MICROBIAL COMMUNITY-LEVEL PHYSIOLOGICAL PROFILES BETWEEN TOKATLI AND SIRÇALI CANYONS, TURKEY

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Abstract. Soil microorganisms are important components of terrestrial ecosystems and play an important role in organic matter fragmentation and nutrient cycling. In our study, microbial diversity of different plant communities and differences in the use of carbon sources in Tokatlı and Sırçalı Canyons were investigated. The canyons were investigated in three stages (tree, shrub and herb). Although there was no significant difference in developmental processes between different vegetation stages, differences between plant communities were significant ($P< 0.05$ and $P< 0.01$). In addition, the groups with the highest variation in carbon sources are *Quercus infectoria* OLIVIER-*Carpinus betulus* L. community in Tokatlı Canyon, and *Quercus pubescens* WILLD-*Pinus nigra* J.F.ARNOLD community in Sırçalı Canyon. The soil carbon content has the most important effect on the diversity of the carbon sources used by microbial communities in both canyons. Carbon source use of microbial communities in soil is affected by dominant species that control environmental factors above the soil.
Keywords: *diversity; plant community; vegetation stages; carbon sources; nutrient cycle*

Introduction

Biodiversity at ecosystem level reveals that not only species or species groups, but also features and processes should be protected (Atik et al., 2010). Soil microorganisms are important components of terrestrial ecosystems. The soil effects nutrient content and primary production. Thus, soil nutrient content and primary production is affected by microorganisms (Rutigliano et al., 2004). Topographic and edaphic factors are significantly influenced by species diversity (Gülsoy and Özkan, 2008). Plants and soil biota have a strong functional relationship as producers and decomposers of terrestrial ecosystems; plants provides organic carbon for soil biota through litter and root exudates, while soil biota decompose organic matter and release mineral nitrogen and phosphorus which are essential for plant growth (Zhang et al., 2010). In general, high plant species richness or functional diversity causes high heterogeneity of resource environments and as a result various microbial groups develop (Sugiyama et al., 2008). Although nitrogen mineralization in soil occurs under control of main soil characteristics, plants also have important functions in this process. Hence, dominant plant species in a region control productivity of an ecosystem by affecting degradation of nitrogen (van Vuuren et al., 1992). Plant species affect soil microbial activity through the quantity and quality of litter they produce. They also affect soil nitrogen conversion rates indirectly. There are important differences in quantity and decomposition characteristics of produced litter between species (Aerts et al., 2006). Also, soil microbial communities play an important to ecological indicator role in maintaining soil fertility and to evaluated soil health (Kumar et al., 2017). Decomposition and mineralization characteristics of organic matter by soil microorganisms are dependent on C / N rate of soil as well as chemical structure of carbon compounds (Krauss et al., 2004).

There are two determinants that shape species diversity in plant communities; species

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pool and ecological connections. Soil organisms such as bacteria, fungi, algae, protozoa and some nematodes play a vital role in preserving soil fertility in organic matter degradation, humus formation and nutrient contents (Smith and Paul, 1990; Lin et al., 2004). Decomposition, cycling and microbial diversity in soil are important indicators of soil microbial function (Fox and Macdonald, 2003). Soil properties are influenced by, for example, soil acidity and nutrient elements of vegetation types (Finzi et al., 1998; van Breemen and Finzi, 1998). Slope differences affect the distribution and development of vegetation and the biological uptake of soil nutrients (Kubota et al., 1998). Soil microorganisms perform in many ecosystem processes, such as nutrient conversion, litter decomposition by regulating the structural and hydrological properties of the soil (Gallardo and Schlesinger, 1994; Kennedy, 1999). Although the effects of soil activity on these processes are well known, we know less about the structure of microbial communities (Derry et al., 1999). Over the last decade, the diversity of soil microbial communities were characterized using commercially available individual Biolog microtiter plates and by production of individual carbon (C) substrates (Insam, 1997). This technique is an ecologically relevant method for measuring microbial biodiversity because it determines the differences in carbon use between communities, which is an important factor. Although the community approach to measure biological diversity is not related to the functioning of the community as a whole, measuring the presence of individuals in the communities can provide more information. Many studies have been carried out on plant diversity in different ecosystems. However, few studies have been undertaken for microbial diversity that contributes to plant diversity for each different habitat (Derry et al., 1999). Canyon ecosystems are special and isolated habitats. Plant diversity of these areas has been studied, but soil microbial diversity and carbon source used has not been studied. This study aimed to determine the effects of biotic factors on diversity of soil microbial communities in isolated canyons and the differences in carbon use between two canyons.

