Institute of Plant Nutrition and Soil Science

Bettina Eichler
Ewald Schnug
Maria Caus
Detloff Köppen

Soil acid and alkaline phosphatase activities in regulation to crop species and fungal treatment

Published in: Landbauforschung Völkenrode 54(2004)1:1-5

Braunschweig
Federal Agricultural Research Centre (FAL)
2004

Soil acid and alkaline phosphatase activities in regulation to crop species and fungal treatment

Bettina Eichler[1], Maria Caus[2], Ewald Schnug[3] and Detloff Köppen[1]

Abstract

Activities of soil acid and alkaline phosphatase (AcP and AlP) were evaluated in relation to various catch crop species and to fungal treatment (*Penicillium bilaii*) in a pot trial under greenhouse conditions. Without fungal treatment the highest activity of AcP was found in soils under serradella, amaranth and maize, by far the lowest for ryegrass. On an average the activity of AlP in the control was higher than in rhizosphere soil associated with the examined crops. With the use of *P. bilaii* the activity of both phosphatases generally decreased. This was especially true for phacelia. Similarly, the double lactate soluble P content in soil (P_{DL}) and pH values decreased under fungal treatment. But there was no correlation between the changes in P_{DL} content, pH value and activity of AcP and AlP. The pH value and P_{DL} content in soil decreased most in the treatments with ryegrass whereas under legume crops (lupin, pea and serradella) only a small decrease of P_{DL} was observed.

Keywords: catch crops, enzymes, Penicillium bilaii, phosphatases, phosphorous, rhizosphere

Zusammenfassung

Aktivitäten der sauren und alkalischen Phosphatase im Boden beim Anbau verschiedener Fruchtarten und in Abhängigkeit von einer Pilzbehandlung

In einem Gefäßversuch unter Gewächshausbedingungen wurden der Einfluss verschiedener Zwischenfrüchte und einer Pilz-Behandlung mit *Penicillium bilaii* auf die Aktivitäten saurer und alkalischer Phosphatase (AcP und AlP) im Boden untersucht. Ohne eine Pilzbehandlung wurde die höchste AcP-Aktivität unter Serradella, Mais und Amarant gefunden, die geringste unter Einjährigem Weidelgras. Im Durchschnitt war in den Kontrollen ohne Bewuchs die Phosphataseaktivität höher als im Rhizospärenboden der Gefäße mit Bewuchs. Durch den Einsatz des Pilzes kam es allgemein zu einer Verringerung der Phosphataseaktivität im Boden, am stärksten aber unter Phacelia. Die doppellactat-löslichen P-Gehalte im Boden (P_{DL}) sowie die pH-Werte verringerten sich bei Zufuhr des Pilzes, wobei sich jedoch kein gesicherter Zusammenhang zwischen diesen Veränderungen und der Aktivität der Phosphatasen feststellen ließ. Unter Weidelgras sanken die pH-Werte und die Gehalte an P_{DL} am stärksten, wohingegen unter Leguminosen (Lupine, Erbse und Serradella) im Vergleich zur Kontrolle keine Verringerungen auftraten.

Schlüsselwörter: Bodenenzyme, Penicillium bilaii, Phosphatasen, Phosphor, Rhizosphäre, Zwischenfrüchte

[1] Institute of Sustainable Plant Production, University of Rostock, J. v. Liebig-Weg 6, 18059 Rostock, Germany
[2] Institute of Plant Physiology, Moldovan Academy of Sciences, Padurii 22, MD-2015 Chisinau, Moldova
[3] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Center (FAL), Bundesallee 50, 38116 Braunschweig, Germany; e-mail: pb@fal.de

