Seasonal variation of acid phosphatase and dehydrogenase activity in natural and artificial habitats of hazel

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Abstract

The hazels (Corylus avellana) are seen in north region of Iran and many stands grow in upper area of northern slop of Alborz Mountains. Soil is an effective living factor in ecosystem balance. There are many biological and biochemical process in soil which rapidly reacts to environmental stresses. Enzymes have essential roles in these processes. The aim of this research was to study the activities of acid phosphatase and dehydrogenase in two natural hazel habitats (Makesh and Fandoghlo) and compare to their activities in an artificial habitat (Alborz). Soil sampling was done in spring and summer. The activity of acid phosphatase and dehydrogenase was evaluated by enzyme-substrate reaction. Enzymes had more activity in summer in all studied habitats. The more activity of acid phosphatase can be related to growth of hazel roots and secretion of enzyme during growth season. Enzymes showed more activity in Fandoghlo habitat in compared to Makesh ones. The amount of nitrogen, phosphorus and organic matter was less in Fandoghlo habitat which causes more activity of microorganisms to supply plants needs and compensation of low nutrients in soil.

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Introduction

Most of natural ecosystems have been damaged during the last decade because of over harvesting, industrial development and agricultural activities. There are some regional and international programs to protect natural ecosystems and rehabilitation of damaged areas. Understanding basic information on different aspects of natural ecosystems as soil quality and function is necessary to succeed in these programs. It is difficult to monitor the long term effects of anthropogenic activities on forest soil. Forest soils are complicated in point of biological, chemical and biological and there are a lot of challenges in determination of soil quality parameters (Staddon et al., 1998).

Measurements of soil enzyme activity have been used extensively for assessment of different process occurring in nutrient cycles in soils (Tabatabai & Dick, 2002). The activity of more than 100 enzymes has been determined in soil (Tabatabai & Dick, 2000). Soil enzyme activities are sensitive to deterioration effects of human and environment and their activity can be used as a tool to assess soil response to management practices and environmental stresses (Dick et al., 1988), since they are highly sensitive to external factors. Measurement of soil enzymatic activity in comparison with other soil properties is cheaper and easier. Soil enzymes have microbial and plant origin and their activity show the activity of intracellular enzymes, extracellular and bound enzymes to clay and organic matters. It is improved that these activities are important to determine soil quality under different usages, anthropogenic and non-anthropogenic destruction and different types of habitats (Waldrop, 2000; Grierson & Adams, 2000; Sinsabaugh et al., 2002; Caldwell et al., 1999; Ajwaet al., 1999).

Phosphatases are key enzymes in phosphorus cycle and their activity is a suitable indicator to determine organic phosphorus mineralization potential and soil biological activity (Tabatabai & Dick, 2002). Dehydrogenase are only found in alive microbial cell and used as a microbial activity indicator (Dick, 1994). Dehydrogenase activity is a good indicator to determine microbial metabolism in soil (Tabatabai, 1994).

Several studies have been done about use of soil to evaluate soil biological potential in Iran. Shirnany (2004) reported decreasing of enzyme activity with increasing soil depth in healthy ecosystem of Ulmusglabra but the pattern was opposite in unhealthy ecosystem of Ulmusglabra. The enzyme activity was studied in touched and untouched habitats of oak in Iran by Matinizadeh et al., 2008. They reported the more enzyme activity in untouched habitats of oak. Moraghebi et al., 2011 showed more acid and alkaline phosphatase activity in summer in compared to spring in hazel habitas. Their results did not show significant difference between natural and artificial habitats of hazel.

Ten years ago hazel seeds was sampled from two natural habitats and sown in Alborz research center known as an artificial habitat. The aim of this study was to measure acid phosphatase and dehydrogenase activity in two natural habitats of hazel and compare to artificial ones.

Materials and methods

Site description

The study was conducted on two natural and one artificial hazel habitats.

