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Original Research Paper

Effects of Forest Communities and Various Depths on Soil Enzyme Activities in the Hyrcanian Forest

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ABSTRACT

Detailed information on soil quality can be ascertained by measuring soil enzymatic activities, which are often affected by soil biological chemical processes and depth. This study investigates the activity of four enzymes, acid phosphatase, alkaline phosphatase, urease and dehydrogenase in three various tree communities; *Parrotio-Fagetum*, *Parrotio-Carpinetum*, and *Parrotietum* at 0-20 cm depth with and without rhizosphere and 20-40 cm depth in the Kheyrud forest, Northern Iran. We found the higher enzyme activities in rhizosphere than without rhizosphere in all the communities. Soil enzyme activities decreased with increasing soil depths. There was a significant difference in acid phosphatase between 0-20 cm without rhizosphere and 20-40 cm. These findings were attributed to the observation that root propagation was reduced across the depths. Alkaline phosphatase and dehydrogenase, both showed a significant difference in activity among the communities, but acid phosphatase and urease did not. Microorganisms producing alkaline phosphatase and dehydrogenase were found to be significantly affected by the vegetation. Alkaline phosphatase activity in both depths and dehydrogenase at 0-20 cm with and without rhizosphere were greater in *Parrotietum* than those of *Parrotio-Fagetum* and *Parrotio-Carpinetum*. It appeared that the microbial community in *Parrotietum* was much greater than the other two types.

INTRODUCTION

The microbiological activity and development of a soil directly influence ecosystem stability and fertility (Smith & Papendick 1993). Activity of soil microorganisms can be evaluated by enzymes activity. Enzymes are biological catalysts of essential processes for the life of microorganisms and the simultaneous measurement of several enzyme activities may be useful for assessing soil microbial activity (Nannipieri et al. 1990). The structure and functional diversity of microbial communities in the soil is tightly related to plant species composition (Grayston & Campbell 1996, Grayston et al. 2001, Priha et al. 1999, Westover et al. 1997). Variation in root system among plant species leads to variation in nutrient utilization and losses and, hence, possibly to changes in soil microbial activity (Niemi et al. 2005).

Soil enzyme activities respond very quickly to the alteration in vegetation (Pei et al. 2008) as extracellular enzymes participating in C, N, P cycling and plant species have significant impacts on the nutrient cycling.

Hyrcanian forest ecosystem is considered to be one of the last remnants of natural deciduous forests in the world. In comparison to European broad-leaved forests, the Hyrcanian forests seem to have remained from the Tertiary and to be relic ecosystem (Sagheb-Talebi 2000).

The potential effects of tree species on soil properties have been a focus of study for a long time (Binkley & Menyailo 2005, France et al. 1989, Kulmatiski et al. 2008, Porazinska et al. 2003, Zinke 1962). However, in Hyrcanian (Caspian) forests, the usage of enzyme for evaluation of forest ecosystems is limited (Shirvany et al. 2004). Caspian forests are an ideal site to investigate the effects of forest communities on soil enzymes because these forests support such important tree communities as *Parrotio-Fagetum* (PF), *Parrotio-Carpinetum* (PC), and *Parrotietum* (P). *Parrotia persica* or Ironwood is an endemic broad leaves species in Hyrcanian forest region (Sagheb-Talebi 2000).

It is well established that root activities of different plant species selectively stimulate growth of different microbial species in the rhizosphere via root exudation of various compounds including sugars, amino acids, organic acids, hormones and vitamins (Bais et al. 2004). Root exudates represent up to 40% of below-ground organic inputs in terrestrial ecosystems (Brimecombe et al. 2001). It appears that nutrient cycling of various species differ extensively and soil enzyme studies could lead us to specify the differences. In fact such differences are expected to be higher in the rhizosphere than outside rhizosphere.