1 Introduction

Phosphorous (P) represents a major nutrient element after nitrogen in higher plants. As an essential macronutrient for plant growth and development, P is found largely as phosphate esthers, which contain pyrophosphate bounds in their molecular structure and posses a huge reserve of free hydrolyzing energy. P is implicated in processes of photosynthesis and respiration, biosynthesis of proteins and complex carbohydrates, and is a component part of nucleic acids, ATP and NADHP. The supply of plants with the necessary P amount for their normal growth and development, first of all, depends on the availability and solubility of P compounds in the soil. In soil, P exists in both organic and inorganic forms. Up to 90 % of the total soil P is found in the non-liable pool (Stevenson 1986) as a result of its immobilisation by soil organic and inorganic components. Besides this, and on a world wide scale, P represents the most often deficient nutrient in soil. A big part (20 % to 90 %) of total soil P exists as organic soil P (Dalal 1977). It is considered that almost only soluble inorganic soil P are available for plant uptake (Joner and Jakobsen 1995). Therefore the organic P compounds have to converted into inorganic P forms through the reactions, that are mediated among others by phosphatases. Two types of phosphatases are known, the acid phosphatase (AcP) and alkaline phosphatase (AlP), which occur in dependence of pH value of soils (Tabatabai and Bremner 1969, Eivazi and Tabatabai 1977). It has been shown that phosphatases are concentrated in the surface layer and rhizosphere where most of the fresh and less rotted organic matter is found (Asmar et al. 1995).

Since soil rhizosphere represents a complex of living communities, it is considered that soil AlP and AcP that are responsible for organic P transformation in soil, might be originating from extracellular and intracellular enzyme activities. AcP activity in soil originates from many sources, including plant roots (Dinkelaker and Marschner 1992), fungi (Tarafdar et al.1988), mycorrhizal fungi (Tarafdar and Marschner 1994) and bacteria (Tarafdar and Claassen 1988). AlP is produced by soil microorganisms and soil fauna, whereas higher plants are devoid of alkaline phosphatase (Tarafdar and Claassen 1988).

The activity of soil AlP and AcP that are responsible for hydrolysis of both esters and anhydrous H_3PO_4 of soil organic matter depends on various factors as soil type and its fertility, type of fertilisation and nutrient management, soil microbiological activity, organic matter, soil pH, soil moisture and varieties of higher plant species (Juma and Tabatabai 1978, Tarafdar and Jung 1987, Haussling and Marschner 1989, Albach et al. 1998, Wright and Reddy 2001, George et al. 2002, Parham et al. 2002).

Plants manifest different adaptive reactions to the impact of environment factors, thereby regulating their supply with necessary nutrients. Numerous data demonstrated that the soil activity of extracelullar AcP and AlP increase during plant growth under P deficient conditions (Tadano et al. 1993, Duff 1994, Li et al. 1997).

Different plants are known to manifest different nutrient requirements for their normal growth and development (Tarafdar and Jung 1987, Marschner 1995).

The objective of this study was to monitor soil AcP and AlP activities under different types of crop species, and fungal treatment as an approach to determine the contribution of various crops in soil organic P breakdown according to other soil living communities as microorganisms and fungi.

2 Material and methods

Several species of catch crops (table 1) were grown in pots containing 6 kg of a poor loamy sand low in plant available P. For the fungal treatment a water-dispersible granulate carrying *Penicillium bilaii* (Prophyta GmbH, Malchow, Germany) was employed. A suspension of the granulate was made with distilled water corresponding to 300 g *P. bilaii* WG 1000 kg^{-1} soil.

Table 1:
Cultivated catch crops in the pot trial (Species, Variety, and number of seeds per pot)

Catch Crops
Oilseed Rape (*Brassica napus*), Evita (10)
Buckwheat (*Fagopyrum esculentum*), Lifago (15)
Phacelia (*Phacelia tanacetifolia*), Lisette (20)
Common Ryegrass (*Lolium westerwoldicum)*, Liflora (50)
Serradella (*Ornithopus sativus*); Lippstädter Sorte, (20)
Maize (*Zea mays*), Florett (8)
Pea (*Pisum sativum*), Duell (10)
Lupin (*Lupinus albus*), Schwako (10)
Amaranth (*Amaranthus hypochondracus)*, Bärenkraft (10)
Control (without catch crops)

In the control pots with and without fungal treated soil, no plants were grown. At the end of the vegetation period, after the plants were harvested, the soil samples from each pot were collected and stored at –20° C. Because at this stage the entire pots were fully explored by the roots, this soil can be considered as rhizosphere soil. Prior to the enzyme assays, the soil samples were air-dried, and subsequently homogenized, ground and passed through a 2-mm sieve and subjected to AcP and AlP determinations.

