Comparison of Variability in Leaves and Roots Nutrient Contents of Persian Oak (*Quercus brantii* Lindl.) in Drought Affected Declining Forests

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Drought-induced crown dieback depends on plant nutrient status. This research examined the leaves and roots nutrients of Persian oak trees (*Quercus brantii* Lindl.) under the effects of drought and tree decline. The leaves and roots of oak trees were sampled randomly on the four main sides of their crown to analyze the N, P, K, Ca, and Mg. In healthy and declining trees, the N, P, and K content in the leaves was higher than in the roots, while the Ca content in the leaves was lower than in roots. The N and P in the roots of healthy trees had decreasing temporal changes, but these elements did not have significant temporal changes in the roots of declining trees. Leaf P showed decreasing temporal changes. The temporal decrease of root N and P and leaf P showed a negative effect of drought on Persian oak trees by reducing their ability to absorb water and nutrients and transfer them to their leaves. The higher concentration of elements in the leaves of oak trees, in addition to showing the decrease in the absorption of elements by the roots from the soil, can indicate a protective mechanism of oak trees against drought stress.

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INTRODUCTION

In the last decade, severe droughts caused by climate change have occurred in the forests of western Iran, and many tree deaths have occurred (Hamzehpour *et al.* 2011; Hosseini *et al.* 2012, 2017, 2018; Hosseini, 2017; Aazami *et al.* 2018; Hoseinzadeh *et al.* 2018). Hosseini *et al*. (2017) stated that long-term reduction in rainfall and increase in temperature happened in the western region of Iran, in the Zagros forests, which led to a drought. Drought has affected the Persian oak (*Quercus brantii* Lindl.) trees by reducing the rainfall and soil moisture and has caused them severe stress. Drought has a great impact on plant nutrients through its effect on water-soil-plant relations (Graciano *et al.* 2005; Sardans *et al.* 2008a). There are several reports indicating the effects of water stress on nutrients in forest trees (Sardans *et al.* 2008a,b; Mohammadzadeh *et al.* 2021; Hosseini and Hosseini 2022). Drought can change the absorption of elements through processes such as reducing the soil moisture content, affecting the properties of the soil and the activity of soil enzymes, reducing plant growth, and changing the status of plant photosynthesis and metabolism and its ability to acquire nutrients (Sardans *et al.* 2008b). In addition, plant elements may increase under drought conditions due to the concentration effect caused by growth reduction or as a result of the accumulation of elements in some plant tissues as a mechanism to avoid drought (Sardans and Penuelas 2007). The effect of drought on the concentration of nutrients in tree organs is diverse and depends on the element, tree species, and forest ecosystem (Sardans *et al.* 2008a,b). The response to drought is different in various plant species, and it is a function of their metabolic and physiological status and adaptability to drought conditions (Sardans *et al.* 2008a). In species more sensitive to drought, the variations in their body are higher and more noticeable. The Persian oak has been one of the most sensitive species to recent droughts; this species exhibits the highest rate of crown dieback and tree death in the Zagros forests in western Iran (Hosseini *et al.* 2017). It is expected that the changes in nutrients in the organs of Persian oak trees will be more noticeable, and in fact, one of the reasons for choosing Persian oak trees for this research was this idea.

Investigating the variations of nutrients contents in different organs of trees is one of the ways to study their response to the stresses (Niinemets 2010; Hosseini 2017). In this field, drought stress has been created artificially, and the status of elements in the body of under stress trees has been compared with trees that are not under stress (Sardans and Penuelas 2007; Sardans *et al.* 2008a). In the present research, all the forest trees located in the habitat were exposed to drought, but some trees remained healthy, and some others suffered from crown dieback, such that the nutrients status of healthy trees could be compared with those of declining trees. In various studies, different organs have been selected to examine the status of elements. Some researchers have investigated the elements in the wood and bark of the tree trunk (Mohammadzadeh *et al.* 2021), while others have investigated the nutrient status in leaves, bark, and soil (Sardans and Penuelas 2007; Sardans *et al.* 2008a). In this research, the tree root has been selected as one of the important organs of the tree that has a direct relationship with the soil and has the task of absorbing water and nutrients, and the status of nutrients and how their concentration changes in the root have been compared with the leaves of Persian oak trees, and at the same time, these comparisons have been made between healthy and declining trees. The results of some studies have shown that the way elements change in different tree species in response to stress is different. For example, Sardans and Penuelas (2007) found that the pattern of element concentration changes in different tree species and in each species in different organs is different under the influence of drought. They stated that the different responses of the species in terms of nutrients status due to drought are consistent with their growth and mortality and reproduction responses. Sardans *et al.* (2008b) also found that drought stress increased leaf Mg concentration in *Phillyrea latifolia*, *Quercus ilex*, and *Arbutus unedo*, but in *Q. ilex* and *A. unedo* was significant. Calcium had a significant decrease in *P. latifolia*. They stated that a decrease in the accumulation of elements in the whole biomass is related to a decrease in soil moisture and that also it is related to the decrease in photosynthesis capacity, sap flow, transpiration, plant growth, and absorption power in drought conditions, which was observed in *Q. ilex* and *A. unedo* species. They concluded that *P. latifolia* has more ability to adapt to drought conditions than the other two species.

