Soil Alkaline Phosphatase and Phosphodiesterase Activities in Relation to Phosphorus Content in a Greenhouse Organic Tomato Crop in Almería (SE Spain)

Fernando del Moral; Juan Antonio Sánchez; Mariano Simón; Sergio de Haro; Inés García.

Department of Edafología y Química agrícola. University of Almería. Crtra Sacramento s/n. 04120-Almería.

E-mail: fmoral@ual.es Tel. +34-950-015924; Fax: +34-950-015319

ABSTRACT

The aim of this work is to assess, in a greenhouse organic tomato crop, the effects of two soil organic amendments (T1:11.0 kg dry manure m^{-2}, and T2: 4.5 kg dry manure m^{-2} + 6.5 kg dry vermicompost m^{-2}) on levels of alkaline phosphatase and phosphodiesterase activities, in relation to available (P*a*), soluble (P*s*) and inorganic phosphorus content (P*i*). Four plots were compared: P1 and P2 had loamy sandy soil (79% illite clay) with saturated hydraulic conductivity (Kfs) of 17.07 mm h^{-1}; P3 and P4 had loamy soil (45 % smectite clay), and Kfs of 3.13 mm h^{-1}. Gravel mulch (5-10 mm) was added to plots P1 and P4, and sand mulch (0.05-2 mm) to P2 and P3. Three replicates per plot of each organic treatment were sampled on a weekly basis over seventeen weeks. There were no statistical differences among treatments for inorganic phosphorus content and phosphodiesterase activity. Available and soluble phosphorus were greater in T2 (901.6 ± 343.27 mg P*a* kg^{-1}; 6.93 ± 4.09 mg P*s* kg^{-1}) than in T1 (312.2 ± 137.69 mg P*a* kg^{-1}; 2.72 ± 1.66 mg P*s* kg^{-1}), while alkaline phosphatase activity was greater in T1 (856.0 ± 406.4 mg PNP kg^{-1} h^{-1}) than in T2 (684.9 ± 324.5 mg PNP kg^{-1} h^{-1}). Mean values for all analyzed variables were significantly higher ($p<0.01$) in plots with gravel mulch (P1 and P4). There were positive and significant correlations ($P< 0.01$) between enzyme activities and forms of phosphorus, except for alkaline phosphatase and available phosphorus content, where no correlation was detected. At plot scale, therefore, phosphorus availability did not inhibit enzyme activity. All values decrease with time in all plots, except in P4 which is the poorest drained.

Keywords: alkaline phosphatase, phosphodiesterase, phosphorus, soil, mulch, greenhouse.

INTRODUCTION

Food and environmental safety are often-cited reasons for the use of alternative soil amendments, but increasingly, economic considerations are becoming important due to a rise in popularity of organically produced foods (Bulluck III, et al, 2002). Greenhouse organic production can increase growers' profits by 12-40%, and this is attracting many new converts. On the other hand, the degradation of greenhouse soils as a result of excessive application of agrochemicals and the growing demand/market for pesticide-free produce have helped to persuade more and more greenhouse growers to adopt environment-friendly production systems based on organic fertilization and biological control. Although this is an area of growing research interest, there are few works on greenhouse ecological systems (Rippy et al. 2004), particularly referring to organic fertilizers applied traditionally or in fertigation systems and to the release of nutrients which is highly dependent on the soil's biological and enzymatic activity.

The application of organic amendments increases the soil's level of biological and enzymatic activity (Fraser et al., 1988; Workneh and van Bruggen, 1994; Satre et al., 1996; Gunapala and Scow, 1998; Colvan et al., 2001). As a result, the level of available nutrients for the edaphic biota and for the crop also increase. However, the overall activity of a single enzyme may depend on enzymes in different locations, including intracellular enzymes from viable proliferating cells, and accumulated or extracellular enzymes stabilized in clay minerals or complexes with humic colloids (Acosta-Martínez et al. 2003). Consequently, not all organic applications are equally effective for al soil types, and the level of humidity is a determining factor. Albiach et al. (2000) have demonstrated that commercial liquid organic amendments are of little use for increasing soil fertility. Edwars (1995), Aira et al. (2003) Chaoui (2003) and Aira et al. (2005) have shown that vermicompost is effective for increasing the levels of organic matter and enzymatic activity, although this enzymatic activity is brought about by the organic fertilizer itself to a great extent (Masciandaro et al. 1997). Díez (1987) demonstrated that manure is much more efficient than compost or peat for increasing phosphorus content, and Saha et al. (2008) showed that the nature and amount of organic fertilizers applied to the soil significantly affect phosphotase activity and available phosphorus. Moreover, mulching, a widespread greenhouse practice, has a great bearing on soil biota and on the evolution of the organic amendments and the mineral salts which they provide for the crop, as it modifies the soil's hydric and thermal balance and improves root growth (Benítez et al., 2000).

