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Enzyme Activities in Undisturbed and Disturbed Forest Soils Under Oak (*Quercus brantii* var. *persica*) as Affected by Soil Depth and Seasonal Variation

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Abstract: The seasonal dynamics of acid phosphatase, alkaline phosphatase and dehydrogenase and the relationship of their activities to soil depth were studied in the undisturbed forest soils under oak and compared with the disturbed one. At each site, four soil samples were randomly taken at depth of 0-20 cm in May, July, September and November and at three different depths (0-10, 10-20 and 20-30 cm) in November. Enzymes were assessed by reaction with substrate and photometrical method. The activity of acid and alkaline phosphatase and dehydrogenase was greater at undisturbed site than disturbed sites. These results signalized a high activity of roots for the secretion of acid phosphatases. High alkaline phosphatase activity reflected a good status of soil microorganisms. Moreover alkaline phosphatase activity was 2.1 to 2.93 times greater than acid phosphatase activity at both sites. Alkaline and acid phosphatase and dehydrogenase activity showed a seasonal pattern with maxima in summer and minimum in autumn. Assay of acid phosphatase activity showed significant ($p < 0.01$) increasing of its activity with soil depth increment at undisturbed site whereas this increasing trend was not observed at disturbed site. The depth pattern of alkaline phosphatase activity was totally different. At undisturbed site, alkaline phosphatase activity significantly ($p < 0.01$) decreased with soil depth and visa versa in the disturbed site. At both sites, dehydrogenase activity significantly ($p < 0.01$) decreased with soil depth increment. In conclusion the higher activity of phosphatase and dehydrogenase at undisturbed sites shows the effect of land use management and for understanding soil ecosystem functioning.

Key words: Dehydrogenase, phosphatase, *Quercus brantii* var. *persica*, seasonal changes, undisturbed and disturbed sites

INTRODUCTION

In the west of Iran in Zagrosian forests (with an area of 5 million hectares), oak manna trees (*Quercus brantii* var. *persica*) are the dominant species in 1000-2300 m above sea level. This forest has the most extensive habitat among oak species in Iran. It is usually found in calcareous soils with alkaline pH. This species has high resistance against drought and low and high temperatures. In the last decades, this natural ecosystem has been disturbed due to intensive harvested, industrial development and agricultural activities. Recently programs have been developed for the protection of natural ecosystems and the rehabilitation of damaged regions in local or global levels. Having fundamental information about different aspects of forest ecosystems such as its soil quality and functioning is an important criteria in the success of management programs for ecosystem preservation. Visser and Parkinson (1992) have

suggested that the biological and biochemical properties that are most useful for detecting the deterioration of soil quality are those that are most closely related to nutrient cycles, including soil respiration, microbial biomass, nitrogen mineralization capacity and the activities of soil enzymes. In particular, enzyme activities are especially significant in soil quality assessments because of their major contribution to the soil ability to degrade organic matter (Taylor *et al.*, 2002; Schloter *et al.*, 2003). The activities of enzymes (i.e., hydrolases) are important soil quality indicators because they are intimately involved in the cycling of nutrients, affect fertilizer use efficiency and reflect in part the microbiological activity in soil (Jordan *et al.*, 1995; Dick *et al.*, 2000; Taylor *et al.*, 2002).

The phosphatases are a group of enzymes key to soil forest P cycling. Phosphatase activities can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils (Speir and Ross, 1978; Dick and Tabatabai, 1993). Soil

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phosphatases, extracellularly secreted by plants and microorganisms, play a key role in the phosphorus cycle, allowing the formation of inorganic phosphorus, the only phosphate-form taken up by plants and microorganisms (Antonietta Rao *et al.*, 2000). Phosphatase activity is related to soil and vegetation conditions (Herbien and Neal, 1990) and changes in response to management practices (Adams, 1992; Clarholm, 1993), soil temperature and moisture (Speir and Cowling, 1991). Phosphatases in soils are derived mostly from the microbial population (Tabatabai, 1994; Li *et al.*, 1997) and have been suggested as a satisfactory index of microbial activity (Tabatabai, 1994; Tarafdar *et al.*, 2001). In the other hand, acid phosphatase is mainly produced by plants but also soil microorganisms release acid phosphatase (Tabatabai, 1994). Alkaline phosphatase is produced by soil microorganisms and soil fauna (Findenegg and Neiemans, 1993; Tarafdar, 1995) whereas higher plants are devoid of alkaline phosphatase (Dinkelaker and Marschner, 1992; Tarafdar *et al.*, 2001).

