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Analysis of Microbial Diversity in Various Forest Communities by Biolog Ecoplate Method: Yenice Hot Spot

Farklı Orman Topluluklarındaki Mikrobiyal Çeşitliliğin Biolog Ecoplate Metodu ile Belirlenmesi: Yenice Sıcak Noktası

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ABSTRACT

The present study identifies the hot spot of Yenice and aims to determine the tree diversity in the *Fagus-Abies*, *Fagus*, and *Quercus-Fagus* forests, to define the microbial community in these forests by the Biolog-Ecoplate method and to reveal the physiological profile differences at the community level between forests. Accordingly, soil samples were taken from these predefined forests and the microbial community in different forest communities was analyzed using the Biolog EcoPlate method. In addition, cover-proportion values of the tree species were determined according to Braun-Blanquet method. As a result, the diversity in microbial communities has been determined as *Fagus-Abies* (3.0033 ± 0.006), *Fagus* (1.2267 ± 0.006), and *Quercus-Fagus* (1.1267 ± 0.012), from highest to lowest, respectively. On the other hand, the fact that the diversity of carbon sources in the *Fagus* forest was quite high and the use of phosphate carbon is seen only in this type of forest is quite significant. In the present study, the Biolog Ecoplate method was applied for the first time to determine the microbial community among forest communities. The results obtained from the present study clearly show the practicability and effectiveness of this method in forest communities. Meanwhile, the determination of the microbial community will contribute to the development of new strategies for establishing ecosystem protection practices.

Key Words

Biolog ecoPlate, forest communities, microbial community, Yenice forest.

ÖZ

Çalışmamızda; Yenice sıcak noktası tanımlanmış *Fagus-Abies, Fagus* ve *Quercus-Fagus* ormanlarındaki ağaç çeşitliliğinin saptanması, bu ormanlardaki mikrobiyal komünitenin Biolog-Ecoplate metodu ile belirlenmesi ve ormanlar arası komünite düzeyinde fizyolojik profil farklılıklarının ortaya konması amaçlanmıştır. Bu kapsamda; Önceden tanımlanmış bu ormanlardan toprak örnekleri alınarak Biolog EcoPlate yöntemi kullanılarak farklı orman topluluklarındaki mikrobiyal komünite analiz edilmiştir. Bunun yanında; ağaç türlerinin örtüş-bolluk değerleri Braun-Blanquet yöntemine göre belirlenmiştir. Sonuç olarak; mikrobiyal komünitelerindeki çeşitlilik en fazla *Fagus -Abies* (3,0033±0,006)'da daha sonra sırasıyla; *Fagus* (1,2267±0,006) ve *Quercus-Fagus* (1,1267± 0,012) şeklinde belirlenmiştir. Öte yandan; *Fagus* ormanında karbon kaynağın çeşitliliğinin oldukça fazla olduğu ve fosfat karbonunun kullanımının yalnızca bu tip ormanında görülmesi de oldukça önemlidir. Çalışmamızda, Biolog Ecoplate yöntemi ilk kez orman toplulukları arasındaki mikrobiyal komünitenin belirlenmesi amacıyla uygulanmıştır. Çalışmamızdan elde edilen bulgular, söz konusu yöntemin orman topluluklarındaki uygulanabilirliğini ve etkinliğini açıkça göstermektedir. Aynı zamanda; mikrobiyal komünitenin de belirlenmesi ekosistem koruma uygulamaları oluşturulmasına yönelik yeni stratejilerin geliştirilmesine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler

