

Responses of epiphytic lichen diversity on *Quercus frainetto* to traffic density in the city of Bursa (Turkey)

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ABSTRACT

In this study, We investigated the differences in epiphytic lichen diversity, species richness and functional group classification on *Quercus frainetto* in areas near roads with low, intermediate and high traffic in Bursa City. A total of 40 epiphytic lichen species were found on *Q. frainetto*. Of these, 16 species were oligotrophic, 15 species were mesotrophic, and 9 were nitrophytic. The lichen diversity value and species richness decreased with increasing traffic density. The mesotrophic species were positively related to Ldv, and were not associated with traffic. The oligotrophic species were positively correlated with traffic, whereas the nitrophytic species were negatively associated with traffic. The eutrophication-tolerant species, such as *Hyperphyscia adglutinata*, *Lecidella elaeochroma*, *Physcia adscendens* and *Xanthoria parietina*, were abundant on oak trees in low and intermediate traffic areas near agricultural areas.

Keywords: Epiphytic lichen, Lichen diversity, Traffic density, *Quercus frainetto*, Environmental quality, Bursa, Turkey

INTRODUCTION

Unlike deciduous plants, especially Angiospermae, the thalli of lichen accumulate in their tissues, elements such as lead, zinc, sulphur, and nitrogen and radionuclides that are present in the air. Lichens do not have a protective cuticle layer like high plants; therefore, they are continuously exposed to pollutants in the air. The aerosols and gases in the air may be absorbed over the entire thallus surface and readily diffuse to the photobiont layer. Therefore, for a long time, lichens have been used as biological indicators to monitor air quality in both urban and rural environments. For over 140 years, lichens have been known to be extremely sensitive to air pollution due to the adverse effects of pollutants on the primer metabolism of both the algal and the fungal partners in the lichen thalli (Brodo et al. 2001).

The epiphytic lichen diversity and community structures have been known to vary based on air pollution and environmental changes (van Herk et al. 2002, Giordani 2007). The main pollutants that cause air pollution in urban environments, roadsides and areas near the road are the gases emitted from motor vehicles, such as sulfur dioxide (SO₂), carbon monoxide (CO) and, in particular nitrogen dioxide (NO₂) (Gilbert et al. 2003, 2007). The traffic level significantly influences the NO₂ concentrations and bark acidity along the roadside. There are significant relations between the NO₂ emitted from motor vehicles and the N content in lichen thalli (Sujetoviené 2010).

The trunk pH of trees is higher in traffic areas due to the presence of pollutants such as alkaline dust particles and/or atmospheric ammonium (Fрати et al. 2006). It is known to cause an increase in eutrophic and basophilous lichens and a decrease in oligotrophic and acidophytic lichens (van Herk 2001, Spier et al. 2010). A high correlation between the accumulation of nitrogen in lichens and their proximity to areas with a dense traffic level has been established in urban areas. The total nitrogen content in lichens varies from species to species with increases in the traffic density on the road. No correlation has been observed between the nitrogen concentration of nitrophytic lichens and traffic density. However, the nitrogen concentration of acidophytic lichens increases significantly with traffic density (Gombert et al. 2003). Traffic is the main cause of the decline in lichen diversity and abundances (Giordani et al. 2002, Loppi et al. 2002, Marmor and Randlane 2007, Llop et al. 2012).

This study aimed to compare the epiphytic lichen diversity on *Quercus frainetto* Ten. near the roadside at three different roads that have a low, intermediate, and high traffic density and to determine the effect of traffic density on epiphytic lichen diversity.

