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Research Paper

Effects of rhizosphere competition on acid phosphatase exudation in pure and mixed planting of Oak and Maple

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Abstract: Root competition is one of the most important factors on the seedlings establishment. Plants have developed their root systems in order to get better water and nutrients. The chemicals ingredients of the root exudates include enzymes are specific to a particular plant species and also effect on mineralization process and nutrients availability. This paper studied acid phosphatase of root exudation in two different species (Oak and Maple) with pure and mixed planting in two seasons (summer and autumn 2015). Seedlings were planted in portable-rhizotron system for monitoring root development and exudations. Results revealed that acid phosphatase of root exudation was significantly difference between species composition and also in two season($p<0.05$) while was more than in autumn. Effects of species and soil sampling depth were significantly difference in autumn although there wasn't significant difference in summer and distance to collar seedlings.

Keywords: Root competition, Acid phosphatase, Rhizosphere, Rhizotron, Oak, Maple

Introduction

Competition between plants is for both aboveground (light) and belowground (water and nutrients) resources (Song et al. 2006). The recently studies revealed that belowground competition was occurred earlier than aboveground competition (Lei et al. 2012) so study of belowground competition is more important and priority matter in for establishment of plant species. Rhizotron is an advanced tool for studying root competition and rhizosphere contents. The investigation of root systems by using rhizotron started for grasses, pasture and swampy plants (Blosfeld et al. 2011;Moorberg et al. 2013) and recently for studying forest trees, seedlings and saplings (Beyer 2012; Fender et al. 2013; Cesarz et al. 2013).

Rhizodeposition is defined as the total output of organic carbon compounds from the plant roots into the soil. Rhizodeposition have a key role in acquiring nutrients in rhizosphere and plant growth (Grayston et al. 1996).Root exudations are the most important compositions to drive microbial activities and rhizosphere is a hot spot for them. The most important effects on rhizosphere as a part of soil had exerted by plant root systems (Ziegler et al. 2013).Roots could directly affect decomposition of soil organic matter by there lease of the extra-cellular enzymes such as phosphatase and cellulose (Oburger et al. 2013).

Tree species effect on soil mineralization process that regulate nutrients cycle in forest ecosystem. This may cause by their effects on substrate quality, physico-chemical soil properties and microbial composition via affecting on enzyme activities (Ushio et al. 2010). Root competition mostly studied in pure stands (Schmid 2002) but mixed planting have more benefits and close to nature.

Soil enzymes are catalysts of organic matter depolymerisation, which is of critical importance for ecosystem carbon (C) cycling. Soil enzymes have essential role in nutrient mineralization. Enzyme activity is a key driver's nutrient stock for plants. Extracellular enzymes are index for quality of soil biology (Kotroczo' et al. 2014).

Phosphatases are groups of enzymes that hydrolyzed phosphomonoesterases releasing phosphate (Hendriksen et al. 2016). Many of phosphatase enzymes are extracellular, acid phosphatase (AcPhos) produce and hide within soil by plants and micro-organisms (Dick and Tabatabai 1992). Phosphatase activity is closely correlated with available phosphorus (Eivazi and Tabatabai 1997). Phosphatases are mediators for phosphorus mineralization that is a critical process in phosphorus biogeochemical cycle and determine ability of accessible soil phosphorus in forest ecosystems (Hou et al. 2015).