Dehydrogenase activity is used to estimate of overall microbial activity due to its presence in all microorganisms (Garcia *et al.*, 1997; Taylor *et al.*, 2002). It is often used as an indicator of microbial activity (Nannipieri *et al.*, 1990; Dick, 1994). The dehydrogenase activity of a soil is the result of the activity of different dehyrogenases, which are an important component of the enzyme system of all microorganisms (enzymes of respiratory metabolism, citrate cycle and nitrogen metabolism). Dehydrogenase activity is thus an indicator of biological redox-systems and can be taken as a measure for the intensity of microbial metabolism in soil (Schloter *et al.*, 2003).

Due to the importance of understanding soil quality in forest ecosystems related to metabolic capacities and biogeochemical cycling, the primary objectives of this study were to investigate selected soil chemical properties of an undisturbed region (UD) and disturbed region (DI) of the Zagrosian forest in Iran and to investigate the activities of dehydrogenase and two phosphomonoesterases (alkaline and acid) activities at the UD and DI forest regions as affected by seasonal variation, soil depth and soil properties.

MATERIALS AND METHODS

Site description: The study was carried out in two different sites in high forest with *Quercus brantii* var.

persica in Monj, Chahar Mahal va Bakhtiari province, Iran at a distance of 696 km from Tehran. Two sampling locations were chosen in Monj: UD in an almost undisturbed site (co-ordinates, 313013N, 503525E and elevation 2056 m) and DI in a disturbed site (co-ordinates, 313015N, 503628E and elevation 1955 m). The climate in Zagrosian forests is semi arid with unpredictably extreme change of temperatures in both summer and winter. In fact disturbed site is a degraded-unmanaged forest that trees are being cut from fifty years ago. The harvesting is done by traditional methods to obtain wood for domestic purposes. Rainfall and snowfall begin from mid-autumn and continue until spring March/April and the climate is dry from May until October. Long-term average precipitation is 520 mm and long-term average of temperature is $17.7°C$. Mean maximum and minimum temperatures are 23 and $5.9°C$, respectively. During the study period, annual temperature was $1.1°C$ below the long-term average and annual precipitation was 61 mm above the long-term average. Soil texture was sand-clay-loamy with pH 7.5-7.7 (Table 1). The DI site had less mature seeding crops and more gaps than UD site because of harvesting.

Soil sampling: At each site, four soil samples were randomly taken at depth of 0-20 cm in May, July, September and November 2003 and at three different depths (0-10, 10-20 and 20-30 cm) in November 2003. Soil samples were placed in tightly sealed plastic bags and transferred immediately to laboratory at $4°C$. The soil samples were passed through a 2 mm sieve and divided into two parts: one fraction for the determination of physical and chemical factors, which was stored at room temperature and the other fraction for measuring of soil enzymes activities which was stored at $4°C$. The soil were analyzed for pH in a 1:2.5 (soil: water) using a glass electrode, organic matter content by wet oxidation (Walkley and Black, 1934), total nitrogen by micro-Kjeldahl digestion procedure (Bremmer and Mulvaney, 1982) and soil texture and particle size analysis by the pipette method (Kilmer and Alexander, 1949). Olsen's bicarbonate extractable P (PO_4^{3-}) was also measured (Olsen *et al.*, 1954). Soil microbial biomass was determined by method as described by Joergensen and Brookes (1990).

Table 1: Some characteristics of soil (0-20 cm) in every site

Sites	pH	Sand (%)	Silt (%)	Clay (%)	Texture	P (ppm)	OC (%)	Microbial biomass (mg C/100 g soil)	Total N (%)
UD	7.81	59.68	16.36	23.96	S.C.L	27.9	2.134	107.61	0.236
DI	7.75	57.68	17.64	24.68	S.C.L	25.6	1.069	75.63	0.160

UD: Undisturbed region; DI: Disturbed region

Phosphatase assay: The activities of both phosphatases (Acid phosphatase and alkaline phosphatase) were determined in the field soil samples using the method described by Ohlinger (1996a).

After the addition of a buffered ρ-nitrophenyl phosphate solution, soil samples are incubated for 1 h at 37°C. The nitrophenol released by phosphomonoesterase activity is extracted and colored with sodium hydroxide and determined photometrically at 400 nm.