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MATERIALS AND METHODS

Study area

The study area was in the northeastern and western regions of Bursa City. The city of Bursa is located between 39°30'-40°37'N and 28°06'-29°58'E in the southeastern part of the Marmara region of Turkey. Bursa is usually dominated by a Mediterranean climate and is a transitional region between the Mediterranean climate and the Black Sea climate. The mean annual temperature (1975-2007) in Bursa (alt. 155 m) is 14.5 °C, and the mean annual rainfall is 674 mm (Öztürk 2010). Lichen samples were collected from *Q. frainetto*, the only oak tree species found at all the stations. *Q. frainetto* is native to the Balkan Peninsula and present in the south of Italy and the northwestern part of Turkey. This oak is a large deciduous tree, reaching more than 30 meters in height and very rarely living more than 200 years. It is a meso-xerophilous species of the sub-Mediterranean flora that occupies the transition climate between the typical Mediterranean climate and the continental climate (Mauri et al. 2016). The study was conducted on *Q. frainetto* near a roadside at three different stations that have a low, intermediate, and high traffic density level on their roads. The first station was located at an asphalt road near Taşpınar village, the Taşpınar – Esence – Zeytinbağı road with intermediate traffic. The second station was located at an asphalt road near Dereköy village, the Çekrice – Mirzaoba road with low traffic; and the third station was at the roadside cemetery near Dürdane village on the Bursa - Istanbul highway with high traffic (Figure 1). The annual average daily traffic values in 2012 (General Directorate of Highways 2013) and 2016 (General Directorate of Highways 2017) for Bursa province, according to the provincial road traffic zones provided by the Traffic Safety Department at the General Directorate of Highways, were used to ascertain the traffic density on the roads in the study area.

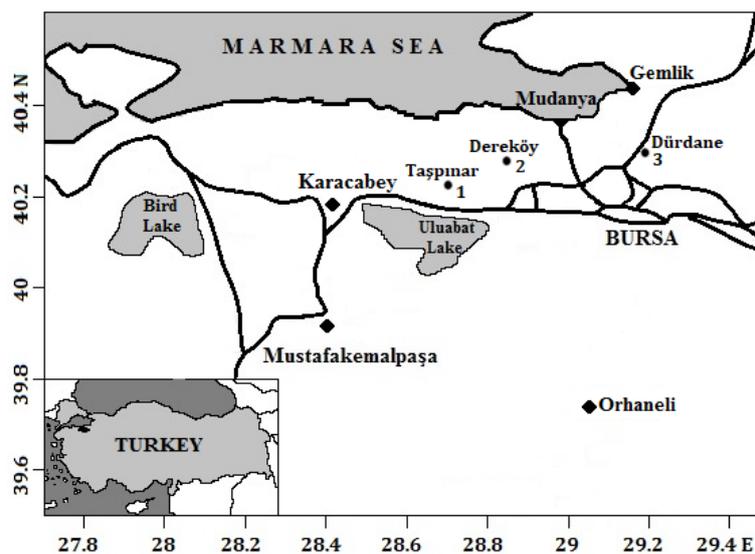


Figure 1. Map of the study area and sampling sites.

Lichen sampling

Epiphytic lichen samples were collected from 9 Hungarian oak in the 3 localities between 2014 and 2015 using the methods specified by Asta et al. (2002). The sampling grid was placed on the north, east, south and west sides of the trunk, with the lowest quadrat beginning at 100 cm above ground level. All lichen species found in each quadrat of the sampling grid were recorded. The frequency and cover of each species were computed as the number of quadrats where it was present and the surface area covered by the species, respectively. The lichen diversity value (Ldv) was calculated based on the frequency values of the species. The lichen diversity value of the locality was the arithmetic mean of the sums of frequencies for each sampling tree in that locality. The circumference of the trunk for each sampled tree was recorded as the diameter at breast height (Dbh).

Statistical analysis

In the statistical analysis, the importance value, which is the sum of the frequency of occurrence % and the cover % values of the lichen species, was used. Standard statistical analyses were conducted using IBM SPSS Statistics 23. The ordination diagram of the epiphytic lichen diversity at the sampling sites was ordinated using an indirect linear model with principal component analysis (PCA) in CANOCO ver. 4.5. The correlation between both the species and traffic and functional groups of the species was obtained using the redundancy analysis (RDA) technique, selecting the linear combination of environmental variables (Ter Braak and Smilauer 2002). Correlations between the ordination axes and other parameters (Traffic density, Ldv, and functional groups) were assessed using Pearson's correlation coefficient.