Biolog EcoPlate, orman toplulukları; mikrobiyal çeşitlilik, Yenice ormanı

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INTRODUCTION

As producers and decomposers of terrestrial ecosystems, biota in plants and soil also have a clear functional connection. Plants provide organic carbon to soil biota through soil litter and root leaks, while soil biota organisms break down organic matter and provide phosphorus and mineral nitrogen for their growth [1]. In addition, microorganisms in the soil form the basis of terrestrial ecosystems in the biochemical cycle of plant nutrients and decomposition of vegetation litter [2, 3]. In general, the high heterogeneity of these habitats is caused by abundant plant species or functional diversity, allowing different microbial communities to evolve in these environments [4]. The strategy of the dominant plant within an ecosystem is driven by environmental conditions. The predominance of plants with high growth rates is linked to the environment's excessive use of light and nutrients. The amount of soil carbon is derived from low-quality litter in biomes with low nutrient availability and short growing seasons, while primary fertility becomes the key factor of soil carbon sequestration in more productive biomes [5].

In comparison to other habitats, forest soils have a very high diversity of prokaryotes [6]. In addition, the amount of organic matter is the major factor limiting the growth in the forest ecosystems and this situation is strongly affected by the processes in the rhizosphere. However, there is limited number of studies on interactions among tree roots, soil, and microbial communities, the microbial community composition is known to vary depending on the tree species in the rhizosphere [7, 8, 9].

Soil microorganisms are an important component of the Earth's biodiversity and play a crucial role in biogeochemical cycles and ecosystem function [10]. By regulating the hydrological and structural characteristics of the soil, these microorganisms perform numerous ecological processes including nutrient transformation and litter decomposition [11]. Therefore, evaluation and understanding of soil microbial diversity and function are very important in terms of evaluating soil quality. Soil microbial diversity is determined by characterizing different carbon (C) substrates using the Biolog EcoPlate Method [12]. This method determines the differences in the utilization of carbon in a microbial community, which is an important factor determining the microbial diversity of the soil [13]. And also, the method is ecologically suitable for measuring microbial diversity. Meanwhile, this method is proposed as a modern technology that allows rapid characterization of the ecological status of soil samples through detected biological functionality.

Yenice Forest includes Kavaklı and Çitdere Nature Conservation Areas. The forest was defined as one of the 100 global forests in urgent need of protection and one of nine forests within the same status in Turkey by the World Wide Fund for Nature (WWF) in 1991. These sites, recognized as "Hot Spots of European Forests," are among the world's most biodiverse forests [14]. Accordingly, the aims of our study are; (i) To determine the tree diversity in different forest communities identified at Yenice hot spot, (ii) to determine the microbial community in these communities by the Biolog EcoPlate method, and (iii) to reveal the community level physiological profile physiological profiles the community level between different forest communities.

MATERIALS and METHODS

This study was conducted in hot spots area Yenice Forest (Çitdere and Kavakli Nature Protection Areas), and Europea-Siberian Provence, Northern Turkey. The sampling was done between 2017-2018. Fresh soil samples taken from three predefined forest types [14], from 0 to 70 cm depth, especially from the A horizon and the dense root zone of dominant trees, were taken in triplicate by mixing in 10 cm increments. Soil samples for analysis of community-level physiological profiling were stored at 4°C.

To determine the diversity of trees in different forest types, the sample parcel sizes of the areas were determined according to the "minimal area method". The minimal area of these areas belonging to the forest community has been determined as 400 m^2. The overlap-abundance values of the tree species were determined according to the Braun-Blanquet method [15] and the species identification of the collected samples was made according to Davis (1965–1988) [16]. Identified forest types from which soil samples were collected and their coordinates are given in Table 1.

The Shannon-Wiener index standardizes the percentage abundance of species proportionally [17] and indicates the proportion of cover of a species in the total sample. Evenness value, which expresses the percentage of

Table 1. Forest communities and their coordinates.

Forest Communities	Species	Coordinate (X)	Coordinate (Y)
Fagus Forest (F)	*Fagus orientalis*	452935	4545317
		453232	4544253
		450702	4557656
Mixed *Quercus-Fagus* Forest (QF)	*Quercus hartwissiana*	452849	4545242
		449892	4556996
		449912	4556758
Mixed *Fagus-Abies* Forest (FA)	*Fagus orientalis - Abies nordmanniana*	452746	4545095
		452671	4545036
		450058	4556644

Table 2. The formulas used to calculate the tree species diversity in Yenice Forest.

