

Foliar resorption of nitrogen and phosphorus in Alcea apterocarpa (Malvaceae) in different habitats types

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ABSTRACT – The study goal is to reveal foliar resorption patterns of *Alcea apterocarpa* populations in different habitats (riverbank, meadow and forest clearing) in the Central Black Sea Region of Turkey. We determined foliar nitrogen and phosphorus resorption, considering proficiency and efficiency. The resorption efficiency was also calculated using the mass loss correction factor (MLCF). Some studies indicated that MLCF provides unbiased resorption values. Phosphorus resorption proficiencies (PRP) of species were intermediate level, while nitrogen resorption proficiencies (NRP) were complete level. Phosphorus resorption efficiencies (PRE) were intermediate level for all habitats considering MLCF, while in meadow habitat, PRE was complete level without MLCF. Nitrogen resorption efficiencies (NRE) were intermediate level for all habitats without MLCF, but NRE values of meadow and forest clearing were incomplete level as using MLCF. Green leaf N/P ratios of *A. apterocarpa* were higher in riverbank, while senescence leaf N/P ratios were higher in meadow habitat. Only, the regression relationship between PRP and green leaf N/P ratio was not significant.

Keywords: endemic herb, nutrient reuse, senescence

RESUMO – Reabsorção foliar de nitrogênio e fósforo em *Alcea apterocarpa* (Fenzl.) Boiss (Malvaceae) em diferentes tipos de habitats. O objetivo do estudo é revelar os padrões de reabsorção foliar de populações de *Alcea apterocarpa* em diferentes habitats (margens de rios, prados e clareiras florestais) na região central do Mar Negro da Turquia. Determinamos a reabsorção foliar de nitrogênio e fósforo por cálculos de proficiência e produtividade. A eficiência de reabsorção também foi calculada usando o fator de correção de perda de massa (MLCF). Alguns estudos indicaram que MLCF fornece valores de reabsorção imparciais. Sua adequação de recaptação de fósforo (PRP) é moderada e sua adequação de recaptação de nitrogênio (NRP) é completa. As eficiências de recaptação de fósforo (PRE) foram moderadas para todos os habitats considerando MLCF, e PRE em habitat de pastagem estava cheio sem MLCF. As eficiências de recaptação de nitrogênio (NREs) foram moderadas para todos os habitats livres de MLCF, mas faltando NREs para pastagem e desmatamento com uso de MLCF. As relações N/P das folhas verdes de *A. apterocarpa* foram maiores na margem do rio, enquanto as relações N/P das folhas em senescência foram maiores no habitat de várzea. Apenas, a relação de regressão entre PRP e relação N/P das folhas verdes não foi significativa.

Palavras-chave: erva endêmica, reutilização de nutrientes, senescência.

INTRODUCTION

Foliar resorption affects the nutrient balance of a particular plant, plant competition and ecosystem processes. Foliar resorption is referred in two ways as resorption efficiency and proficiency. Resorption efficiency represents the percentage reduction of a nutrient concentration between the green and senesced leaves, while resorption proficiency is just the amount of a nutrient that resides in senesced leaves (Killingbeck 1996, Kilic *et al.* 2010). Resorption proficiency also may indicate biochemical limits of nutrient reuse in species adapted to diverse soil fertility conditions (Aerts & Chapin 1999, Diehl *et al.* 2008, Du *et al.* 2011, Kilic *et al.* 2012).

The strategy of foliar nutrient resorption is usually related to habitat types. Significantly, soil nutrients effect to foliar nutrient resorption (Drenovsky & Richards 2006, Brant & Chen 2015). Soil nitrogen (N) and phosphorus (P) availability plays a key role in the foliar nutrient resorption (Kozovits *et al.* 2007, Huang *et al.* 2012). N and P have high mobility between tissues during the growth and development period, and their mobility decreases as a result of resorption at the end of the vegetative period (Tecimen & Makineci 2007). N participates in the structure of proteins, while the element phosphorus plays an important role in cellular energy transfer. However, both elements are components of nucleic acids (Darrah 1993, Hinsinger 1998, Marchner 2011). Therefore, soil nutrient

availability is critical in understanding changes in foliar nutrient absorption, plant nutrient conservation strategies, and nutrient cycling.

Plants competent of high nutrient resorption have a high aptitude to reuse their intimate nutrients, rather than losing more of them via litter. Many extraneous and essential variables, including environmental and biological factors, influence nutrient resorption capacity of plants in natural environments. For a particular habitat type, the leaf traits may coincide to make the equilibrium between growth and environmental conditions (Chang *et al.* 2017, Yan *et al.* 2017).

