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ORIGINAL PAPER

Quantitative assessment of hydrolase production and persistence in soil

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Abstract The aim of this work was to calculate indices of hydrolase production (Pr) and persistence (Pe) through simple arithmetical calculations. Changes in acid and alkaline phosphomonoesterase, phosphodiesterase, urease, protease, and β-glucosidase activities were monitored under controlled conditions in seven soils with a wide range of properties, in which microbial growth was stimulated by adding glucose and nitrogen. Glucose mineralization was monitored by CO_2–C evolution, and microbial growth was quantified by determining the soil adenosine triphosphate (ATP) content. Hydrolase Pr and Pe indices were numerically quantified by the following relationships: $Pr=H/t_H$ and $Pe=(r/H)\Delta t$, respectively, where H indicates the peak value of each measured hydrolase activity, t_H is the time of the peak value, r indicates the residual activity value, and Δt is the time interval $t_r - t_H$, where t_r is the time of the residual activity value. Addition of glucose and N-stimulated soil respiration increased ATP content and stimulated the production of the measured hydrolase activities in all soils; the measured variable reached a maximum value and then decreased, returning to the value of the control soil. Apart from β-glucosidase activity, whose activity was not stimulated by glucose and N addition, the other measured hydrolase activities showed a trend that allowed us to calculate the Pr and Pe indices using the above-mentioned equations. Acid phosphomonoesterase and protease Pr values were significantly higher in soils under forest or set aside management; the alkaline phosphomonoesterase and phosphodiesterase Pr values were generally higher in the neutral and alkaline soils, and the urease Pr values showed no obvious relationships with soil pH or management. Concerning the persistence of enzyme activities, Pe values of the acid phosphomonoesterase activity were significantly higher in the acidic soils, and those of urease activity were higher in acidic soils and the Bordeaux neutral soil. No relationships were observed between Pe values of alkaline phosphomonoesterase, phosphodiesterase, or protease activities and soil pH or management. The different responses of hydrolases were discussed in relation to soil properties, microbial growth, and regulation at the enzyme molecular level.

Keywords Hydrolase activity · Production · Persistence · Microbial growth · Soil type

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Introduction

Hydrolytic enzymes make nutrients available to plants and soil microorganisms from a wide range of complex substrates (Nannipieri et al. 2001) and are influenced by a wide range of soil properties such as pH, organic matter content and texture, and also by management (Speir and Ross 1978; Dick and Tabatabai 1992; Dick et al. 2000).

The increase in hydrolase activity during microbial growth induced by additions of organic compounds and nutrients to soil is long and well documented (Drobnik 1957; Zantua and Bremner 1976; Nannipieri et al. 1978, 1979, 1983; McCarty et al. 1992; Dilly and Nannipieri 2001; Allison and Vitousek 2005). Past studies have shown that enzyme production peaks and persistence depend on the amount and quality of substrate (Balasubramanian et al. 1972; Zantua and Bremner 1976; McCarty et al. 1992).

This behavior can be summarized as reported in Fig. 1. Some hydrolase activities can be produced in high amounts and display low persistence (enzyme A), some can be produced in lower amounts but persist longer after substrate consumption (enzyme B). Other trends (e.g., higher production and longer persistence or low production and short persistence) are also possible but, for simplicity, are not shown in Fig. 1.

The aim of our work was to calculate some indices of hydrolase production (Pr) and persistence (Pe) related to the catalytic capacity of soils with different properties through simple arithmetical calculations using a substrate-induced microbial response approach. Glucose and NH_4NO_3 were chosen as C and N sources, as they are readily used by a large fraction of soil microorganisms within 24–36 (Sparling 1995). This makes possible the estimation of hydrolase persistence within relatively short incubation periods. Small amount of N was added to avoid N-limiting conditions previously observed during microbial growth in some of the studied soils (Landi et al. 2006).

Materials and methods

Soil characteristics and treatments

Four Italian, one French, and two Mexican soils with a wide range of physical and chemical characteristics were used in this experiment. Three of the Italian soils were sampled from the long-term Vicarello experimental fields, located in Tuscany, Central Italy, under forest (Vic F), permanent grassland (Vic G), and continuous wheat crop (Vic W) management. The fourth Italian soil was sampled from the Vallombrosa (Val) fir forest also located in Tuscany, Central Italy. The French soil (Bor) was sampled from the AGIR long-term field trials (Bordeaux, France) under "set-aside" regime covered by a mixed grassland. The two Mexican soils were sampled from Sierra Norte of Oaxaca in Talea de Castro (Oax) under a coffee crop and from Puebla, Ahuatamimilotl, Municipio Tlatauquitepec, (Pue), under a mixed *Pinus-Quercus* spp. forest.

Soils were sampled from the A_0 horizon (forest and grassland soils) or from the A_p horizon (arable soils), and their main characteristics are reported in Table 1.

All soils were sieved (<2 mm) and stored at 4°C before use. Soils were then preincubated at 25°C and 40% WHC for 7 days to stabilize the microbial activity before treatment. After preincubation, soil samples equivalent to 500 g dry weight in triplicates were amended with 2 mg glucose-C g^{-1} and 0.2 mg NH_4NO_3-N g^{-1} dry weight soil as aqueous solutions and mixed thoroughly to achieve an even distribution of the amendment. Soils treated with deionized water only and mixed in the same way served as controls. Initial soil samples were immediately split in subsamples of 25 g (dry weight equivalent) each, incubated at 25°C separately, and destructively sampled for analysis so as to avoid the effects of disturbance at any sampling time. After soil amendment, the moisture content was 50% of WHC for all soils. Soil respiration, enzyme activities, and ATP content were measured after 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, 14, 20, 30, and 40 days.