Soil phosphorus cycling and availability is controlled by a combination of biological processes (mineralization-immobilization) and chemical processes (adsorption-desorption and dissolution-precipitation) (Chen, et al., 2003). As a part of the P-cycle, alkaline and acid phosphatase activities catalyze the hydrolysis of both organic P esters and anhydrides of phosphoric acid into inorganic P. However, it has been suggested that the rates of synthesis, release and stability of acid and alkaline phosphatases by soil microorganisms are dependent on soil pH, so alkaline phosphatase activity is induced in high pH soils (Deng and Tabatabai, 1997; Acosta-Martínez and Tabatabai, 2000; Acosta-Martínez et al. 2003) but only from microorganisms, not from plants (Böhme, Böhme, 2006).

The aim of this work is to assess, in a greenhouse organic tomato crop, the effects of two soil organic amendments on levels of alkaline phosphatase and phosphodiesterase activities, in relation to available (P*a*), soluble (P*s*) and inorganic phosphorus content (P*i*).

MATERIALS and METHODS.

Experimental Site

The experiments were carried out in a greenhouse (20 m x 6 m x 3.8 m) at the University of Almería (southeastern Spain 36º50'N 2º27'W, 5 m above mean sea level). The climate is Mediterranean semiarid with less than 300 mm annual rainfall. The average temperatures inside the greenhouse were between 12.3 ºC and 24.8 ºC, and average soil temperature varied from 15 ºC to 18.7 ºC during the growing season.

Experimental Design

The greenhouse was divided into four plots (P1, P2, P3 and P4). Plots P1 and P2 had a *Hortic Anthrosol* (FAO, 2006) with a loamy sandy texture (79% illite clay), and saturated hydraulic conductivity (Kfs) of 17.07 mm h^{-1} (S1, Table 1); Plots P3 and P4 had a *Hortic Anthrosol* with loamy texture (45% smectite clay), and Kfs of 3.13 mm h^{-1} (S2, Table 1). Gravel mulch with dominant particle size > 5mm and water retention capacity at -33kPa of less than 3% p/p (M1, Table 2) was added to plots P1 and P4. Sand mulch with dominant particle size 0.1 mm – 1.0 mm and water retention capacity at -33kPa of 20.4% p/p (M2, Table 2) was added to plots P2 and P3. All soil types and mulches are of common use in greenhouses of southeastern Spain. Two organic amendments with three randomized replicates were applied per plot, mixed with soil to a depth of 10 cm. In T1 we applied 11.0 kg m^{-2} dry manure (30% w/w total organic matter, 4% w/w P_2O_5) in the crop row, while in T2 we applied 4.5 kg m^{-2} dry manure (30% w/w total organic matter, 4% w/w P_2O_5) mixed with 6.5 kg m^{-2} dry vermicompost (35% w/w total organic matter, 1.5% w/w P_2O_5) (Table 3). All combinations of soil, mulch and organic amendments are summarized in Table 4.

Table 1.- Particle size distribution (% w/w) of the soils used in the assays. Standard deviation in parentheses (n=4). Particle size in mm.

