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Araştırma Makalesi - Research Article

Leaf Nitrogen, Phosphorous, Carbon Variation, and N and P Resorption in *Diospyros kaki* L. along an Altitudinal Gradient

Yükseklik Gradiyenti Boyunca *Diospyros kaki* L.'de Yaprak Azot, Fosfor, Karbon Değişimi ve N ve P Rezorbsiyonu

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ABSTRACT

In the present study, nitrogen (N), phosphorus (P), carbon (C) levels and nutrient contents, N and P resorption were investigated in *Diospyros kaki* L., whose leaves has a strong antioxidant property and is cultivated in the Eastern Black Sea Region. The research was carried out at four different locations where *Diospyros kaki* was grown within the provincial borders of Trabzon. Leaf samples were taken from these selected locations monthly from May to December. Soil samples were taken from a depth of 0-20 cm from each locality. Soil N, P and C analysis were also determined. There were important differences in N (%) concentration and N (g dm^{-2}) content values in terms of localities. N, P and C (%) concentration values significantly varied among months. Similarly, there were notable differences in N and P (g dm^{-2}) values depending on months. Nitrogen and phosphorus resorption efficiency (RE) values were below stated boundaries at only 796 m. Nitrogen and phosphorus resorption proficiency (RP) values significantly varied between localities.

Keywords- Diospyros kaki L., Leaves Nutrient Content, Persimmon, Resorption

ÖZ

Bu çalışmada; Doğu Karadeniz Bölgesi'nde yetişen ve güçlü antioksidan içeriğine sahip *Diospyros kaki* L.'nin azot (N), fosfor (P), karbon (C) düzeyleri ile besin içerikleri, N ve P rezorbsiyonu incelenmiştir. Çalışma Trabzon il sınırları içerisinde yer alan *Diospyros kaki*'nin yayılış gösterdiği dört farklı lokalitede gerçekleştirilmiştir. Belirlenen lokalitelerden Mayıs-Aralık ayları boyunca her ay yaprak örneği toplanmıştır. Her bir lokaliteden 20 cm derinlikten toprak örnekleri alınarak N, P ve C analizleri yapılmıştır. N (%) ve N (g dm^{-2}) değerleri lokaliteler arasında, N (%), P (%), C (%), N (g dm^{-2}) ve P (g dm^{-2}) değerleri ise aylar arasında $P < 0.01$ seviyesinde önemli farklılıklar göstermiştir. N ve P rezorbsiyon kullanım verimliliği değerleri sadece 796 m'deki lokalitede belirtilen sınırların altındadır. N ve P rezorbsiyon kullanım yeterlilik değerlerinin ise lokaliteler arasında önemli oranda farklılık gösterdiği tespit edilmiştir.

Anahtar Kelimeler- Diospyros kaki L., Rezorbsiyon, Trabzon Hurması, Yaprak Besin İçeriği

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I. INTRODUCTION

Resource availability is an important driver of ecosystem structure and function, including species diversity and nutrient cycling. Plants have developed a number of strategies to respond to changes in nutrient availability by maximizing the acquisition and retention of the most restrictive nutrients. One of these strategies is the nutrient resorption. Resorption is an important mechanisms used by plants to conserve nutrients before period of senescence [1-4]. Resorption is the transfer of elements from leaves to durable tissues before the leaf senescence usually in trees and shrubs [5]. Resorption improves the adaptation and continuity of the plant [6].

The foliar nutrient resorption is an important strategy for new leaf production in the following year and has a tight cycle in ecosystems [7]. In addition, resorption reduces the possibility of the plant's nutrient loss through annual litter fall, and thus also reduces its dependence on nutrients in the soil [4]. Resorption efficiency (RE) is expressed as the mineral element transported from senescent leaves to green leaves or resistant tissues before absorption, and is usually determined by measuring the nutrient pools of mature leaves. Herein, the nutrient pool is known as the weight of the leaf or the amount of elements per leaf area. Resorption proficiency (RP) is the amount of elements remaining in fully aged leaves [2]. RP is more objective than resorption efficiency. Low element concentration in senescent leaves means high proficiency, and vice versa. The lower the element concentration in senesced leaves, the lower the element loss by defoliation and higher the N resorption proficiency [1,8,9].