Griffiths et al. (2003) showed a reduction in metabolic diversity and change in dominant microbial species with soil depth. Very little information is available on the activity of enzymes found throughout the soil profile (Venkatesan &

Senthurpandian 2006). Most studies in soil enzymology have concentrated particularly on the surface soils, where enzyme activities are expected to be higher (Zaman et al. 2002, Venkatesan & Sudhahar 2004, Sudhahar & Venkatesan 2004). Hence, in the current study it was decided to determine the activity of important enzymes like acid phosphatase, alkaline phosphatase, urease and dehydrogenase under three different forest communities, namely, *Parrotio-Fagetum* (PF), *Parrotio-Carpinetum* (PC), and *Parrotietum* (P), at depths of 0-20 cm with and without rhizosphere and 20-40 cm. As tree types and depths impact on soil chemical properties, we measured such properties as soil pH, soil organic-carbon (SOC), available phosphorus and total nitrogen to evaluate the conditions of the microbial community. In conclusion, detailed information was provided to help evaluate the chemical and biological conditions of the three tree communities across depths.

MATERIALS AND METHODS

Study site and soil sampling: The study was conducted in Kheyrud forest located in Mazandaran province, Northern Iran (from 36°36'N to 36°40'N and from 51°32'E to 51°43'E). Three sites were chosen: *Parrotio-Fagetum*, *Parrotio-Carpinetum*, *Parrotietum* (co-ordinates, 36°36' N, 51°34'E, and elevation 390m above sea level). The climate is humid with mean annual air temperature of 12°C and mean annual precipitation of 1450mm. During the study, average rainfall and temperature was 160 mm and 19°C respectively in September. The vegetation was dominated by *Parrotia persica*, *Fagus orientalis*, *Carpinus betulus*, *Ruscus hyrcanus*, *Ilex spicigera*, *Crataegus* spp. The sampling area is established on the lime stone and soil texture is loamy-clay with pH 5.74 to 6.84. At each site, three soil samples were randomly taken at depths of 0-20 cm with and without rhizosphere and 20-40 cm in the late summer 2009. Soil samples were placed in tightly sealed plastic bags and transferred immediately to the laboratory at 4°C. The soil samples were passed through at 2 mm sieve and divided into two parts: one fraction for the determination of chemical factors, which was stored at room temperature and the other fraction for measuring of soil enzyme activities which was stored at 21°C.

Soil chemical and physical properties: Soil dry matter was measured following the method of Schlichting & Blume (1966). The soil samples were analysed for a variety of chemical characteristics to determine the gradients in pH (Black 1973), organic carbon by wet oxidation (Walkley & Black 1934), total nitrogen by micro Kjeldahl digestion procedure (Bremmer & Mulvaney 1982) and available phosphorus (Olsen & Sommers 1982).

Enzymes assays: The activities of acid phosphatase, alkaline phosphatase, urease and dehydrogenase were determined. The activities of both the phosphatases (activity acid phosphatase, alkaline phosphatase) in the field soil samples were measured using the method described by (Margesin 1996). Phosphatases activity was assayed by mixing 1 g of air-dried soil with 4 mL modified universal buffer (pH: 6.5 for acid phosphatase, pH: 11 for alkaline phosphatase), 1 mL substrate (p-nitrophenyl phosphate). After incubation for 1 h at 37°C under shaking conditions, the enzyme reaction was stopped by adding 1 mL 0.5 M $CaCl_2$, 4 mL 0.5 M NaOH and 90 mL distilled water. After shaking briefly and paper-filtering, the absorbance was measured at 400 nm. Calculated enzyme activity was expressed in µg of p-nitrophenol released per gram per hour.

Urease activity was estimated by mixing 5 g of air-dried soil and 2.5 mL of 79.9 mM substrate solution (urea) followed by incubation for 2 h at 37°C (Kandeler 1996). The amount of NH_4^+ radical released during the incubation period was calculated by shaking the contents of the flask with 50 mL KCl solution (2M) for 30 min. The activity is expressed in µg of NH_4^+-N released per gram per hour.

Dehydrogenase activity was measured using the TTC method (Ohlinger 1996). 5 grammes of air-dried soil sample was incubated with 5 mL of 0.1 M Triss buffer and 5 mL of substrate solution (triphenyltetrazolium chloride) at 25°C for 16h. The enzyme activity, expressed in µg triphenylformazon (TPF) g^{-1} h^{-1},was determined by spectrophotometry at 546 nm after extraction with acetone.

Statistical analysis: Each sample had three replicates. Duncan and James Howell tests were used based on the statistical distribution. All statistical analyses were performed using the SPSS version 17 statistical package.