Phosphatase activity is affected by the many factors in forest soil such as pH(Herold et al. 2014;Hendriksen et al. 2016), soil humidity (Baldrian et al. 2010; Ushio et al. 2010; Hendriksen et al. 2016), organic carbon (Ushio et al. 2010; Herold et al. 2014;Hendriksen et al. 2016), total nitrogen (Ushio et al. 2010; Hendriksen et al. 2016), C/N ratio (Ushio et al. 2010; Hou et al. 2015), C/P and N/P ratios (Hou et al. 2015),amount of Al and Fe in mineral soil (Herold et al. 2014), plant species (root exudates, rhizosphere, litter) (Ushio et al. 2010; Adamczyk et al. 2014; Kotroczó et al. 2014; Hou et al. 2015), season (Baldrian et al. 2010; Kotroczó et al. 2014), soil depth (Herold et al. 2014), soil horizons (Herold et al. 2014; Hou et al. 2015), climate (Hendriksen et al. 2016), parent material (Herold et al. 2014), soil texture (Herold et al. 2014) and forest management (Herold et al. 2014). In this paper we studied AcPhos activity between two forest species include maple *(Acer velutinum)* and oak *(Quercus castaneifolia)* in two seasons. We applied a new rhizotron system designed similar to double- split- root rhizotron introduced by Fender et al. 2013 and Cesarz et al. 2013.

Martial mad Methods

Soil and litter was collected from oak-maple stand, Shastkalate forest (36º 41'N -36º 45' N, 54º 20' E – 54º 24' E) about 250m altitude from sea level. The soil texture was loam with composed of 39%sand, 36% silt, and 25%clay. Seedlings (*Quercus castaneifolia* and *Acer velutinum*) had respectively collar diameter 12.01±0.32 and 10.38±0.14 mm (means ± 1 SE) and mean height 55.23±0.83 and 35.07±0.29mm(means ± 1 SE), selected from Ghorogh nursery (about 120 m a.s.l). The collected fresh soil was mixed, homogenized then gravel, pebble and roots were separated by sieving with a mesh size of 10 mm. Rhizotrons was filled with mentioned soil in and litter then seedlings were planted in January 2015. During the test, rhizotrons were irrigated with regard to maintaining humidity in field capacity level.

Rhizotron was designed based on double- split- root rhizotron (from Cesarzet al. 2013 and Fender et al. 2013) with some changes. Rhizotron was placed in the 30º angle caused to growing roots on the back side. The 12 raster access ports (RAPs) were embedded in each rhizotron. The schematic design of the rhizotron was shown in figure1. Rhizotron was filled with mentioned soil and 4% litters in 2 shallow RAP depth and with mineral soil in 2 deep RAP depth.

