DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

Comissão 2.1 - Biologia do solo

PHOSPHATASE ACTIVITY IN SANDY SOIL INFLUENCED BY MYCORRHIZAL AND NON-MYCORRHIZAL COVER CROPS[1]

Alceu Kunze[2], Murilo Dalla Costa[3], Jailso Epping[4], Jean Carlos Loffaguen[5], Rafael Schuh[6] & Paulo Emílio Lovato[7]

SUMMARY

Cover crops may difffer in the way they affect rhizosphere microbiota nutrient dynamics. The purpose of this study was to evaluate the effect of mycorrhizal and non-mycorrhizal cover crops on soil phosphatase activity and its persistence in subsequent crops. A three-year experiment was carried out with a Typic Quartzipsamment. Treatments were winter species, either mycorrhizal black oat (*Avena strigosa* Schreb) or the non-mycorrhizal species oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg) and corn spurry (*Spergula arvensis* L.). The control treatment consisted of resident vegetation (fallow in the winter season). In the summer, a mixture of pearl millet (*Pennisetum americanum* L.) with sunnhemp (*Crotalaria juncea* L.) or with soybean (*Glycine max* L.) was sown in all plots. Soil cores (0–10 cm) and root samples were collected in six growing seasons (winter and summer of each year). Microbial biomass P was determined by the fumigation-extraction method and phosphatase activity using p-nitrophenyl-phosphate as enzyme substrate. During the flowering stage of the winter cover crops, acid phosphatase activity was 30–35 % higher in soils with the non-mycorrhizal species oilseed radish, than in the control plots, regardless of the amount of P immobilized in microbial biomass. The values of enzyme activity were intermediate in the plots with corn spurry and black oat. Alkaline phosphatase activity was 10-fold lower and less sensitive to the treatments, despite the significant relationship between the two phosphatase activities. The effect of plant species on the soil enzyme

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[2] Teacher at Instituto Federal de Educação, Ciência e Tecnologia Catarinense. C. P. 21, CEP 89245-000 Araquari (SC). E-mail: akunze@gmail.com

[3] Researcher at Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Lages Experimental Station. Rua João José Godinho s/n, Morro do Posto, CEP 88502-970 Lages (SC). E-mail: murilodc@gmail.com

[4] Agricultural Extensionist at Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Escritório Municipal de São Martinho. Rua Praia Redonda, s/n, Centro, CEP 88765-000, São Martinho (SC). E-mail: jaisoepping@epagri.sc.gov.br

[5] Agricultural Extensionist. Engenheiro Agrônomo, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Escritório Municipal de Alfredo Wagner. Praça da Bandeira, s/n, Centro, CEP 88450-000, Alfredo Wagner (SC). E-mail: loffaguen@epagri.sc.gov.br

[6] Agricultural Extensionist. Rua Henrique Kappke, 109 . Irani – SC. CEP 89680-000

[7] Associate Professor, Departamento de Engenharia Rural, Universidade Federal de Santa Catarina – CCA-UFSC. C. P. 476, CEP 88.040-970 Florianópolis (SC). E-mail: plovato@mbox1.ufsc.br

profile continued in the subsequent periods, during the growth of mycorrhizal summer crops, after completion of the life cycle of the cover crops.

Index terms: ***Avena, Raphanus, Spergula,*** **phosphorus mobilization.**

RESUMO: *ATIVIDADE DE FOSFATASES NO SOLO AFETADA POR CULTURAS DE COBERTURA MICORRÍZICAS E NÃO MICORRÍZICAS*

Culturas de cobertura do solo podem afetar de forma distinta a microbiota rizosférica e a dinâmica de nutrientes. Com o objetivo de avaliar o efeito de culturas de cobertura micorrízicas e não micorrízicas sobre a atividade de fosfatases no solo e a persistência dessa atividade na sucessão de culturas, um experimento foi conduzido durante três anos em um Neossolo Quartzarênico. Os tratamentos foram culturas de cobertura de inverno, sendo uma espécie micorrízica (aveia-preta, Avena strigosa *Schreb) e duas não micorrízicas: o nabo forrageiro (*Raphanus sativus *L. var.* oleiferus *Metzg) e a espérgula ou gorga (*Spergula arvensis *L.). O controle foi constituído pela vegetação espontânea (pousio de inverno). No verão, todas as parcelas foram semeadas com uma mistura de milheto (*Pennisetum americanum *L) e crotalária (*Crotalaria juncea *L.) ou com soja (*Glycine max *L). Amostras de solo (0–10 cm) e de raízes foram coletadas durante seis estações de crescimento (inverno e verão de cada ano). Foram avaliados o teor de fósforo (P) microbiano, pelo método de fumigação-extração, e a atividade de fosfatases, utilizando p-nitrofenilfosfato como substrato enzimático. No florescimento das culturas de inverno, a atividade de fosfatase ácida foi 30–35 % maior em solos com nabo forrageiro, em comparação com as testemunhas, independentemente da quantidade de P imobilizado na biomassa microbiana. As parcelas com espérgula e aveia-preta tiveram valores intermediários de atividade enzimática. A atividade de fosfatase alcalina foi 10 vezes menor e menos sensível aos tratamentos, embora a correlação entre as atividades das duas classes de fosfatase tenha sido significativa. O efeito das culturas de cobertura no perfil enzimático do solo continuou nos períodos subsequentes, durante o crescimento das culturas de verão, após as plantas de inverno terem completado seu ciclo.*