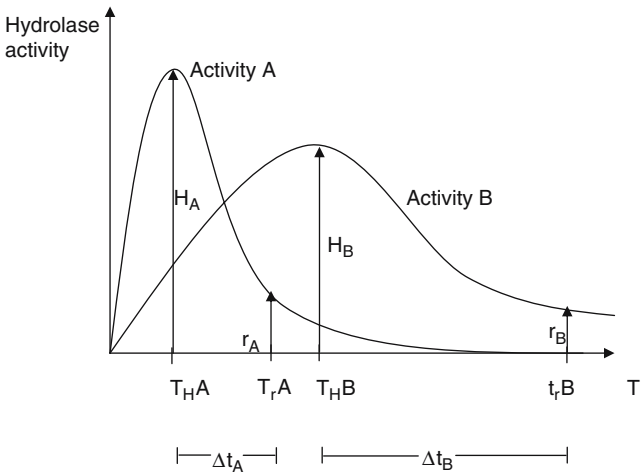

Fig. 1 Schematic representation of the hypothetical behavior of enzyme activities after substrate-induced microbial stimulation to illustrate the parameters used for the estimation of the hydrolase production and persistence indices. Values H_A and H_B are the peaks enzyme activities A and B, respectively; T_HA and T_HB are the incubation times at which the enzyme activities A and B show their peaks; r_A and r_B are residual activities of enzymes A and B, respectively; and T_rA and T_rB are the longest incubation times at which the enzyme activities A and B of the substrate amended soils are significantly higher than those of control soils

Soil respiration and ATP content measurements

Soil respiration was measured by placing soil samples equivalent to 25 g (dry weight equivalent) in 1-l air-tight conical flasks provided with three-way valves and incubated at 25°C in the dark. The CO_2–C evolution was measured by head-space gas sampling and gas chromatographic analysis by a gas chromatograph (HP 6890) equipped with a gas-sampling valve, a packed column (Porapack Q), and a thermal conductivity detector according to Blackmer and Bremner (1977). Unamended soils served as controls and empty flasks as blanks accounting for the CO_2–C background concentration. The CO_2 evolution was calculated by taking into account the CO_2–C solubility in soils with different pH values. No detectable changes of soil pH values occurred in the treated soils.

The ATP content of control and amended soils was measured according to Ciardi and Nannipieri (1990).

Table 1 Main properties of soils

Soil	Soil type[d]	Clay (%)	Silt (%)	Sand (%)	$pH_{(H2O)}$[a]	TOC[b] (%)	N_{tot}[c] (%)
Vic F	Vertic Xerochrept	42.2	33.0	24.8	8.0 (0.3)	2.2 (0.2)	0.22 (0.1)
Vic G	Vertic Xerochrept	42.2	33.0	24.8	8.1 (0.1)	1.8 (0.2)	0.19 (0.02)
Vic W	Vertic Xerochrept	42.2	33.0	24.8	8.0 (0.3)	1.1 (0.1)	0.09 (0.1)
Bor	Arenic Udifluvent	17.5	15.0	67.5	7.0 (0.1)	0.6 (0.1)	0.08 (0.01)
Val	Fragic Dystrudept	2.2	20.0	77.8	5.1 (0.2)	3.7 (0.8)	2.1 (0.2)
Oax	Anthropic Kandihumults	63.0	22.0	15.0	3.4 (0.2)	4.1 (0.3)	1.8 (0.4)
Pue	Andic Palehumults	50.0	26.0	24.0	3.7 (0.3)	14.8 (2.1)	4.1 (0.6)

Values in brackets are the standard deviation of the means (n=3).
[a] $pH_{(H2O)}$ values were measured using a soil-to-water ratio of 1:2.5.
[b] Total organic C was measured according to Walkley and Black (1938).
[c] Total N was measured by solid chromatography (Perkin Elmer CHNS/O 2400).
[d] Soil Survey Staff (1998)

Hydrolase activities measurements

Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969) and phosphodiesterase activity as reported by Browman and Tabatabai (1978). The β-glucosidase activity was measured according to Tabatabai (1982). The urease activity was assayed by the method of Nannipieri et al. (1978) by using 0.1 M of phosphate buffer at pH 7. Protease activity was determined by hydrolysis of N-benzoylargininamide (BAA) according to Ladd and Butler (1972). All enzyme activities were assayed at 37°C for 1 h with centrifugation of soil slurries at 6,000×g at 4°C. The concentration of p-nitrophenol (p-NP) produced in the assays of acid and alkaline phosphomonoesterase and phosphodiesterase and β-glucosidase activities was calculated from a p-NP calibration curve after subtraction of the absorbance of controls at 400 nm wavelength, using a spectrophotometer Lambda 2 (Perkin Elmer). The NH_4^+ produced by urease and BAA-hydrolyzing activities was determined by a flow injection analyzer (FIAStar, Tecator, SE). Recovery of NH_4^+ solutions shaken with soil for 1 h at 37°C and extracted with 2 M KCl was evaluated to account for the NH_4^+-fixing capability of clays of soils. The NH_4^+-N concentrations were in the range of those produced by urease and protease activities; this recovery varied from 92 to 98% (data not shown). Alkaline and acid phosphomonoesterase and phosphodiesterase activities were expressed as mmol kg^{-1} h^{-1} of p-NP at 37°C and at optimal pH, whereas urease and protease activities were expressed as mmol kg^{-1} h^{-1} of NH_4^+-N at 37°C and at optimal pH.

Net C mineralization rates, net hydrolase activities, with the exception of β-glucosidase activity, and net ATP contents were calculated by subtracting the values of the control soils from those of the glucose+N-amended soils. All treatments and measurements were replicated three times. The values of the β-glucosidase activity of the glucose+N-amended soils are reported as the proportion (%) of the β-glucosidase activity of the control soils.