RESULTS

A total of 40 epiphytic lichen species were found on the *Q. frainetto* (Table 1). Of these, 16 species were oligotrophic, 15 species were mesotrophic, and 9 were nitrophytic. The number of species on the oak trees in Dereköy, which were close to the road with low traffic was 26 (oligotrophic: 7, mesotrophic: 12, nitrophytic: 7); in Taşpınar, Which had intermediate traffic, that number was 16 (oligotrophic: 5, mesotrophic: 5, nitrophytic: 6); and in Dürdane, which had high traffic, that number was 17 (oligotrophic: 10, mesotrophic: 4, nitrophytic: 3). *Lecidella elaeochroma* was found in all the localities in the study area. The other common species, which each had an importance value

Table 1. Percentage of the mean frequency and cover values of the epiphytic lichens found in the study area and the functional group classification according to Nimis and Martellos (2018).

		LOCALITIES								
		Dereköy		Taşpınar		Dürdane				
Altitude (Meter)		127		110		328				
Latitude		40°18'40" N		40°15'02" N		40°20'02" N				
Longitude		28°47'51" E		28°38'12" E		29°05'30" E				
Mean circumference of tree trunk (Dbh)		114.0±11.1		130.3±34.7		104±6.2				
Lichen Diversity Value (Ldv)		108		71		75				
Annual Average Daily Traffic (AADT) ^{a, b}		60		1052		49331				
Traffic density of sampling sites		Low traffic		Intermediate traffic		High traffic				
Species	Abr.	Frequency (%)	Cover (%)	Frequency (%)	Cover (%)	Frequency (%)	Cover (%)	Total (%)	E	Functional Group
<i>Hyperphyscia adglutinata</i> (Flörke) H. Mayrhofer & Poelt	Hype adg	53.33	1.88	85.00	8.83			149.1	3-5	Nitrophytic
<i>Xanthoria parietina</i> (L.) Beltr.	Xant par	55.00	3.87	45.00	1.85			105.7	3-4	Nitrophytic
<i>Lecidella elaeochroma</i> (Ach.) M. Choisy	Leci ela	35.00	1.32	10.00	0.10	53.33	2.35	102.1	2-4	Mesotrophic
<i>Physcia adscendens</i> (Fr.) H. Olivier	Phys ads	66.67	2.28	28.33	0.80			98.1	3-5	Nitrophytic
<i>Parmelina tiliacea</i> (Hoffm.) Hale	Parm til					71.67	23.88	95.6	2-3	Mesotrophic
<i>Bacidia rosella</i> (Pers.) De Not.	Baci ros	41.67	0.85	38.33	0.88			81.7	1-3	Oligotrophic
<i>Physconia grisea</i> (Lam.) Poelt	PSCO gri	1.67	0.03	68.33	8.48			78.5	4-5	Nitrophytic
<i>Parmelia sulcata</i> Taylor	Parm sul	10.00	0.50			38.33	9.53	58.4	1-3	Oligotrophic
<i>Lecanora chlarotera</i> Nyl.	Leca chl	30.00	0.57			25.00	0.60	56.2	2-5	Mesotrophic
<i>Physconia enteroxantha</i> (Nyl.) Poelt	PSCO ent	28.33	2.43			18.33	2.17	51.3	3-4	Nitrophytic
<i>Candelariella vitellina</i> (Hoffm.) Müll. Arg.	Cand vit			43.33	0.80	1.67	0.03	45.8	2-5	Nitrophytic
<i>Candelaria concolor</i> (Dicks.) Arnold	Cand con	36.67	0.82					37.5	3-5	Nitrophytic
<i>Physcia stellaris</i> (L.) Nyl.	Phys ste	31.67	3.13	1.67	0.03			36.5	2-4	Mesotrophic
<i>Arthothelium spectabile</i> A. Massal.	Arte spe					35.00	0.53	35.5	1	Oligotrophic
<i>Lepraria incana</i> (L.) Ach.	Lepr inc	6.67	0.07			26.67	1.72	35.1	1-2	Oligotrophic
<i>Alyxoria varia</i> (Pers.) Ertz & Tehler	Alyx var			31.67	0.47			32.1	1-2	Oligotrophic
<i>Amandinea punctata</i> (Hoffm.) Coppins & Scheid.	Aman pun			1.67	0.02	26.67	2.03	30.4	2-4	Nitrophytic
<i>Buellia disciformis</i> (Fr.) Mudd	Buel dis	23.33	0.35					23.7	1-2	Oligotrophic
<i>Phlyctis argena</i> (Ach.) Flot.	Phly arg	16.67	0.73			3.33	0.13	20.9	1-2	Oligotrophic
<i>Catillaria nigroclavata</i> (Nyl.) J. Steiner	Cati nig	8.33	0.13	8.33	0.15			17.0	2-3	Mesotrophic
<i>Melanelixia glabrata</i> (Lamy) Sandler & Arup	Mela gla	15.00	0.18					15.2	2-3	Mesotrophic
<i>Lecanora carpinea</i> (L.) Vain.	Leca car	8.33	0.08			6.67	0.08	15.2	1-3	Oligotrophic