Termos de indexação: Avena, Raphanus, Spergula*, mobilização de fósforo.*

INTRODUCTION

Cover crops are used to prevent soil degradation and improve fertility. Their use, associated with zero tillage, affects organic matter decomposition rates and improves soil chemical properties (Carneiro et al., 2004). However, more information is needed on the interactions between cover crops, soil biological factors and nutrient cycling. Soil biological activity plays an important role in P cycling, especially through solubilisation, mineralization of organic forms catalyzed by phosphatases (Juma & Tabatabai, 1988), and by the action of mycorrhizas on nutrient uptake by plants. Phosphatase activity is enhanced by the increased addition of organic material and by root system concentration in systems with zero tillage (Curci et al., 1997), as well as by the use of organic fertilizers (Oberson et al., 1996). Besides soil tillage and fertilization, temporal distribution of species in crop rotation may affect phosphatase activity (Rao et al., 1995). Organic P can be mineralized by hyphae of arbuscular mycorrhizal fungi through excretion of extra-cellular enzymes (Koide & Kabir, 2000), but the quantitative significance of this process for plant nutrition does not seem to be relevant (Joner et al., 2000).

Interactions between the effects of fertilizer use and crop rotation on enzyme activity and also soil biomass and P supply to subsequent crops have already been studied (Kabir & Koide, 2002; Dodor & Tabatabai, 2003), but little information is available on the effect of the mycorrhizal or non-mycorrhizal character of crops on P cycling. Although almost 90 % of all plant families are mycorrhizal, some important cover crop species form no mycorrhizal association, so P mineralization can be important for the nutrition of such species.

Cover crops change the soil physical and chemical properties in their rhizosphere, and consequently regulate soil microbial populations. The different effects that mycorrhizal and non-mycorrhizal plants have on the dynamics of soil P, especially on organic fractions of this nutrient can be hypothesized.

The aim of this study was to evaluate the effect of mycorrhizal and non-mycorrhizal cover crops, on phosphatase activity and the persistence of such effects in the subsequent growing seasons.

MATERIAL AND METHODS

The experiment was carried out in Florianópolis-SC, Brazil, on a hydromorphic Quartzipsamment soil, with pH (in water) 4.8; SMP index 5.8; P (Mehlich) 10.1 mg dm^{-3}; K (Mehlich) 15 mg dm^{-3}; organic matter, 14 g dm^{-3}; Al^{3+}, 9.0 $mmol_c$ dm^{-3}; Ca^{2+}, 6.0 $mmol_c$ dm^{-3}; Mg^{2+}, 1.0 $mmol_c$ dm^{-3}; H + Al, 42.7 $mmol_c$ dm^{-3}; cation exchange capacity (CEC), 50.1 $mmol_c$ dm^{-3}; base saturation (V %), 14.8 %.

Three winter cover crops were used: black oat (*Avena strigosa* Schreb), a mycorrhizal plant; and oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg) and corn spurrey (*Spergula arvensis* L.), both non-mycorrhizal plants. Control treatments consisted of fallow plots, with resident vegetation composed of grasses and *Gnaphalium spicatum* Lam. (Asteraceae). In the first summer season, a mixture of two mycorrhizal crops, pearl millet (*Pennisetum americanum* L.) and sunnhemp (*Crotalaria juncea* L.), was grown in all plots. In the second summer, sunnhemp was sown and soybean (*Glycine max* L.) in the third in all plots (six 40 cm rows per 2.0 x 2.0 m plot). The experiment was evaluated in a randomized block design with eight replications. The soils were limed (dolomitic lime 300 kg ha^{-1}) and disked and after 30 days fertilized with rock phosphate (from Arad, Israel), potassium chloride and urea, corresponding to 33 kg ha^{-1} P_2O_5, 43 kg ha^{-1} K_2O and 50 kg ha^{-1} N, respectively.