The Fandoghlo habitat
This natural habitat locates in 45 km distance from Ardabil, Iran. The habitat is next to Fandoghlo village and its height from sea level is 1450. Its climate according to Dumbarton is very humid and extremely cold. There are 3 dry months according to rain-temperature curve. The texture of soil is loam-silt with 6.8 acidity.

Makesh habitat
This natural habitat has 35 km distance from Talesh, Iran. Its height is 1400 to 1500 m above sea level. Its climate according to Dumbarton is very humid and extremely cold and there is no dry season. The soil
has mainly loam texture loam-silt with pH 6.9.

Alborz habitat

The hazel seeds were collected about 8 years from mentioned natural habitats and planted in Alborz Research Center. This research center with 80 ha area locates in 15 km far from of Karaj, Iran. The mean of annual rain, minimum and maximum annual temperatures are 25 mm, 21.7 and 41°C, respectively, and its climate is semi dry. The soil has mainly loam texture loam and loam-clay silt with pH 8.2.

Soil sampling and analysis

Soil samples were taken randomly from. Soil samples were collected in spring (May) and summer (September). Samples were placed in tightly sealed plastic bags and transferred immediately to the laboratory at 4°C. The soil samples were passed through a 2 mm sieve and divided into two fractions: one fraction for the determination of physical and chemical factors, which were kept at room temperature and the other fraction for measuring of soil enzymes activities which was stored at 4°C.

Chemical properties determination

Chemical analyses were done on air-dried and sieved (2 mm) soil samples. Soil pH and EC was measured with a glass electrode in 1:2.5 soil/water suspension. Total soil N was determined by Kjeldahl digestion (Bremmer&Mulvaney, 1982), and the organic-C was resolute by dichromate digestion (Walkley & Black, 1934). Olsen’s bicarbonate extractable P (PO₄³⁻) was also measured (Olsen et al., 1954).

Enzyme activities

Acid phosphatase activity

The activity of acid phosphatase (EC 3.1.3.2) was determined based on the method of (Ohlinger, 1996). The reaction mixtures consisted of 1.0 g soil, 1.0 ml PNP (disodium ρ-nitrophenyl phosphate 0.115 MM) and 2.0 ml MA buffer (maleate buffer 0.1 M, pH 6.5). The reaction mixtures were incubated at 37°C for 1 h. After incubation, the reaction was stopped by adding 1.0 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH. Concentration of p-nitrophenol (NP) produced in the assays of phosphotase activities was calculated from a p-NP calibration curve after subtracting the absorbance of the control at 400 nm wavelength using a UV-VIS spectrophotometer Bausch &Lambd (spectronic 21). Two analytical replicates and one control were analyzed for each soil sample. Soil moisture content was determined from the loss in weight after drying at 105°C for 24 h. Enzymes activities are expressed as microgram (µg) ρ-nitrophenol per gram (g) soil per hour at 37°C.

Dehydrogenase assay

By using enzyme- substrate reaction, dehydrogenase changes its buffered substrate triphenyl tetrazolium chloride to triphenyl formazan at 25 ºC. This product is extracted by acetone and its absorbance is measured at 546 nm by a spectrophotometer (Ohlinger, 1996b). The enzyme activity expressed as µg triphenyl formazan g⁻¹ h⁻¹ at 25 ºC.

Statistical analysis

All data analyzed by T-test.

Results

Table 1 shows the several chemical properties of soil. As shown in Table 1, the amount of available P was 56.8, 23.21 and 32.41 in Makesh,Fandoghlo and Alborz habitats, respectively. The nitrogen percentage was 0.43 and 0.17 and 0.25 in Makesh,Fandoghlo and Alborz habitats, respectively.