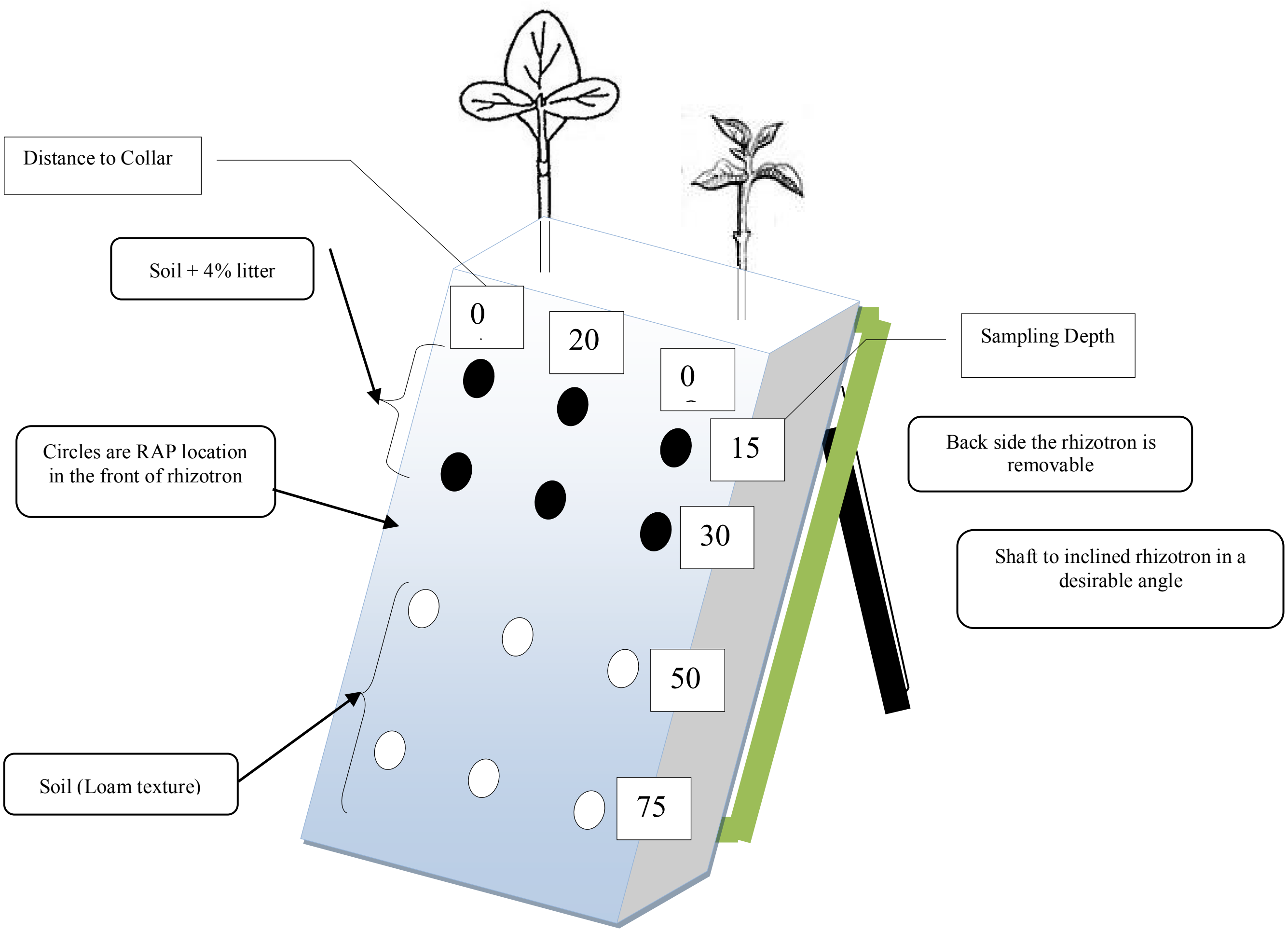

Fig1- schematic design of rhizotron

A factorial experiment design was setup with three different species composition (oak, maple and mixed) through eighteen rhizotrons. The experiment factors were consisted of species, soil (RAP) depth and distance to collar. Soil adjacent root seedlings were sampling in summer and autumn 2015. Samples immediately conserved in -20 ºCand then AcPhos activity determined with colorimetric method by spectrophotometer according to Tabatabai and Bremner (1969); Eivazi and Tabatabai(1977) in Margesin (1996) modification. Soil moisture was determined by weighting method.

Statistical analysis

All the analyses were done with SPSS16 for windows. Data normality distribution was tested by the Shapiro-wilks and homogeneity of variances by the leven's test. Multivariate analyses were employed to test the effects of species composition, vertical and horizontal distance from collar seedling and season on AcPhos root exudations. Analysis of contrast was used to compare means between soil humidity in two seasons. Pearson coefficient was characterized correlation between soil moisture and AcPhos activity.

Results

Acid phosphatase (AcPhos) activity was significantly differences between species and seasons ($P<0.05$)(Table 1) while was more in autumn than summer. The most amount of the AcPhos activity were maximized in mixed rhizotron while in the order of oak > maple > control respectively. There are significant differences in AcPhos activity between summer and autumn whereas the most amount of AcPhos activity was in autumn. On average, AcPhos activity was 14% higher in autumn (table 1).

Table 1.Effect of species composition and season on AcPhosactivity (ugpNp h-1)

Species composition	Summer	Autumn
Control	1183.60Bb	1389.69Ac
Mixed	2139.47Ba	2370.37Aa
Pure maple	1666.15Ba	1861.78Ab
Pure oak	1848.30Ba	2161.62Aab

Different small letters showed significantly difference (p<0.05) between species composition while different big letters showed differences between seasons.

