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Soil Biology & Biochemistry 38 (2006) 795–802

Soil Biology & Biochemistry

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# Phosphomonoesterase production and persistence and composition of bacterial communities during plant material decomposition in soils with different pH values

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Received 8 July 2005; accepted 15 July 2005
Available online 24 August 2005

**Abstract**

The aim of this work was to study the synthesis and persistence of acid and alkaline phosphomonoesterases in three soils with different pH values amended with ryegrass residues. The organic input increased soil respiration, as estimated by $CO_2$–C evolution in all soils. The ATP content of the three soils showed a 3–7-fold increase between 7 and 10 d in the different soils since the amendment. The dsDNA content of the three amended soils also peaked between 7 and 10 d, increasing by 2.5–3.5 times in the different soils. The bacterial species richness increased in the amended as compared to the control soils during the early stages (7–10 d) of organic matter decomposition, as indicated by the decreasing values of the Sørensen similarity index between the treatments in this period. Soil amendment increased the alkaline phosphomonoesterase activity by 6, 8 and 15 times in the Vallombrosa acidic, Romola neutral and Vicarello alkaline soil, respectively, whereas the acid phosphomonoesterase activity showed a 6-, 2- and 10-fold increase in the Vallobrosa acidic, Romola neutral and Vicarello alkaline soil, respectively. Phosphatase activities peaked between 4 and 10 d, depending on the activity and the soil considered, but activity of alkaline phosphomonoesterase was higher in alkaline soils and persisted longer than the acid phosphomonoesterase activities; the opposite occurred in the acid soil. During a 180 d decomposition period, both acid and alkaline phosphomonoesterase activities were related to dsDNA and ATP contents in all soils. Peaks of phosphmonoesterase activity coincided with the changes in the composition of the bacterial microflora, as detected by 16S-rDNA analysis, although no relationship between bacterial community composition and persistence of the phosphomonoesterase activities could be shown. It was concluded that acid and alkaline phosphomonoesterases are produced in greater amounts during plant residue decomposition, and that in acid soils acid phosphomonoesterase activity predominates and in neutral and alkaline soils alkaline phosphomonoesterase activity predominates. However, the persistence of newly produced enzymes is determinated by other factors such as soil texture, organic matter content and formation of soil colloid–enzyme complexes.


*Keywords:* Phosphomonoesterase activity; Organic matter decomposition; Soil pH; Microbial growth; Microbial community structure

## 1. Introduction

Phosphomonoesterase activity has been measured in soils with pH values ranging from 4 to 9 (Speir and Ross, 1978). Usually, in acidic soils the acid phosphomonoesterase activity dominates whereas in neutral and alkaline soils the alkaline enzyme activity dominates (Speir and Ross, 1978; Dick et al., 1988, 2000; Eivazi and Tabatabai, 1977; Dick and Tabatabai, 1992; George et al., 2002). This behaviour may depend on the different composition of soil microflora at different soil pH value, with microflora of acidic and alkaline soils producing greater amounts of acid and alkaline phosphomonoesterase activity, respectively. However, the link between microbial community composition and enzyme activities in soil is still unclear (Nannipieri et al., 2003).

Enzymes can have different locations in soils being present in viable cells, cell debris, as extracellular enzymes stabilised by soil colloids, or as free enzymes in the soil solution (Burns, 1982; Nannipieri et al., 2002). Short-term enzyme assays that exclude microbial growth and limit the movement of substrate into cells, are believed to measure extracellular enzyme activities (Burns, 1982; Klose and Tabatabai, 1999).

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0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.07.005

The relationship between enzyme synthesis and microbial growth has been studied by measuring changes in enzyme activity during microbial growth induced by additions of organic compounds to soil (Nannipieri et al., 2002). Changes in microbial biomass have been monitored by measuring the ATP content or the microbial biomass by fumigation–extraction method. However, both techniques are not accurate for measuring microbial growth immediately after the addition of available organic C to soil (Nannipieri et al., 2003). This problem can been solved by monitoring the dsDNA content after the addition of available C to soil (Marstorp and Witter, 1999). However, this technique has not been applied to study the link between enzyme synthesis and microbial growth in soil.