Material and methods

Field study and sampling

The study was carried out in Tokatlı and Sırçalı Canyons, (Tokatlı Canyon: 41° 16′ 0.49″ N - 32° 41′ 0.85″E; Sırçalı Canyon: 41° 15′ 1.20″N - 32° 46′ 9.67″E), mainly covered by enclave mediterranean maquis, rocky slopes, stream coasts and meadows (*Fig. 1*). The vegetation of the area is a mosaic of plant patches of different ages, such as low maquis, high maquis, grassland and rock surface plants (*Fig. 2*). There were a total of 7 different plant communities in canyons, 4 communities in Tokatlı Canyon (*Sedum pallidum, Quercus infectoria-Carpinus betulus, Arbutus andrache-Cistus creticus, Corylus avellana*) and 3 communities in Sırçalı Canyon (*Fumana aciphylla- Helianthemum nummularium, Quercus pubescens-Pinus nigra, Juniperus excelsa*). Soil samples (n = 21) were collected in autumn, 2016. Representative surface soil samples (0–10 cm) and total 21 soil sample were taken from plots. All samples were kept at 4 °C from field to laboratory. Soil C was usually oxidized by combustion or wet oxidation and measured by conductivity. Soil pH values were measured in 1:2.5 soil/water extract (Richards, 1954). For electrical conductivity assessment with 1:2.5 ratio, 20 g soil sample was weighed within a conical flask. 50 ml distilled water was added and shaken on horizontal shaker for 15 minutes. Then, the solution in the conical flask was transferred into 100 beaker and left for 15 minutes. At the end of this period, a conductimeter electrode was soaked into clear upper phase and electrical conductivity (EC) value was read when it was stabilized.

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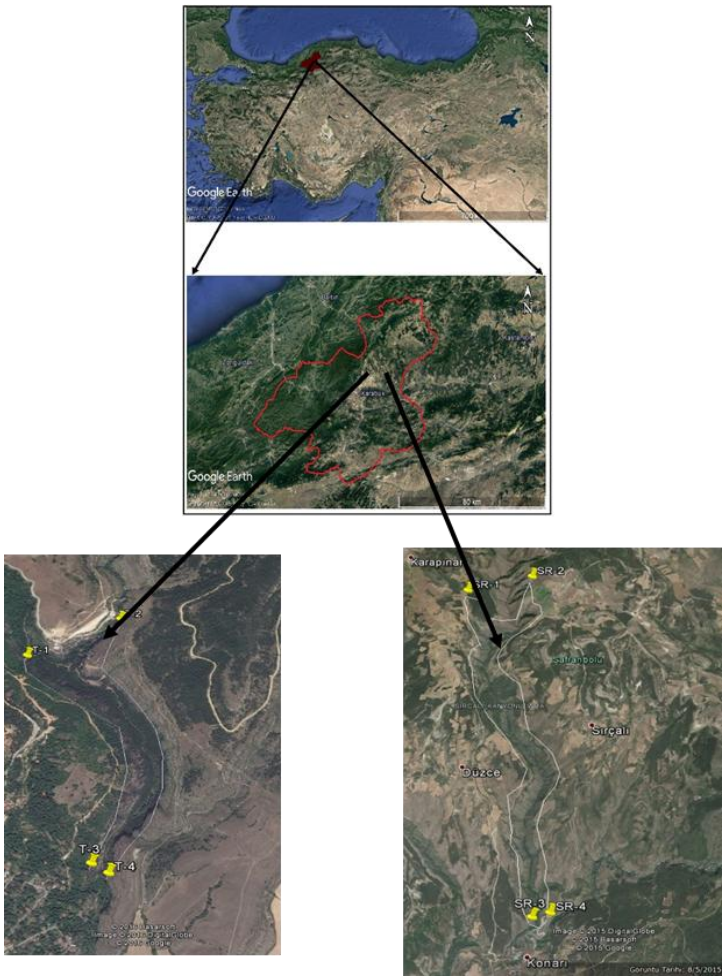

Figure 1. *Location of study area.*

Figure 2. *Habitat photos of study area (a-b: Sırçalı Canyon; c-d: Tokatlı Canyon).*