<i>Melanelia subaurifera</i> (Nyl.) Essl.	Mela sub	5.00	0.15			8.33	0.22	13.7	1-3	Mesotrophic
<i>Scoliciosporum chlorococcum</i> (Graewe ex Stenh.) Vězda	Scol chl	13.33	0.28					13.6	1-3	Mesotrophic
<i>Strigula affinis</i> (A. Massal.) R.C. Harris	Stri aff			13.33	0.22			13.6	2-3	Mesotrophic
<i>Pleurosticta acetabulum</i> (Neck.) Elix & Lumbsch	Pleu ace	11.67	1.50					13.2	2-3	Mesotrophic
<i>Pertusaria albescens</i> (Huds.) M. Choisy & Werner	Pert alb	5.00	0.10			5.00	0.20	10.3	1-3	Oligotrophic
<i>Lecanora argentata</i> (Ach.) Röhl.	Leca arg			6.67	0.12			6.8	1-2	Oligotrophic
<i>Pertusaria leioplaca</i> DC.	Pert lei					6.67	0.10	6.8	1-2	Oligotrophic
<i>Physcia aipolia</i> (Ehrh. ex Humb.) Fűrnr.	Phys aip	5.00	0.17					5.2	3-4	Nitrophytic
<i>Opegrapha herbarum</i> Mont.	Opeg her			5.00	0.07			5.1	1-2	Oligotrophic
<i>Ramalina farinacea</i> (L.) Ach.	Rama far					3.33	0.10	3.4	1-2	Oligotrophic
<i>Melanohalea elegantula</i> (Zahlbr.) O. Blanco et al.	Mela ele	3.33	0.05					3.4	2-3	Mesotrophic
<i>Anaptychia ciliaris</i> (L.) Körb. ex A. Massal.	Anap cil	1.67	0.30					2.0	2-3	Mesotrophic
<i>Ramalina pollinaria</i> (Westr.) Ach.	Rama pol	1.67	0.08					1.8	2-4	Mesotrophic
<i>Ramalina fastigiata</i> (Pers.) Ach.	Rama fas					1.67	0.05	1.7	1-3	Oligotrophic
<i>Blastenia crenularia</i> (With.) Arup, Søchting & Frödén	Blas cre	1.67	0.03					1.7	2-4	Mesotrophic
<i>Physconia perisidiosa</i> (Erichsen) Moberg	PSCO per			1.67	0.03			1.7	2-3	Mesotrophic
<i>Arthonia leucopellaea</i> (Ach.) Almq.	Arth leu					1.67	0.02	1.7	1	Oligotrophic
<i>Gyalecta truncigena</i> (Ach.) Hepp	Gyal tru			1.67	0.02			1.7	1-2	Oligotrophic

Abbr.: Abbreviations of species names, **E:** Eutrophication (**1:** no eutrophication, **2:** very weak eutrophication, **3:** weak eutrophication, **4:** rather high eutrophication, **5:** very high eutrophication). **a:** General Directorate of Highways (2013), **b:** General Directorate of Highways (2017)

exceeding 50 %, were *Bacidia rosella*, *Hyperphyscia adglutinata*, *Lecanora chlarotera*, *Parmelia sulcata*, *Parmelina tiliacea*, *Physcia adscendens*, *Physconia grisea*, *P. enteroxantha* and *Xanthoria parietina*.