Variation of acid phosphatase activity in spring and summer

The acid phosphatase activity was measures in spring and summer in all studied habitats. It is evident from Figures 1 that acid phosphatase activity changed with sampling time in all studied habitats. Acid phosphatase activity ranged from 112.24 (±8.23) µg ρ-nitrophenol g⁻¹ h⁻¹ in spring to 295.54 (±15.64) µg ρ-nitrophenol g⁻¹ h⁻¹ in summer in Makesh habitat (Figures 1). This enzyme activity varied from 288.53(±18.79) µg ρ-nitrophenol g⁻¹ h⁻¹ in spring to 678.02 (±29.52) µg ρ-nitrophenol g⁻¹ h⁻¹ in summer in Fandoghlo.Acid phosphatase activity ranged from 268.40 (±29.52) µg ρ-nitrophenol g⁻¹ h⁻¹ in spring to
620.23 (±21.73) μg p-nitrophenol g⁻¹ h⁻¹ in summer in Alborz habitat (Figures 1). Acid phosphatase activity was more in summer samples in all studied habitats compared to spring samples. In addition, acid phosphatase activity was more than two times higher in Fandoghlo samples in compared to Makesh ones in both seasons. The acid phosphatase activity in spring and summer in Makesh habitat was less and showed significant difference (p<1%) with two other habitats (table 2). There was no significant difference between Fandoghlo and Alborz acid phosphatase activity in spring but difference was significant (p<5%) in summer samples activity (table 2).

Table 1. Some chemical characters of soil in studied sites.

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>Organic matter %</th>
<th>K (ppm)</th>
<th>N%</th>
<th>Ca</th>
<th>CEC (ppm)</th>
<th>P (ppm)</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malesh</td>
<td>3.9</td>
<td>6.7</td>
<td>989</td>
<td>0.43</td>
<td>26.4</td>
<td>270</td>
<td>56.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Fandoghlo</td>
<td>3.71</td>
<td>4.64</td>
<td>279.5</td>
<td>0.17</td>
<td>30.4</td>
<td>300</td>
<td>23.21</td>
<td>18.4</td>
</tr>
<tr>
<td>Alborz</td>
<td>3.65</td>
<td>5.2</td>
<td>340</td>
<td>0.25</td>
<td>32.5</td>
<td>290</td>
<td>32.42</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Variation of Dehydrogenase activity in spring and summer
Dehydrogenase activity changed with sampling time in all studied habitats. Its activity ranged from 251.15 (±16.28) μg TPF g⁻¹ h⁻¹ in spring to 351.72 (±11.53) μg TPF g⁻¹ h⁻¹ in summer in Makesh habitat (Figures 2). This enzyme activity varied from 434.32 (±32.29) μg TPF g⁻¹ h⁻¹ in spring to 502.36 (±21.38) μg TPF g⁻¹ h⁻¹ in summer in Fandoghlo habitat (Figures 2). Dehydrogenase activity was more in Fandoghlo in both seasons in compared to Makesh habitat. The dehydrogenase activity in Alborz was very similar to Fandoghlo ones. There was a significant difference (p<5%) between Makesh and Fandoghlo habitats in dehydrogenase activity (table 3) but among them difference (p<1%). There was no significant difference in dehydrogenase activity between Fandoghlo and Alborz habitats in both seasons (table 3).

Table 2. Statistical comparison of acid phosphatase activity among habitats.

<table>
<thead>
<tr>
<th></th>
<th>Fandoghlo-spring</th>
<th>Alborz-spring</th>
<th>Makesh –summer</th>
<th>Fandoghlo-summer</th>
<th>Fandoghlo- Alborz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makesh –spring</td>
<td>**</td>
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<tr>
<td>Fandoghlo- spring</td>
<td>-</td>
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<tr>
<td>Alborz- spring</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>**</td>
</tr>
<tr>
<td>Makesh –summer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Fandoghlo- summer</td>
<td>**</td>
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</tr>
</tbody>
</table>

In all studied habitats both enzymes had more activity in summer in compared to spring (figures 1, 2 and Table 2 and 3).