RESULTS

General soil data: Soil pH ranged from 5.74 to 6.84, due to the accumulation of organic matter on the forest floor , the pH of the soils beneath *Parrotio-Fagetum* was slightly lower than that of soils beneath *Parrotio-Carpinetum* and *Parrotietum* at all depths. The SOC with the highest content at 0-20 cm with rhizosphere revealed a tendency to decrease with increasing soil depth. For all the tree communities, there were significant differences ($P<0.05$; Duncan and James Howell) in SOC fractions along depths. A similar pattern was seen in concentrations of soil total N. However, soil total N was generally low in all soils.

Available phosphorus showed a different result from SOC and total N and showed no significant difference ($P<0.05$) among the depths. Soil properties such as SOC, total N and available phosphorus did not differ significantly among tree communities except for SOC in *Parrotio-*

Carpinetum at 20-40 cm depth, which was significantly ($P<0.05$) lower than other tree communities (Table 1).

Enzyme activity as affected by soil depth: Generally, at all sites, enzymes activity was greater at 0-20 cm with rhizosphere than without rhizosphere depths. However, acid phosphatase activity only in *Parrotio-Carpinetum* was significantly different ($P<0.05$), (639.64 ± 6.03% to 452.72 ± 43.58%; mean ± SD). There were depths-related changes in acid phosphatases activity and decreased from 0-20 cm without rhizosphere to 20-40 cm depths. Acid phosphatases activity, for both *Parrotio-Carpinetum* and *Parrotietum* showed a significant difference ($P<0.05$), 452.72 ± 43.58% to 305.20 ± 44.17% and 421.16 ± 36.02% to 319.10 ± 23.65% respectively (Fig. 1a).

Alkaline phosphatase activity only in *Parrotio-Fagetum* was significantly different ($P<0.05$), at 0-20cm with rhizosphere (282.98 ± 47.39%) compared to 0-20 cm without rhizosphere (161.29 ± 21.99%). Alkaline phosphatase varied with increase in sampling soil. However, there was no significant difference between 0-20 cm without rhizosphere and 20-40 cm depth in all communities (Fig. 2b).

Among 0-20 cm with and without rhizosphere, urease activity under *Parrotio-Carpinetum* and *Parrotietum* exhibited significant differences ($P<0.05$), 132.29 ± 23.91% to 62.65 ± 11.21% and 119.45 ± 15.65% to 67.26 ± 9.60% respectively. Urease activity showed a decreasing trend among 0-20 without rhizosphere and 20-40 cm. However, only in *Parrotio-Fagetum* a significant difference ($P<0.05$) was observed, 81.84 ± 10.41% to 42.90 ± 6.66% (Fig. 1c).

The pattern of dehydrogenase activity was almost same as alkaline phosphatase activity. The difference in dehydrogenase activity, among 0-20 cm with and without rhizosphere except *Parrotio-Carpinetum* was significant ($P<0.05$). Dehydrogenase activity beneath *Parrotio-Fagetum* and *Parrotietum* was from 26.12 ± 4.04% to 15.25 ± 1.93% and from 65.71 ± 12.09% to 33.12 ± 7.85% respectively. Dehydrogenase did not differ significantly ($P<0.05$) with increase in soil depths (Fig. 1d).

Effect of forest communities on soil enzyme activity: The activity of acid phosphatase and alkaline phosphatase differed among communities. In terms of acid phosphatase, the soil beneath *Parrotio-Fagetum* in all depths showed higher activity than the soil underneath other communities. However, acid phosphatase did not differ significantly ($P<0.05$) among communities at all depths (Fig. 2a). In contrast to acid phosphatase, alkaline phosphatase activity in *Parrotietum* was higher than others at all depths. Alkaline phosphatase differed significantly ($P<0.05$) at 0-20 cm with and without rhizosphere among communities. The significant difference ($P<0.05$), in rhizosphere, was among *Parrotietum* with *Parrotio-Carpinetum* and *Parrotio-Fagetum*, 584.28 ± 6.89%, 376.30 ± 41.84% and 282.98 ± 47.39%. At 0-20 cm without rhizosphere depth, between *Parrotietum* with *Parrotio-Fagetum* was also seen significantly different ($P<0.05$) 398.81 ± 1.01% and 161.29 ± 21.99% respectively (Fig. 2b).