AcP and AlP activities in soil samples were analyzed, using p-nitrophenol phosphate, a synthetic compound as substrate. Both enzymes hydrolyzed p-nitrophenol phosphate to p-nitrophenyl (p-NP) and inorganic phosphate. The yellow color intensity of p-NP was read spectrophotometrically at 400 nm for both AcP and AlP. A standard curve was made for calculation of the results. Statistical

evaluations were conducted by means of the DUNCAN test.

3 Results and discussion

It was observed that in the soil samples from the controls, without catch crops the activity of AcP was about 5 fold higher than that of AlP. But also in the treatments with vegetation where the soil is considered as rhizosphere soil, the activity of AcP was higher than the one of AlP (table 2).

According to studies from Pannikow and Minejew (1980) and Helal and Sauerbeck (1981) maize has the ability to utilise organic phosphates whereas the activity of phosphatase seems to be decisive. For maize this would be one possibility to satisfy a temporary very high P demand. Amann and Amberger (1989) and Tarafdar and Jung (1987) showed for buckwheat an increased activity of AcP under P deficiency and consequently a better use of organic P. Other studies demonstrated also that, especially under P deficiency the rhizosphere soil had higher amounts of phosphatases compared to non-rhizosphere

Table 2:
Activity of acid and alkaline phosphatase activity in soil (µg p-nitrophenyl g^{-1} soil h^{1}) in dependence of the cultivated crop and addition of the fungus Penicillium bilaii in a pot trial (6 kg of loamy sand pot^{-1} (low in plant available P)) after 7 weeks of vegetation time under greenhouse conditions

	Acid Phosphatase		**Alkaline Phosphatase**	
Crop	without *P. bilaii*	with *P. bilaii*	without *P. bilaii*	with *P. bilaii*
Oilseed rape	227.8 c	236.2 c	33.8 bc	33.7 cd
Maize	276.7 d	279.0 e	27.0 b	43.6 ef*
Phacelia	122.6 b	70.9 a *	50.6 e	23.1 b*
Buckwheat	262.1 d	204.8 b *	33.8 bc	51.2 g*
Common Ryegrass	73.0 a	77.8 a	6.8 a	15.2 a*
Lupin	217.1 c	246.6 cd	48.4 de	38.8 de
Pea	231.8 c	266.0 de *	41.6 cd	34.9 cd*
Serradella	302.6 e	256.5 cde	38.2 c	49.5 fg*
Amaranth	301.5 e	262.1 cde*	56.0 e	29.2 bc*
Control (without)	225.0 c	191.2 b	55.1 e	24.8 b*

alphanumeric (a-e) indicate significant differences between the crops
* indicates a significant difference between the treatments without and with fungi (Duncan, $p < 0.05$)

Without fungal treatment, the highest activity of AcP was found in rhizosphere soil under serradella, amaranth and maize but the by far lowest under ryegrass.

For AlP the highest activity was found under phacelia and amaranth, but again the lowest under common ryegrass. The comparison of the results for AcP and AlP activity in the controls with the soil from the vegetated treatments, which is considered to be under the effects of the rhizosphere, reveals that the AcP activity is less affected by the plants. Comparable levels of AcP in the non-rhizosphere and rhizosphere soils for the tested crops lead to the conclusion, that the majority of the AcP activity under these plants originates from the soil microflora, indicating that the contribution of the soil microflora to the organic P breakdown is larger than the one of the plants.

In contrast to this, the AcP activity in rhizosphere soil of maize, serradella and amaranth, showed enhanced values of the enzyme activity than the control. This indicates that the increase of the rhizosphere AcP activity under these types of crops can be attributed to the plant influence. This crop influence could be direct, through the secretion of extracelullar enzyme into the soil rhizosphere by root exudates or indirect, through the stimulation of microbiological activity and/ or depletion of P.

soil where the enzyme accumulation was related to the type of crop (Tarafdar and Claassen 1988, Duff et al. 1994). Though, a study (George et al. 2002) showed that phosphatase activity did not increase under maize plants compared to that in the fallow, root free soil.

The results of the research work presented here showed indirectly, that probably the amount of available P in the soil, was not sufficient for maize, serradella and amaranth crops. These plants are likely to require higher amounts of P for their normal growth and development, compared with other tested crop species, and therefore developed an increased AcP activity, thereby providing their P nutrient needs.