Kobe *et al.* (2005) and Vergutz *et al.* (2012) indicated that leaves with high concentrations of a particular nutrient have lower resorption efficiency for that specific nutrient, but some inconsistencies were also found mainly at regional scales (Enoki & Kawaguchi 1999, Norby *et al.* 2000, Lal *et al.* 2001). It has been stated that foliar N and P resorption vary comparable to each other over gradients of N and P limitation in different ecosystems. To indicate this trend N/P ratios between green and senesced leaves should be compared (Richardson *et al.* 2008). Both resorption efficiency and resorption proficiency calculations and N/P ratios showed that foliar resorption as an adaptation mechanism for plant species to balance their need for these elements in response to alterations in environmental availability (See *et al.* 2015).

Alcea apterocarpa (Fenzl.) Boiss. (Malvaceae) is a perennial herb and an endemic species belonging to Irano-Turanien phytogeographical region. Its natural habitats are stony, fields and maquis areas. It is in "LC" category according to red list of threatened species (IUCN) (Kutbay et al. 2014, Sürmen et al. 2016). This plant is generally used in folk medicine, landscape and ornamental studies. In recent years, many studies have been done about ethnomedicinal researches (Demir et al. 2017).

There were many studies on foliar resorption in woody species (Killingbeck et al. 1990, Côté et al. 2002, Niinemets & Tamm 2005, Covelo et al. 2008, Blanco et al. 2009, Kilic et al. 2010, Kilic et al. 2012, See et al. 2015, Yan et al. 2017), while foliar absorption studies of herbaceous plants, especially in endemic species, are very few in temperate ecosystems (Boerner 1986, Urgenson et al. 2009, Li et al. 2012, Gilliam et al. 2016).

In this study, especially resorption efficiency values were calculated according to two methods. These are the methods using the standard and the mass loss correlation factor (Killingbeck 1996, Vergutz *et al.* 2012). Mass loss correlation factor is specifically the ratio of the dry mass of senesced leaves and the dry mass of green leaves (van Heerwaarden *et al.* 2003). Some studies indicated that MLCF defines quantify unbiased resorption values

(Yan et al. 2016, Ji et al. 2018, Jiang et al. 2019). On a global scale, N and P nutrient elements have a limiting effect for plant species because of geological history and soil type (Walker and Syers 1976, Reich & Oleksyn, 2004). We hypothesized that there would be significant differences in foliar N and P resorption levels of *Alcea apterocarpa* in different habitats (riverbank, meadow and forest clearing). We think that the main reason for this difference is that each habitat has different soil types and vegetation structure.

MATERIALS AND METHODS

The study area was selected in Samsun/Turkey in Centre Black Sea Region. This region belongs to Euxinian sector of Euro-Siberian phytogeographical region. It has an oceanic type climate with a mean annual precipitation of 885.2 mm; summer drought is not observed in the area. Mean annual temperature is 13.8 °C. Summer rainfall is 152.2 mm. Mean maximum for the hottest month and mean minimum for the coldest month are 27.7 and 2.1 °C, respectively. The precipitation regime in Samsun is East Mediterranean-type (autumn, winter, spring, summer).

This study was carried out in three habitats in Samsun province. Habitat types are riverbank (H1), meadow (H2) and forest clearing (H3). The main differences between habitats are soil types and vegetation structures. Riverbank soil type is alluvial, meadow soil type is grey-brown podzolic and forest clearing soil type is brown forest soils. Each habitat characterized by different plant species. Riverbank habitat is characterized by *Phillyrea latifolia* L., Cistus creticus L., Ferula communis L. subsp. communis, Spartium junceum L. Meadow habitat is characterized ruderal species which are Cynodon dactylon (L.) Pers., Ficus carica L., Sinapis alba L., Sonchus asper (L.) Hill, Senecio vernalis Valdst. & Kit., Malva parviflora L., Xanthium strumarium L. Forest clearings habitat is characterized by cut oak which are *Quercus petraea* (Mattuschka) Liebl. subsp. iberica (Steven ex M. Bieb.) Krassiln., Quercus cerris L. var. cerris L. forests. Plant species compete with each other against shading. Altitude, slope and direction are effective in shading periods of plants. Considering the shading features in habitats; Riverbank habitat is under shadow in the early and late hours of the day, meadow habitat is almost non-shaded, and finally, forest clearing habitat is under the influence of shadowing late in the day.