There were not significant ($P<0.05$) effects of forest communities on soil urease activity. Urease activity beneath *Parrotio-Fagetum* at 0-20 with and without rhizosphere showed higher activity than the soil underneath other communities (Fig. 2c). Both the soils, with and without rhizosphere, under *Parrotietum* exhibited higher dehydrogenase activity. The remarkable difference ($P<0.05$) in rhizosphere was among *Parrotio-Fagetum* with *Parrotio-Carpinetum* and *Parrotietum* 26.12 ± 4.04%, 39.52 ± 5.24% and 65.71 ± 12.09%. There was also a significant difference ($P<0.05$), at 0-20cm without rhizosphere among *Parrotio-Fagetum* with *Parrotio-Carpinetum* 15.25 ± 1.93% and 28.71 ± 5.23% respectively (Fig. 2d). There were not significant effects of communities on soil enzymes activities at 20-40 cm depth (Fig. 2.a,b,c,d).

DISCUSSION

Enzyme activities as affected by soil depth: In the rhizosphere very close to the roots, plant exudates including enzymes are probably more pronounced (Niemi et al. 2005).

Table 1: Soil chemical properties for depths 0-20cm without rhizosphere (WR), 0-20cm with rhizosphere (R) and 20-40cm. Different letters indicate statistically significant differences at $p < 0.05$.

Depth cm	Tree Type	SOC%	Total N%	Available Pmg kg^{-1}	pH
0-20 WR	PF	3.06±0.20a	0.20±0.02a	4.87±0.86a	5.74
	PC	2.74±0.12a	0.17±0.04a	6.53±1.28 a	6.15
	P	2.91±0.26a	0.23±0.05a	5.22±1.03a	6.61
0-20 R	PF	3.56±0.17b	0.26±0.06b	5.48±1.09a	5.90
	PC	3.44±0.22b	0.31±0.02b	4.56±0.85a	6.01
	P	3.60±0.22b	0.30±0.01b	5.14±0.88a	6.84
20-40	PF	1.98±0.39c	0.13±0.03c	6.02±1.01a	5.83
	PC	0.35±0.15d	0.11±0.03c	4.49±0.74a	6.00
	P	1.51±0.21c	0.12±0.03c	4.85±1.20a	6.43