Dehydrogenase assay: Dehydrogenase activity was determined in the soil samples according to Ohlinger (1996b). In brief, field moist soils were suspended in a triphenyl tetrazolium chloride solution and incubated for 16 h at 25°C. The triphenyl formazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm.

Statistical analyses: The results were analyzed with the SAS program and the means presented in this study for all measurements represents means (n = 4). All data in the study were subjected by analysis of variance and means were separated by Duncan's multiple range tests.

RESULTS

Phosphatase activities as affected by seasonal variation: Alkaline and acid phosphatase activities were significantly different (p<0.01) in both undisturbed and disturbed sites throughout all sampling times (Table 3). In this study, average alkaline phosphatase activity was 2.1-2.9 times higher than average acid phosphatase activity. Alkaline phosphatase activity ranged from 542.1 at disturbed site to 934.7 μg ρ-nitrophenol g^{-1} soil h^{-1} at undisturbed site (Table 4). Acid phosphatase activity ranged from 187.6 μg ρ-nitrophenol g^{-1} soil h^{-1} at disturbed site to 427.0 μg ρ-nitrophenol g^{-1} soil h^{-1} at undisturbed site (Table 4). Both enzyme activities followed a similar seasonal pattern at undisturbed and disturbed sites. The enzyme activities increased a few from fall to spring, continued increasing through spring and early summer, reached to the maximum activity in summer and then dropped again in fall.

Seasonal changes of alkaline phosphatase activity were not significantly different in undisturbed site (Table 2, 4). The highest activity was 934.7 μg ρ-nitrophenol g^{-1} soil h^{-1} in July and the lowest one was 762.7 μg ρ-nitrophenol g^{-1} soil h^{-1} in November in undisturbed site. In disturbed site the highest activity was 710.1 μg ρ-nitrophenol g^{-1} h^{-1} in September and the lowest was 542.1 μg ρ-nitrophenol g^{-1} soil h^{-1} in May. In this site, alkaline phosphatase activity in November was significantly lower than other sampling times. Acid phosphatase activity had significant differences at both sites in November in comparison to summer (Table 2, 4). The highest activity was 427.0 and 418.7 μg ρ-nitrophenol g^{-1} soil h^{-1} in September and July and the lowest one was 263.0 in November. In disturbed site the highest activity

Table 2: Mean comparison of season effect on soil enzymes activities

Months	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
May	290.67[b]	672.13[b]	33.60[b]
July	357.46[a]	813.37[a]	37.34[ab]
September	347.58[a]	799.19[a]	45.88[a]
November	225.26[c]	656.84[b]	28.56[c]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan. Alphanumeric indicates significance differences between treatments

Table 3: Mean comparison of region effect on soil enzymes activities

Region	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
UD	365.39[a]	846.89[a]	44.50[a]
DI	245.10[b]	623.87[b]	28.18[b]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan; UD: Undisturbed region; DI: Disturbed region. Alphanumeric indicates significance differences between treatments

Table 4: Mean comparison of interaction between season and region on soil enzymes activities

Month region	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
May×UD	352.87[b]	802.15[ab]	40.18[b]
May×DI	228.48[e]	542.12[d]	27.02[d]
July×UD	418.72[a]	934.72[a]	44.21[b]
July×DI	296.19[c]	692.02[c]	30.46[c]
Sep×UD	426.99[a]	888.26[a]	58.30[a]
Sep×DI	268.18[d]	710.12[c]	33.45[c]
Nov×UD	262.96[d]	762.44[b]	35.31[bc]
Nov×DI	187.56[f]	551.23[d]	21.80[e]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan; UD: Undisturbed region; DI: Disturbed region. Alphanumeric indicates significance differences between treatments

Table 5: Mean comparison of depth effect on soil enzymes activities

Depth (cm)	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
0-10	275.51[a]	701.49[a]	43.29[a]
10-20	209.08[b]	701.54[a]	13.82[b]
20-30	175.00[c]	669.02[b]	4.92[c]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan. Alphanumeric indicates significance differences between treatments

Table 6: Mean comparison of region effect on enzymes activities in different depths of soil

Region	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
UD	242.89[a]	677.60[b]	24.56[a]
DI	196.84[b]	703.76[a]	16.79[b]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan; UD: Undisturbed region; DI: Disturbed region. Alphanumeric indicates significance differences between treatments

Table 7: Mean comparison of interaction between season and region on soil enzymes activities