The resorption of mineral elements is a factor that limits the nitrogen and phosphorus loss of the plant and increases the nitrogen and phosphorus utilization efficiency. Resorption is a very important internal control mechanism that protects the plant from being dependent on environmental factors, especially soil [8].

The homeland of Trabzon persimmon, which is a fruit type grown in subtropical climate conditions in the world, is China. Since the entrance of the commercially grown date type to our country is via Trabzon, it has been named "Trabzon Persimmon" [10]. This research was carried out to determine the foliar nitrogen and phosphorus resorption and nutrient contents of *Diospyros kaki*, which has an economical importance for our country along with elevation and throughout one growing season. Besides, the effect of soil factor on foliar nutrient resorption was investigated.

II. MATERIAL AND METHODS

A. Study Area and Plant Samples

This study was undertaken in Barışlı Village, located in the northeastern of Turkey, Trabzon. 5 (20 m × 20 m) plots were selected in homogeneous places at each altitude of 170, 344, 485 and 796 m (Table 1). In sampling area, 5 healthy plants were randomly chosen and marked. Each plant was taken ≥ 2.5 m from the stems of neighboring canopy trees to avoid potential microsite variation [11]. Green leaf samples were collected in the first weeks from May to December 2015. Senesced leaves were collected in the first week of December. Senescent leaves were collected by shaking lightly the same branches from which green leaves were collected. Leaf samples were collected from the middle part of the canopy for each plant from the five replicate plots. The collected samples were mixed with each replicate plots.

Table 1. The geographic coordinates of all localities.

Altitudes	N	E
170 m	40° 52′ 839″	39° 42′ 080″
344 m	40° 51′ 979″	39° 42′ 339″
485 m	40° 51′ 910″	39° 42′ 899″
796 m	40° 51′ 588″	39° 43′ 172″

B. Laboratory Analyses

The leaf samples were oven-dried at 60 °C, grounded and sieved. N and C (%) concentrations of samples were detected by Dumas method [12]. P (%) concentration was detected by using standard method. The absorbance was measured at 430 nm with a Biochrom Libra S70 Double Beam Spectrophotometer [13].

Soil samples were collected from a depth of 0-20 cm, were also collected from each altitude during the growing season. On each sample date, soil and plant samples were taken from each altitude simultaneously during the growing season. The soil samples were air-dried and sieved to pass through a 2-mm screen. Soil pH values (1:1) were measured by pH-meter (Thermo Scientific Orion 3 Star, Singapore). Soil nitrogen and phosphorus

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content was spectrophotometrically detected by Kjeldahl method and by extraction with ammonium acetate, respectively.

C. Calculation of Specific Leaf Area (SLA), Nutrient Contents, Resorption Efficiency and Proficiency

Leaf area of each species was measured with a leaf-area meter (LI-3000, LICOR-USA). SLA was computed with respect to Cornelissen et al. [14]:

$$SLA = \Sigma\ LA\ (dm^2) / \Sigma\ LDW\ (g) \tag{1}$$

$$LA\text{: Leaf area } (dm^2) \tag{2}$$

$$LDW\text{: Leaf dry weight } (g) \tag{3}$$

N, C and P (g dm^{-2}) were computed with respect to the following formulas.

$$\text{Element contents}=\Sigma LDW(g)\times\text{crude element concentration} / SLA = g\ dm^{-2} \tag{4}$$

N-RE, P-RE, N-RP and P-RP were computed [15,16]:

$$RE = [(\text{Nutrient in mature leaves} - \text{Nutrient in senesced leaves}) / \text{Nutrient in mature leaves}] \times 100 \tag{5}$$

N-RP and P-RP were the concentrations of nutrient in senesced leaves [1].

D. Statistical Analyses

Statistical analysis was performed by using SPSS version 21 (IBM SPSS Statistics for Windows, Armonk, NY). One-way ANOVA used to expose whether foliar N, P and C concentrations, resorption efficiency and proficiency changed with respect to months and altitudes. After the analysis of variance, post–hoc multiple comparisons were made using Tukey HSD test.

III. RESULTS

A. Leaf N, P, C Dynamics Across All Altitudes and Months

Descriptive statistics for leaf N, P, C dynamics, N and P resorption parameters are provided in Table 2. Significant differences were not found in C (%), P (%) and P (% - g dm^{-2}) elements between localities overall, but N (%) and N (g dm^{-2}) element showed a significant difference ($P< 0.01$). All elements among the months showed notable differences.