Average height of adult individuals is 1.5 m. Individuals closest to 1.5 m were selected from each habitat. Leaf samples were collected on the same day. Five fully expanded leaves were randomly collected from 10-15 marked individuals to the leaf base in each habitat on 2018. When a leaf or at least two-thirds of its area turned

yellow or brown, it was considered senesced (Ren et al. 2011, Kilic et al. 2012). July and November leaves were used as "green" and "senesced" leaves, respectively. These leaves were removed by a gentle flicking of the branch or leaf (Yuan et al. 2005, Luo et al. 2010, Yilmaz et al. 2012). In late July (flourishing period), 5 g of fully expanded green leaves were randomly collected from 10–20 marked individuals of each species. Leaf samples were oven-dried at 60° C until they reached a constant weight and then weighed.

Seven soil samples of 0-30 cm depth were collected in each habitat types using a drill. The soil samples were air-dried and then sieved to pass through a 2-mm screen. Soil N was determined by the micro Kjeldahl method. Soil available P (g.kg⁻¹) was spectrophotometrically determined by Olsen method following extraction by sodium bicarbonate (Allen *et al.* 1976).

N and P concentrations per leaf area were used to calculate nutrient resorption (Kilic *et al.* 2012). Nitrogen and phosphorus (%) resorption efficiency (NRE and PRE) were calculated considering two methods. The first method is the percentage of N, and P recovered from senescing leaves (Killingbeck 1996) and the second method is by using a mass loss correction factor (MLCF) suggested by Vergutz *et al.* (2012). MLCF is defined as the ratio of the dry mass of senesced leaves to the dry mass of green leaves (van Heerwaarden *et al.* 2003).

Nutrient RE =
$$(1 - \frac{Nutrient \ in \ senescent \ leaves}{Nutrient \ in \ green \ leaves}) \times 100$$

$$\mathit{MLCF} = \frac{\mathit{Senescent leaves mass}\left(g\right)}{\mathit{Green leaves mass}\left(g\right)}$$

$$\textit{Nutrient RE} = (1 - \frac{\textit{Nutrient in senescent leaves}}{\textit{Nutrient in green leaves}} * \textit{MLCF}) \times 100$$

Resorption proficiency (RP) is defined as the level to which a nutrient (N and P) is reduced in a senescing leaf or in short as the concentration of a nutrient in senesced leaves (Killingbeck 1996, Hoch *et al.* 2003).

Statistical analyses were performed by SPSS software version 25.0 (SPSS Inc. 2017). One-way ANOVA investigated the differences among habitat types. Dependent variables were green and senescent N:P ratios, NRE, PRE, NRE_{mlcf}, PRE_{mlcf}, NRP, PRP and soil traits (N, P), respectively. Independent variables were habitat types. Tukey's honestly significant difference (HSD) test was used to rank means. Regression analyses investigated the relationships between resorption values and green/senescent

N:P ratios. The correlation test determined the direction of the relationship between parameters.

RESULTS

PRP values for all habitat types were found between 3 $\mu g/cm^2$ and 8 $\mu g/cm^2$ and at intermediate level. PRP values are 3.5 $\mu g/cm^2$ in riverbank, 5.02 $\mu g/cm^2$ in meadow and 5.60 $\mu g/cm^2$ in forest clearing habitat. Nitrogen resorption proficiency (NRP) values for all habitat types were found to be less than 50 $\mu g/cm^2$ and at complete level. NRP values are 10.11 $\mu g/cm^2$ in riverbank, 22.70 $\mu g/cm^2$ in forest clearing and 23.59 $\mu g/cm^2$ in meadow habitat (Fig. 1).

Phosphorus resorption efficiency (PRE) values without MLCF are 49.52 % in riverbank, 57.51 % in forest clearing and 78.15 % in meadow habitat. NRE values without MLCF are 44 % in forest clearing, 49.49 % in meadow habitat and 57.23 % in riverbank and were found at intermediate level. PRE values with MLCF were found at intermediate level for all habitats (45.38 % in riverbank, 53.18 % in forest clearing and 68.82 % in meadow). NRE values with MLCF were incomplete level in meadow and forest clearing (27.92 % in meadow and 38.29 % in forest clearing). NRE was intermediate (53.73 %) in riverbank when MLCF was used. Significant differences were found among studied habitats concerning RE and RP patterns (Fig. 1).

Green leaf N/P ratios were found to be higher in riverbank (35.66) than meadow (20.28) and forest clearing (31.26) habitats, while senescence leaf N/P ratios were found to be higher in meadow (47.75) than riverbank (29.29) and forest clearing (41.42) habitats. Statistically differences were found for green and senescence leaf N/P ratios among habitat types (Fig. 2).