The experiment lasted six growing seasons (three years), including seven samplings. In the first winter season, the soil was sampled 41 and 86 days after sowing; the latter date corresponded to cover crop flowering. In the following season (summer) all plots were grown with pearl millet and sunnhemp, and soil was sampled 86 days after sowing (flowering). In the second winter, plots were sown again with the same cover crops, but due to a lack of chemical reagents, the corn spurry plots were not sampled. Soil was sampled at flowering (100 days after sowing). During the second summer season, when all plots were sown with sunnhemp, one sample was taken at the end of the season, since heavy rains occurred during flowering. In the third winter, samples were taken at 100 days after sowing (cover crop flowering). The third summer sampling was performed 15 days after sowing soybean.

Each sample was composed of 10 sub samples, taken with a core sampler 10 cm deep, 5 cm away from the row center. Soil samples were sieved (2mm mesh) and maintained at 4 °C until analyses. Acid and alkaline phosphatase activities and microbial biomass P were evaluated. Acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatase activities were assessed as proposed by Tabatabai (1994). Soil samples were incubated for 60 min at 37 °C in buffer solution, at pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase. To the soil-buffer mixture, 0.05 mol L^{-1} p-nitrophenyl-phosphate (Merck, Darmstadt, Germany) was added. Absorbance was measured using a visible light spectrophotometer (λ 410 nm) and expressed as µg p-nitrophenol h^{-1} g^{-1} dry soil.

Soil microbial P was evaluated in the first winter and summer seasons, by the fumigation-extraction method (Brookes et al., 1982). Soil samples were fumigated with chloroform (CH_3Cl) for 24 h and P was extracted with Mehlich-1 solution (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}). Non-fumigated soil samples were used to determine soil extractable P. The difference between fumigated and non-fumigated soil P was considered as microbial P, using a 0.40 factor (Brookes et al., 1982).

Plants from the two central rows were sampled to evaluate mycorrhizal colonization. Root samples were washed with 5.0 % KOH and stained with 0.5 % Trypan Blue in glycerol and mycorrhizal colonization intensity was evaluated according to Giovanetti & Mosse (1980).

Acid and alkaline phosphatase activity and mycorrhizal colonization values were transformed to $x^{1/2}$, and microbial biomass P values were transformed to log10 (x), for analysis of variance. If F values ($p \leq 0.05$) were significant, means were compared by Tukey´s test ($p \leq 0.05$). Relationships between the different variables were obtained by the Pearson correlation coefficient and tested for significance ($p \leq 0.05$ and $p \leq 0.01$).

RESULTS

Activity of acid phosphatase, stimulated by roots and soil microbiota (Juma & Tabatabai, 1988; Dakora & Phillips, 2002), was influenced by the winter cover crops (Table 1). In the first growing season, differences increased over time, and there were significant effects of plants species at flowering. The highest values for acid phosphatase activity were observed in soil with oilseed radish, which were significantly higher than in the fallow soil with resident vegetation. Plots with corn spurry and oat had intermediary values, which did not differ significantly from the other treatments. In the second winter, acid phosphatase activity was significantly higher in soil with oilseed radish than in all other treatment, and in the third winter, treatment results were similar to the first season. The phosphatase activity of oilseed radish plots was higher than of control plots, and intermediate in plots planted with oat and corn spurry. In the first summer season, results from the winter season tended to persist, since the values of acid phosphatases of the treatments had the same ratio between, and the error probability (0.06) was slightly higher than expected (0.05). In the second summer, heavy rains delayed sampling, which was performed late in the season.

Table 1. Acid phosphatase and alkaline phosphatase activity in sandy soil under plant rotation systems including mycorrhizal (black oat) or non-mycorrhizal (oilseed radish and corn spurry) cover crops or with resident vegetation (control) in winter, followed by mycorrhizal crops in summer

Year	Season	Stage	Oilseed radish	Corn spurry	Black oat	Control	*p*
			μg p-nitrophenol h^{-1} g^{-1} soil				
			Acid phosphatase				
1	Winter	Vegetative	225	200	-	179	0.115
		Flowering	315 a	273 ab	258 ab	233 b	0.042
	Summer (pearl millet and sunnhemp)	Flowering	344	-	299	255	0.056
2	Winter	Flowering	287 a	232 b	228 b	220 b	0.073
	Summer (sunnhemp)	Senescence	247 ab	256 a	247 ab	213 b	0.022
3	Winter	Flowering	204 a	178 ab	167 ab	155 b	0.010
	Summer (soybean)	Early	211 a	201 ab	195 ab	174 b	0.049
			Alkaline phosphatase				
1	Winter	Vegetative	24	21	-	20	0.169
		Flowering	39	34	33	29	0.115
	Summer (pearl millet and sunnhemp)	Flowering	38	-	29	30	0.186
2	Winter	Flowering	45 a	42 a	33 ab	28 b	0.015
	Summer (sunnhemp)	Senescence	14	16	16	14	0.449
3	Winter	Flowering	22 a	20 ab	15 b	16 ab	0.009
	Summer (soybean)	Early	19	22	18	19	0.625

Means followed by the same letter in each line do not differ significantly according to Tukey's confidence test at $p \leq 0.05$.