	S1	**S2**
Sand (2 mm - 0.05 mm)	75.1 (1.2)	52.1 (0.6)
Silt (0.05 mm - 0.002 mm)	11.1 (0.4)	35.3 (1.4)
Clay (< 0.002 mm)	13.8 (0.5)	12.6 (0.4)
pH	7.73 (0.18)	7.70 (0.15)

Table 2.- Particle size distribution (% w/w) of materials used as mulches. Particle size in mm

Particle size	**Gravel mulch (M1)**	**Sand mulch (M2)**
>= 5	83.38	4.95
5-4	6.37	0.84
4-3	2.92	0.94
3-2	1.78	1.42
2-1	1.51	2.78
1-0.5	1.02	15.70
0.5-0.25	1.20	41.08
0.25-0.1	1.11	22.16
0.1-0.075	0.30	3.71
0.075-0.05	0.19	2.36
<0.05	0.22	4.05

Table 3.- Properties of the organic amendments used in the assays. All values are expressed in percentages. Data from manufacturers.

	Manure	Vermicompost
Oxidizable organic matter	30	35
Total P_2O_5	4	1,5
Total humic extracts	15	10
Fulvic acids	9	3
Humic acids	6	7
Humins	15	25

Table 4.- Combinations of soil type, mulch type and organic amendment applied per treatment.

Plot	Soil type	Mulch type	Organic amendment	Treatment
P1	S1	M1	T1	1
			T2	2
P2	S1	M2	T1	3
			T2	4
P3	S2	M2	T1	5
			T2	6
P4	S2	M1	T1	7
			T2	8

The greenhouse tomato "Marmande Raf" was used in all treatments. Tomato seedlings were transplanted into the greenhouse on 15/10/02 and organic amendments had been mixed with the soil one week before. Crop management, i.e. staking, pruning suckers and leaves and pollination, was similar in both treatments. A drip irrigation method was used to supply crop water requirements. The drip flow was 3 L h-1 in a 1 m x 0.5 m arrangement.

Data Collection

Soil samples were collected on a weekly basis. Each sample, one per replicate, was a composite mixture from three points per crop row (2.9 m x 1.0 m) to represent better the treatment studied (Böhme et al. 2004). The mulch was removed before collecting the soil (0-10 cm). After sampling, soil samples were air-dried at room temperature and ground to pass a 2 mm sieve.

Soil Analyses

The pH values were measured in air-dried soil (<2mm) by using a glass combination electrode (soil:water ratio, 1:2.5). The particle size distribution of soils was determined by sieving and sedimentation, applying Robinson's pipette method (Soil Conservation Service, 1972). Field saturated hydraulic conductivity (Kfs) was measured in-situ with a Gelph permeater (Reynolds and Elrick, 1985). Mulch water content was measured in the laboratory with a ceramic pressure plate (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) at air pressure of 33 kPa (Richards, 1954). Mulch particle size distribution was determined by sieving. The alkaline phosphatase (EC 3.1.3.1) and phosphodiesterase (EC 3.1.4.1) activities were assayed (<2 mm air-dried soil) at their optimal pH values including one control. The assays are described in Tabatabai (1994). Total soil organic carbon (*SOC*) was determined by wet oxidation with potassium dichromate (Tyurin, 1951). Available phosphorus (*Pa*) was determined by the Olsen method (Olsen *et al.* 1954). For inorganic phosphorus (*Pi*) determination we used the method described in Kuo (1996) after extraction with concentrated sulfuric acid and dilute sodium hydroxide. Soluble phosphorus (*Ps*) was determined in saturation extract by ion chromatography (Dionex ICS-1000)

Statistical analysis

Software package SPSS from SPSS Inc. v.15 was used for statistical analysis. Data were assessed by Duncan's multiple range test, with a probability $p < 0.05$. Differences between means were evaluated by univariant general linear model for each of the variables studied, analyzing the effect of the fixed factors, soil type, mulch type, organic treatment applied and their interactions. Pearson's bivariate linear correlation coefficient was calculated for all pairs of variables.

RESULTS and DISCUSSION.