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Community level physiological profiling (CLPP) analysis

Biolog EcoPlates that are 96-well plates, containing 31 different carbon sources plus a control well, in three replications. The carbon utilization rate was determined with the purple color change of the colorless microorganisms by tetrazolium violet redox. Soil samples (10 g) were shaken for 60 min in 20 ml of a 10 mM Bis–Tris solution (pH 7) and then solutions were inoculated on microplates (100 µl per well) and incubated at 28 °C and allowed to settle for 30 min. Substrate utilization was measured with a spectrophotometer (Multiskan Microplate Photometer–Thermo Fisher Scientific) at 590 nm after 24, 48, 72, 96, 120 and 144 h incubation (Boivin, 2005). ODi (optical density) values obtained at 96 h incubation represented the optimal range of optical density values, so 96 h incubation readings were used for the assessment of microbial functional diversity and determination of carbon sources utilization. Microbial activity in each microplate was determined as average well color development (AWCD). Substrate richness values (R) were calculated as number of wells with color development (Zak et al., 1994). The AWCD was calculated using the equation: AWCD = Σ ODi / 31; the Shannon diversity (H') was calculated using the equation: $H = -\Sigma$ pi (lnpi) (Pi was called proportional color development of the well over total color development of all wells of a plate); and Evenness (E) was calculated using the equation: E = H / lnS (S = Richness was called number of wells with color development) (Garland, 1997).

Results

AWCD values of metabolized substrates in Biolog EcoPlates were obtained during 144 h for both study areas in two canyons. AWCD generally followed the same pattern of incubation time in both canyons (*Figs. 3* and *4*). There is no difference (*P value* = 0.065) in microbial diversity between different successive processes in Tokatlı Canyon. Significant differences in species diversity (H ') and richness (R) ($P < 0.001$) were found among different communities, while there was no difference between the processes (*P value* = 0.917). The group that caused the difference is *Arbutus andrache* plant community, which is the Mediterranean enclave. In Sırçalı Canyon, there is no significant difference in terms of successions processes and communities. In Tokatlı and Sırçalı canyons, significant differences were found ($P < 0.05$) in succession process levels in terms of AWCD values (*Table 1*). Significant differences ($P < 0.001$) and ($P < 0.05$) were found in R, H and AWCD between different plant communities in Tokatlı Canyon.

Regarding carbon source use in Tokatlı and Sırçalı Canyons, no differences were found in terms of successional processes. However, significant differences were found ($P < 0.0$, $P < 0.05$) for different plant communities. The differences in the use of complex carbons, carboxylic acids, amino acids and carbohydrates in the Tokatlı were found to be highest in *Quercus infectoria - Carpinus betulus* community. The use of amines and phosphate carbon in *Arbutus andrache* community has been found to be at minimum rate. Significant differences were found ($P < 0.01$, $P < 0.05$) in the use of complex carbons, carbonic acids, amino acids and amines as carbon source in Sırçalı Canyon. According to the Tukey's test, the differences were found to be highest in *Quercus pubescens - Pinus nigra* communities in terms of their complex carbon, amino acids, carboxylic acids and amine amounts (*Table 2* and *Figs. 5, 6*).

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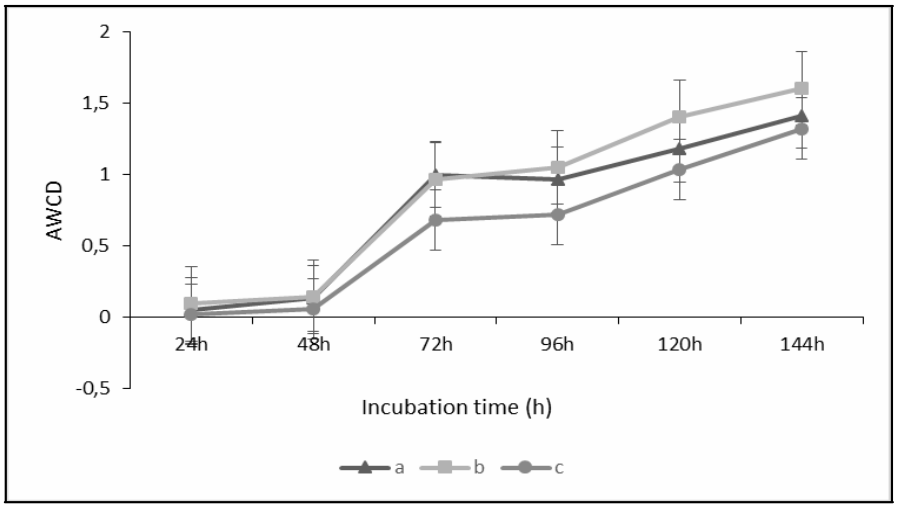