Month region	Acid phosphatase (μg ρNP g^{-1} soil h^{-1})	Alkaline phosphatase (μg ρNP g^{-1} soil h^{-1})	Dehydrogenase (μg TPF g^{-1} soil h^{-1})
$A_1 \times$UD	346.60[a]	850.71[a]	57.15[a]
$A_1 \times$DI	204.43[b]	552.26[d]	29.43[b]
$A_2 \times$UD	202.74[b]	674.68[c]	13.47[c]
$A_2 \times$DI	215.41[b]	728.39[b]	14.17[c]
$A_3 \times$UD	179.33[c]	507.42[d]	3.07[e]
$A_3 \times$DI	170.68[c]	830.62[a]	6.76[d]

ρNP: ρ-nitrophenol; TPF: Triphenyl formazan; UD: Undisturbed region; DI: Disturbed region. A_1, A_2 and A_3: Soil depths of 0-10, 10-20 and 20-30 cm, respectively. Alphanumeric indicates significance differences between treatments

was 296.2 μg ρ-nitrophenol g^{-1} soil h^{-1} in July and the lowest one was 187.6 μg ρ-nitrophenol g^{-1} soil h^{-1} in November.

Phosphatase activities as affected by soil depth: At undisturbed site, acid phosphatase activity was greater at 0-10 cm depth 346.6 μg ρ-nitrophenol g^{-1} soil h^{-1} compared to 202.7 μg ρ-nitrophenol g^{-1} soil h^{-1} at 10-20 and 179.3 μg ρ-nitrophenol g^{-1} soil h^{-1} at 20-30 (Table 5, 7). At disturbed site, acid phosphatase activity did not vary with soil depths. Acid phosphatase activity was significantly different between undisturbed and disturbed sites at 0-10 and at 20-30 cm.

Alkaline phosphatase activity remarkably decreased with depth in soil profile from 850.7 to 507.4 μg ρ-nitrophenol g^{-1} soil h^{-1} at 0-10 and 20-30 cm depths. But at the disturbed site, this enzyme activity increased with depth in soil profile from 552.3 to 830.6 μg ρ-nitrophenol g^{-1} soil h^{-1} at 0-10 and 20-30 cm depths, respectively. In 10-20 cm depth, alkaline phosphatase activity was not significantly different between undisturbed and disturbed sites.

Dehydrogenase activities as affected by seasonal variation: Dehydrogenase activity were significantly different (p<0.01) in both undisturbed and disturbed sites throughout all sampling times (Table 6). Dehydrogenase activity ranged from 21.8 at disturbed site to 58.3 μg triphenyl formazan g^{-1} soil h^{-1} at undisturbed site. Same

as acid and alkaline phosphatase, dehydrogenase followed a similar seasonal pattern at undisturbed and disturbed sites. Activities increased a few from fall to spring, continued increasing through spring and early summer, reached to the maximum activity in summer and then dropped again in fall.

The highest activity was 58.3 μg triphenyl formazan g^{-1} soil h^{-1} in September that were significantly different (p<0.01) with other sampling times. The lowest one was 35.31 μg triphenyl formazan g^{-1} soil h^{-1} in November at the undisturbed site. At the disturbed site the highest activity was 33.46 μg triphenyl formazan g^{-1} soil h^{-1} in September and the lowest was 21.8 μg triphenyl formazan g^{-1} soil h^{-1} in November. In this site dehydrogenase activity in November was significantly (p<0.01) lower than September.

Dehydrogenase activities as affected by soil depth: Dehydrogenase activity remarkably (p<0.01) decreased with depth increment from 57.15 to 13.47 and to 3.06 μg triphenyl formazan g^{-1} soil h^{-1} at 0-10, 10-20 and 20-30 cm, respectively (Table 5). This trend was also observed in the disturbed site. The enzyme activity decreased with depth in soil profile from 29.43 to 14.18 and to 6.76 μg triphenyl formazan g^{-1} soil h^{-1} at 0-10, 10-20 and 20-30 cm depths, respectively. Dehydrogenase activity at 10-20 and 20-30 cm depths at the disturbed site was more than the undisturbed site. This difference was significant (p<0.01) at the 20-30 cm depth (Table 7).

DISCUSSION

In our study, the undisturbed site represented the undisturbed system with the higher levels of microbial biomass C and total C content when compared to the disturbed site. Similarly, other studies have found higher microbial biomass in undisturbed systems like native pasture in compared to touched ones like the agricultural systems (Acosta-Martínez *et al.*, 2004). The undisturbed site showed higher total C content and microbial biomass than disturbed site most likely due to positive impact surface cover, vegetation, on soil properties (Acosta-Martínez *et al.*, 2004) and due to differences in their root systems and root exudates of different systems (Fang *et al.*, 2001).