A distinct difference in both, AcP and AlP activities associated with the rhizosphere of phacelia and especially ryegrass was found. Their enzyme activity values are much lower than that in soil of other tested crops variants and even lower than the control (without crops). Probably, these crops exude biological molecules into the rhizosphere soil that decreased the activity of both AcP and AlP through the suppression of the microbiological activity in soil. It was demonstrated that plant exudes consisted of various biological compounds (Gardner et al. 1983, Jones and Darrah 1994, Schilling et al. 1998, Uren and Reise-

Table 3:
P_{DL} content and pH value in soil in dependence of catch crops and fungal treatment with *Penicillium bilaii* in a pot trial (6 kg of loamy sand pot^{-1} (low in plant available P)) after 7 weeks of vegetation period under greenhouse conditions

	P_{DL} content (mg P 100 g^{-1} soil)		**pH value**	
Crop	without *P. bilaii*	with *P. bilaii*	without *P. bilaii*	with *P. bilaii*
Oilseed rape	3.22 b	3.30 b	5.32 ab	5.27 ab
Maize	3.65 d	2.86 a*	5.39 bc	5.25 a*
Phacelia	3.68 d	3.37 b*	5.56 de	5.35 c*
Buckwheat	3.46 c	2.88 a*	5.38 bc	5.24 a*
Common Ryegrass	2.71 a	2.80 a	5.28 a	5.25 a
Lupin	4.18 e	3.70 c*	5.47 cd	5.34 c*
Pea	4.20 e	3.99 d	5.69 f	5.29 b*
Serradella	4.18 e	3.45 b*	5.59 e	5.26 ab*
Amaranth	3.32 b	3.23 b	5.39 bc	5.51 d*
Control (without)	4.39 e	4.39 e	5.53 de	5.61 e

alphanumeric (a-e) indicate significant differences between the crops
* indicates a significant difference between the treatments without and with fungi (Duncan, $p < 0.05$)

nauer 1998, Wright and Reddy 2001) can regulate the soil enzyme activity and soil microflora biomass accumulation.

No relation was found between the activity of AcP and AlP and plant P-uptake.

The difference between the AcP and AlP of the *P. bilaii* treated and non-treated soil wasn't large but significant. Usually a decrease of enzyme activity was found when soil was treated with *P. bilaii*. This was especially true for phacelia. Only under ryegrass the AcP enzyme activity was twice as high as in rhizosphere soil without fungal treatment. The production of AcP by fungal hyphae has been discussed throughout the literature. A positive correlation was for instance found for phosphatase activity and mycelial hyphae by Jagadish et al. (2001), whereas an other study reported no difference in the activity between soil with or without fungal mycelium (Joner and Jakobsen 1995).

The pH value and the lactat-soluble P content (P_{DL}) showed significant differences between the treatments (table 3). The highest value was found for the control. The plant which decreased the content of P_{DL} and the pH values mostly was ryegrass.

Usually the pH values were lower under fungal treatment. Probably because of excretion of organic compounds by the fungus. This decrease did not cause an increase of the activity of AcP. Gleddie (1993) found an increased solubility of rock phosphates due to the reduction of pH values caused by the fungus *P. bilaii*.

In the data presented here no relation between the changes in P_{DL} content of soil and the activity of AcP and AlP could be found. The decrease of P_{DL} under fungal treatment compared to that without treatment is supposedly related to the fungal P utilisation.

4 Conclusion

The results of the research work presented underline the large influence of different catch crops on the activity of AcP and AlP. However, these observations do not provide a direct evidence on the role of plant and soil microflora in soil P metabolism. Further work is required to determine whether root exudates released into the rhizosphere soil are responsible for the decrease in soil AcP and AlP under phacelia and ryegrass plants. A better understanding for such processes in soil is important for an adequate use of catch crops in accordance of soil conditions.