Index	Formulae	Definitions
Shannon–Wiener index	$H' = \sum_{i=1}^{s} p_i ln p_i$	*H'* = Shannon-Wiener index *pi* = percent cover proportion of the species. *s*= number of the species
Shannon and Simpson evenness	$EH = H' / H' max$	E_H= *Evenness*

plant species found in a plant gathering or a sample plot relative to each other is calculated with Shannon and Simpson (Dominance index) [17, 18]. The formulas used to determine the tree species and calculate their diversity in forest types from which soil samples were taken are given in Table 2.

Community-level physiological profiling

The diversity of microorganisms in soil samples taken from different forest communities at the Yenice hot spot was determined using the Biolog Ecoplate (Biolog, USA) containing different carbon substrates in the soil. The 96-well plates contain 31 different carbon sources in triplicates. In addition, substrates were subdivided into six groups, according to Weber and Legge (2009) [19] (Table 3). For the extraction of soil samples, after mixing 10 g of soil sample with 20 ml of 10 mM Bis-Tris buffer (pH 7) for 60 minutes, the samples were centrifuged at 2,600 g for 10 minutes. 150 µL of supernatant was applied to the Biolog EcoPlate and the plates were incubated at 28 °C. Oxidization of the carbon sources by microorganisms causes the colorless tetrazolium dye to reduce into violet formazan [20]. The use of carbon substrates was determined by measuring the absorbance at 590 nm every 24 hours for 120 hours using the microplate reader (Multiskan FC, Thermo) [21].

The value of optical density (OD) obtained in 72 hours of incubation represent the optimum range of OD values. Therefore, Statistical analysis of Shannon diversity (H) and Shannon evenness (E) values were calculated due to the incubation time [22]. Total substrate (R) and E were calculated according to Zak et al. (1994) [23] (Table 4).

RESULTS and DISCUSSION

Different microbial communities live in the soils of different forest types. Several studies have shown that the composition of the microbial community may vary when the microbial biomass is similar among forest types in a climate zone [24, 25]. In our study, tree diversity in different forest types (*Fagus* Forest, Mixed *Quercus-Fagus* Forest, and Mixed *Fagus-Abies* Forest) defined in the Yenice hot spot was determined, and the microbial diversity and differences in carbon sources between these different forests were revealed.

Table 3. BiologEcoPlate carbon substrates classification.

Groups	C-substrats
AM	Phenylethylamine, Putrescine
AA	Glycyl-L-glutamic acid, L-arginine, L-asparagine, L-phenylalanine, L-serine, L-threonine
*C	D,L-alpha-glycerol phosphate, Glucose-1-phosphate, Pyruvic acid methyl ester
C	Alpha-D-lactose, Beta-methyl-D-glucoside, D-cellobiose, D-mannitol, D-xylose, i-erythritol, N-acetyl-D-glucosamine
CA	D-galactonic acid-gamma-lactone, D-galacturonic acid, D-glucosaminic acid, Gamma-hydroxybutyric acid, 2-Hydroxy benzoic acid, 4-Hydroxy benzoic acid, Itaconic acid, Alpha-ketobutyric acid, D-malic acid
P	Tween 40, Tween 80, Alpha-cyclodextrin, Glycogen

AA: Amino acids, AM: Amines/amides, *C: *Carbohydrate, C: Carbohydrates, CA: Carboxylic and Acetic acids, P: Polymers

Table 4. The Formulas for index calculations.

Index	Formulae	Definitions
H	$H = -£\mathrm{pi}(\ \mathrm{npi})$	H = Shannon diversity index
E	$E = H / lnS$	S = the number of colored wells
R	*R*	R = total substrate

Table 5. Mean values of H, E and R of three different forest communities based on 72 hour incubation.