The highest and the lowest N values (%) were at 796 m in May and 485 m in December, respectively. The highest and the lowest P values (%) were at 344 m in May and 170 m in December, respectively. The highest and the lowest C values (%) were at 796 m in December and 344 m in July, respectively (Figure 1). For N and P concentrations (g dm^{-2}), the highest value was at 344 m in September and the lowest value was at 485 m in May (Figure 2).

Table 2. Nitrogen, carbon and phosphorus values for *Diospyros kaki* according to altitudinal gradients and months (Mean ± Standart Error).

		N %	P %	C %	N ($g\ dm^{-2}$)	P ($d\ dm^{-2}$)
Altitudes	170 m	2.01±0.05**b**	0.41±0.26**a**	38.64±0.58**a**	8.52±0.73**a**	1.58±0.15**a**
	344 m	2.17±0.04**b**	0.61±0.78**a**	38.32±0.52**a**	9.00±0.91**a**	1.79±0.18**a**
	485 m	1.83±0.08**b**	0.54±0.59**a**	39.82±0.49**a**	5.25±0.44**b**	1.36±0.10**a**
	796 m	2.55±0.16**a**	0.57±0.50**a**	40.03±0.47**a**	6.93±0.56**ab**	1.61±0.21**a**
Months	May	3.11±0.23**a**	0.94±0.09**a**	41.71±0.55**ab**	1.87±0.15**d**	0.67±0.12**d**
	June	2.91±0.12**a**	0.60±0.04**b**	37.62±0.31**c**	6.50±0.27**bc**	1.54±0.17**abc**
	July	1.87±0.05**bc**	0.49±0.04**bc**	32.78±0.48**d**	8.26±0.85**bc**	2.13±0.21**ab**
	August	2.03±0.04**b**	0.62±0.11**b**	38.41±0.40**c**	7.20±0.67**bc**	1.69±0.17**abc**
	September	2.18±0.04**b**	0.48±0.06**bc**	41.26±0.28**b**	12.14±1.10**a**	2.34±0.32**a**
	October	1.88±0.03**bc**	0.43±0.02**bc**	38.24±0.33**c**	9.54±0.95**ab**	1.99±0.13**ab**
	November	1.58±0.06**c**	0.48±0.09**bc**	40.26±0.46**b**	6.03±0.68**c**	1.32±0.17**bcd**
	December	1.57±0.04**c**	0.20±0.01**c**	43.34±0.46**a**	7.84±0.24**bc**	0.97±0.03**cd**

Different letters denote significant differences between the groups according to Tukey's HSD test (rejection level 0.05)