Figure 3. *In Biolog EcoPlates AWCD of metabolized substrates based on 96 h incubation in Tokatlı Canyon.*

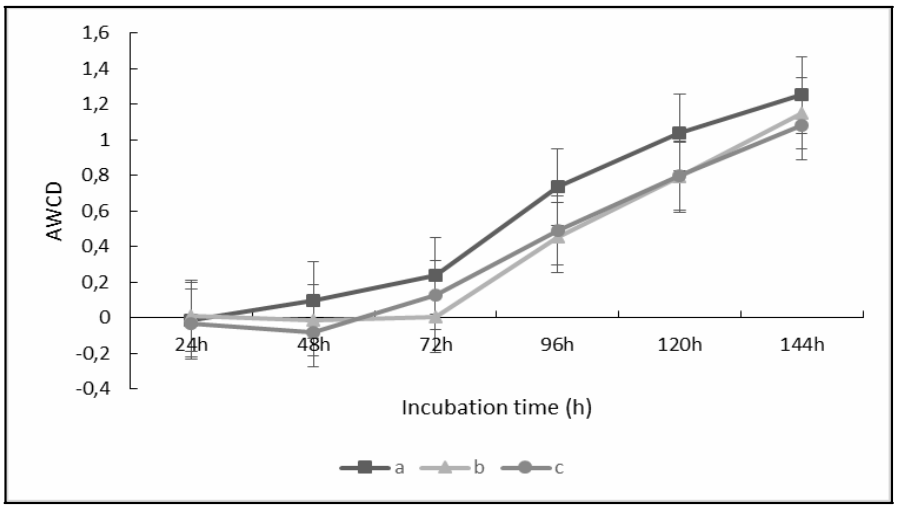

Figure 4. *In Biolog EcoPlates AWCD of metabolized substrates based on 96 h incubation in Sırçalı Canyon.*

Table 1. *Average well-color development (AWCD), richness (R), Shannon–Weaver index (H), Evenness (E) index calculated on carbon substrate use in Biolog EcoPlate (TC: Tokatlı canyon; SC: Sırçalı Canyon). Correlation is significant at the 0.05 and 0.01 level (**P<0.01 and P<0.05).*

	R	**H'**	**E**	**AWCD**
TC	7^{b} *0,000****	1.907^{b} *0,000****	0,713ns	$1,4789^{a}$ *0,015**
SC	0,425ns	0,264ns	0,419ns	$1,1798^{a}$ *0,016**

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Table 2. *Carbon usage differences of different plant communities ANOVA test result in two canyons (TC: Tokatlı Canyon; SC: Sırçalı Canyon; Community 2T: Quercus infectoria- Carpinus betulus C Community 3T: Arbutus andrache- Cistus creticus Community 2S: Quercus pubescens- Pinus nigra . Correlation is significant at the 0.05 and 0.01 level (**P< 0.01 and P< 0.05).*

	TC			SC		
Complex carbons	1,9694[a]	*0,024**	Community 2^T	1,4631[a]	*0,041**	Community 2^S
Carboxylic acids	1,5746[a]	*0,002***		1,1915[a]	*0,019**	
Amino acids	1,4470[a]	*0,046**		1,1789[a]	*0,009***	
Carbohydrates	1,8487[a]	*0,040**		1,1223[a]	*0,015**	
Phosphate carbons	1,0237[b]	*0,017**	Community 3^T		*0,080*ns	
Amines	0,7620[b]	*0,003***			*0,101*ns	