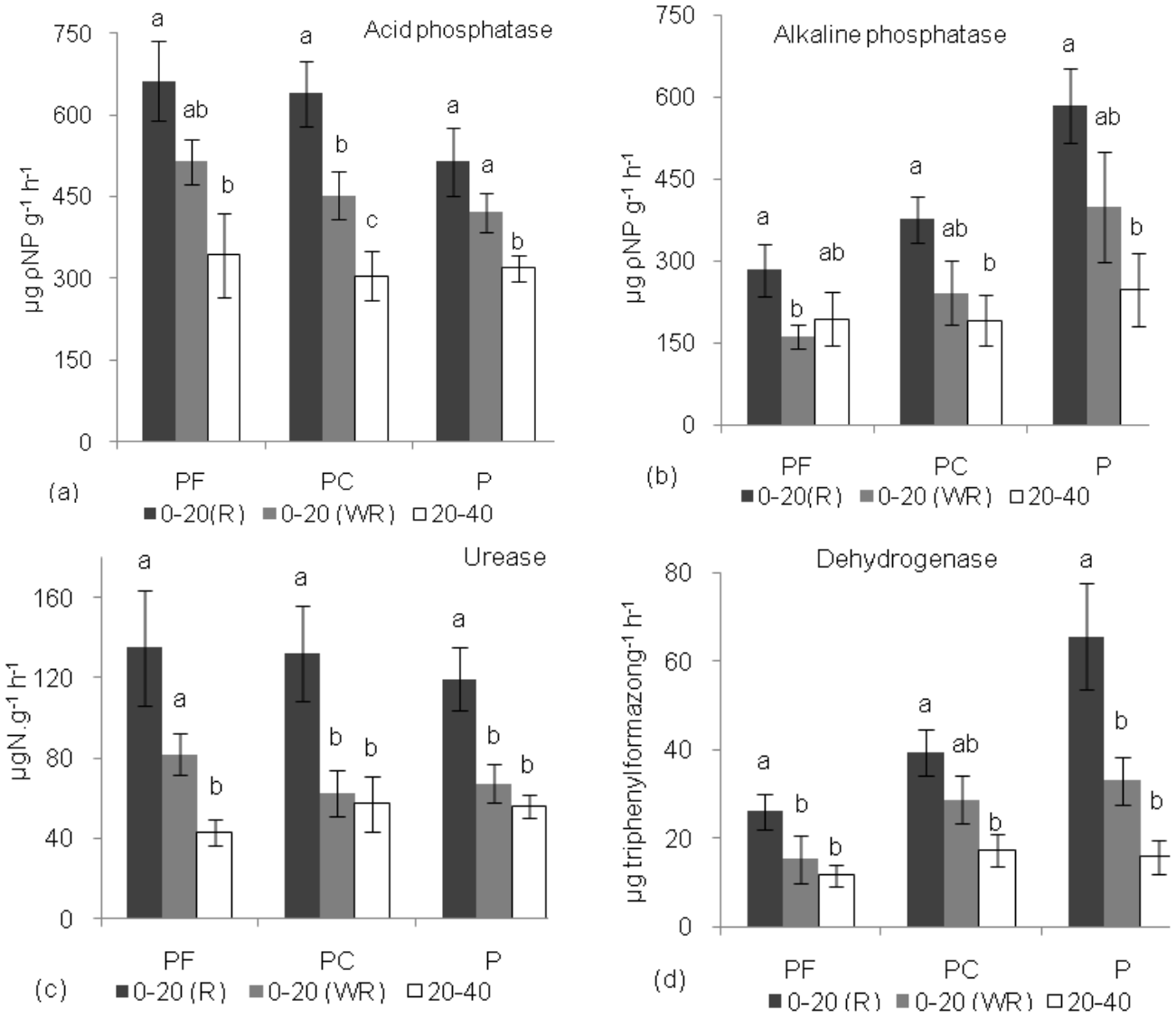

Fig. 1: Relationship between soil depths 0-20 cm with rhizosphere (R), 0-20 cm without rhizosphere (WR), 20-40cm and soil enzymes acid phosphatase (a), alkaline phosphatase (b), urease (c), dehydrogenase (d) across communities *Parrotio-Fagetum* (PF), *Parrotio-Carpinetum* (PC), *Parrotietum* (P). Data are the means of three replicates with bars representing the standard errors of the means. Different letters indicate statistically significant differences at $p < 0.05$.

In our study, we observed that enzyme activities were greater in the rhizosphere. Between, with and without rhizosphere, remarkable difference was found in acid phosphatase only under *Parrotio-Carpinetum* (Fig. 1a), which may have been caused by the root system of *Carpinus betulus*. Niemi et al. (2005) found that plants with diverse root systems can have various effects on the microbial and enzymatic activities.

Alkaline phosphatase activity only determined a significant difference in *Parrotio-Fagetum* (Fig. 1b). Alkaline phosphatase is produced by the microorganisms, and it may be concluded that beech (*Fagus orientalis*) root exudates play an important role in the rise of the microbial population producing alkaline phosphatase. A significant difference was not seen in available phosphorus with soil depth (Table 1). Adams (1992) showed that phosphatases activity are not related to the mineralization of phosphorus, and instead of phosphatases enzymes, the solubility of organic phosphorus played a vital role in the process of phosphorus mineralization (Adams & Pate 1992).