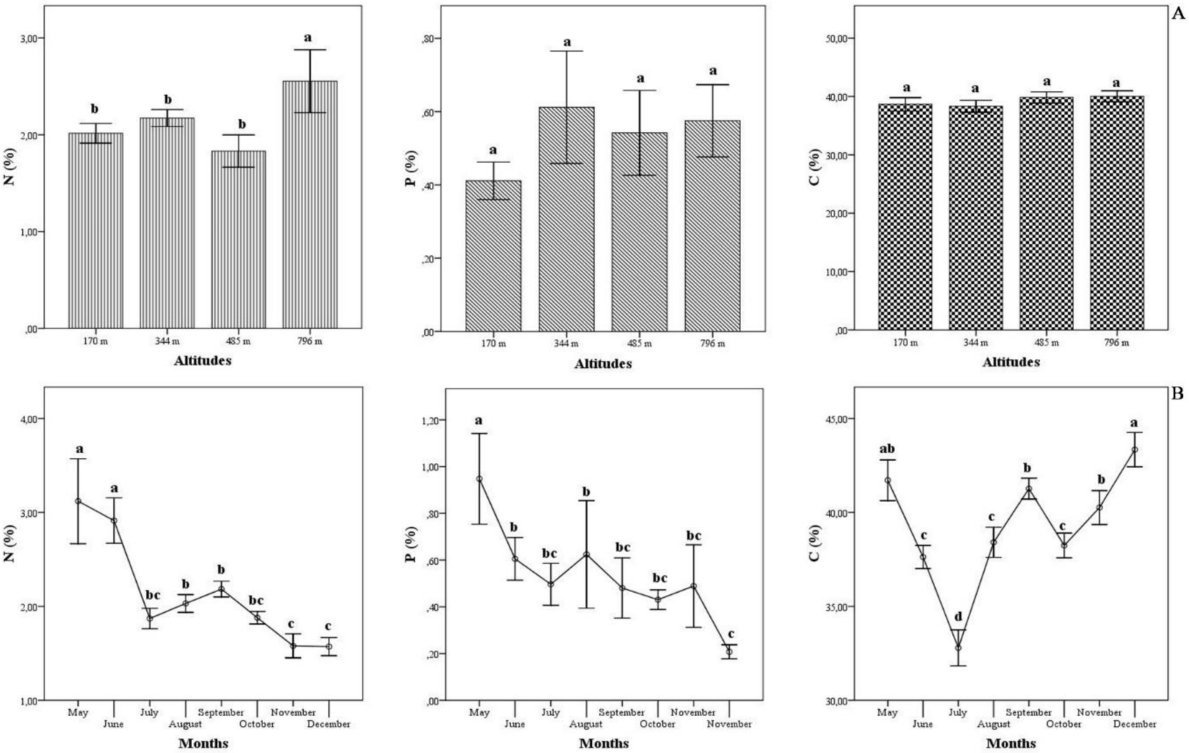

Figure 1. Nitrogen, phosphorus and carbon (%) concentrations in leaves of *Diospyros kaki* depending on altitudes (A) and months (B).

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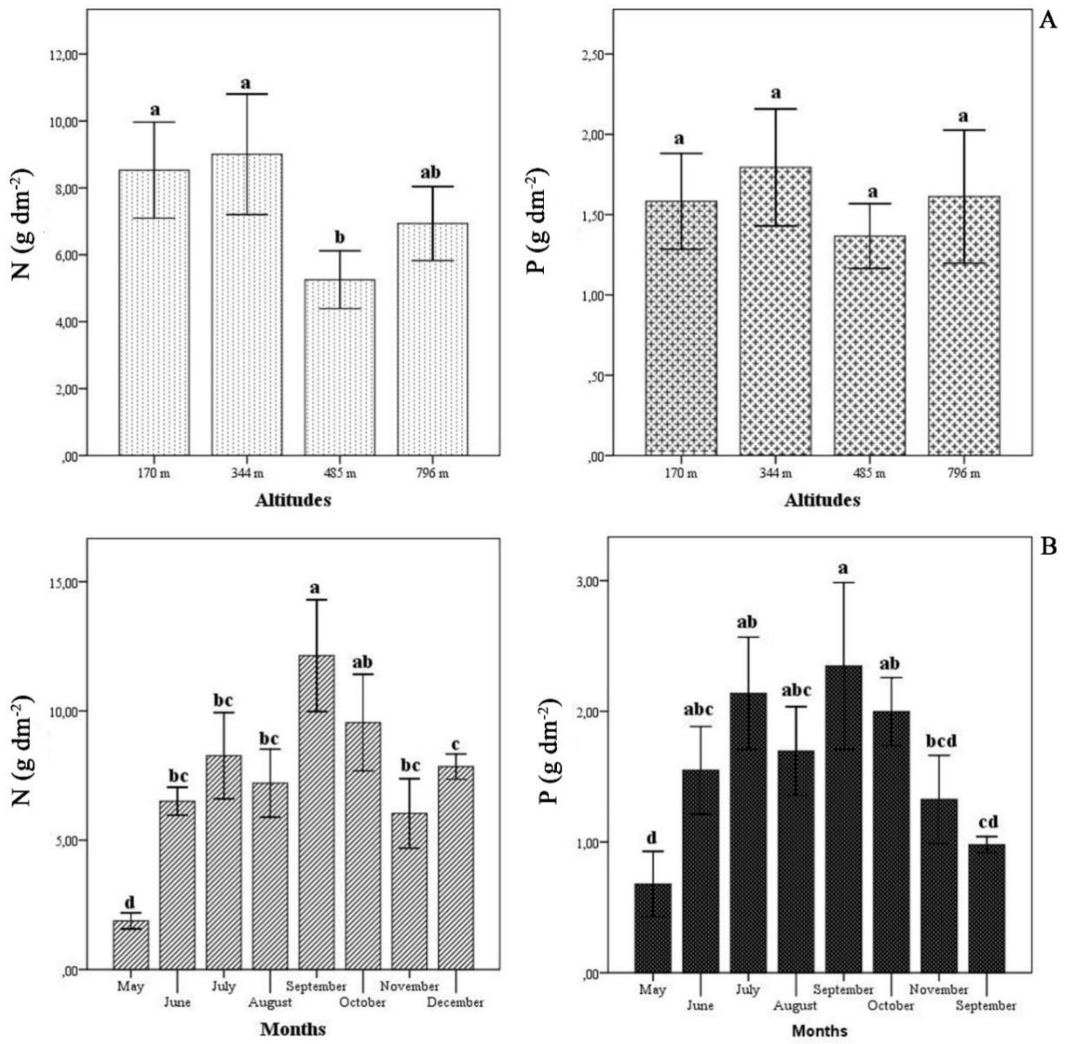