AcPhos activity between soil depth and distances to collar were not significantly differences (P >0.05) in summer (Table2). Although the most amount of AcPhos activity was in intermediate between two collar seedlings and 1st (15cm depth) shallow RAP depth. Enzyme activity decreased with increasing soil depth so AcPhos activity was 1947.98 in the average of first RAP depth and 1592.73, 1467.91 and 1560.21 in next RAP depth respectively. On average, AcPhos activity was 17% higher in 20 cm distance to collar seedlings compared with adjacent collar seedlings (table 2).

Table 2.Effect of soil rhizoton depth and distances to collar seedling on AcPhos activity (ugpNp h-1) on summer

Species composition	Soil depth (cm)				Distance to Collar seedlings(cm)		
	15	30	50	75	0A	20	0Q
control	1235.34a	1220.47a	1140.26a	1153.41a	1223.82a	1205.82a	1127.16a
mixed	2649.20a	1817.20a	1704.73a	1561.82a	2219.09a	2087.27a	2059.33a
Pure maple	1947.09a	1534.42a	1558.74a	1739.85a	1343.92*	1669.42*	1697.06*
Pure oak	1960.29a	1798.85a	**-------	1785.76a	1658.36a	2942.21a	1791.20a

* Comparison of means was not performed because at least one group has fewer than three observations.
** There was not sample. Same small letters shows there are not significantly differences between columns.

AcPhos activity between soil depth and distances to collar were significantly differences (P >0.05) in autumn. The most amount of AcPhos activity was in shallow RAP depth whereas 2.6% was more than deep RAP depth. On average, AcPhos activity was 21.2% higher in adjacent collar seedlings compared with the intermediate between two seedlings planted (table 3).

Table 3.Effect of soil rhizoton depth and distances to collar seedling on AcPhos activity (ugpNp h-1) on autumn

Species composition	Sampling depth (cm)				Distance from Collar seedlings (cm)		
	15	30	50	75	0A	20	0Q
control	1525.27*	1108.69*	1322.96*	1459.69*	1534.46a	1195.60a	1326.05a
mixed	1843.30b	2948.59a	2250.79ab	2205.16ab	2524.07a	2079.54a	2614.51a
Pure maple	2017.97a	1669.17a	1892.11a	1911.86a	1976.06a	1426.32b	2320.89a
Pure oak	2338.37a	2615.38a	1615.98a	2019.94a	2550.80a	1932.94a	1989.75a

* Comparison of mean was not performed because at least one group has fewer than three observations. Different small letters shows significantly differences between columns.

Soil moisture between rhizotrons werenot significantly differences while was significantly more in summer than autumn (P<0.05),although there was not significantly correlation between soil moisture and AcPhos activity in summer and autumn (Table 4).

Table 4.Soil humidity characteristics and correlation coefficient between soil moisture with AcPhos activity

Season	Soil moisture percent	Pearsoncoefficient	Stdv	CV	Minimum	Maximum	N
Summer	44.02	0.144ns	5.225	0.1187	30%	58%	54
Autumn	41.11	0.217ns	5.9986	0.14560	29%	59%	54

ns there are not significant correlation between soil humidity with AcPhos activity

Conclusion

The amount of AcPhos was significantly difference between species composition that showed effect of tree species on root competition and exudation. The last studies also revealed that enzyme activity was differentin different tree species (Ushio et al. 2010; Adamczyk et al. 2014; Herold et al. 2014). The most AcPhos activity was in mixed rhizotron (Oak -Maple seedling).We conclude Oak-Maple seedlings have synergism relation and no root competition existence in 6 month time after planting so mixed planting effect was greater than the sum of individual effects in pure plantation.