Enzyme activities under undisturbed and disturbed sites:
In this study, the activities of acid phosphatase, alkaline phosphatase and dehydrogenase were higher at undisturbed forest sites compared to the disturbed forest sites due to the higher organic C content in the undisturbed forest sites. In addition, microbial biomass which is the principal source of enzymes in soils was greater at undisturbed site than disturbed forest soils (Acosta-Martínez *et al.*, 2007). Previously, it has been reported that greater microbial biomass correlates with organic carbon matter content (Moore *et al.*, 2000). Generally, enzyme activities are correlated to soil organic matter content because the latter plays a key role as a precursor for enzyme synthesis and in enzyme physical stabilization (Tabatabai, 1994).

During the time of this study, alkaline phosphatase activities were up to 2.1 to 2.93 times greater than acid phosphatase at both studied sites. The observed differences are related to different origin of these enzymes in soils. For example plant roots are major source of acid phosphatase in soil (Speir and Cowling, 1991; Dinkelaker and Marschner, 1992) while soil microorganisms (including bacteria, fungi and fauna) are major source of alkaline phosphatase in soil (Findenegg and Neiemans, 1993; Tarafdar, 1995). The higher activity of alkaline phosphatase activity of undisturbed site may be related to the higher microbial biomass (Table 1). Furthermore, alkaline phosphatase generally exceeds acid phosphatase activity in high pH soils (Eivazi and Tabatabai, 1977). Therefore, soil microbes appear to be the important producers of phosphatase in these oak forest soils.

Enzyme activities as affected by seasonal variation:
Alkaline and acid phosphatase activity showed a seasonal pattern with maxima in summer and minimum in autumn, suggesting that temperature might be one of the major controlling factors for these enzymes activities. Similar results were reported to find maximum microbial population and enzyme activities in the summer (Tiwari *et al.*, 1989; Rastin *et al.*, 1988; Kaiser and Heinemeyer, 1993). But Boerner *et al.* (2005) did not observe seasonal effects on the activities of all of enzymes whose activity they measured such as acid phosphatase. Dick *et al.* (1988) observed little seasonality in acid phosphatase or b-glucosidase activity in North American agricultural fields. The differences in seasonal variation among the enzyme activities studied in this forest are due to their different origin. For example, dehydrogenase and alkaline phosphatase activities were generally higher in September compared to acid phosphatase activity, which was generally higher during July. These findings may demonstrate the beneficial effects of higher soil moisture and lower soil temperatures during September by the microbial populations, which are more directly, correlated to dehydrogenase and alkaline phosphatase activities than acid phosphatase. In the other hand, acid phosphatase, which is thought to be more extracellular in nature, could have retained it activity due to the organic matter protection during July.

Enzyme activities as affected by soil depth: The decreases observed in alkaline phosphatase, acid phosphatase and dehydrogenase activities with soil depth at the undisturbed sites may be related to the higher abundance of soil microorganisms and organic matter content at shallower soil layers of non-disturbed sites. Especially, forests soil surface tend to have high organic matter content compared to other systems such as agricultural and pasture soils (Lavahun *et al.*, 1996). These results were in agreement with the literature data in forest and agriculture soils (Ekelund *et al.*, 2001; Aon *et al.*, 2001; Taylor *et al.*, 2002; Zaman *et al.*, 2002; Turner *et al.*, 2002; Baum *et al.*, 2003; Chen, 2003). At the disturbed forest site, acid phosphatase activity did not vary with soil depths while alkaline phosphatase activities increased with soil depth. The opposite trend found at the disturbed site may demonstrate an irregular distribution of soil microorganisms in disturbed regions compared to undisturbed regions.

CONCLUSION

Our study found differences in microbial biomass, organic matter, acid and alkaline phosphatase and dehydrogenase activities between undisturbed and disturbed forest soils under oak that may be related to the disturbing of forest areas influencing the soils quality

negatively. Thus, the significant reductions in soil enzyme activities under undisturbed area compared to disturbed areas should be taken in consideration as indicators of soil quality in the forest areas. Higher soil enzyme activities and microbial biomass were encouraged by the preserving management that may lead to increases in other soil quality parameters such as organic matter content, aggregation and soil water infiltration, soil sustainability and productivity and consequently soil and ecosystem functioning.

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