Considering the different behavior of tree species in terms of the nutrient concentration variability in their organs in response to stress, investigating the response of Persian oak trees to drought and tree dieback events is an important step towards understanding the effects of drought on the physiological characteristics of the roots and leaves of Persian oak trees, which in this regard, the affected nutrients by drought will be identified and used as biological indicators in future studies. Paying attention to the extent of tree decline in the vast forests of western Iran highlights the importance of this research. The goals of this research include: 1) determining how the amounts of elements change in the leaves and roots of healthy and declining Persian oak trees, 2) comparing the elements contents in the leaves and roots of healthy and declining oak trees, and 3) determining the temporal changes of nutrients contents in the roots and leaves of oak trees.

EXPERIMENTAL

Research Area

This research was accomplished in the Meleh-siah forested area, located the north of Ilam province, Western Iran (3735102 N and 612610 E, UTM) (Fig. 1). This region is a part of the Zagros Mountains, which has a Mediterranean to semi-Mediterranean climate with cold winter (Asakereh 2007). The average annual temperature in the region is 16 °C, and the average annual rainfall is 509 mm (Asakereh 2007). Meteorological data for this research were available from the climate station of Ilam (10 km from the research area). This area is located on the northern aspect of Meleh-siah Mountain, and its topography conditions at the sampling sites were homogenous in slope, aspect, and elevation. The general slope of the research area is gentle. It is located at an altitude of 1,460 to 1,490 m above sea level. The bedrock is limestone. The soil texture in the research area is clay-loam, and soil pH varies slightly across this area, an average of about 7.5 (Hosseini 2017). Persian oak is the dominant tree species, accompanied by other tree and shrub species, such as *Pistacia atlantica*, *Acer monspessulanum*, and *Crataegus pontica*. There are two forms of oak trees in the research area: standard and coppice, where the frequency of coppice trees is more than that of standard trees.

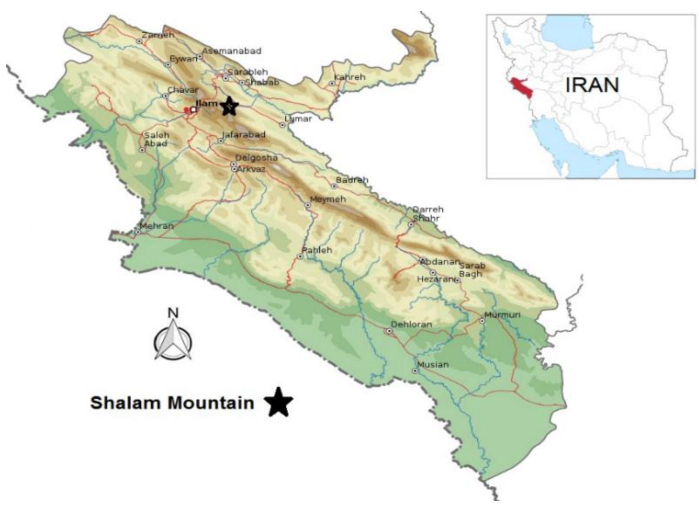

Fig. 1. The study area: Meleh-siah Mountain in the northern half of Ilam province, in western Iran