When all habitat types considered, only the regression between PRP and green leaf N/P ratio was not significant (Fig. 3).

A similar pattern was found the regression between senescence leaf N/P ratio and PRP (Fig. 4).

NRE and NRE (MLCF) were positively correlated with green leaf N/P ratio, while PRE, PRE (MLCF), NRP negatively correlated with green leaf N/P ratio. Positive correlations were found between PRE, PRE (MLCF), NRP and senescence leaf N/P ratio, while negative correlations were found between NRE, NRE (MLCF) and senescence leaf N/P ratio (Tab. 1).

The highest soil N and P concentrations were found in meadow, while the lowest soil N and P concentrations were found in riverbank and forest clearing, respectively. Statistically differences were found for all soil traits among habitat types (Tab. 2).

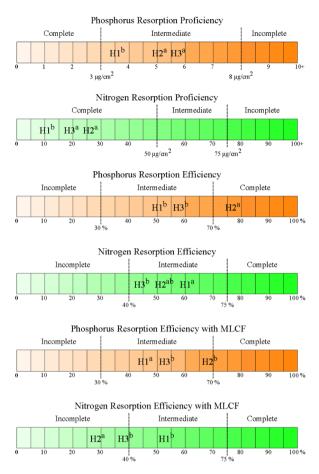


Figure 1. Resorption proficiency and efficiency levels of *A. apterocarpa* according to all patterns. Habitat abbreviations are H1: riverbank, H2: meadow and H3: forest clearing. Minor letters followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test.

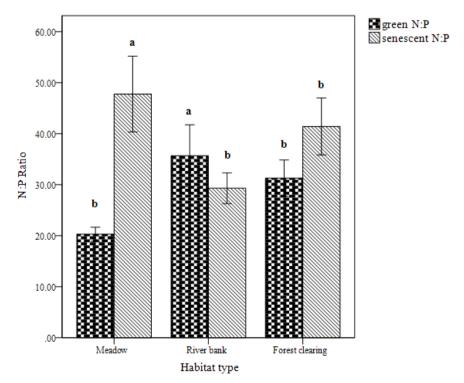


Figure 2. Green and senescence N:P ratio of A. apterocarpa in different habitat types. Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test.

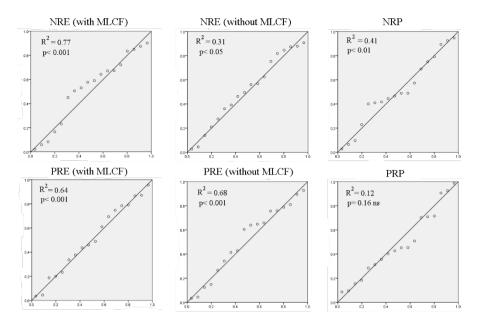


Figure 3. Regression between green leaf N/P ratio and RE and RP of A. apterocarpa.

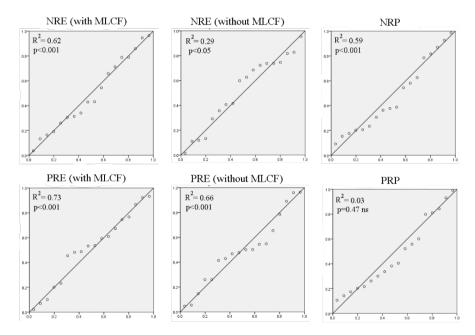


Figure 4. Regression between senescence leaf N/P ratio and RE and RP of A. apterocarpa.

 $\textbf{Table 1.} \ Correlation \ coefficients \ between \ leaf \ N/P \ ratios \ and \ resorption \ patterns \ of \ \textit{Alcea apterocarpa}.$

Resorption parameter	Green leaf N/P ratio	Senescence leaf N/P ratio
NRE	0.554*	-0.538*
NRE(MLCF)	0.877**	-0.786**
PRE	-0.825**	0.810**
PRE(MLCF)	-0.799**	0.852**
NRP	-0.642**	0.766**
PRP	-0.348	0.180

Table 2. Soil traits in studied habitats (mean ± SE). Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test.