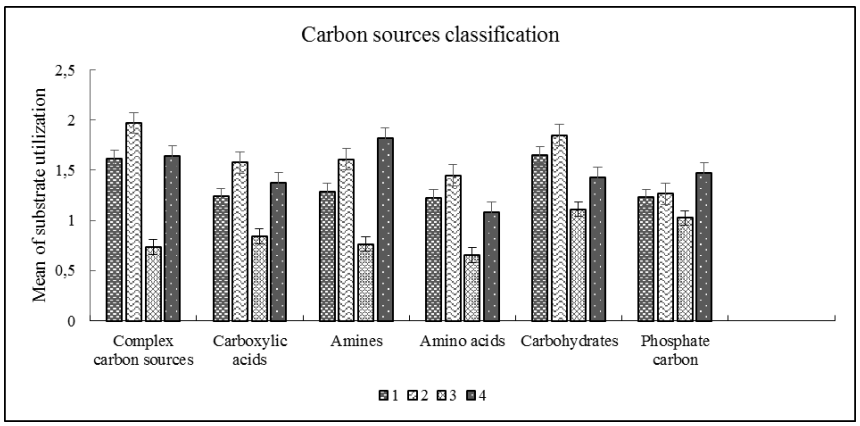

Figure 5. *Mean of substrate utilization carbon substrates from different plant communities* (Community 1: *Sedum pallidum; Community 2: Quercus infectoria-Carpinus betulus; Community 3: Arbutus andrache- Cistus creticus; Community 4: Corylus avellana) in Tokatlı Canyon based on 96-h incubation.*

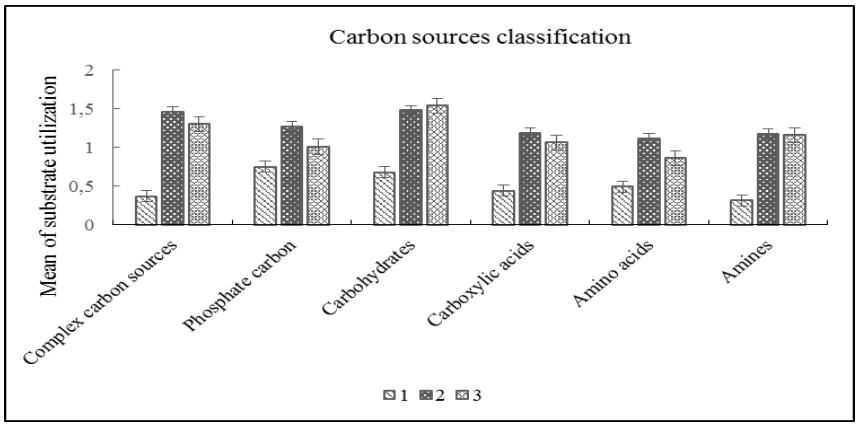

Figure 6. *Mean of substrate utilization carbon substrates from different plant communities (Community 1: Fumana aciphylla- Helianthemum nummularium; Community 2: Quercus pubescens-Pinus nigra; Community 3: Juniperus excelsa) in Sırçalı Canyon based on 96-hincubation.*

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In surface soil samples of both canyons, there are large variations between the carbon sources used by different plant communities on rocky surfaces (*Figs. 7, 8*). This proofs that microbial community varies depending on plant community structure. The first and second factors (PC1 and PC2) accounted for 28.31 and 20.21% of the variance, with a cumulative variance sum of 48.52%, respectively to Tokatlı Canyon. The effectiveness of ecological parameters contributing to the separation of carbon sources used by microbial communities in the field. CCA is indicated by the ordination analysis (*Fig. 9* and *Table 8*).

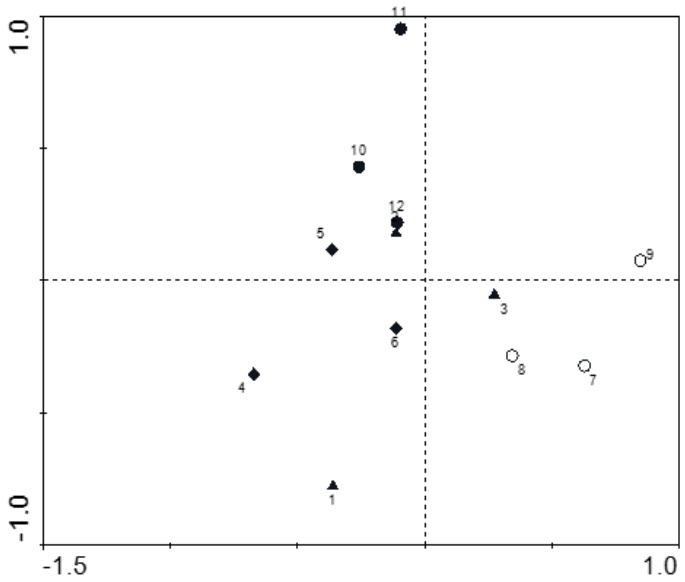

Figure 7. *PCA plot of substrate utilization patterns of microbiological communities estimated using Biolog EcoPlates data in Tokatli Canyon.*