Research Methods

The crown condition of trees was evaluated with respect to the percentage of crown dieback (Kabrick *et al.* 2008; Hosseini *et al.* 2017; Hosseini and Hosseini 2022). According to Kabrick *et al.* (2008), there were four classes of crown condition in the research area including: healthy (0 to 5% crown dieback), low (5 to 33% crown dieback), moderate (33 to 66% crown dieback), and severe (more than 66% crown dieback). Six trees were randomly selected from the healthy and moderate classes as replications (a total of 12 trees were sampled). The selected trees were all in the form of standard and had approximately the same or similar DBH. A random sampling of leaves in the four main sides of the tree crown and of the end branches in the middle part of the crown was done. 20 mature leaves were collected from each tree and transported to the laboratory for element analyses. Also, holes up to 70 cm deep were dug in the four main sides of the tree, and tree root samples were collected for each tree from these holes and were taken to the laboratory for element analyses (a total of 12 combined root samples were collected). After the extraction process, the concentration of elements of roots and leaves, including nitrogen, phosphorus, potassium, calcium, and magnesium was measured. Leaf and root sampling was done during two years in the summer season and in August. The concentrations of nitrogen and phosphorus in roots and leaves were measured by the Kjeldahl (Isaac and Johnson 1984) and Olsen method by spectrophotometer (Jones 2001). Potassium, calcium, and magnesium of leaves and roots were measured using the atomic absorption method and spectrophotometer (Isaac and Johnson 1984).

Statistical Analysis

After measuring the concentration of N, P, K, Ca, and Mg for roots and leaves samples, their values were compared between root and leaf. The independent-samples t-test was used to compare the nutrients concentrations between root and leaf. Also, in order to investigate the temporal changes in the concentrations of root and leaf elements of oak trees, the values of leaf and root elements were compared separately between the first and second year of sampling. The paired-samples t-test was used to compare the values of root and leaf elements between sampling years. The Kolmogorov-Smironov test was used to check the normality of the data. All statistical analysis was performed using SPSS software.

RESULTS AND DISCUSSION

Comparing the Elements Concentrations between the Persian Oak Roots and Leaves

Analysis of the nutrient concentrations in leaves and roots of healthy and declining Persian oak trees showed that there were significant differences between leaves and roots, which indicated the effect of drought on Persian oak trees, although some nutrients did not show any statistical difference (Tables 1 and 2). In agreement with the negative effects of drought stress on the status of nutrients in tree trunks, this has been demonstrated in many previous studies (Sardans *et al.* 2008a,b; Sardans and Penuelas 2007; Hosseini and Hosseini 2022). Examining the concentration of elements in the leaves and roots of Persian oak trees showed a decrease in the concentration of N (Healthy: 0.553, 1.028; Declining: 0.660, 0.672, Tables 1 and 2), P (Healthy: 0.366, 0.103; Declining: 0.490, Tables 1 and 2), and K (Healthy: 0.385, 0.372; Declining: 0.405, 0.550, Tables 1 and 2) in the roots compared to the leaves, and these reductions were seen in both classes of crown dieback. Variations in the nutrients concentrations of leaves compared to the roots are a reflection of the physiological changes in the tree in response to the effect of drought and crown dieback (Hosseini and Soleimani 2022). Reducing the concentration of root elements compared to leaves is probably due to the decrease in the absorption power of water and elements by the roots of Persian oak trees under the influence of drought stress, so that the concentration of elements in the roots is reduced and the roots do not have the ability to osmotically absorb water and elements in a sufficient amount, which results the transfer of water and nutrients to the crown being slow or reduced, contributing to the tree crowns' wilting and dieback. This result is consistent with the findings of Sardans *et al.* (2008a) and Hosseini and Hosseini (2022) regarding the effect of drought on the concentration of elements by changing the tree's ability to absorb and transport elements. In the present research, the amount of reduction in the concentration of root elements compared to leaves was more evident in declining trees, which supports the relationship between drought induced-crown dieback and the relative decrease in the element concentrations in the roots. The reduction rate of N, P, and K in roots compared to leaves was higher in declining oak trees than in healthy trees (Tables 1 and 2). This actually indicates a decrease in the ability of the roots to absorb elements from the soil. This result is consistent with previous findings (Martinez-Vilalta *et al.* 2002; Hosseini *et al.* 2018). Hosseini *et al.* (2018) found that the relative leaf water content of declining Persian oak trees was less than those of healthy trees. Martinez-Vilalta *et al.* (2002) concluded that crown dieback of *Q. ilex* trees is due to disrupting soil-plant water relations. However, the higher concentration of elements in the oak tree leaves compared to their roots can be a protective mechanism against drought stress. In drought conditions, nutrients accumulate in leaves due to the protective mechanisms, and as a result, this accumulation prevents the continuation of water flow and the transfer of nutrients to the crown (Sardans and Penuelas 2007). In a sudden drought condition, the amount of N is increased in roots and reduced in leaves due to low transition from root to the upper part of the plant, but under constant conditions of water shortage, amount of N in leaves is more than usual (Dhindsa and Cleland 1975). Increasing the leaf P concentration is related to an increase of water use efficiency, which is a mechanism to cope with drought (Egilla *et al.* 2005; Sardans and Penuelas 2007). Increasing of leaf K concentration suggested that it has been more absorbed by oak trees under drought conditions to enhance water use efficiency or defense mechanisms (Sardans and Penuelas 2007; Hawkesford *et al.* 2012).