Habitat	Soil type	Soil N content (%)	Soil P content (g kg ⁻¹)
Riverbank	Alluvial	$0.30 \pm 0.09 b$	$0.008 \pm 0.005a$
Forest clearing	Brown forest	$0.33 \pm 0.04b$	$0.003 \pm 0.001b$
Meadow	Gray-brown podzolic	$0.61 \pm 0.07a$	$0.008 \pm 0.003a$

DISCUSSION

NRE and PRE were found to be lower than that of the NRE and PRE values reported by Brant & Chen (2015) who found NRE and PRE 66% and 63%, respectively, for different life forms. However, PRE was found to be higher in meadows than the reported values. PRE was found to be higher as compared to NRE in meadows and forest clearings, while NRE was found to be higher in riverbank. P fractions in a leaf are more easily broken up and reabsorbed than N fractions, and hence PRE provides more than to nutrient use efficiency than NRE. High PRE also indicated that the less P availability in a particular ecosystem (Covelo et al. 2008, Salazar et al. 2011, Martinez-Sánchez 2005, Yilmaz et al. 2012). McGroddy et al. (2004) also proposed that the different patterns in NRE and PRE result from differences in the biochemistry of the two elements. The part of plant N that is organized in cell walls cannot be re-allocated, and this sets a line on the N resorption. In contrast, most of the P is in models that can be retranslocated, but at a cost, and P resorption will, therefore, be more flexible (Ågren 2008).

De Aldana & Berendse (1997) found that the N-use efficiency of species at constant levels of nutrient supply tends to increase with increasing nutrient availability in their preferred habitat. Soil N concentration could influence leaf N status along a habitat gradient (Yuan et al. 2005, Luo et al. 2010). The highest NRP was found in A. apterocarpa populations in meadows where soil N concentration was the highest. Animal feces are the main reason for high soil nitrogen in meadow habitats (Sürmen et al. 2015). The plants in this environment are exposed to higher rates of solar radiation and therefore need a higher concentration of N for the production of chlorophylls (Turkis & Ozbucak 2010, Karavin 2013). The duration and spectral properties of light are effective in leaf fall and growth (Burrows 1990). Yan et al. (2016) stated that species in the nutrient-poor soil are generally expected to be high RE and RP. Riverbank populations were more proficient regarding RP than meadows and forest clearings

An increase in the N/P ratio during senescence indicates higher PRE relative to NRE (Wood *et al.* 2011, Gilliam *et al.* 2016). However, the N/P ratio was decreased in senescence leaves in riverbank populations. NRE was also found to be higher in riverbank populations. Foliar N/P ratio below 14 indicated N limitation (Aerts & Chapin 1999, Finzi *et al.* 2004). Güsewell (2004) suggested if

N<10 N-limitation occurred, while if P>20 P-limitation happened in a particular habitat. Based on the recommended threshold values, P-limitation occurred in all of the studied habitats. It has also been found that P was more proficiently resorbed than N in all of the studied habitats. This may probably be related to the low soil P concentrations in all of the studied habitats. The different patterns among studied habitats might be associated with the different types of nutrient limitation, and the high reuse proficiency of P could have favored moderating P limitation in the study area (Li *et al.* 2012, Pang *et al.* 2018).

Resorption efficiency should be calculated by using MLCF because intermediate NRE was found in all of the studied habitats when MLCF was not used. Similarly, intermediate PRE was found in forest clearings and riverbank, while complete PRE was found in meadows when MLCF was not used. However, different results were found when MLCF was used. Intermediate NRE was found in riverbank populations, while incomplete resorption was found in a forest clearing and meadow populations when MLCF was used. PRE was intermediate in all of the studied habitats when MLCF was used.

Foliar RE and RP patterns were changed in A. apterocarpa individuals across different habitat types due to the changes in soil N and P concentrations. Alluvial soils are deep and permeable soils. It is rich in plant nutrients. They are easily processed as they consist of sand, clay and gravel. Brown forest soils thrive under broad-leaf deciduous forests in winter in temperate climates. It is rich in organic matter (humus) because it is formed under the forest. Soil is acidic. Gray - brown podzolic soils have a thin organic layer and light colored mineral soil beneath it. Soil reaction is usually medium acid. The fertility of these soils varies greatly depending on the main material (Şahin 2012). This may be an indication of multiple-element control foliar resorption, and foliar resorption is potentially crucial for the plant nutrient budget (Freschet et al. 2010, See et al. 2015). In addition to this, the relationships between the N/P ratio of green and senesced leaves and RE and RP were also changed in different habitat types.

These results emphasize that the relationship between resorption processes and plant economics, which is potentially influenced by several plant physiological and structural adaptations to environmental factors other than nutrient stress. Leaf N resorption patterns were mainly determined by soil N status across habitats, and there were some consistent patterns among life forms.

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