Calculation of the hydrolase Pr and Pe and data analysis

Hydrolase Pr and Pe indices were calculated using net enzyme activity values calculated by subtracting the values of the control soils from those of the glucose+N-amended soils by the following relationships: $Pr=H/t_H$ and $Pe=(r/H)\Delta t$. In the two relationships, H indicates the peak value of the measured hydrolase activity, t_H is the time at which the peak value is detected, r is the residual net enzyme activity value, and Δt is the time interval $t_r - t_H$ (Fig. 1), where t_r is the time of the residual enzyme activity value. The t_r for each enzyme activity was taken as the last sampling time at which the net enzyme activity values were significantly greater than 0. Units used for calculation of the Pr and Pe indices were millimole of products and days for enzyme activities and incubation times, respectively.

The significance of differences of the means were calculated by a post hoc mean separation through the Tukey HSD test (P level <0.05). In the results section, symbols = and > indicate no significant differences and significant differences at the $P<0.05$ level between soils, respectively.

Results and discussion

To calculate the soil hydrolase Pr and Pe indices, it was essential to increase microbial activity and induce microbial growth in all soils. Results showed the addition of glucose and N-stimulated soil respiration and ATP content in all soils (Figs. 2 and 3). The daily respiration rates peaked after 1 day for the Vic F, Vic G, and Bor soils, after 2 days for the Val and Vic W soils, and after 3 and 4 days for the Pue and Oax soils, respectively (Fig. 2). The ranking order of

the soil net respiration rates for the different soils was as follows: Vall>(Vic F=Vic G Bor)>(Vic W=Pue)>Oax. Net ATP content peaked after 2 days in the Vic F and Vic G soils, after 4 days in the Bor and Pue soils, after 5 days in the Val soil, and after 10 days in the Vic W and Oax soils (Fig. 3). The ranking order of net soil ATP content for the different soils was: Vic F>Bor>Vic G>Vic W>Val>Pue=Oax (Fig. 3).

Hydrolase activities

Apart from β-glucosidase activity, all hydrolase activities measured in the amended soils showed a behavior similar to that proposed in Fig. 1, allowing the calculations of Pr and Pe indices within the incubation period.

Net acid phosphomonoesterase activity peaked after 6 days in the Vic F and Vic G, Val, Bor, and Oax soils, and after 7 days for Vic W and Pue soils (Fig. 4). The ranking order of net acid phosphomonoesterase activity for the different soils was as follows: (Pue=Val)>(Vic F=Bor)>Oax>Vic G>Vic W (Fig. 4). The last significant difference in the residual acid phosphomonoesterase activity between amended and control soils was detected after 12 days in the Vic F, 14 days in the Vic W soil, 16 days in the Bor soil, 20 days in the Vic G soil, and 30 days in the Val, Pue, and Oax soils (Fig. 4).

Net alkaline phosphomonoesterase activity peaked after 1 day in the Bor soil, 3 days in the Vic F soil, 5 days in the Vic W soil, 6 days in the Val soil, 7 days in the Vic G and Oax soils, and 10 days in the Pue soil (Fig. 5). The ranking order of net alkaline phosphomonoesterase activity for the different soils was as follows: (Vic W=Vic F=Vic G)>(Val=Bor)>Pue>Oax (Fig. 5). The last significant difference in the residual alkaline phosphomonoesterase activity between amended and control soils was detected after 12 days in the

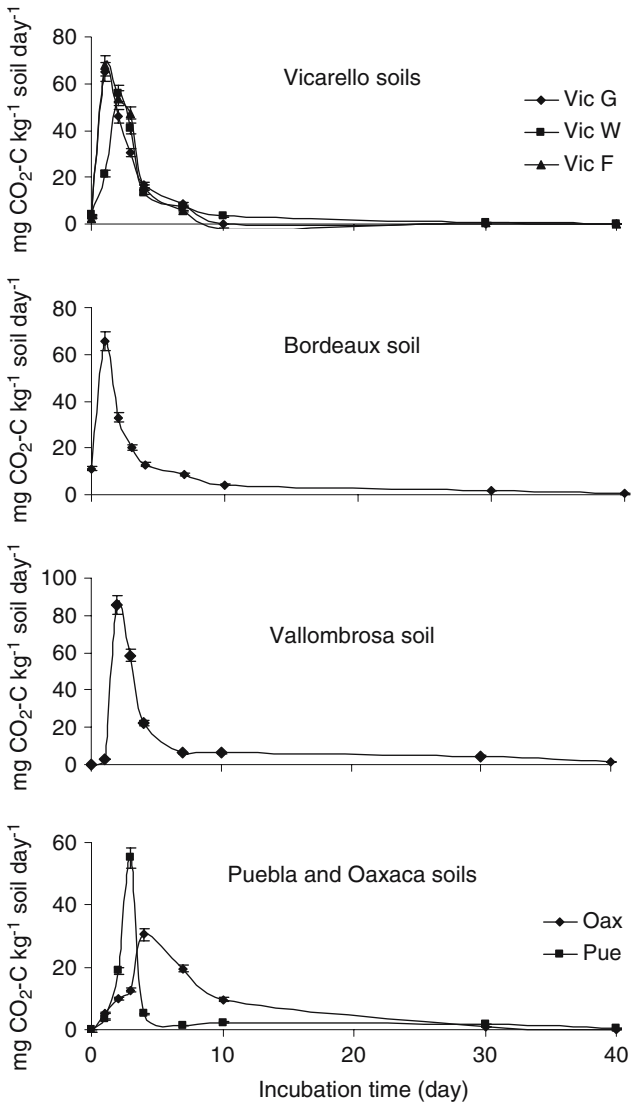

Fig. 2 Net CO_2–C evolution from the different soils. The *error bars* are the standard deviation of the means (n=3)