Due to the granulometry of mulch type M1, the movement of water is primarily determined by gravitational potential. The flow of water through this type of mulch is mainly vertical and occurs extremely rapidly, and so the flow provided by the drip outlet reaches the soil surface practically unaltered. This flow exceeds the infiltration capacity of both soil types, causing temporary flooding, more notably in S2, which had a lower percentage of sand, a higher percentage of silt, smectite clays and a saturated hydraulic conductivity six times less than S1. As a result, for the same amount of water supplied the surface area moistened is greater and the depth of the moistened front is less in S2 than in S1, and this in turn affects both organic amendments in different ways. For mulch type M2, with much finer particles, the matric potential modifies the flow of water. Part of the water is retained permanently by the mulch and the rest is released more slowly into the soil. In this case the flow received by the soils is less than that emitted by the drip outlet and there is no temporary flooding. Also, the nature of the organic amendments and its P_2O_5 content differ, and this will affect each of the treatments in a different way. Manure provides more labile organic matter (with a higher percentage of less condensed fractions) than vermicompost, which apart from the composting process, has undergone the digestive process of the earthworms.

Therefore, the different treatments gave rise to significant differences in the variables studied (table 5). For the inorganic phosphorus (Pi) and phosphodiesterase variables the treatments gave rise to two significantly different groups, coinciding with the differences in the mulch type. For the remaining variables, however, the treatments cannot be grouped so clearly.

Table 5.- Average values for available phosphorus (Pa, mg kg^{-1}), soluble phosphorus (Ps, mg kg^{-1}), inorganic phosphorus (Pi mg kg^{-1}), alkaline phosphatase (mg pNP kg^{-1} h^{-1}), phosphodiesterase (mg bispNP kg^{-1} h^{-1}) and soil organic carbon (SOC, % w/w). In each column values sharing the same letter are not significantly different ($p<0.05$)

Treatment	Pa	Ps	Pi	Alkaline phosphatase	Phosphodiesterase	SOC
1	403.51^{b}	3.29^{b}	6645.23^{b}	983.02^{d}	929.63^{b}	13.04^{c}
2	1019.12^{d}	7.39^{e}	6962.59^{b}	805.84^{c}	892.94^{b}	11.96^{bc}
3	284.19^{a}	1.66^{a}	4177.37^{a}	579.61^{ab}	551.98^{a}	10.76^{b}
4	792.49^{c}	4.59^{cd}	4015.63^{a}	544.64^{a}	607.07^{a}	8.48^{a}
5	261.16^{a}	2.06^{a}	4088.67^{a}	691.36^{bc}	645.05^{a}	8.85^{a}
6	728.91^{c}	5.43^{d}	3946.88^{a}	565.66^{ab}	623.16^{a}	8.09^{a}
7	298.07^{a}	3.80^{bc}	6571.32^{b}	1156.57^{e}	974.25^{b}	14.78^{d}
8	1065.73^{d}	10.31^{f}	6387.85^{b}	823.40^{c}	871.31^{b}	12.85^{c}

In order to determne which factors (soil type, mulch type, organic amendment), or combination of factors, are responsible for said differences, we applied a univariant general linear model for each of the variables, also taking into account the time since the crop was planted. The soil type only had a significant effect on the average levels of Ps and alkaline phosphatase activity (table 6), and S2, with the worst drainage, had the highest values. The values of Pa and Ps are significantly greater on average when vermicompost is applied, and all the variables showed higher average levels with the mulch of larger particle size (M1).

Table 6.- Average values obtained for available phosphorus (Pa, mg kg^{-1}), soluble phosphorus (Ps, mg kg^{-1}), inorganic phosphorus (Pi mg kg^{-1}), alkaline phosphatase (mg pNP kg^{-1} h^{-1}), phosphodiesterase (mg bispNP kg^{-1} h^{-1}) and soil organic carbon (SOC, % w/w) as a function of the main factors analyzed. * significant at $p<0.01$

	Soil Type		Mulch type		Organic amendment	
	S1	S2	M1	M2	T1	T2
Pa	624.43	589.36	695.70*	518.09	312.22	901.56*
Ps	4.24	5.41*	6.20*	3.45	2.72	6.93*
Pi	5536.00	5328.89	6723.06*	4141.83	5465.62	5399.27
Alkaline phosphatase	728.82	812.07*	944.16*	596.74	856.01*	684.88
Phosphodiesterase	746.08	780.16	919.01*	607.22	777.61	748.62
SOC	11.08	11.15	13.18*	9.05	11.87*	10.36

The combination of the different factors analyzed does not give rise to significant differences for Pi or phosphodiesterase, whose average values only depend on the type of inorganic mulch used. However, the remaining variables analyzed are clearly determined by the combination of different factors. The highest values for Pa and Ps are found with the M1T2 combination, although in the case of Ps the values are systematically greater in soil type S2, which indicates an accumulation due in the main to worse drainage. Alkaline phosphotase activity is also greater in the worse drained soil and with gravel mulch. However, its highest values, and those of SOC, are found in treatment T1 without vermicompost, which is probably due to the more labile nature of the organic matter.