Our results showed that amount of AcPhos activity was significantly more in autumn(1945.86ugpNp h-1) than summer(1709.38ugpNp h-1).Other studies showed climate conditions have important effects on soil enzyme activity (Ushio et al. 2010;Kotroczó et al. 2014; Hendriksen et al. 2016). Baldrian et al (2010) andHou et al (2015) have expressed that more soil humidityeffect on more enzyme activity. In this study, rhizotrons humidity were controlled in field capacity level by investigators so more humidity in autumn was not because of season effects and also not significant differences created so we conclude more AcPhos activity in autumn because of seedling physiology.

Baldrian et al (2010) revealed that variability of AcPhos activity in different horizons (L and H) was because of the different humidity. They expressed that more humidity result in more microbial biomass (fungi and bacteria) is reason of more AcPhos activity. Their results showed that litter horizon had more humidity so more enzyme activity. This study showed any differences in soil humidity by depth and horizons but more enzyme activity was on shallow depth (soil+litter) so we suggested that litter as a substrate may increase AcPhos activity. Hendriksen et al (2016) expressed that organic matter is a substrate for microbial growth and there was a positive correlation between OM, extracellular enzyme activity and soil respiration. According to results from this study shallow depth with litter as an addition substrate associated with root exudation had more AcPhos activity than depth horizon but this wasn't significantly difference.

Herold et al (2014) showed enzyme activities such as AcPhose activity decreased with increasing depth in soil profiles on forest sites. They expressed that the most important factor for this actual is quality of OM – C:N ratio – and others are soil properties, microbial biomass, total N, OC. They suggests that lower level of microbial biomass associated with different quality and quantity of fresh carbon inputs are reasons for decreasing enzyme activity on deeper soil layers. Hou et al (2015) also revealed that Acid phosphomonoesterase and phosphodiesterase activity were more in L and F/H layer than mineral soil. This study couldn't show effects of soil properties on enzyme activity but reasons for reducing AcPhos activity by increasing depth could be litter additions on upper layer that influenced on quality and quantity substrates.

Adamczyk et al (2014) studied AcPhos and alpha-glugosidase activities under three tree species. They found that enzyme activity was similar on all species. They suggested that inorganic P had a negative response regulation under different tree species while there was no significant difference. Ushio et al (2010) unlike revealed that AcPhos and alpha-glugosidase activities were significantly different under tree species. They suggested that lower level of available P had result in more AcPhos activity. Adamczyk et al (2014) and Ushio et al (2010) showed that enzyme activity may effect on nutrient availability in forest soils. Their results showed that different litter quality is responsible for different microbial community and enzyme activity. They suggested that tree roots could be affected on enzyme activity by influence on substrate quality but their results couldn't show effect of the roots separately. Present study showed significant differences between tree species. More AcPhos activity on species composition than control could be expressed effect of the roots separately; therefore root exudation probably is a major force for increasing enzyme activity.

Kotroczó et al (2014) revealed that removal of roots had more significantly effects on decreasing AcPhos activity than other treatments (Double litter addition, Double wood addition, No litter, No inputs). These results expressed that roots had the most important effect on soil AcPhos activity. Results of Kotroczo et al (2014) confirmed present study because control treatment had the lowest amount of AcPhos activity and its difference with species compositions was important.

More AcPhos activity results more mineralization of phosphorus, therefore we suggest mixed planting (oak - maple) could prepared more availability of mineral P for seedlings, more positive effect for height increment of plants and suggest to afforestation.

Herold et al (2014) showed that there is less enzyme activity in A horizon (organic layer) with more organic carbon although they showed phosphatase activity decreased with increasing depth. They showed labile material inputs could be cause of the more enzyme activity.Because of the Al and/or Fe hydroxides adsorption ,more leaching from organic layer didn't necessarily cause to more resource for microbial activity.Our results showed there were not significant differences between enzyme activities in soil depths in summer.

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