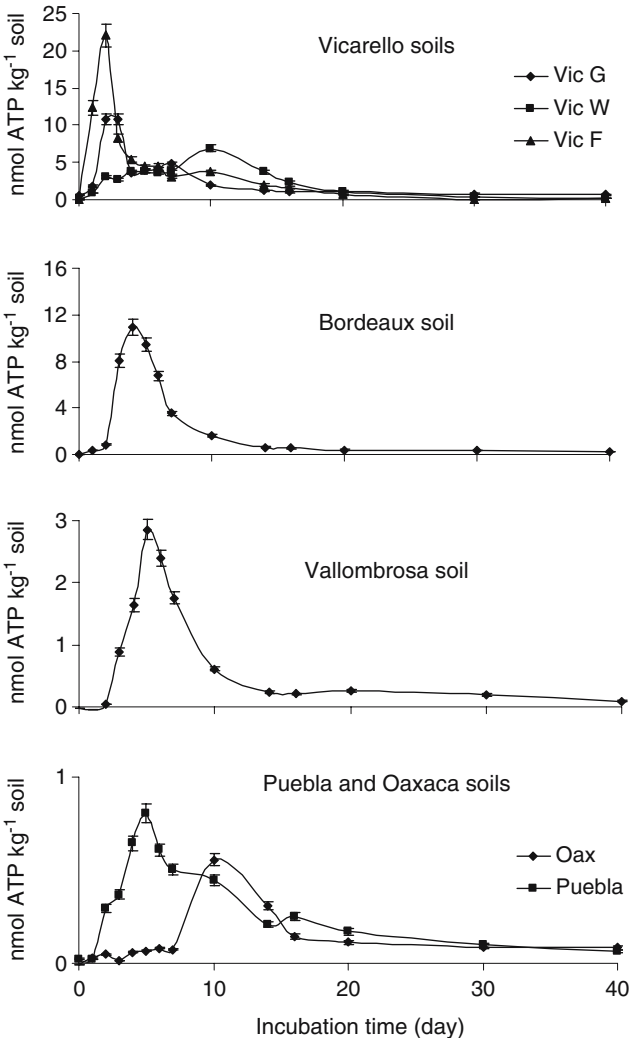

Fig. 3 Net ATP content of the different soils. The *error bars* are the standard deviation of the means (n=3)

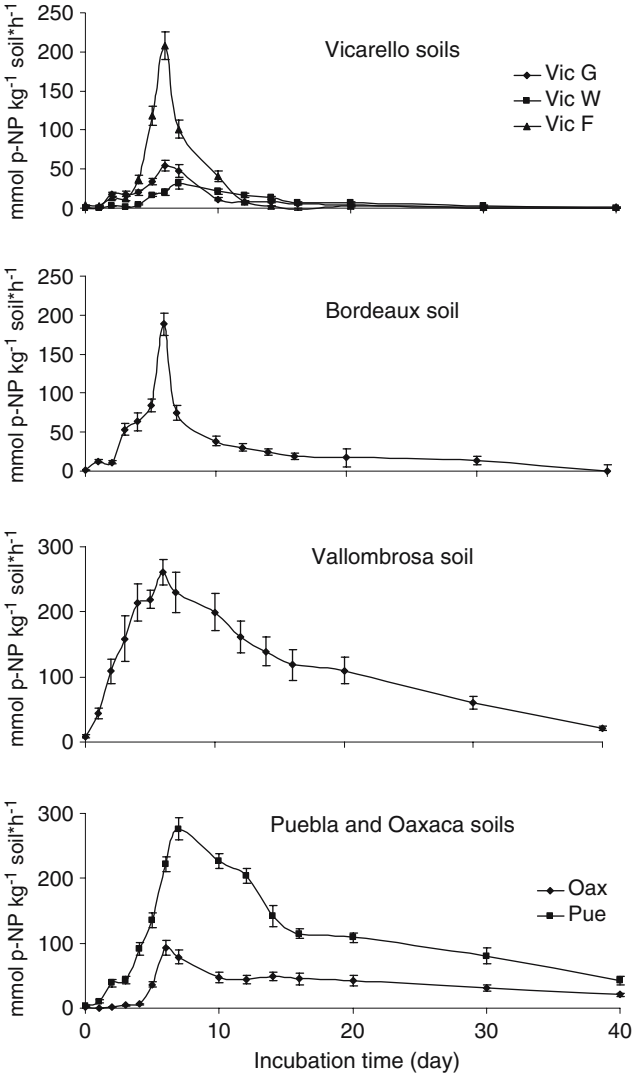

Fig. 4 Net acid phosphomonoesterase activity of the different soils. The *error bars* are the standard deviation of the means (n=3)

Vic F and Vic W soils, 14 days in the Bor and Vic G soils, 16 days in Val and Oax soils, and 20 days in Pue soil (Fig. 5).

Net phosphodiesterase activity peaked after 3 days in the Vic F and Bor soils, 4 days for the Vic W and Oax soils, 6 days for the Vic G soil, and 7 days in the Val and Pue soils (Fig. 6). The ranking order of net phosphodiesterase activity for the different soils was as follows: (Vic F=Vic G)>(Vic W=Val)>Pue>(Oax=Bor; Fig. 6). The last significant difference in the residual phosphodiesterase activity between amended and control soils was detected after 10 days in the Bor soil, 12 days in the Oax soil, 14 days in the Vic G and Vic W soils, 16 days in the Vic F and Pue soils, and after 20 days in Val soil (Fig. 6).