Alyxoria varia, *Gyalecta truncigena*, *Lecanora argentata*, *Opegrapha herbarum*, *Physconia perisidiosa* and *Strigula affinis* were only on oak trees near Taşpınar village. Likewise, *Anaptychia ciliaris*, *Blastenia crenularia*, *Buellia disciformis*, *Candelaria concolor*, *Melanelixia glabratula*, *Melanohalea elegantula*, *Physcia aipolia*, *Pleurosticta acetabulum*, *Ramalina pollinaria* and *Scoliciosporum chlorococcum* were only on oak trees near Dereköy village, while *Arthonia leucopellaea*, *Arthothelium spectabile*, *Parmelina tiliacea*, *Pertusaria leioplaca*, *Ramalina farinacea* and *R. fastigiata* were only found near Dürdane village in Bursa province.

Interestingly, *Arthothelium spectabile*, *Bacidia rosella*, *Gyalecta truncigena* and *Strigula affinis* were recorded for the first time in Bursa province, according to the literature (Doğru and Güvenç 2016).

The cluster analysis divided the localities that were exposed to different traffic densities into three groups at the second level based on the lichen species diversity on the trees (Figure 2). Dürdane, which was exposed to a high traffic density and located on the Bursa-Istanbul highway, was separated from the other sites at the first level.

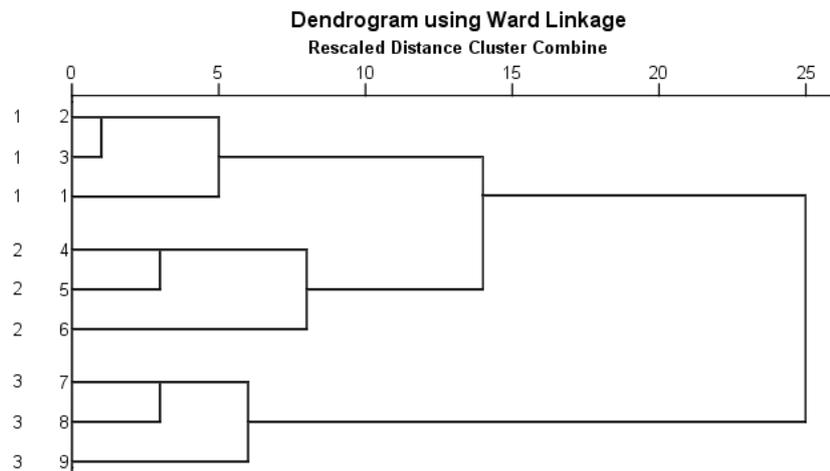


Figure 2. Dendrograms of the localities according to the cluster analysis using Ward's method with Euclidean distance. (1: Taşpınar (Intermediate traffic) 2: Dereköy (Low traffic) 3: Dürdane (High traffic))

The PCA results, which were based on the linear responses of localities and species to different traffic densities, are shown in Figure 3. The first two axes of the PCA represented 69.9% of the total variance (the value for the first axis was 47.8% and the value for the second axis was 22.1%). The first axis was associated with an increasing gradient of traffic density of the localities. The localities exposed to a low traffic density and their species are located at the upper left side of the first axis, and the localities exposed to an intermediate traffic density are at the lower left side. In contrast, the localities exposed to a high traffic density are on the right side of the first axis.