Both, dehydrogenase and urease activity, showed a sig-

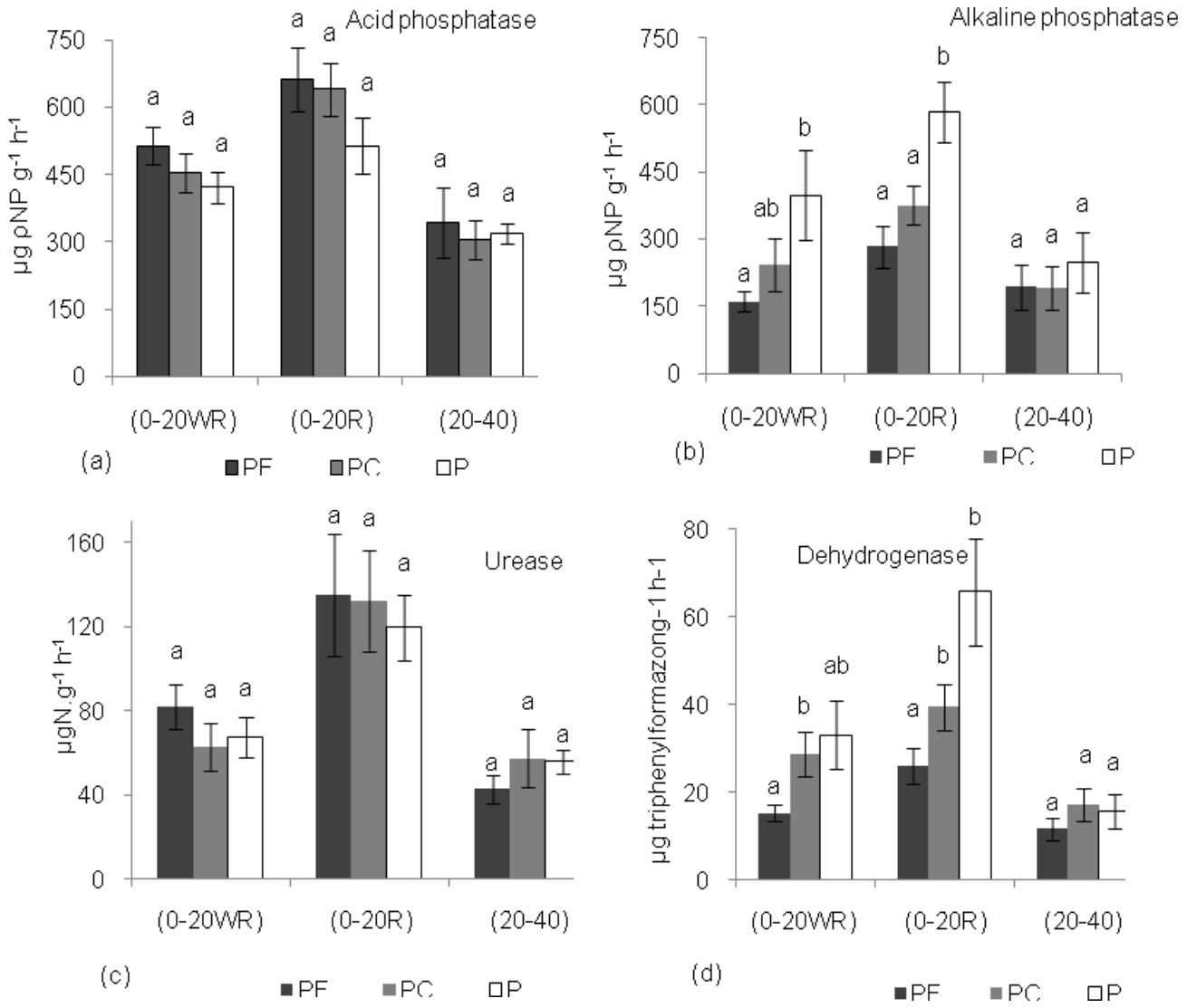

Fig. 2: Relationship between soil enzymes acid phosphatase (a), alkaline phosphatase (b), urease (c), dehydrogenase (d) and communities *Parrotio-Fagetum* (PF), *Parrotio-Carpinetum* (PC), *Parrotietum* (P) across soil depths 0-20 cm with rhizosphere (R), 0-20 cm without rhizosphere (WR), 20-40 cm. Data are the means of three replicates with bars representing the standard errors of the means . Different letters indicate statistically significant differences at $p < 0.05$.

nificant difference in *Parrotietum*. The source of carbon is the limiting factor in the growth of the microbial population (Wardle et al. 1992). In our study, SOC and total N were significantly greater in rhizosphere than without rhizosphere and this supports microbial activity producing such enzymes in rhizosphere.