Net urease activity peaked after 2 days in the Bor and Pue soils, 3 days in the Vic F soil, 4 days in the Vic G and Vic W soils, 7 days in the Val soil, and after 16 days in the Oax soil (Fig. 7). The ranking order of net urease activity for the different soils was as follows: Val>Bor>(Pue=Vic G=Vic W)>Vic F>Oax (Fig. 7). The last significant difference in the residual urease activity between amended and control soils was detected after 7 days in the Vic G, Vic W, and Pue soils, 12 days in the Vic F soil, 14 days in the Val soil, 16 days in the Bor soil, and after 30 days in the Oax soil (Fig. 7).

Net protease activity peaked after 2 days in the Val, Vic F, and Pue soils, 3 days in the Vic G, Vic W, and Bor soils, and 10 days in the Oax soil (Fig. 8). The ranking order of net protease activity for the different soils was as follows: (Pue=Vic F)>(Bor=Val)>(Vic G=Vic W=Oax; Fig. 8). The last significant difference in the residual protease activity between amended and control soils was detected after 4 days in the Vic W soil, 5 days in the Vic G soil, 6 days in the Val soil, 7 days in the Bor and Pue soils,

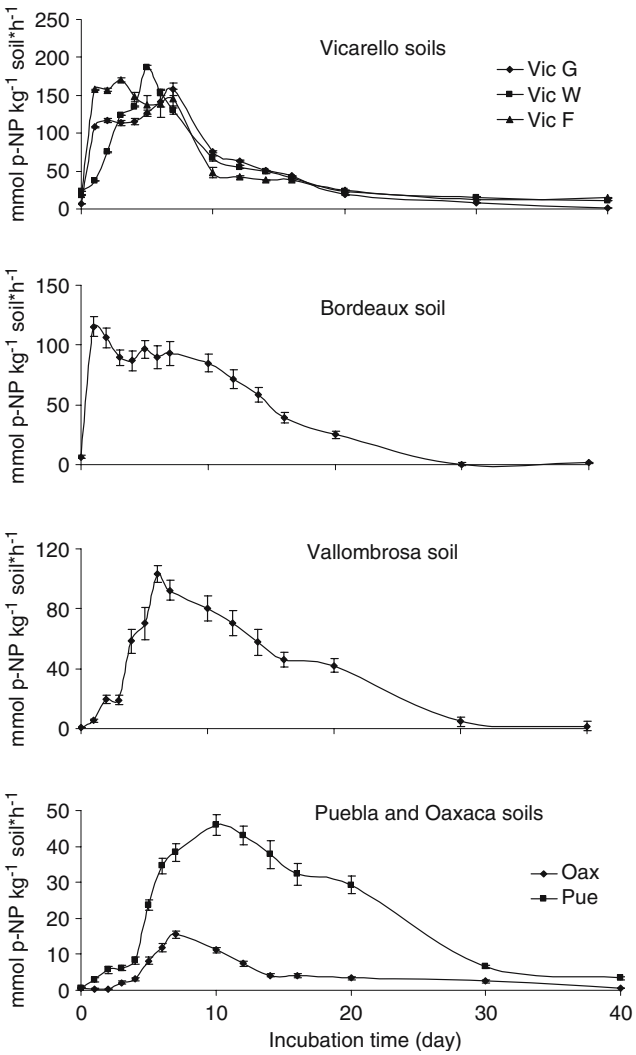

Fig. 5 Net alkaline phosphomonoesterase activity of the different soils. The *error bars* are the standard deviation of the means (n=3)

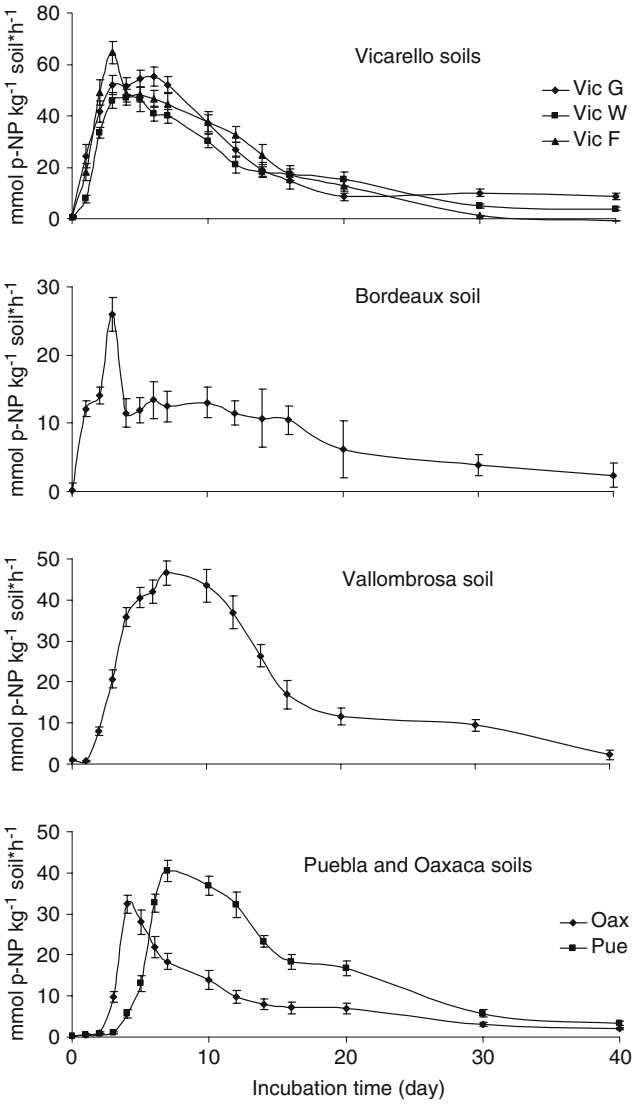

Fig. 6 Net phosphodiesterase activity of the different soils. The *error bars* are the standard deviation of the means (n=3)

10 days in the Vic F soil, and after 20 days in the Oax soil (Fig. 8).