Index	QF	F	FA
H	1,1267± 0,012	1,2267±0,006	3,0033±0,006
E	0,410±0,010	0,429±0,145	0,957±0,002
R	17,33±0,333	15,66±0,333	23,33±0,881

(QF: *Quercus -Fagus* Forest; F: *Fagus* Forest; FA: *Fagus -Abies* Forest; means ± standard errors, n=3)

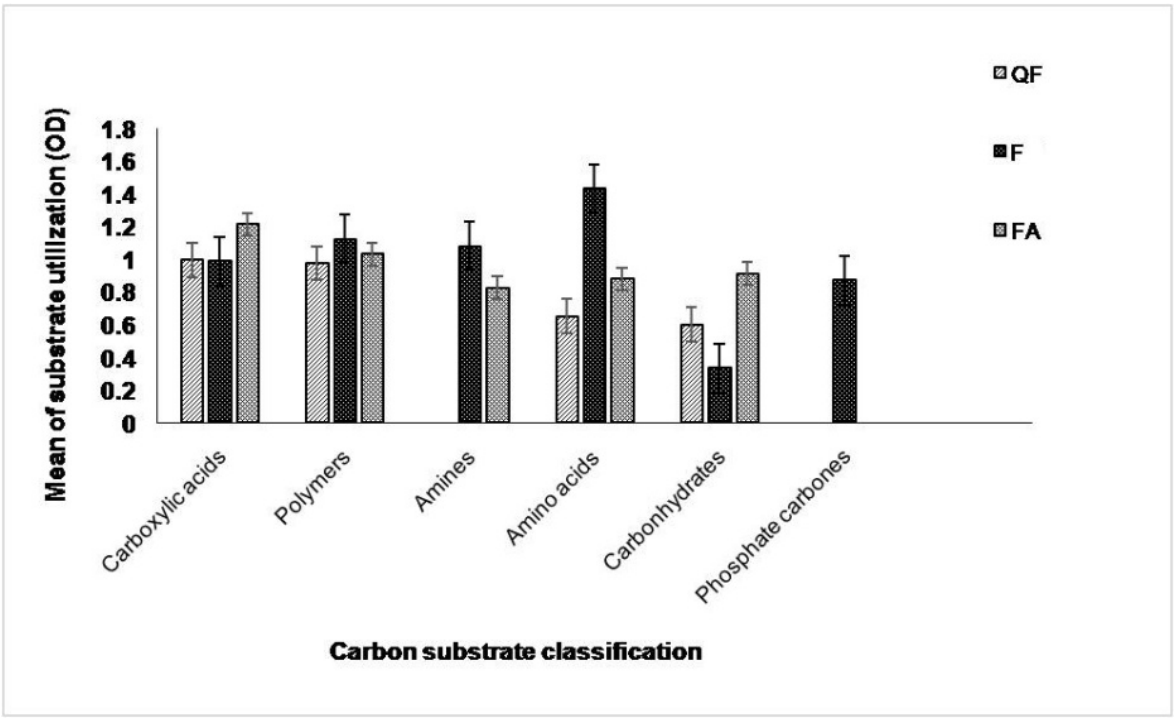

Figure 1. Means of carbon substrate group utilization from different by forests microorganisms based on 72 h incubation from the QF, F, and FA (n=3).

Table 5 shows the H, E, and R values determined by the Biolog Ecoplate method. A high richness index indicates that the number of oxidized carbon substrates is high [26]. For this reason, the "total substrate utilization" value was used for this index value in the present study (Table 4). Meanwhile, this index is a very sensitive evaluation tool for determining microbial activity [26]. The higher Shannon diversity (H) value for the *Fagus-Abies* forest shows that the diversity of microbial communities is greater in this forest than in the others (Table 5).