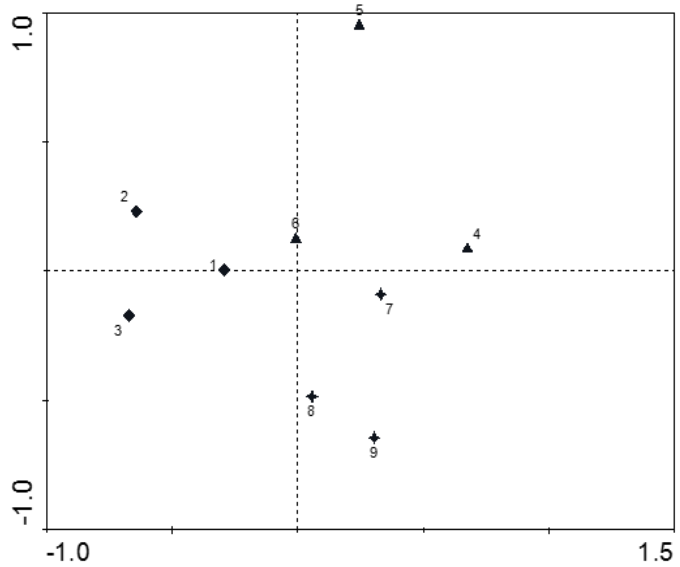

Figure 8. *PCA plot of substrate utilization patterns of microbiological communities estimated using Biolog EcoPlates data in Sircali Canyon.*

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Table 3. *Results for the first and second canonical correlation analysis based on plant andenvironmental data for Tokatli and Sircali Canyons. Significant values were defined in bold.*

	Tokatli		Sircali	
	I. axis	**II. axis**	**I. axis**	**II. axis**
Eigenvalues	0.004	0.001	0.043	0.008
Species-Environment correlation	0.771	0.801	1.000	1.000
Percent of restricted cumulative	35.2	46.5	84.6	100
Environmental variables with highest contribution to the axes				
pH	-0.079	0.494	0.296	**0.955****
EC	0.306	**0.734***	**-0.704***	**0.709***
Carbon content	0.256	**-0.566***	**-0.731***	**0.681***

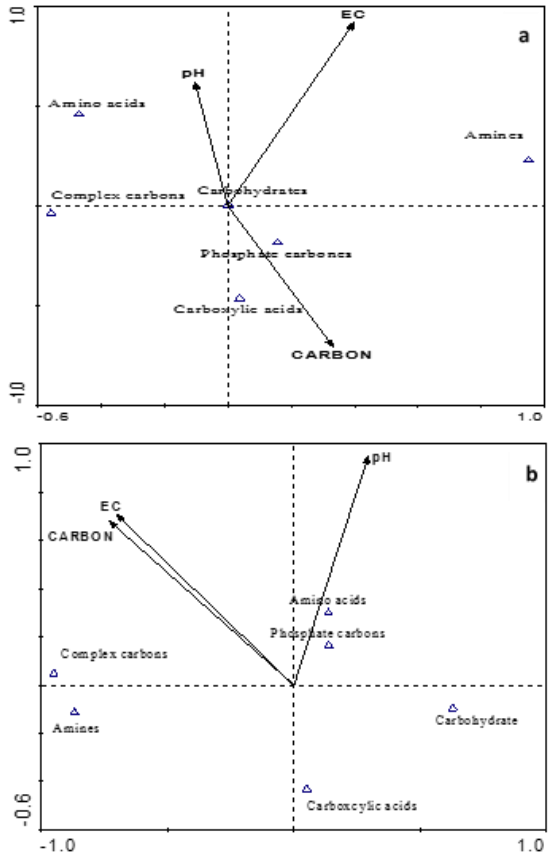

Figure 9. *CCA ordination biplot of Biolog substrates and environmental variables for Sircali and Tokatli Canyons. Triplots indicate the directions and relative importance (arrow lengths) of the three environmental variables. Soil carbon content, soil pH and soil electrical conduktivity (EC). a- Substrates with approximate correlations of $p < 0.05$ to either soil carbon content, electrical conductivity are indicated in Tokatli. b- Substrates with approximate correlations of $p<0.05$ to either carbon contents, soil pH, electrical conductivity are indicated in Sircali.*