The assessment of microbial diversity in soil using traditional techniques has been problematic because only a minor proportion of soil microorganisms is culturable on synthetic media (Torsvik et al., 1994). Thus information on microbial diversity provided by plate counts or by the metabolic fingerprinting-approach (BIOLOG) is limited (Garland and Mills, 1991; Insam, 1997). Unculturable soil microorganisms can be determined by molecular techniques based on direct extraction of DNA from soil, its amplification by polymerase chain reaction (PCR) and its characterization by the denaturing or temperature gradient gel electrophoresis (DGGE and TGGE) (Muyzer and Smalla, 1998).

The aim of this work was to study the mechanisms responsible for the prevailance of alkaline and acid phosphomonoesterase activities in alkaline and acidic soils. Phosphomonoesterase activities were monitored upon addition of decomposable organic C and compared with microbial growth determined by either dsDNA or ATP contents. The bacterial diversity was determined by the PCR–DGGE technique. Ryegrass residues were used because it has been reported that it induces and sustains longer the growth of a larger fraction of soil microflora than simple substrates such as glucose (Wu et al., 1993).

## 2. Materials and methods

### 2.1. Soil characteristics and ryegrass treatments

Three forest soils with contrasting pH and texture were used in this experiment (Table 1). Soils were sampled from the $A_0$ horizon, sieved ($<2$ mm) and incubated at 25 °C and 50% WHC for 7 d prior to use. After pre-incubation soils were split in two subsamples: one was amended with 20 g $kg^{-1}$ soil dried-milled ryegrass (48.1% C, 3.4% N) and mixed by hand to achieve an even distribution of the organic amendment; the other was mixed in the same way, but without the organic amendment. Soils were assayed after 6 h and 1, 2, 4, 7, 10, 30, 60, 90 and 180 d.

### 2.2. Soil respiration, phosphomonoesterase activities and ATP and dsDNA content

Soil respiration was measured by placing soil samples equivalent to 50 g (dry matter equivalent) in 1 L air-tight conical flasks provided with three-way valves and incubated at 25 °C in the dark. Unamended soils served as controls and empty flasks as blanks accounting for the $CO_2$ background concentration. The $CO_2$ evolution was measured by headspace gas sampling and gas chromatographic analysis (Blackmer and Bremner, 1977) and the pH-dependence of $CO_2$ evolution from soils with different pH values was taken into account using $CO_2$ solubility coeffiecients.

The acid (EC 3.1.3.2) and alkaline phosphomonoesterase (EC 3.1.3.1) activities were assayed according to Tabatabai and Bremner (1969), using modified universal buffer (MUB) at pH 6.5 and 11, respectively. The ATP content was measured according to Ciardi and Nannipieri (1990).

The soil total dsDNA was extracted with a bead-beating method (FastDNA SPIN Kit for soil, Bio 101 Inc., USA) using 0.5 g soil samples (dry weight equivalent) and quantified by fluorometer (Hoefer™ DyNA Quant™ 200) using bisbenzimide as the fluorochrome (Hoechst H 33258).

### 2.3. Bacterial community structure

The bacterial community structure was studied by polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE). Whole-community DNA was extracted as described above. The 16S rDNA was amplified using the eubacterial primers GC-968f: 5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TA-3′ and 1401r: 5′-GCG TGT GTA CAA GAC CC-3′ (Felske et al., 1997), with a Perkin–Elmer 2400 thermocycler, under reaction conditions of 94 °C for 90 s followed by 33 cycle of 95 °C for 20 s, 56 °C for 30 s, 72 °C for 45 s, and final extension at 72 °C for 7 min. The DGGE profiles were obtained using the Dcode System (Universal Mutation Detection System, Biorad)

Table 1
Soil properties

| Soil type | pH ($H_2O$) | Sand (%) | Silt (%) | Clay (%) | TOC[a] (%) | N tot (%) |
|---|---|---|---|---|---|---|
| Vallombrosa (fragic distrudept) | 5.1 | 77.8 | 20.0 | 2.2 | 3.7 | 2.1 |
| Romola | 7.2 | 81.9 | 6.7 | 11.4 | 0.7 | 0.07 |
| Vicarello (vertic haploxerept) | 8.1 | 20.5 | 33.0 | 42.2 | 2.2 | 0.22 |