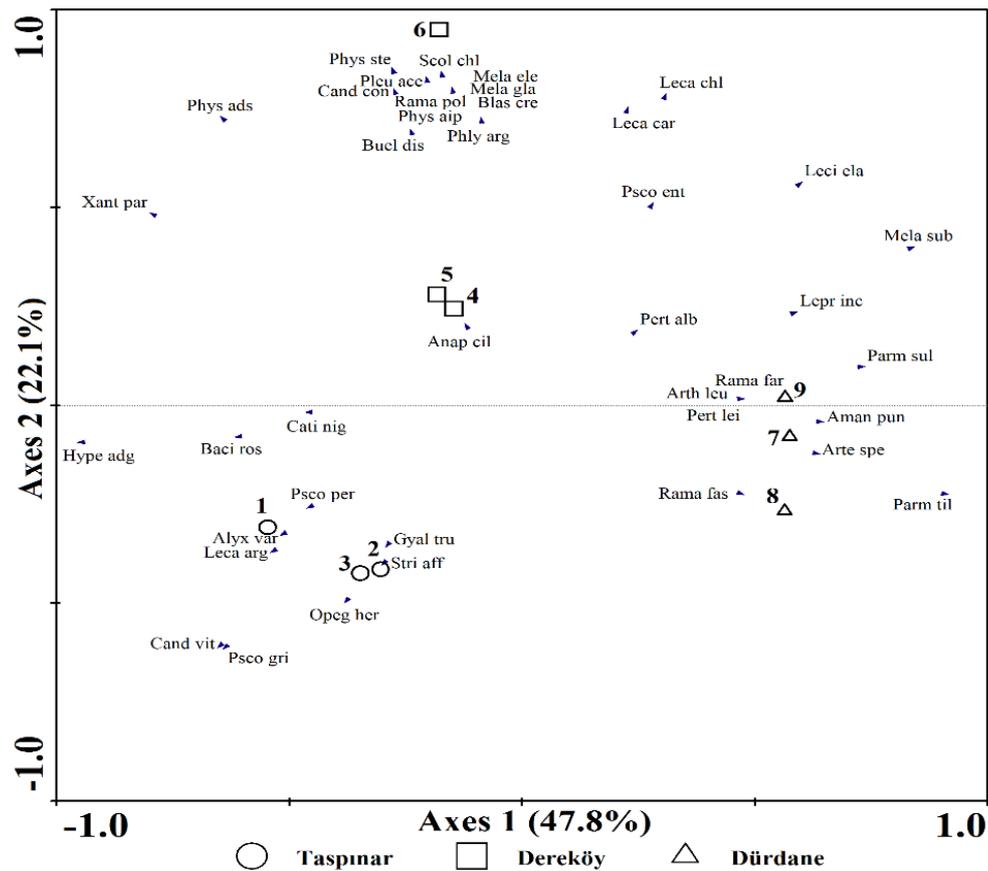


Figure 3. Ordination diagram of the PCA of localities and species in relation to different traffic density levels. Taşpınar (Intermediate traffic) Dereköy (Low traffic) Dürdane (High traffic)

Correlations between the ordinate axes and the other parameters (traffic density, Ldv, and the functional group classification of the epiphytic lichen species) are given in Table 2. The first axis was positively correlated with the localities, and oligotrophic species (Oligo) and negatively correlated with the nitrophytic species (Nitro). In contrast, the second axis was positively correlated with the lichen diversity value (Ldv), and mesotrophic species (Meso).

Table 2. Pearson's correlation coefficients of the ordination axes and other parameters (traffic density, Ldv, and functional groups).

	Axis1	Axis2	Traffic	Ldv
Traffic	0.95**	- 0.19		
Ldv	- 0.19	0.93 **	- 0.32	
Oligotrophic	0.68*	0.34	0.68 *	0.19
Mesotrophic	- 0.25	0.91 **	- 0.40	0.89 **
Nitrophytic	- 0.88**	0.34	- 0.95 **	0.42

Significances: P < 0.05 (*) and P < 0.01 (**)

The mesotrophic species were strongly and positively related to the Ldv. These species were not associated with traffic. The oligotrophic species were positively correlated with traffic, whereas the nitrophytic species were negatively associated with traffic.

DISCUSSION

In this study, the influence of traffic density on epiphytic lichen diversity was examined in oak trees along roads with different vehicle density levels. Among the causes of air pollution, the air pollutants emitted from the motor vehicles are as important as the pollutants resulting from residential and industrial facilities (Mitreski et al. 2016). Pollutants such as NO_x, CO₂, CO and SO₂ released by vehicle traffic are among the main sources of air pollution in urban and roadside areas (Gilbert et al. 2003, 2007). Phytotoxic gases emitted from motor vehicles have reduced the diversity and changed the composition of epiphytic lichens in urban environments and roadside areas (Giordani et al. 2002, Loppi et al. 2002). Similarly, this study showed that the Ldv value decreased with increasing traffic density. In a previous study, 30 species on *Q. frainetto* were identified from localities (altitudes of 180 - 650 meters) of the rural areas of Bursa Province (Oran and Öztürk 2012). In our study, the epiphytic lichen diversity on *Q. frainetto* near the roadside was quite different from those in rural areas. Of the 41 species in this study, 27 were different from those identified in rural areas in a previous study.