Nevertheless the results showed a positive trend of enhanced acid phosphatase activity by the cover crops. This trend was observed again in the third summer growing season.

The values of alkaline phosphatase activity, produced by soil microbiota (Dakora & Phillips, 2002), were 10 times lower than those of acid phosphatases (Table 1). This enzyme activity was also affected by the cover crops, although they differed significantly only in the second and third seasons and its activity was no longer observed in the following summer seasons.

The mycorrhizal colonization intensity (Figure 1) varied among treatments, and highest values were found in the plots with wild oat, a mycorrhizal species, while roots in the plots with non-mycorrhizal cover crops had colonization values under 5 %. Mycorrhizal colonization was found in roots belonging to resident plants forming this association. In the control plots, values were around 15.0 %. In the second summer season, mycorrhizal colonization indexes did not vary among plots with different cover crops, showing that the presence of non-mycorrhizal plants did not affect the number of propagules of arbuscular mycorrhizal fungi in the soil. Similar rates of mycorrhizal colonization were found in resident plants and in pearl millet and sunnhemp growing after the winter species (Figure 1), which suggests that the persistence of phosphatase activity levels in plots previously planted with non-mycorrhizal species was not affected by the presence of the association in summer-growing plants.

No differences in microbial biomass P were found among treatments during the winter or summer seasons (Table 2). There was a weak correlation between soil microbial biomass-P and phosphatase activity (Table 3), and a weaker, but still significant correlation between microbial P and extractable P, which is used to estimate the nutrient availability to plants.

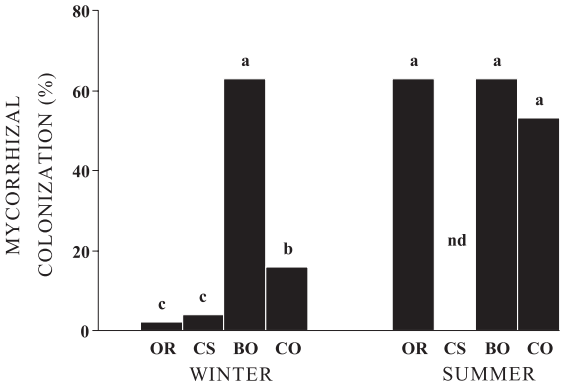

Figure 1. Mycorrhizal colonization indexes in roots collected in plots grown with oilseed radish (OR), corn spurry (CS), black oat (BO) or under fallow (CO) during winter cover crop growth and in the subsequent summer (pearl millet and sunnhemp). (Means followed by the same letter do not differ significantly according to Tukey's test p≤ 0.05). nd = not determined.

Table 2. Microbial biomass phosphorus in sandy soil under plant rotation systems during the first growing season of mycorrhizal (black oat) or non-mycorrhizal (oilseed radish and corn spurry) cover crops or with resident vegetation (control) in winter, followed by mycorrhizal crops in summer (pearl millet and sunnhemp)

Season	Stage	Oilseed radish	Corn spurry	Black oat	Control
		μg g^{-1} soil			
Winter	Early	3.6 [ns]	3.4	...	4.6
	Vegetative	6.0	4.8	...	5.6
	Flowering	6.8	7.3	6.1	5.9
	Senescence	5.1	7.8	6.5	6.6
Summer	Early	6.8	...	6.7	7.2
	Flowering	5.6	...	6.8	4.6

[ns] F test ($p \leq 0.05$) not significant.

DISCUSSION

These results show that changes in acid phosphatase activity were associated with cover crops and a persistency of the plant effects. In a similar study, higher acid phosphatase activity was observed under oilseed radish and white lupine (*Lupinus albus* L.), which are both non-mycorrhizal, and this effect persisted under corn grown in the following season (Dalla Costa & Lovato, 2004). The persistence in this enzyme activity may be linked to its immobilization in complexes formed with mineral and humic colloids, which can become stable in the soil, depending on their molecular composition and chemical nature (Rao et al., 2000), and the soil organic matter, which plays an important role in providing substrate for P-mobilizing enzyme reactions from soil biota (Curci et al., 1997). The amount and quality of plant material incorporated into the soil are directly related to the nutritional conditions for the activity of microbial populations (Dick, 1992) and non-mycorrhizal crop residues may be associated with phosphatase activity and P mobilization in oilseed radish (Pavinato et al., 2008).