[a] TOC is the soil total organic C determined according to Walkley and Black (1938).

after loading 300 ng of DNA onto a 6% polyacrylamide gel with a denaturant gradient ranging from 46 to 56%, where 100% denaturant contains 7 M urea and 40% (vol./vol.). The gels were run at constant temperature (60 °C) and voltage (75 V) for 16 h and stained for 2 h with SybrGreen I nucleic acid gel stain 1× (FMC Bio Products, Rockland, ME, USA) afterward. The DNA bands were visualised and photographed using a UV-light gel transillumination. A schematic DGGE profile was manually designed relying on the DGGE banding patterns. The DNA extraction, amplification and fingerprinting were replicated two times for each soil, treatment and incubation time. Generally high similarity and reproducibility were observed between two DGGE replicates.

### 2.4. Data analysis

The similarity of the DGGE profiles was assessed by the Sørensen index of similarity (Sørensen, 1948) as: $S_{1,2}=2a/(2a+b+c)$, where $a$ is the number of bands shared by both samples, $b$ is the number of bands in sample 1 and $c$ is the number of bands in sample 2. Therefore, a Sørensen index value approaching 0.5 indicates high similarity in the composition of eubacterial communities (Nakatsu et al., 2000). Our working definition was that two bands are in common if they migrated at the same distance; marker bands of *B. subtilis* amplicons of known migration profiles were used as reference, as suggested by Griffiths et al. (2002).

All treatments and biochemical measurements were replicated three times. The net mineralisation rates, net phosphomonoesterase activities, and net ATP and dsDNA contents were calculated by subtracting the values of the control soils from those of the ryegrass amended soils. The significance of differences of the means were calculated with the Tukey–Kramer test (Statwiew 5, SAS Institute).

## 3. Results

The amount of dsDNA associated with the ryegrass and added to soil was 0.45 mg $kg^{-1}$ soil. This value was subtracted from total DNA values of the ryegrass-amended soils throughout. The DGGE-fingerprinting of DNA extracted from the plant residues showed three-bands (Fig. 4, lane R), whereas no ATP, acid and alkaline phosphomonoesterase activity were decteted in plant residues (data not shown).

### 3.1. Vallombrosa acidic soil

About 45% of the organic C added was evolved as $CO_2$–C during the 180 d of incubation (Fig. 1). The net ATP content peaked between 7 and 30 d after soil amendment and declined to the initial value at the end of the incubation (Fig. 1).

The net alkaline phosphomonoesterase activity showed a rapid 6-fold increase within 4 d after the plant residue addition and then declined rapidly to reach the initial value at the end of the incubation (Fig. 1). The net acid phosphomonoesterase activity peaked after 10 d, reaching a 6-fold increase and then slowly declined and was twice the initial value at the end than that measured at the beginning of the incubation (Fig. 1).

The net dsDNA did not significantly increase during the first 2 d after the plant residue amendment, and showed a 6-fold increase from d 4 to 10; then it decreased reaching the initial value after 90 d of incubation (Fig. 1).

The DGGE fingerprints of both amended and control soils showed five–six bands, in the 0–7 d incubation period; this number increased to 11 at both 90 and 180 d (Fig. 4). In both treatments five bands were visible throughout whereas only a few bands were specific to the ryegrass-amended soils in the 0–7 d incubation period (see the arrows in Fig. 4). The Sørensen index of similarity ranged from 0.44 to 0.50, with the latter value reached after 90 d, indicating a high similarity between eubacterial communities of the ryegrass-amended and control soils. Of the three-bands probably attributable to bacterial populations associated with ryegrass (Fig. 2, lane R), only one persisted throughout whereas the other two disappeared once the residue was incorporated into soil. However, they both reappeared after 180 d (Fig. 2).

### 3.2. Romola neutral soil

About 40% of the C added as plant residues was evolved as $CO_2$–C after 6 months (Fig. 2). The net ATP content peaked after 7 d and then declined slowly throughout (Fig. 2).