The first and second axes of the RDA explained 46.9 % and 22.1 %, respectively, of the total variance in the species data (Figure 4). The total variance percentage of the relation between the species data and environmental variables of the first two axes was 73.6 % (the first axis: 50 %, the second axis: 23.6 %). *Amandinea punctata*, *Arthonia leucopellaea*, *Arthothelium spectabile*, *Lepraria incana*, *Melanelia subaurifera*, *Parmelia sulcata* and *Parmelina tiliacea*, *Pertusaria leioplaca* and *Ramalina farinacea* were positively correlated with traffic, whereas *Bacidia rosella*, *Hyperphyscia adglutinata*, *Physcia adscendens* and *Xanthoria parietina* were negatively correlated with traffic.

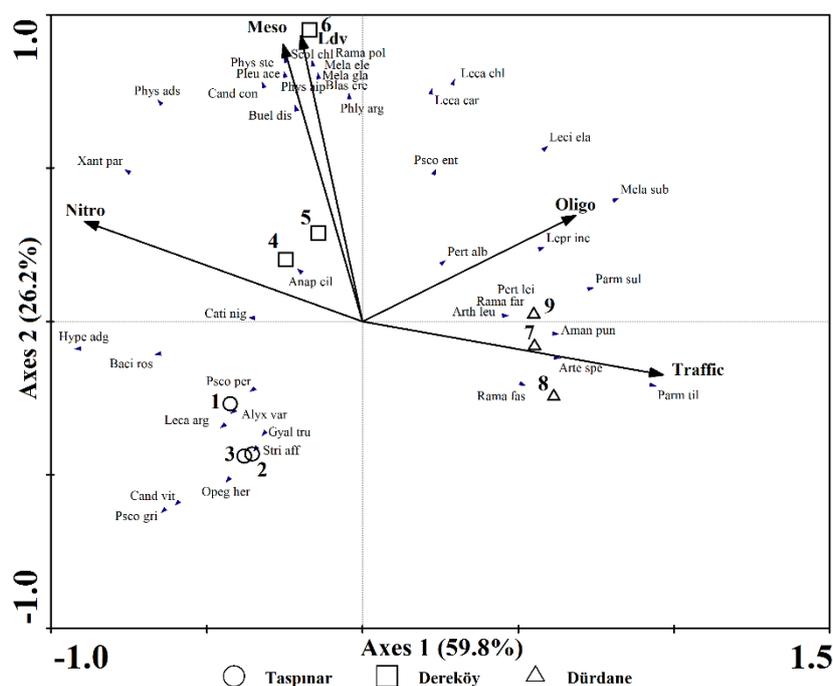


Figure 4. RDA ordination diagram of the species data and environmental variables of the localities.

In addition, *Blastenia crenularia*, *Buellia disciformis*, *Candelaria concolor*, *Lecanora carpinea*, *L. chlarotera*, *Melanelixia glabratula*, *Melanohalea elegantula*, *Physcia adscendens*, *P. aipolia*, *P. stellaris*, *Pleurosticta acetabulum*, *Ramalina pollinaria*, *Scoliosporum chlorococcum* and *Xanthoria parietina* were positively correlated with Ldv.

The Ldv value has been reported to decrease in areas near roads compared to areas far from roads (Llop et al. 2012). It has been determined that the number of species decreases as the distance from a motorway decreases (Marmor and Randlane 2007). The Ldv values decreased with increasing traffic density in this study

(Table 1). In our study, nitrophytic species such as *Hyperphyscia adglutinata*, *Physcia adscendens*, *Physconia grisea*, *P. enteroxantha* and *Xanthoria parietina* were found with high importance values on oak trees in low traffic areas (Dereköy) and intermediate traffic areas (Taşpınar). There are agricultural areas in the vicinity of the roads in these regions. Recently, it has been shown that eutrophication-tolerant species such as *Hyperphyscia adglutinata*, *Lecidella elaeochroma*, *Physcia adscendens* and *Xanthoria parietina* were abundant on holm oak trees in cultivated areas (Garrido-Benavent et al. 2015).

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