The β-glucosidase activity was inhibited to a different extent in the studied soils immediately after the soil amendment, showing different trends in the various soils throughout the incubation (Fig. 9).

Production of hydrolytic enzymes by soil microorganisms is likely to be a major event when microbial growth is initiated by availability of organic substrates. However, microorganisms do not synthesize enzymes if their potential substrates are not present in the environment (Allison and Vitousek 2005). In the studied soils, not all of the measured hydrolases were produced to the same extent, and generally, enzyme production was not precisely synchronized with microbial growth, as peaks of phosphatase, urease, or protease production preceded or followed those of soil ATP content in the different soil types. Correlations between hydrolase activities and microbial production in soil have been previously reported (Dilly and Nannipieri 2001).

Indices of hydrolase production and persistence

Apart from β-glucosidase activity, hydrolase activities showed a trend that allowed us to calculate the Pr and Pe indices using the above-mentioned equations. Values of the indices of enzyme production and persistence are reported in Table 2.

The ranking order of Pr values was (Vic F=Val=Pue)> Bor>Oax>Vic G>Vic W for acid phosphomonoesterase, Bor>Vic F>Vic W>(Vic G=Val)>Pue=Oax for alkaline phosphomonoesterase, Vic F>(Vic G=Vic W=Bor)>Oax> (Val=Pue) for phosphodiesterase, Bor>(Va=Pue)>(Vic F=

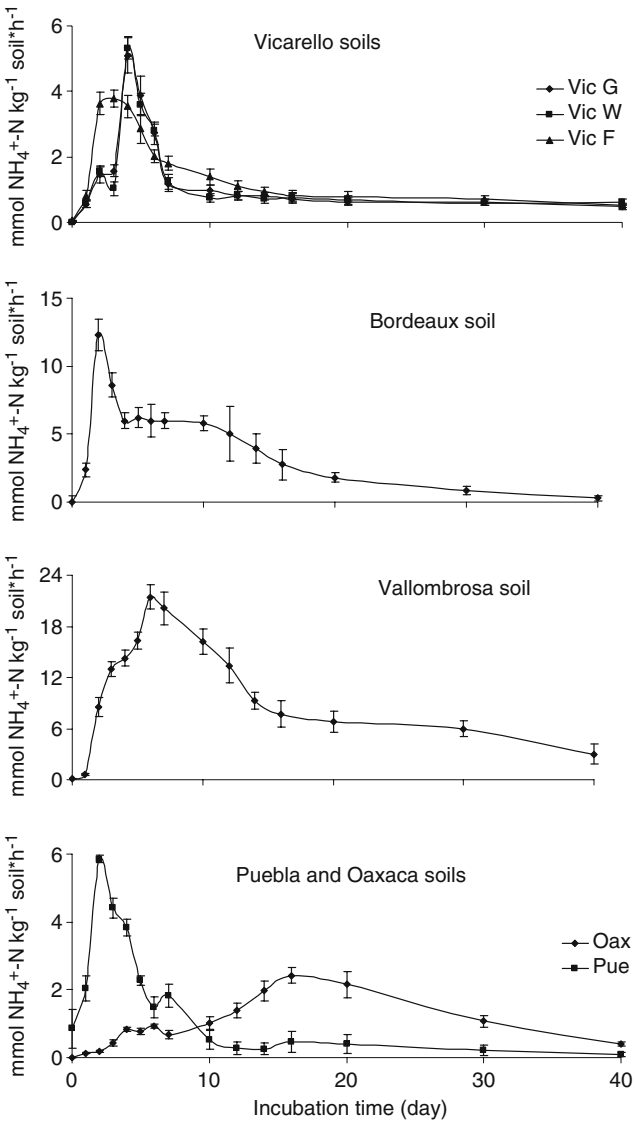

Fig. 7 Net urease activity of the different soils. The *error bars* are the standard deviation of the means (n=3)