According to Venkatesan & Senthurpandian (2006), enzyme activities were greater in the top layer of soils. Our results showed the decline of enzyme activities as a function of soil depth. However, among 0-20 cm without rhizosphere and 20-40 cm depths, only acid phosphatase was more affected by depth and indicated a significant difference (Fig. 1a). This is in agreement with the findings of Kandeler (2002). There can be two arguments proposed for such an observation: First, acid phosphatase had been mostly produced by the plant roots and its amount had decreased with distance from the root zone. While, dehydrogenase, urease and alkaline phosphatase were largely produced by microorganisms present in deeper layers making it possible for these enzymes to appear in those depths. Secondly, lighter organic carbon can also migrate down into the bottom layers and become a source of carbon for microorganisms present

there (Kandeler 1994). Therefore, it could make a balance between upper and lower layers and so not to be observed any significant difference between them.

Effects of forest communities on soil enzyme activity: Soil microbial communities producing soil enzymes are affected by plant species and soil biochemical properties. A high acid phosphatase activity was found in soil samples under *Parrotio-Fagetum*. Because soil pH has significant impact on soil phosphatase activity (Speir & Ross 1978), we suggest that more acid phosphatase activity in *Parrotio-Fagetum* is primarily due to lower pH (Fig. 1, Table 1). The higher alkaline phosphatase activity was seen in *Parrotietum* (Fig. 2); alkaline phosphatase generally exceeds acid phosphatase in high pH soil (Eivazi & Tabatabai 1977). Katarzyna et al. (2009) found that greater activities of enzymes can be attributed to more nutrient resources and microbial biomass. Therefore, high alkaline phosphatase in *Parrotietum* may result from a higher microbial biomass and some nutrients resources in comparison with the mixed communities.

In this study, unlike acid phosphatase, there was communities-specific differences in alkaline phosphatase activity (Fig. 2 ab). The observed differences are related to different origin of these enzymes in soils (Matinizadeh 2008). It seems that microorganisms are the only source of alkaline phosphatase which have more been affected by vegetation in comparison with root exudates as the most important source of acid phosphatase.

Previous studies suggested that poorly decomposable litter often decrease the fertility of soils (Hobbie 1992, Vitousek 2006). The lower dehydrogenase activity under *Parrotio-Fagetum* is related to the slower decomposition of beech (*Fagus orientalis*) (Fig. 2d). Baudoin et al. (2002) found that plant species richness might influence dehydrogenase activity, since the quantity and quality of root exudates can vary between different plant species. Therefore, we observed a significant relationship between communities and dehydrogenase at 0-20 cm with and without rhizosphere with higher activity under *Parrotietum*.

In the present study, the weak influence of communities on urease activity can be attributed to the proximity of the habitats of the studied types which according to Hoult & McGanty (1986) would result in similar litter entry as one of the effective factors on the activity of urease.

Our research revealed that there was no significant difference in the level of nutrients among the types (Table 1). We concluded that nitrogen, organic carbon and phosphorus requirements in all the three types were basically the same. This result may also support previous study that plant species would not affect the soil organic carbon level (Bastida et al. 2008). Although, the microelement variation threshold may differ from that of microorganisms, as such, even though there are not significant differences in the nutrients, there are significant differences in the soil enzymes being the evidence for the presence of microorganism activity.

CONCLUSION

Alkaline phosphatase and dehydrogenase was more affected by tree types than acid phosphatase and urease. Our results revealed that the activities of alkaline phosphatase at all depths and dehydrogenase at 0-20 cm with and without rhizosphere were significantly higher in *Parrotietum* than *Parrotio-Fagetum* and *Parrotio-Carpinetum*. Enzymes activities were higher in the rhizosphere than without rhizosphere in three forest communities. We also observed that enzymes activities decreased from the surface soil with increasing soil depth. However, dehydrogenase, alkaline phosphatase and urease were found to be less affected by soil depth. On the contrary, acid phosphatase was more affected by depth. The impact of forest communities was less than the impact of soil depth on nutrient cycling. SOC and total N decreased with increasing soil depth. Future investigation on enzyme activities may throw more light on complete soil biology of Hyrcanian forest.

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