The net alkaline phosphomonoesterase activity increased rapidly after the organic amendment reaching a 8-fold increase 7 d after the soil amendment (Fig. 2). The increase in the net acid phosphomonoesterase activity was less pronounced and peaked (2-fold increase) at 10 d (Fig. 2).

The net dsDNA content peaked at 10 d but declined thereafter and was twice the initial value after 180 d (Fig. 2).

Five bands characterised the 16S rDNA-DGGE fingerprint at the beginning of the incubation (Fig. 4). The amendment with plant residues increased the number of dominant bands to 10 after a 7 d incubation (Fig. 4, lane 7+). The calculated Sørensen index value of 0.13 indicates low similarities between bacterial communities in the control and the ryegrass-amended soils. An increase in the number of bands, representing numerically dominant bacterial species, was observed in both the amended and control soils on prolonged incubation (Fig. 4, lanes 90−, 90+, 180− and 180+). Both fingerprints were similar in the 90–180 d incubation period, as Sørensen index values were 0.50 and 0.49, respectively. The three-bands associated with ryegrass (lane R) disappeared in the 0–7 d incubation period but reappeared after 90 d.

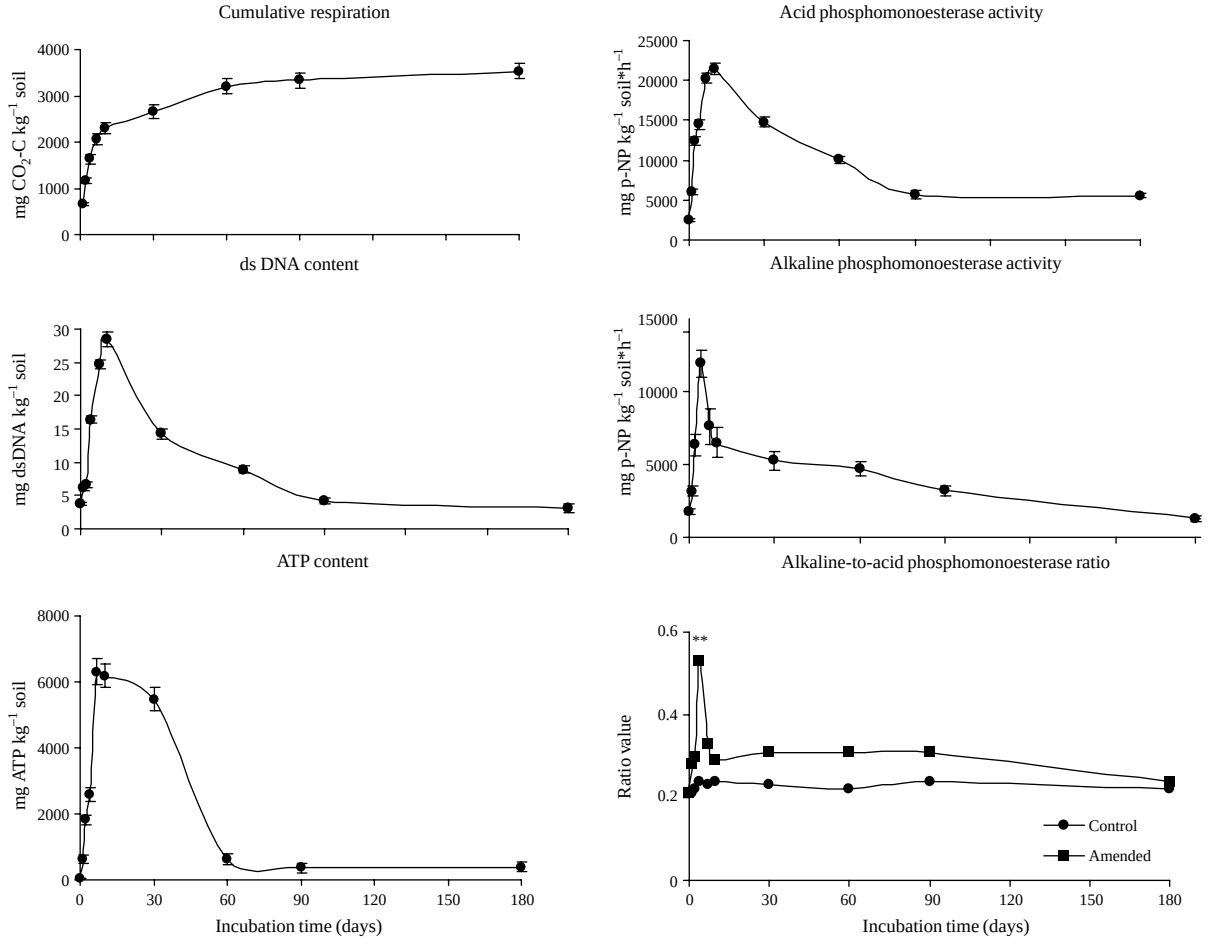


Fig. 1. Cumulative respiration, alkaline and acid phosphomonoesterase activities, alkaline-to-acid phosphomonoeseterase activity ratio, and ATP and dsDNA contents of the Vallombrosa acidic soil. Values were calculated by subtracting the values of the control soils from those of the ryegrass-amended soils. Error bars represent the standard deviation of the means ($n=3$). Symbol ** in the alkaline-to-acid phosphomonoeseterase activity ratio chart indicate significant differences at $P$ level of 0.01.

### *3.3. Vicarello alkaline soil*

About 40% of the added organic C was evolved as $CO_2$–C by the end of the incubation period (Fig. 3). The net ATP content peaked 30 d after the organic amendment and then declined reaching the initial value after 6 months (Fig. 3).