Another reason for the decrease in the concentration of N, P, and K in the root is the function of these elements (Paoli *et al.* 2005; Hawkesford *et al.* 2012). These elements, especially N and K, are transported easily. Because it does not require much water or moisture content to move them in the tree body, their concentration is higher in the leaves than in the roots. However, the water relations are weaker in the declining trees, so that in declining oak trees there were lower amounts of these elements in the leaves and roots, which shows that the declining trees are less powerful than healthy trees in absorbing water and minerals from the soil and transferring them to the crown.

The calcium status was the opposite, and it was higher in the roots of oak trees than in their leaves. Calcium is one of the elements that is moved and transferred slowly, and this is probably one of the reasons for the higher concentration of Ca and its accumulation in the roots. Considering that the bedrock of the study research is limestone and the amount of Ca in the soil is high, and it is likely that the high concentration of Ca in the roots and leaves reflects the amount in the soil. If the Ca content in the plant increases, the intensity of plant transpiration decreases (Heidari Sharifabad 2001). Calcium plays an important role in the turgor pressure (Takagi and Nagai 1992) and is important in the water loss process (Sardans *et al*. 2008a). These results suggest the role of this element in the efficiency of water consumption in trees (Sardans *et al*. 2008a). Therefore, the higher concentration of Ca in the leaves of Persian oak trees is probably related to the reduction of transpiration in dry conditions and helps to absorb more N. Therefore, the most variability of elements in leaves and roots are related to N, P, and K. Magnesium showed the least variability between organs.

Table 1. Mean Values (n = 6 trees) of Leaf and Root Nutrients in Healthy and Declining Persian Oak Trees in First Sampling Year

		t-test for Equality of Means		Means Comparison	
Nutrients	Crown condition	t	Sig.	Leaf	Root
N (% d.w.)	Healthy	6.041	0.000	1.733	1.180
	Declining	3.340	0.007	1.617	0.957
P (% d.w.)	Healthy	3.669	0.004	0.543	0.177
	Declining	22.764	0.000	0.575	0.085
K (% d.w.)	Healthy	6.576	0.000	0.682	0.297
	Declining	6.403	0.001	0.662	0.257
Ca (% d.w.)	Healthy	-2.201	0.062	2.767	4.487
	Declining	-3.459	0.006	2.522	5.902
Mg (% d.w.)	Healthy	-0.638	0.538	0.513	0.575
	Declining	0.604	0.559	0.585	0.520

Independent-samples t test was used to examine statistical differences between leaf and root for each mineral element separately in healthy oak trees and in declining oak trees. Significant differences were considered at sig. < 0.05. d.w. is an abbreviation of dry weight.

Table 2. Mean Values (n = 6 trees) of Leaf and Root Nutrients in Healthy and Declining Persian Oak Trees in Second Sampling Year

		t-test for Equality of Means		Means Comparison	
Nutrients	Crown condition	t	Sig.	Leaf	Root
N (% d.w.)	Healthy	4.476	0.002	1.677	0.649
	Declining	4.174	0.003	1.476	0.804
P (% d.w.)	Healthy	5.773	0.002	0.137	0.034
	Declining	-0.727	0.505	0.104	0.125
K (% d.w.)	Healthy	6.141	0.000	0.620	0.248
	Declining	8.619	0.000	0.766	0.216
Ca (% d.w.)	Healthy	-4.310	0.002	3.482	5.304
	Declining	-2.917	0.019	3.052	4.234
Mg (% d.w.)	Healthy	1.073	0.311	0.758	0.644
	Declining	-0.179	0.863	0.484	0.510

Independent-samples t test was used to examine statistical differences between leaf and root for each mineral element separately in healthy oak trees and in declining oak trees. Significant differences were considered at sig. < 0.05. d.w. is an abbreviation of dry weight.

Comparison of Temporal Changes in the Elements Concentrations between the Persian Oak Leaves and Roots

The concentration of N and P in the roots, as well as the concentration of leaf P demonstrated significant temporal changes. Nitrogen and phosphorus in the healthy tree roots decreased during the sampling years, but these elements showed no significant temporal change in the declining tree roots. Potassium, calcium, and magnesium of roots also did not show significant differences between the sampling years. In the roots, phosphorus in the healthy and declining oak tree leaves showed a decrease in concentration in the second sampling year, although the reduction rate of P concentration in the leaves of declining trees was higher than the leaves of healthy trees. Nitrogen, potassium, calcium, and magnesium in leaves did not show any significant difference between the two sampling years.