Soil moisture variations in Yenice Forest are caused by changes in forest layers. In the study conducted by Türkiş and Elmas (2018) in Yenice Forest, the lowest value of soil moisture was found in the regions where *Fagus orientalis* communities are located, however, the highest value was found in *Fagus-Abies* forests [14]. Soil moisture is known to support nitrogen mineralization by affecting microbial activity. Thus, the soil moisture level that is suitable for both plant growth and microbial activity [27]. This situation explains the greater diversity of microbial communities in the *Fagus-Abies* forest compared to others in our study (Table 5).

The variation of carbon sources evaluated in six categories within the scope of the Biolog EcoPlate technique is shown in Figure 1. There is a diversity of microbial communities using all types of carbon sources in *Fagus* forest. On the other hand, the diversity of the microbial community in the *Fagus-Abies* forest is in the form of carboxylic acid, polymer, amines, amino acid, and carbohydrate groups; in the *Quercus-Fagus* forest, this variety is in the form of carboxylic acid, polymer, amino acid, and carbohydrate groups. The fact that the use of Glucose-1-phosphate and D,L-alpha-glycerol phosphate (phosphate carbons) is seen only in the *Fagus* forest is remarkable (Table 3) (Figure 1).

In soil, fungi are defined as primary decomposers of complex compounds, while bacteria are primary decomposers of simple carbohydrates, amino acids, and organic acids [28]. Fungi are also tolerant to low soil matrix potential (binding of water to materials such as soil particles, cellulose and proteins and retention of water in capillaries or other fine pores) and especially in oak forest areas, relatively coarse-textured xeric soils contribute more to the abundance of mushrooms in this ecosystem [29]. Therefore, the dominance of fungi over the area can suppress the microbial diversity in the same area. Although various substrates belonging to carboxylic acid, polymer, amino acid and carbohydrate groups were detected in the *Quercus-Fagus* forest, this