To investigate the relationship between variables, data were subjected to correlation analysis (table 7). There were positive and significant correlations between all variables under study except for available phosphorus and alkaline phosphatase, and for available phosphorus and SOC, where no correlations were observed. Nevertheless, when data were assessed for individual organic amendment, no correlation was observed between phosphatase activities and available phosphorus in T1 amended soils, but T2 amended soils produced positive and significant ($p<0.01$) correlations between Pa and alkaline phosphatase ($r = 0.57$) and phosphodiesterase ($r=0.64$). In any case, whether for lack of correlation or for positive correlation, no type of enzymatic inhibition was observed for the amounts of Pa at plot level.

Table 7.- Pearson's correlation coefficients (bilateral) for available phosphorus (Pa), soluble phosphorus (Ps), inorganic phosphorus (Pi), alkaline phosphatase, phosphodiesterase and soil organic carbon (SOC). * significant at p<0.01.

	Ps	Pi	Alkaline phosphatase	Phosphodiesterase	SOC
Pa	0.764*	0.265*	0.032	0.234*	0.071
Ps		0.431*	0.33*	0.472*	0.35*
Pi			0.68*	0.755*	0.566*
Alkaline phosphatase				0.876*	0.684*
Phosphodiesterase					0.63*

Masciandaro et al. (1997) state that, in the case of treatments with vermicompost, a considerable part of available phosphorus and phosphatase activity is supplied by the organic fertilizer itself. The results of the above-mentioned correlations agree with this statement.

Time since planting gives rise to a significant decrease in the average values of all the variables ($p<0.01$) (Fig. 1).

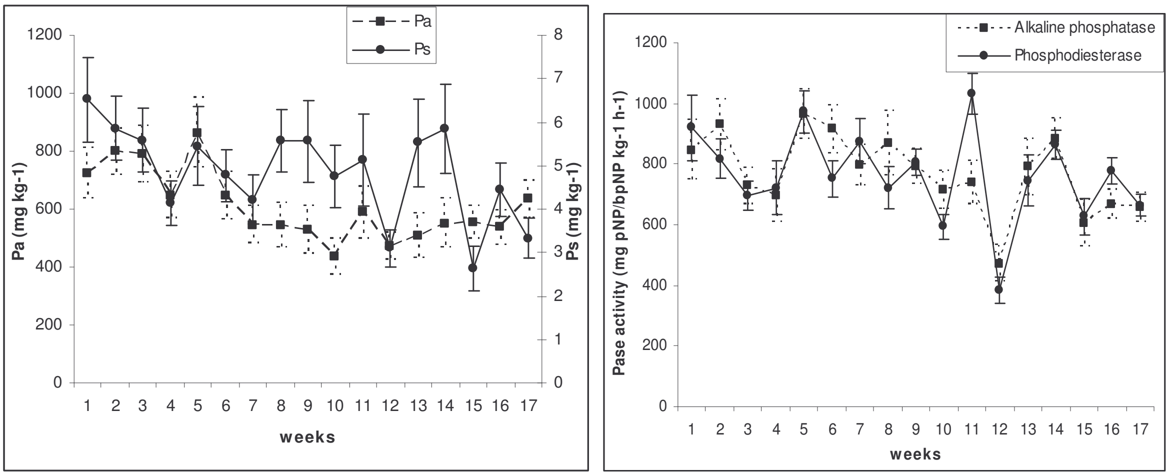

Fig. 1.- Evolution with time of average values for available phosphorus (Pa, mg kg^{-1}) and soluble phosphorus (Ps, mg kg^{-1}) (left) and Alkaline phosphatase (mg pNP kg^{-1} h^{-1}) and phosphodiesterase (mg bispNP kg^{-1} h^{-1}) activities (right) over the seventeen weeks of the assays. Error bars show one typical error for the mean.