Figure 2. Nitrogen, phosphorus and carbon (g dm^{-2}) concentrations in leaves of *Diospyros kaki* depending on altitudes (A) and months (B).

B. N and P Resorption Across All Altitudes

Depending on altitudes, there were meaningful differences ($P< 0.01$) in N-RE, P-RE and N-RP (%) values, only P-RP (%) value showed a difference ($P< 0.05$).

N-RE (%) and N-RP values were high at 170 m. The lowest N-RE (%) and N-RP (%) values were at 796 and 485 m, respectively. P-RE (%) values were high at 344 m and low at 796 m. the highest and lowest P-RP (%) values were at 796 and 485 m, respectively (Table 3).

Table 3. Nitrogen and phosphorus resorption efficiency and proficiency (%) in *Diospyros kaki* across all the altitudes by using Tukey's HSD test.

Altitudes (m)	N-RE (%)	N-RP (%)	P-RE (%)	P-RP (%)
170	31.40±0.73**a**	6.86±0.12**a**	33.63±1.77**b**	1.03±0.05**ab**
344	30.43±0.32**a**	6.08±0.24**ab**	43.97±0.50**a**	0.98±0.36**ab**
485	27.54±0.42**a**	2.96±0.55**c**	32.57±1.34**b**	0.74±0.19**b**
796	19.89±1.64**b**	4.92±0.47**b**	25.31±1.20**c**	2.00±0.20**c**

Different letters in the same column indicate statistically significant differences among parameters for the mean of altitudes. Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test

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C. Soil Total N, P and C (%) Concentrations Across All Altitudes and Months

Soil N, P and C (%) concentrations of *Diospyros kaki* had valuable significant differences. The highest and lowest N and C (%) values were at 485 m and 344 m, respectively. P (%) values were high at 485 m and were low at 170 m (Table 4).

Table 4. Soil physicochemical properties of all sampling localities.

Altitudes (m)	N (%)	P (%)	C (%)
170	0.12±0.006**b**	0.06±0.015**a**	1.68±0.04**b**
344	0.04±0.001**c**	0.09±0.008**ab**	0.99±0.20**b**
485	0.34±0.02**a**	0.12±0.01**a**	4.67±0.31**a**
796	0.11±0.004**b**	0.06±0.005**b**	1.34±0.10**b**

Different letters in the same column indicate statistically significant differences among parameters for the mean of altitudes. Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test.

IV. DISCUSSION

In the present study, N, P and C dynamics of *Diospyros kaki* from Turkey according to localities and growing period are reported. In addition, N and P resorption values of *D. kaki* were investigated based on localities.

The concentrations of nutrients vary depending on their physiological functions, chemical nature, nutrient supply levels and other factors throughout the life of the leaves [17].

N and P (%) concentrations of *Diospyros kaki* reached the highest values in May when the leaves first started to emerge. This situation may have been caused by the high rate of photosynthesis with the first formation of leaves at the beginning of the growing season. The lowest N and P (%) concentrations were observed in December, when the leaves entered the senescence period. The decrease in photosynthetic activity in the leaves during the senescence period and the transport of leaf nutrients back to the durable tissues may be the cause of this situation [18].

Özbucak et al. [19], Turkis and Özbucak [20] stated in their study that N and P concentrations increased with increasing height. As similarly, it was found that N, P and C (%) concentrations of *Diospyros kaki* increased with increasing height (Figure 1A). The reason for this may be that the humidity decreases depending on the altitude and the N and P mechanisms are activated as a result [19].

As a result, it was determined that the leaf nutrient contents of *Diospyros kaki* changed according to the changing environmental conditions and showed differences according to the growing season.