The net alkaline phosphomonoesterase activity peaked at d 10 and this value was 15 times higher than the initial value (Fig. 3); the acid phosphomonoesterase activity also peaked at d 10 when it was 10 times greater than at d 0 (Fig. 3).

The net dsDNA content increased up to 7 d to values 12-fold greater than at 0 d and then decreased to a value approximately five times higher than the initial value after 180 d (Fig. 3).

The DGGE profiles of the ryegrass-amended soil at the beginning of the incubation were characterised by two additional bands (see the arrows of lane 0+ in Fig. 4). The DGGE profiles of the amended and the non amended soils showed a different number of bands (8 and 9, respectively) after 7 d, resulting in a similarity index of 0.45. In both control and ryegrass-amended soils, the number of bands increased to 13 during the 90–180 d incubation period; however, both soils showed very similar bacterial communities (Sørensen index values of 0.5). The three-band pattern of the bacterial population associated with ryegrass disappeared during 0–7 d, but one band reappeared after 90 d.

### *3.4. Alkaline to acid phosphomonoeseterase activity ratio values*

In the Vallobmrosa acidic soil the alkaline to acid phosphomonoeseterase activity ratio increased after the ryegrass amendment, with a significantly higher ($P<0.05$) value after 4 d. Then, the ratio value declined reaching a value similar to that at the beginning of the incubation period after 180 d (Fig. 1). In the Romola neutral soil the alkaline to acid phosphomonoeseterase activity ratio increased rapidly after the ryegrass amendment and were significantly ($P<0.05$) higher from 2 to 90 d (Fig. 2). The ratio had declined by 180 d but was still greater than the initial value (Fig. 2). In the Vicarello alkaline soil,