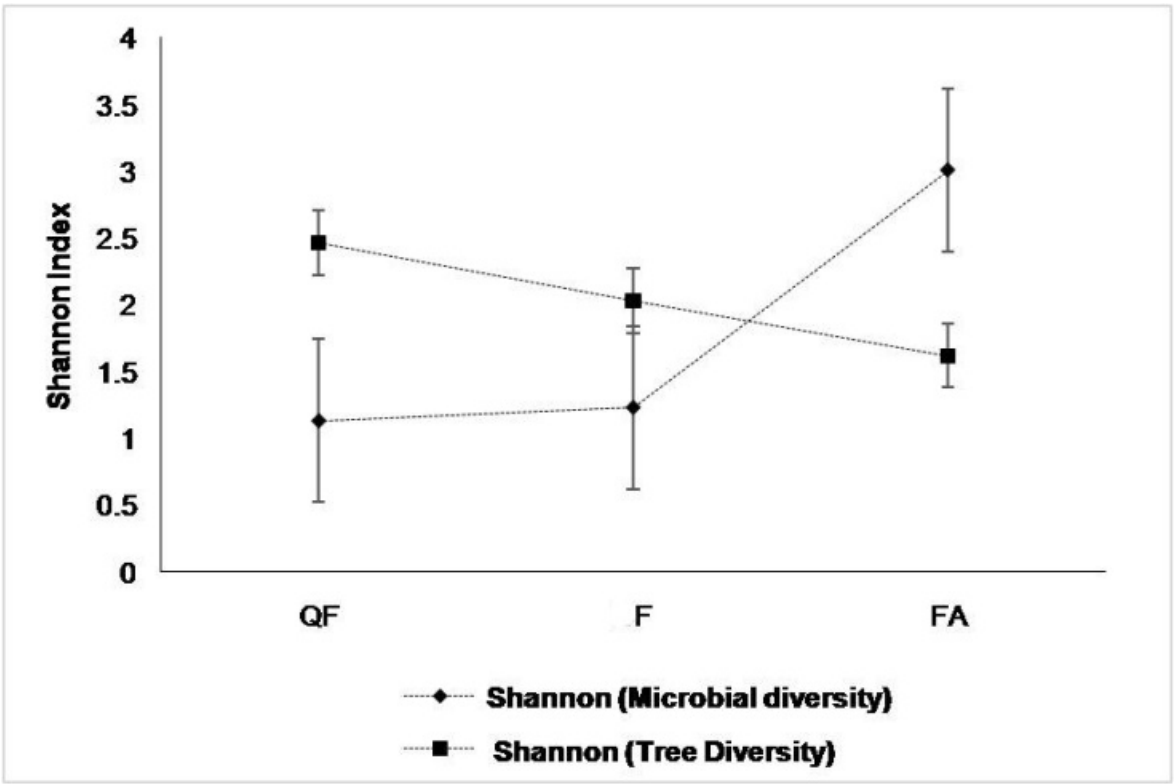

Figure 2. Comparison of microbial diversity and tree diversity in three different forest types of Yenice Forest.

situation explains the fact that the microbial diversity is the lowest compared to other forest types in our study.

When the microbial diversity in QF, F and FA forest types and the tree diversity in these forest types are compared, the order of microbial diversity was observed as *Fagus-Abies*, *Fagus* and *Quercus-Fagus*, while the order of tree diversity was observed as *Quercus-Fagus*, *Fagus,* and *Fagus-Abies*, from highest to lowest, respectively (Figure 2). In our study, microbial diversity was found lower in *Quercus-Fagus*, where tree diversity is high, compared to other forest types. The dominance of trees in forests forms a gradient from the ecological effect of various factors. Thus, the type and composition of tree species affect plant biodiversity, which in turn affects microbial diversity [30].

Tannins constitute an important part of carbon pools in forest ecosystems. Tannins can affect the nutrient cycle by inducing toxicity to microbial populations, limiting decomposition, inhibiting enzyme activities, and producing complex proteins [31]. The number of tannins vary with respect to tree species and can be found in more concentrated amounts in tree species such as beech, oak, scots pine, and black cherry [32]. In our study, although the highest carbon type variation was determined in *Fagus* forest areas, the highest microbial diversity was determined in *Fagus-Abies* forest areas. This situation supports the fact that the tannins, which exist in higher amounts in beech and oak trees, affect on microbial diversity.

The role of aboveground and underground substrate inputs in the development of soil microbial communities is poorly understood. While the soil in the root zone of the plant has more microbial biomass, the soil in other parts of the plant has a different composition [33]. Although this information has long been acknowledged, uncertainties for the ecosystem processes of the rhizosphere and soil microbial communities are still not fully explained. Plant-derived inhibitory compounds that are released into the soil by plants affect on soil microorganisms. Different compounds may be used as substrates, inhibit digestive enzymes, precipitate nutritive proteins, and induce direct or indirect toxic effects on microorganisms, depending on the microorganism [34].

The northern forest ecosystem, in which our study site is included, stores a significant amount of soil organic matter that can act as a resource, and the enzymatic degradation of this organic matter causes an increase in carbon dioxide. Some plants, such as beech and oak in our study, contain high concentrations of tannins. Although tannins negatively affect microbial diversity, high tannin concentration may act reversely by reducing this enzymatic activity [35].

In the present study, the community level physiological profile of microbial communities in the *Fagus-Abies*, *Fagus,* and *Quercus-Fagus* forests in the Yenice hot spot, which is among the most biodiverse forests in the world, were determined using Biolog EcoPlates. The amount of use and variety of substrates analyzed with Biolog EcoPlate has shown that they are significantly affected by the tree species that constitute different forest types. The results obtained from the present study are considered to contribute to the development of new strategies for establishing ecosystem protection practices by determination of the microbial community, which is one of the factors affecting forest ecosystems.

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