The lower levels of alkaline phosphatase acitivity may be due to the acidic soil conditions (pH 4.8), which may have restricted the activity of these enzymes, regulated by soil pH (Acosta-Martinez & Tabatabai, 2000). Furthermore, changes in the enzyme structural conformation and in the environment surrounding it, due to soil acidity or other conditions, may affect the activity and accessibility of enzyme active sites, and therefore enzyme activity (Rao et al., 2000).

No differences in microbial biomass P were found among treatments during the winter or summer seasons. In a short period of time, changes in microbial functional diversity may be more sensitive to changes in biomass- or soil organic matter- nutrient content, induced by plant management (Bending et al., 2000). The weak relationship (Table 3) between soil microbial biomass P and phosphatase activity shows that higher levels of enzyme activity were not linked primarily to higher microbial biomass, and that cover crops have some effect on functional structure and diversity of the soil microbial community. Enhancement of microbial population growth by the rhizosphere activity of oilseed radish might be linked to the amount and composition of metabolites exuded to soils by the roots of these plants. Merbach et al. (1999) found higher amounts of exudates, especially organic acids and amino acids, in the soil solution around *Raphanus oleiformis*, as compared to wheat, which exuded mostly carbohydrates. Acid phosphatases and other root exudates from oilseed radish may have regulated the microbial community composition and functional diversity in the plant rhizosphere, which must have been different from the communities around the roots of the resident vegetation (Marschner et al., 2004).

Under the conditions of this study, changes in the soil enzyme profile were not limited to the growing season of the cover crop, but affected the enzyme activity dynamics related to the transformation of organic P and P availability in the soil. Plant activity, through the rhizosphere effect, stimulates some populations in the microbial community; this effect may persist in the soil in the following season, under another plant population (Kowalchuk et al., 2002). Enzyme activity was more affected by plant diversity and productivity than by tillage of soils with low plant diversity (Dick, 1992). Managed plant populations may affect the microbial community structure and their ability to mineralize nutrients more than they can affect microbial biomass as a whole. Changes in the soil management system or plant population affect the microbial community composition and physiological patterns, and consequently change enzyme activity in the soil (Waldrop et al., 2000).

Limitations in soil P availability, as shown by the low level of Mehlich-extractable P (CQFSRS/SC., 2004) in the soil, may induce an increase in acid phosphatases of plant origin, with an increased presence of these

Table 3. Pearson correlation coefficients between acid and alkaline phosphatase activity levels, extractable P, microbial P in sandy soil sampled during the first winter growing season (n=160)

	Acid phosphatase	Alkaline phosphatase	Extractable P	Microbial P
Acid phosphatase	1	0.55**	0.04ns	0.19*
Alkaline phosphatase		1	-0.01ns	0.20*
Extractable P			1	0.34**
Microbial P				1

ns: not significant, *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$.

enzymes in root exsudates (Dakora & Philips, 2002). Therefore, the higher P mineralizing activity in the plots with the non-mycorrhizal oilseed radish suggests the existence of a strategy to increase the rates of soil P mobilization. This would be a mechanism to compensate for the absence of mycorrhizas and their contribution to P nutrition (Marschner & Dell, 1994). Production and release of acid phoshatases by the roots, besides increasing hydrolysis of organic P, could be associated to the release of plant metabolites regulating microbial populations in the rhizosphere, which would further contribute to P mineralization. (Tarafdar et al., 2001; Klose & Tabatabai, 2002). The assessment of other functional groups and quantification of other nutrients in the microbial biomass may provide more information on the changes in the microbial community promoted by different plant species and their management.

The results show that soils with non-mycorrhizal cover crops had higher levels of phosphatase activity, and that these effects persisted in the following seasons, even under mycorrhizal plants, regardless of the amount of P immobilized in microbial biomass. Changes in the enzyme profile might be part of a strategy to mobilize P by some non-mycorrhizal species and these changes persist beyond the life span of the crop itself.

CONCLUSIONS

1. Mycorrhizal and non-mycorrhizal cover crops have different influences on the temporal dynamics of soil phosphatase activity.

2. Oilseed radish, a non-mycorrhizal cover crop species, increases the activity of enzymes related to soil organic P mineralization, and this a high level persists in the following crop seasons.

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