However, when the amount of P was examined, it was noticed that the P stock value in the litterfall was lower than in the soil. In this situation, while the amount of P stock in the soil (2.68 tons ha⁻¹) was higher than the stock in litterfall, the P stock value in litterfall could be deemed more significant (Table 4). That's being said, P concentrations change depending on the change of microbial activity in the litterfall (Ilg et al., 2009).

Table 4. C-N-P stocks in soil layer (0-30 cm) and litterfall (ton ha⁻¹)

	n	Mean	Min.	Max.	S.D.	Cv
Soil C stock	10	157.68	139.08	171.39	8.79	5,6
Litterfall C stock	10	5.90	2.87	10.77	2.23	37,8
Soil N stock	10	24.60	18.38	30.12	3.97	16,1
Litterfall N stock	10	1.34	0.82	2.19	0.50	37,3
Soil P stock	10	2.68	0.88	4.56	1.42	53,0
Litterfall P stock	10	0.24	0.11	0.36	0.07	29,2

The soil pools of C, N, and P and their stoichiometric properties play a vital role in maintaining the sustainability of ecosystems. Since the C, N, and P pools in the soil are both vast and vulnerable to

environmental influences in terrestrial ecosystems, detecting changes in these pools can promote adaptation to climate change by helping to the sustainable management of the ecosystem (Bui and Henderson, 2013; L. Liu et al., 2020; Reysers et al., 2017; Tipping et al., 2016).

N concentrations increased as the mean C concentrations increased depending on soil depth. This could be explained by the fact that the N dynamics of soil play an essential part in controlling C retention over the long term (Liu et al., 2018; Luo et al., 2004; Rastetter et al., 1997). Once more, decreasing P concentrations with increasing N concentrations depending on depth showed a negative correlation. As a result, by promoting P uptake and plant formation, N accumulation leads to a decline in soil P concentrations.

The C-N ratio varies drastically depending on forest type, management, and environmental factors. C-N ratio in soil and litterfall is one of the significant indicators of soil productivity and soil organic matter. The ratio of C: N that ranges from 12 to 16 suggests that the organic matter is well degraded. C: N ratios below 10 generally were observed in subsoil. (Bui and Henderson, 2013; Paul, 2015; Rayment and Higginson, 1992; Zeller et al., 2000). Besides, at values below 15, the litterfall is decomposed by oxidation, therefore, carbon is emitted into the air as CO₂ (Kantarci, 2000). C: N ratios in the soil of the research area were greater than 10 at some points, but the mean score was less than 10. On the other hand, the mean score of this ratio was 4 in the litterfall. Decreases in this ratio could be an indicator of 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 compared to the rain season. (García-Oliva et al., 2006).

The C:P ratio of soil and litterfall also offers details on the origins and condition of decomposition of organic matter. Generally, the condition of C: P <200 is explained by net mineralization, while the C: P > 300 refers to net immobilization. C: P between 200 and 300 refers to the change in soluble P concentrations (Paul, 2015). High C:P ratios also suggest the net immobility of nutrients (Bui and Henderson, 2013; Güsewell and Verhoeven, 2006). In this research, this ratio ranged between 514 and 20 in the soil, with a mean of 112. In the litterfall, this ratio was 39.1. Commonly, when the ratio of C: P is >100, phosphorus is immobilized by microorganisms. Yet again, the change in the C: P ratio is considered to be a result of the high carbon and low phosphorous intake combination by microorganisms.

N and P concentrations and stoichiometry are the main limitations for plant development in terrestrial environments. In general, the N:P mass ratio of less than 14 indicates that plant growth is restricted to N, while the N:P mass ratio of greater than 16 signifies that plant growth is restricted to P (Koerselman and Meuleman, 1996; Xie et al., 2019). The mean N: P ratios were higher than 16 in this research, suggesting that the possible P limit for plant growth is found in the area under examination. However, this value was lower than 14 at the 0-10 cm depth level. This situation could be explained by washing away. Moreover, to analyze the scale of change with time, long-term analysis is needed.

N and P content generally affects productivity. The amounts of N and P in litterfall potentially suggest productivity. This increases the organic matter input into the soil. (Tang et al., 2018; Wu et al., 2018). C and N in the soil are highly dependent on the physical and chemical properties and composition of the litter. Also, the N contribution to the soil is impacted by litterfall, which is the primary source of soil organic matter (Song et al., 2016). In the compilation work conducted to measure the amount of organic carbon in forest soils in Turkey, the weighted mean of carbon for mixed forests was estimated as 161.4 tons ha⁻¹ and soil organic carbon stock was calculated as 158.6 tons ha⁻¹ (Tolunay and Çömez, 2007). Although there are several studies on the stock status of nutrients (especially carbon) in forest

environments, the subject remains unclear due to the lack of sufficient research on whether C-N-P stoichiometry changes according to parameters such as age, soil depth and climate.

CONCLUSION

The findings of this study clearly indicated that forests could be a tool to be used as a mechanism for mitigating climate change in the short and long term. In particular, the positive correlation between the C-N ratios and the decrease in this ratio depending on the depth is an important factor that is taken into account. Also; C: P needs to be evaluated in long-term follow-up studies as it can be indicative of nutrient limitation in a forest. Policies need to be established that recognize the carbon accumulation capacity of forests which are the primary sources of carbon storage when establishing forest ecosystem management and silviculture implementations. In order to bond more carbon to forest soils, it is essential to improve the conditions particularly in disturbed forest areas, increase effective afforestation and prevent soil erosion. Particularly for forest ecosystems, there is no standard soil nutrient stoichiometry. In this sense, it is possible to consider C: N: P stoichiometries as an indication of ecosystem nutrient constraints, soil degradation and drought.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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