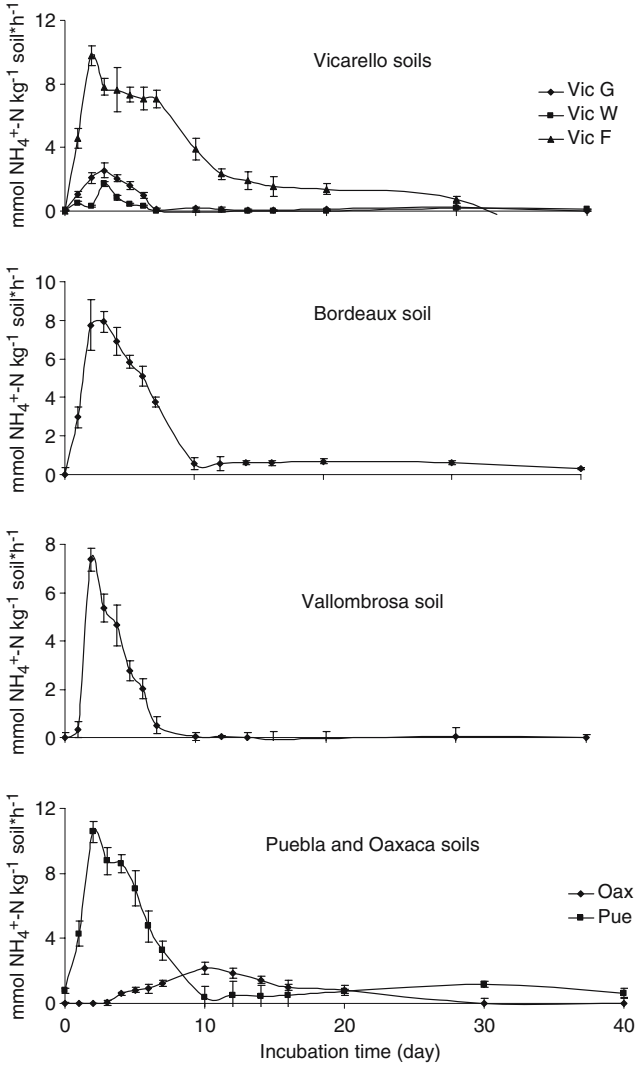

Fig. 8 Net protease activity of the different soils. The *error bars* are the standard deviation of the means (n=3)

Vic G=Vic W)>Oax for urease, and (Vic F=Pue)>Val> Bor>(Vic G=Vic W)>Oax for protease (Table 2).

The ranking order of Pe values was (Pue=Oax)>Val> Vic W>(Vic G=Bor)>Vic F for acid phosphomonoesterase, Pue>(Vall=Bor)>(Vic F=Vic G=Vic W=Oax) for alkaline phosphomonoesterase, (Pue=Vic F=Vic W= Bor=Val)>(Vic G=Oax) for phosphodiesterase, (Val= Bor)>(Vic F=Oax)>Pue>(Vic G=Vic W) for urease, and (Vic F=Oax)>(Vic G=Bor=Val=Pue)>Vic W for protease activities (Table 2).

Concerning the relationship between the calculated hydrolase Pr and Pe indices and soil properties and management, our results showed that urease activities showed significantly greater Pr values in acidic and neutral soils and significantly greater Pe values in acidic soils (Table 2); this probably resulted from the need to mobilize N in these N-limited soils (Landi et al. 2006), despite the addition of NH_4NO_3. It has been demonstrated that the urease synthesis by soil microorganisms is not only dependent on the availability of mineral N but by the N metabolites produced by the immobilization of N taken up by microbial cells (McCarty et al. 1992). The acid phosphomonoesterase and protease activities were produced significantly more in forest than in other soils probably because of the quality of the soil organic matter (Lahdesmaki and Piispanen 1988) or to the greater microbial growth in these soils (Dilly and Nannipieri 2001). Moreover, the alkaline phosphomonoesterase and phosphodiesterase activities showed significantly greater Pr values in alkaline than other soils (Table 2), confirming previous findings that the phosphomonoesterase activity with a pH optimum close to soil pH was produced in higher quantities and persisted longer than the phosphomonoester-

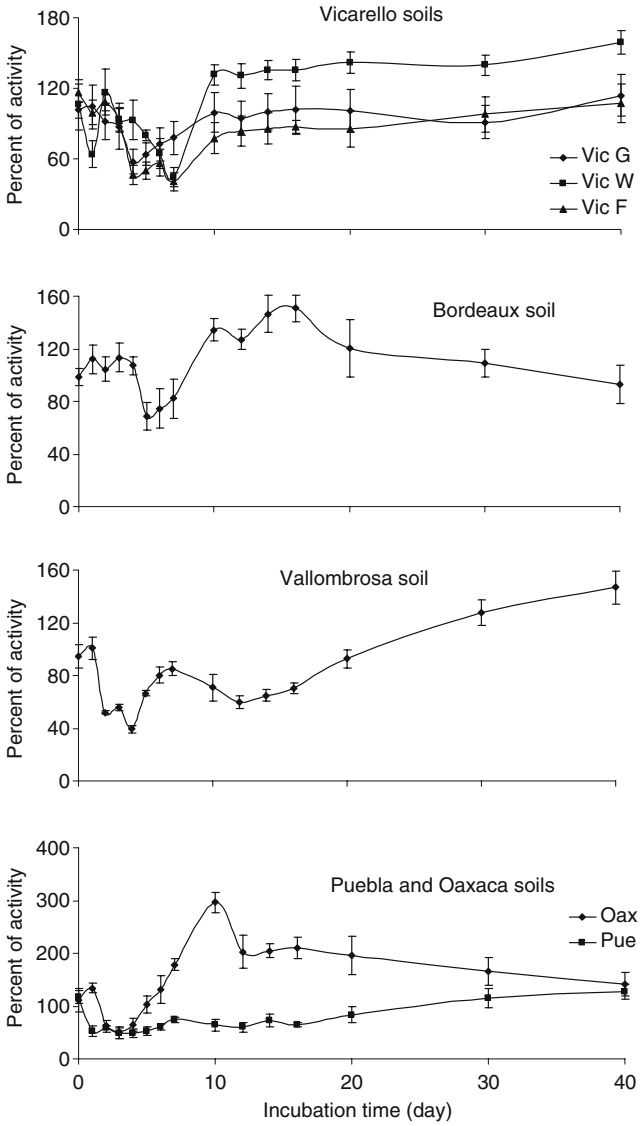

Fig. 9 β-glucosidase activity of the glucose+N-amended soils. The values on the *y-axis* represent the percent of the β-glucosidase activity as compared to that of unamended soils. The *error bars* are the standard deviation of the means (n=3)

Table 2 Production and persistence indices values of the different hydrolase activities