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Discussion

As the succession stages progress, symbiotic interactions, mineral cycles, energy and nutrients are increasingly being used effectively. These events lead to the emergence of potential niches. While increasing the number of niches increases species diversity, competition and life span are also increasing. As this situation dominates some living species in the ecosystem, there are emerging elements of balancing species diversity. For this reason, the change of species diversity is not a direct effect of succession but a consequence of its indirect effects. The level of diversity reached determines the energetic relationships in the environment (Odum, 1997). The 95 substrate in the Biolog microplate provides prediction of the relative metabolic potential and responses of the microbiological community (Gomez et al., 2004). Nutrient availability is the main limiting factor for growth in forest ecosystems, and this is highly influenced by the processes in the rhizosphere. In rhizosphere the structure of microbial communities has been shown to vary depending on the type of tree. Microbial growth in soil is limited by carbon sources. As a result of rhizodeposition, carbon in rhizosphere increases microbial activity at high concentrations. Microbial diversity in different plant rhizospheres arise from the diversity of compounds leak from the plants. The data on the interactions between the microbial community and the soil is still insufficient (Graystone and Campbell, 1996). The carbon source utilization diversity of the samples in the Biolog microplate is related to the amount of carbon in the soil, soil pH and electrical conductivity. The soil carbon content is the most important effect on the diversity of the carbon sources used by microbial communities in both canyon areas. These results are similar to Bossio and Scow (1995) and Ritz et al. (1992). Tree layer in Tokatlı Canyon and grass layer in Sırçalı Canyon were responsible for these differences. Long carbon residence times in soil depend on quality and low quantity of litter and lower mineralization rate. Besides, at community level traits that support primary productivity and increase the availability of nutrients are slow degradation and traits that support soil carbon stability. This situation is related with coexistence of species with different growth rates (Hooper et al., 2005). Significant difference was found in terms of carbon use between communities.

Complex carbons, carboxylic acids, amino acids and carbohydrates in Tokatlı canyon were used most effectively by *Quercus infectoria* - *Carpinus betulus* communities. A mediterranean enclave *Arbutus andrache* community has the lowest use rate in amine and phosphate use as carbon sources. Drought and stress factors reduce the rate of carbon use in mediterranean enclaves while *Quercus* - *Carpinus* communities form more humid environment and thus affect mineralization level and provide more efficient use of carbon sources. Microbial communities in soil are controlled by climate, edaphic and topographic factors and even by fauna of the area (Zornoza et al., 2009). Dominant plant strategy within an ecosystem depends on the environmental conditions because dominance of species with high growth rates require highly available light and nutrients. Therefore, soil carbon input will be mainly derived from poor-quality litter in biomes with a short growing season and low nutrient availability, whereas primary productivity will be the main driver of soil carbon sequestration in more productive biomes (de Deyn et al., 2008). In this study, factors affecting substrate use in Sırçalı Canyon were pH, EC and soil carbon content. Among these factors, pH caused the most significant effect. Therefore in the area is controlled by nitrate use, because soil pH is influential in mineralization of nitrogen by

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determining the activity of microorganisms that decompose organic matter. In general, nitrate is formed in slightly acidic and slightly alkali (pH 6.0-8.0) soil and an increase in ammonium is seen due to increasing acidity (Doğan, 2012). In Tokatlı Canyon, factors influencing substrate use are EC and soil carbon content, respectively. Microbial markers of soil quality involves various variable parameters at ecosystem, community and population levels. Chemical properties are accepted as essential measurements although they are not sensitive markers as C and N contents (Ros et al., 2006). In our study, the difference in communities affects carbon sources, microbial diversity and richness, while no difference is found in terms of microbial communities for different successive processes (tree, shrub and grassy). Because, in the study areas in two canyons, the formation processes of plant communities on rock surfaces were at the beginning stages, indicating that the development in these areas were at early stages. At canyon surfaces, different plant communities show different carbon use rates. This proofs that microbial communities vary depending on plant community structure. The difference between communities is evidence that the soil microbial content is changing by plant communities settled in the area. Global changes are affecting carbon cycle pathways. Regarding this issue only a few biomes has been studied to date. Therefore, the number of studies on this topic should increase in order to determine the effects of climate change (de Deyn et al., 2008).

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