The simultaneous decreasing trend of N and P concentrations in roots and leaf P during the sampling years shows the negative effect of drought on Persian oak trees. Because during the two sampling years, under the influence of drought, the ability to absorb water and nutrients by tree roots and transfer elements from roots to leaves have decreased. This is probably due to the climatic conditions of the sampling years, which is actually under drought. In this condition, the soil moisture required to absorb water and elements by the tree roots is reduced, and the reduction of soil moisture has a negative effect on the water relations between the soil and plant (Sardans and Penuelas, 2007; Sardans *et al.* 2008a,b). The effect of drought on declining trees was greater. In healthy oak trees, the movement and transfer of N and P from roots to leaves were done in a better way, so that the concentration of these elements in the roots was less in the second sampling year. The N and P absorbed by the roots moved to the leaves or were consumed, but in declining trees, these elements did not have significant temporal variations due to disruption of water relations. This result is consistent with previous findings (Martinez-Vilalta *et al*. 2002; Hosseini *et al*. 2018).

Table 3. Mean Values (n = 6 trees) of Leaf Nutrients in Healthy and Declining Persian Oak Trees in Both Sampling Years

		Paired Samples Test		Means Comparison	
Nutrients	Crown condition	t	Sig.	First year	Second year
N (% d.w.)	Healthy	0.338	0.749	1.733	1.677
	Declining	0.917	0.411	1.660	1.476
P (% d.w.)	Healthy	33.721	0.000	0.543	0.137
	Declining	21.689	0.000	0.570	0.104
K (% d.w.)	Healthy	1.631	0.164	0.682	0.620
	Declining	-1.106	0.331	0.670	0.766
Ca (% d.w.)	Healthy	-1.268	0.261	2.767	3.482
	Declining	-1.476	0.214	2.306	3.052
Mg (% d.w.)	Healthy	-1.925	0.112	0.513	0.758
	Declining	0.802	0.468	0.622	0.484

Paired-samples t test was used to examine statistical differences between first year and second year for each leaf mineral element separately in healthy oak trees and in declining oak trees. Significant differences were considered at sig. < 0.05. d.w. is an abbreviation of dry weight.

Table 4. Mean Values (n = 6 trees) of Root Nutrients in Healthy and Declining Persian Oak Trees in Both Sampling Years

		Paired Samples Test		Means Comparison	
Nutrients	Crown condition	t	Sig.	First year	Second year
N (% d.w.)	Healthy	3.632	0.022	1.154	0.649
	Declining	1.086	0.339	0.942	0.804
P (% d.w.)	Healthy	5.316	0.006	0.078	0.034
	Declining	-1.289	0.267	0.088	0.125
K (% d.w.)	Healthy	1.167	0.308	0.312	0.248
	Declining	0.934	0.403	0.248	0.216
Ca (% d.w.)	Healthy	-0.772	0.483	4.724	5.304
	Declining	1.139	0.318	5.090	4.234
Mg (% d.w.)	Healthy	-0.482	0.655	0.602	0.644
	Declining	-0.400	0.710	0.458	0.510

Paired-samples t test was used to examine statistical differences between first year and second research year for each root mineral element separately in healthy and in declining oak trees. Significant differences were considered at sig. < 0.05. d.w. is an abbreviation of dry weight.

CONCLUSIONS

1. Variations in nutrients concentration were observed between root and leaf in Persian oak trees, as well as variations in root and leaf nutrients concentration, were observed when comparing contrasting crown dieback of Persian oak trees. The drought-induced crown dieback in the research area had a strong effect on leaf and root N, P, and K contents.
2. Given the significant variations of some elements in the leaves and roots of Persian oak trees, this species has no considerable ability to absorb some nutrients and use them in vital activities, so it is a sensitive species to stress, especially to drought.
3. The intra-species variability between crown dieback classes is attributed to acclimation of foliar morphology with drought occurred in this area. More changes in N, P, and K than the other studied elements within the leaves and roots of Persian oak trees is referred to the defense mechanisms of this species which has absorbed more amounts of these elements to cope with the occurred stresses, to absorb more water from soil and to prevent further crown dieback.

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Competing Interests

The authors have declared that no competing interests exist.

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