Soil	Enzyme activity									
	Phosphomonoesterase acid		Phosphomonoesterase alkaline		Phosphodiesterase		Urease		Protease	
	Pr	Pe	Pr	Pe	Pr	Pe	Pr	Pe	Pr	Pe
Vic F	34.7^{a}	0.22^{e}	56.9^{b}	2.24^{c}	21.5^{a}	3.48^{a}	1.26^{c}	2.62^{b}	4.90^{a}	3.19^{a}
Vic G	9.0^{d}	0.92^{d}	22.5^{d}	2.23^{c}	9.18^{b}	2.75^{b}	1.27^{c}	0.68^{d}	0.85^{d}	1.28^{b}
Vic W	4.4^{e}	3.06^{c}	37.3^{c}	2.08^{c}	11.8^{b}	3.87^{a}	1.33^{c}	0.70^{d}	0.57^{d}	0.49^{c}
Bor	31.4^{b}	1.00^{d}	115.3^{a}	4.12^{b}	8.66^{b}	3.51^{a}	6.15^{a}	3.13^{a}	2.64^{c}	1.91^{b}
Val	43.4^{a}	5.57^{b}	17.2^{d}	4.44^{b}	6.65^{d}	3.24^{a}	3.58^{b}	3.45^{a}	3.68^{b}	1.10^{b}
Oax	15.5^{c}	8.09^{a}	2.21^{e}	2.33^{c}	8.09^{c}	2.42^{b}	0.15^{d}	2.49^{b}	0.22^{e}	3.70^{a}
Pue	39.4^{a}	6.66^{a}	4.60^{e}	6.38^{a}	5.78^{d}	4.05^{a}	2.93^{b}	1.56^{c}	5.30^{a}	1.54^{b}

Different superscripts denote significant differences ($P<0.05$) between different soils within each column. The Pr values are in millimole of hydrolase activity product *day^{-1}; the Pe values are in millimole of residual hydrolase activity *day^{-1}.

ase activity with a pH optimum further away from soil pH (Renella et al. 2006). Significant correlations between the hydrolase Pr and Pe indices and some soil properties were found (e.g., alk phosphomonoesterase Pr—clay content, $r=0.70$; ac phosphomonoesterase Pe—soil pH, $r=0.87$). However, enzyme production and stabilization in soil unlikely depend on a single factor but more likely by the interaction of more soil factors. In this contest, a more valid conclusion might be drawn by analyzing a larger set of soils.

Differences in Pr values of the studied hydrolytic enzymes in the different soils were unlikely related to the microbial community composition because such enzymes are produced by different species, unless we hypothesize that different microbial species synthesize different amounts of hydrolytic enzymes. No relationship between phosphomonoesterase production and persistence and bacterial community structure were found in different soils by Renella et al. (2006). Microorganisms can control enzyme synthesis at different levels, e.g., regulation of gene transcription, posttranscriptional modification of mRNA, and protein cytoplasmic processing after protein synthesis. Allison and Vitousek (2005) showed that regulation at the level of transcription in soil microorganisms (e.g., enzyme induction, enzyme repression) may be important. This was likely the case with β-glucosidase activity in our experiment; its synthesis was inhibited in all soils until all the added glucose had been exhausted (Fig. 9). It is difficult to assess the induction or repression of enzyme synthesis in soil because the available enzyme assays do not distinguish the activity of intracellular (either inducible or constitutive) enzymes from that of enzymes stabilized by soil colloids, which do not respond to addition of nutrients (Burns 1982; Nannipieri et al. 2001). These methodological limitations do not allow investigation of the regulation of the activity at the level of enzyme molecule (e.g., allosteric regulation, feedback inhibition, catabolite repression). However, it has been reported that by increasing nutrient availability, the activity of different soil enzymes is reduced (Clarholm 1993; Sinsabaugh and Moorhead 1994). Possibly, both enzyme production and persistence may depend on the quality of substrate used to induce microbial growth (Balasubramanian et al. 1972; Allison and Vitousek 2005). In vitro studies have shown that glucose represses many inducible enzymes in different microorganisms, mainly blocking the synthesis of cyclic AMP (cAMP) required to activate the catabolite activator protein, which in turn binds to different RNA polymerase promoters (Busby and Ebright 1999). Generally, at high glucose availability, the intracellular levels of cAMP are low, and several positively controlled promoters are not expressed thus preventing indirectly the synthesis of enzymes that metabolize other substrates for growth. The C:N ratio values of the soil amendment can also affect the hydrolase production, as demonstrated by the work by Zantua and Bremner (1976), McCarty et al. (1992), and Allison (2005).

Hydrolase persistence in soils is likely influenced by several abiotic and biotic factors. It is generally assumed that soil factors such as texture, organic matter content, and pH value play major roles in stabilizing the released proteins and that association with clays and/or humic substances can protect the extracellular enzymes from the microbial decomposition (Burns 1982; Nannipieri 1995). In our study, no significant stabilization of the measured hydrolase activities was observed after the microbial growth phase (Figs. 4, 5, 6, 7, 8, and 9). With the used enzyme assays, it is not possible to determine the contribution of extracellular enzymes stabilized by soil colloids or pericellular enzymes associated to the surviving microbial cells to the residual measured enzyme activity (Skujins 1978; Burns 1982). The Pr values of different hydrolases responded to different soil management for a given soil type. In fact, in the Vic F soil, the Pr values of acid and alkaline phosphomonoesterase, phosphodiesterase,

and protease were significantly higher than in Vic W and Vic G soils as well as in the two Mexican Ultisols; the Pr values of acid phosphomonoesterase, urease, and protease were significantly higher in the Pue (forest) than in the Oax (coffee crop) soil. This leads us to suggest that the hydrolase Pr index might be used to assess the impact of management practices on different soils.

In conclusion, our work showed that it was possible to quantitatively assess the hydrolase production and persistence in soils with different properties through simple arithmetical calculations using a substrate-induced microbial response approach. The presented approach might be used for assessing either the effects of substrates stimulating microbial growth or the effects of management practices on the enzyme activity of soils.

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