Resorption of nutrients in plants, it is determined by calculating the resorption efficiency and resorption efficiency values [21]. It has been stated that in deciduous species, N-RE and P-RE varies between 40-75% for N and 30-70% for P [22]. In other studies, it has been reported that the N-RE and P-RE values in deciduous species vary between 26-64% and 56-71%, respectively [23,24]. The mean N-RE and P-RE values (27.31% and 38.87%, respectively) of *Diospyros kaki* were nearly the same as those reported. Based on the obtained data, the N-RE and P-RE values were in accordance with the values stated in the literature. Our findings show that all studied altitudes effectively resorbed N and P nutrients except for 796 m (Table 3). Wang et al. [6] stated that an average N-RE and P-RE (%) values of lucerne (*Medicago sativa*) were 16.2 and 27.3, respectively. Stackpoole et al. [25] found that resorption efficiency of cranberry was 35.7 (%). Roley et. al [26] determined that N resorption efficiencies averaged 53% for lowland versus 29 % for upland switchgrass cultivars. Aerts [22] indicated that the N-RE and P-RE differed among evergreen shrubs and trees, deciduous shrubs and trees, graminoids and forbs, and also varied by sites.

Rejmankova [16] reported that the RP was more effective than the RE in determining nutrient availability. Resorption proficiency is determined by the lowest nutrient concentrations in leaves and is the nutrient concentration in completely senescent leaves [1]. During the senescence period, the amount of nutrients decreases significantly as a result of the aging of the leaves and the breakdown of chlorophyll [15]. If the lowest N and P concentrations are below 0.7% and 0.05% in senesced leaves respectively, N and P resorption is accepted sufficient [1]. In our study, NR-P and PR-P values of *Diospyros kaki* are above the values of 0.7% and 0.05%, respectively, resorption is not biochemically sufficient.

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There have been many studies in the literature stating that leaf nutrient resorption efficiency and proficiency values change with altitude. Bilgin and Güzel [27] stated that NR-E and PR-E (%) values of *Tilia rubra* subsp. *caucasica* increased with the increasing altitude, but NR-P and PR-P (%) values decreased. Similarly, Bilgin et al. [4] found that the leaf N and P resorption efficiency of *Vaccinium myrtillus* increased with the altitude, while the N-RP and P-RP reduced. Also, Bilgin et al. [4] pointed out that N resorption efficiency values changed with height. In the current study, while NR-P decreased depending on the altitude, PR-P decreased until 485 m and the highest PR-P level was found in the locality at 796 m. It was determined that N and P resorption efficiency values decreased depending on the height (Table 3). As a result, it was determined that leaf N and P resorption values varied with the height. This may be due to changes in leaf or soil nutrient content.

It is thought that the species growing in low fertile soils have higher nutrient resorption efficiency values. It is also known that high nutrient resorption efficiency values depend on changes in nutrient availability. This shows us that although nutrient resorption is a sign important protection strategy, change in soil nutrients is effective in the distribution of species. In this work, it was found that the % N values in the soil samples taken from the research areas were between 0.05 and 0.38, while the % P values varied between 0.03 and 0.14 (Table 4). This situation shows us that the N content of the soils in the study area is higher than the P content. Short-term increases in soil fertility reduce or increase resorption or have no effect. For example, in *Larrea tridentata* D.C., it has been reported that fertilization does not have an effect on resorption, whereas in *Typha latifolia* L., one of the marsh plants, fertilization decreases the resorption. On the other hand, short-term fertilization increased the resorption of *Pinus radiata* in D. Don [28, 29]. For resorption efficiency, mulching decreased P-RE by an average of 3.0%, while P fertilization increased the N-RE and P-RE values in alfalfa by an average of 6.8% and 6.2% over two years, respectively [30]. In the current research, N-RE and P-RE values were generally determined between the limits specified in the literature, but N-RP and P-RP values were found to be higher than other deciduous species. Similarly, Oikawa et al. [31] reported that legume species were not sufficient to resorb nitrogen from aging leaves. N and P resorption use adequacy could not be achieved due to the excessive elements in the soil and the plant could not obtain a recovery that could feed itself. It is thought that the main reason for this may be the fertilizers used around the work area. This situation may adversely affect the development of persimmon plant in adverse conditions such as climate changes and competition that may occur in the future.

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