References

Albiach R, Gómez A, Pomares F, Canet R (1998) Efecto del tipo de fertilización sobre la actividad biológica del suelo en reconversión a la agricultura ecológica : una alternativa para el mundo rural del tercer milenio [online]. Valencia, Sep.1998, zu finden in http://www.agroecologia.net/congresos/valencia/24.pdf [zitiert am 03.11.2003]

Amann C, Amberger A (1989) Phosphorus efficiency of buckwheat (Fagopyrum esculentum). Z Pflanzenernähr Bodenkd 152:181-189

Asmar F, Gahoonia TS, Nielsen NE (1995) Barley genotypes differ in activity of soluble extracelullar phosphatase and depletion of organic phosphorous in the rhizosphere. Plant Soil 172:117-122

Dalal R (1977) Soil organic phosphorous. Adv Agron 29:83-117

Dinkelaker B, Marschner H (1992) In vivo demonstration of acid phosphatase in the rhizosphere of soil grown plants. Plant Soil 144:199-205

Duff SM, Sarath G, Plaxton WC (1994) The role of acid phosphatase in plant phosphorous metabolism. Physiol Plant 90:791-800

Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9:167-172

Gardner WK, Parberry DG, Barber DA (1983) The acquisition of phosphorous by Lupinus albus. III. Some characteristics of the soil/root interface. Plant Soil 68:19-32

George TS, Gregory PJ, Wood M (2002) Phosphatase activity and organic acids in the rhizosphere of potential agroforesty species and maize. Soil Biol Biochem 34:1487-1494

Gleddie SC (1993) Response of pea and lentil to inoculation with the phoshate-solubilizing fungus Penicillium bilaii (PROVIDE.). Agric Sci Proc 328:47-52

Haussling M, Marschner H (1989) Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [Picea abies (L.) Karst.] trees. Biol Fertil Soils 8:128-133

Helal HM, Sauerbeck DR (1981) Phosphatumsetzungen im Wurzelraum von Pflanzen. Mitteilg Dtsch Bodenkundl Gesellsch 32:295-304

Jagadish C, Tarafdar JC, Yadav RS, Niwas R (2001) Relative efficiency of fungal intra- and extracelullar phosphatases and phytase. J Plant Nutr Soil Sci 165:17-19

Joner EJ, Jakobsen I (1995) Growth and extracelullar phosphatase activity of arbuscular mycorryzal hyphae as influenced by soil organic matter. Soil Biol Biochem 27:1153-1159

Jones DL, Darrah PR (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil 166: 247-257

Juma MG, Tabatabai MA (1978) Distribution of phosphomonoesterases in soils. Soil Sci 126: 101-108

Li M, Shinano T, Tadano T (1997) Distribution of exudates of lupin roots in the rhizosphere under phosphorous deficient conditions. Soil Sci Plant Nutr 43:237-245

Marschner H (1995) Mineral nutrition of higher plants. Amsterdam : Academic Press, XV, 889 p, ISBN 0-12-473543-6

Pannikow WD, Minejew WG (1980) Boden, Klima, Düngung und Ertrag. Berlin : Dt Landwirtschaftsverl, 62 p

Parham J, Deny SP, Braun WR, Johnson GV (2002) Long term cattle manure application in soil. I. Effect on soil phosphorous levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biol Fertil Soils 35:328-337

Schilling G, Gransee A, Deubel A, Lezovic G, Ruppel, S (1998) Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Z Pflanzenernähr Bodenkd 161:465-478

Stevenson FJ (1986) Cycles of soil : carbon, nitrogen, phosphorus, sulfur, micronutrients. New York : Wiley, XVIII, 380 p, ISBN 0-471-82218-3

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenol phosphate for assay of soil phosphatases. Soil Biol Biochem 1:301-307

Tadano T, Ozawa K, Sakai H (1993) Secretion of acid phosphatase by the roots of crop plants under phosphorous deficient conditions and some properties of the enzyme secreted by lupin roots. Plant Soil 156:95-98

Tarafdar JC, Jung A (1987) Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorous. Biol Fertil Soil 3:199-204

Tarafdar JC, Rao AV, Bala K (1988) Production of phosphatase by fungi isolated from desert soils. Folia Microbiol 33:453-457

Tarafdar JC, Claassen N (1988) Organic phosphorous compounds as a phosphorous source through the activity of phosphatase produced by plant roots and microorganisms. Biol Fertil Soil 5:308-312

Tarafdar JC, Marschner H (1994) Phosphatase activity in the rhizosphere and hyposphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorous. Soil Biol Biochem 26:387-395

Uren NC, Reisenauer HM (1998) The role of root exudates in nutrient acquisition. Adv Plant Nutr 3:79-114

Wright AL, Reddy KR (2001) Phosphorous loading effects on extracelullar enzyme activity in Everglades Wetland soils. Soil Sci Soc Am J 65:588-595