Nevertheless, there are significant interactions between the factors studied and time. Figure 2 shows the gradual drop in Ps content over the sampling time, except for the M1S2 combination (P4), with worse drainage.

CONCLUSIONS

The effect of organic amendments depends on the nature of the fertilizer and the characteristics of the medium in which it is applied. On average the treatments with vermicompost gave rise to the highest values of available phosphorus and soluble phosphorus, although the type of mulch determined the final result of the organic amendments to a great extent, since it conditions the hydric dynamics of the underlying soil. The average values of the variables analyzed are greater in the plots with mulch of larger particle size. The lack of correlation, or the positive correlation between phosphatase activity and the concentrations of available or soluble phosphorus, do not permit the observation of enzymatic inhibition on the scale of this study.

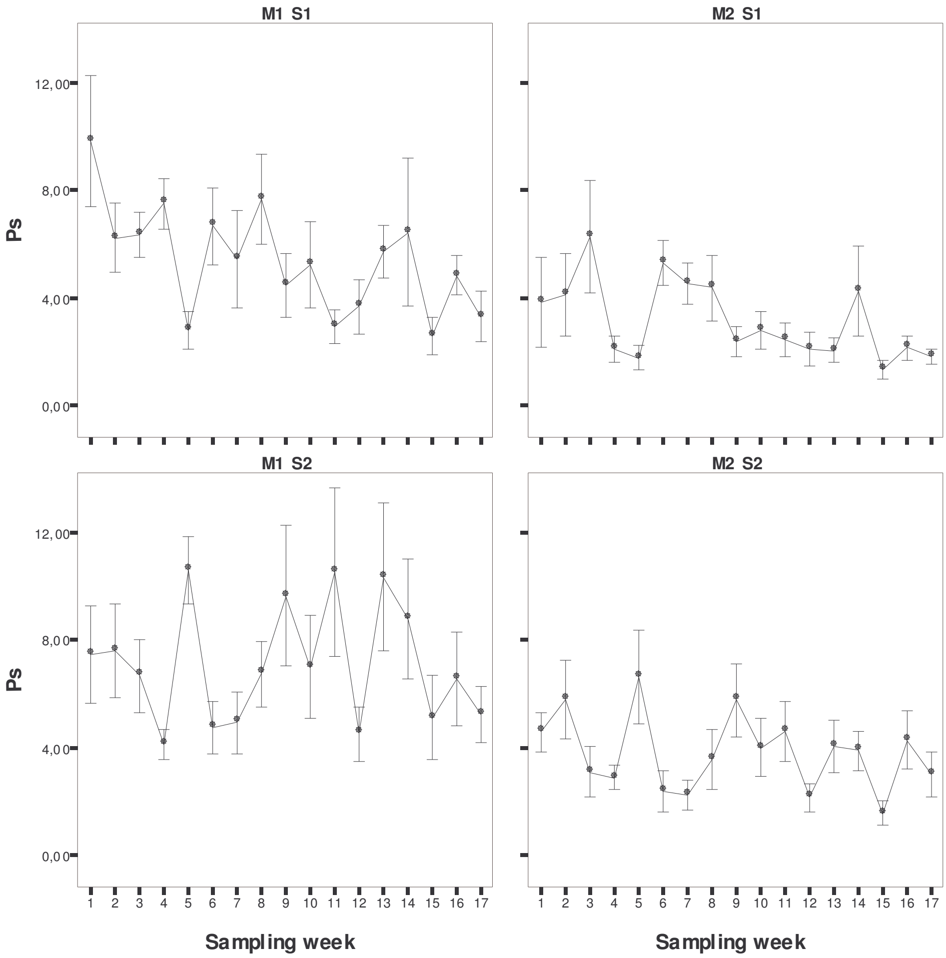

Fig. 2.- Evolution over time for soluble phosphorus (Ps, mg kg^{-1}) grouped by mulch type (M1 and M2) and soil type (S1 and S2). Error bars show one typical error for the mean.

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