Discussion
Assessing the long term effects of human activities on forest soil is difficult. Forest soils are complicated as regards the physical, chemical and biological point of view and there are a lot of challenges in determination of soil quality parameters (Staddon et al., 1998). Monitoring of these effects by use of trees growth or soil organic matter is time consuming and can’t be suitable indicators (Dick, 1994; Turco et al., 1994).

Biochemical and biological properties of soil enzyme

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activity, changing in response to environmental stresses, can be used to assess the soil potential and monitor the effects of anthropogenic activities or environmental stresses (Klein et al., 1985). Soil enzyme activities are sensitive to deterioration effects of human and environment and measurement their activity can be used as a valid tool to assessment of soil metabolically response to management practices, climate changes and environmental stresses and bring valuable information on nutrient cycles (Tabatabai & Dick, 2002; Sinsabaugh et al., 2002; Kandeler, 2007).

Table 3. Statistical comparison of dehydrogenase activity among habitats.

<table>
<thead>
<tr>
<th></th>
<th>Fandoghlo- spring</th>
<th>Alborz- spring</th>
<th>Makesh –spring</th>
<th>Fandoghlo- summer</th>
<th>Alborz- summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makesh –spring</td>
<td>**</td>
<td>*</td>
<td>ns</td>
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<tr>
<td>Fandoghlo- spring</td>
<td>-</td>
<td>ns</td>
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<td>*</td>
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<tr>
<td>Alborz- spring</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>**</td>
<td></td>
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<tr>
<td>Makesh –summer</td>
<td>**</td>
<td>-</td>
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<td></td>
<td>**</td>
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<tr>
<td>Fandoghlo- summer</td>
<td>*</td>
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<td>ns</td>
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</tbody>
</table>

Fig. 1. Acid phosphatase activity in Fandoghlo, Makesh and Alborz habitats in spring and summer.

The effect of season on acid phosphatase activity
The key to understanding seasonality in enzyme activity may be in the factors that regulate various enzyme systems. Some of soil enzymes are regulated primarily by microclimate and soil chemical factors, whereas other enzymes are more regulated by substrate availability (Sinsabaugh et al., 2002). Acid phosphatase are produced mainly by plants root although microbial community produce but in less extent (Tabatabai, 1994). The acid phosphatase activity in Fandoghlo habitat in both seasons was higher significantly than in Makesh. The more density of plants in Fandoghlo causes better distribution of roots. Although the phosphorus content was less in Fandoghlo but microorganisms have better growth and activity and balanced situation in temperature and humidity resulting in more activity of microorganisms which are source of these enzymes. There is a direct relationship between extracellular enzyme activity and plant cover as with increasing of plant cover enzyme activity increase (Amiaud&Benizi, 2005; Bastidaet al., 2006).

Then the more activity of this enzyme in summer can be related to more分泌ary activity of root in summer during their growth season that is in agreement with Kaiser&Heinemeyer (1993) finding. In addition enzyme activity was almost same that in conformity with Moraghebi et al., (2010) finding.

Fig. 2. Dehydrogenase activity in Fandoghlo, Makesh and Alborzin spring and summer.

The effect of season on dehydrogenase activity
Dehydrogenase is only produced by alive cells (Dick, 1994) and is a good indicator of microbial metabolism in soil (Tabatabai, 1982).

The enzyme activity in Fandoghlo and Alborz habitat in both seasons was higher than in Makesh. The more density of plants in these habitats causes more balanced situation in temperature and humidity.
resulting in more activity of microorganisms which are source of these enzymes. Although organic matter and carbon content in Fandoghlo is less than two other habitats but according to dehydrogenase activity, microorganisms have more activity which has conformity with Kramer & Green, (2000), Sedia & Ehrenfeld, (2006) and Moraghebi et al.,(2012) findings.

Summer samples had more activity in all studied habitats that shows suitable moderation in temperature and humidity. These conditions are suitable for microorganisms and increase their activity and consequently soil enzyme activity. Kaiser & Heinemeyer(1993)reported increase activity of soil enzyme activity in the end of summer.

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References


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