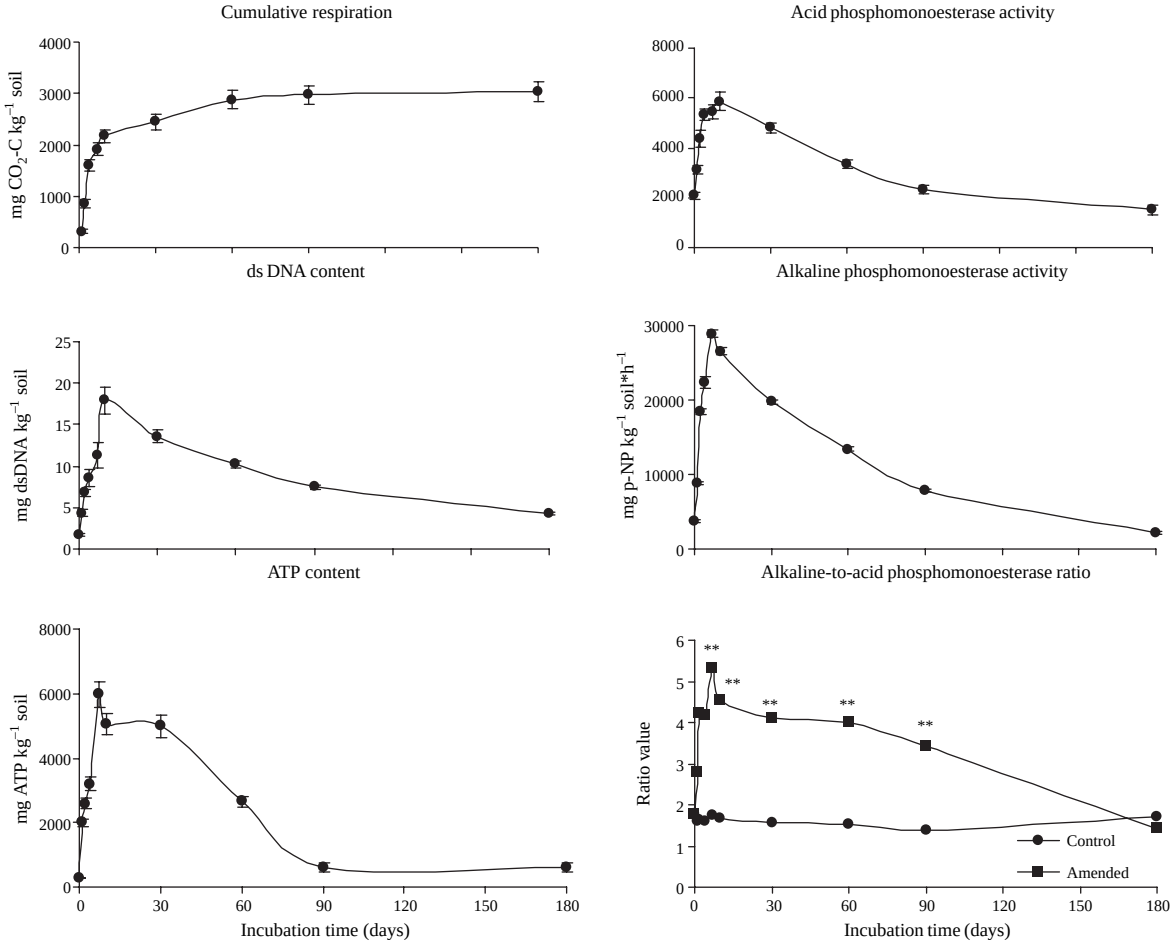


Fig. 2. Cumulative respiration, alkaline and acid phosphomonoesterase activities, alkaline-to-acid phosphomonoeseterase activity ratio, and ATP and dsDNA contents of the Romola neutral soil. Values were calculated by subtracting the values of the control soils from those of the ryegrass-amended soils. Error bars represent the standard deviation of the means ($n=3$). Symbols *, **, and *** in the alkaline-to-acid phosphomonoeseterase activity ratio chart indicate significant differences at $P$ levels of 0.05, 0.01 and 0.001, respectively.

the alkaline to acid phosphomonoeseterase ratio after the ryegrass amendment was only significantly ($P<0.05$) higher after d 2 and remained higher until d 7. After 180 d the ratio declined to values not significantly different from the initial ones (Fig. 3). It is noteworthy to underline that in the acid soil the ratio ranged from 0.2 and 0.6 whereas in the neutral and alkaline soils it was always $>1$.

## 4. Discussion

The rapid increase in enzyme activity after plant residue additions to soils has been generally attributed to enzyme activities directly associated to plant material and to microbial response to soluble sugars of the added residue (Nannipieri et al., 1983). However, neither acid nor alkaline phosphomonoesterase activity were detected in the ryegrass residues, probably due to desiccation and milling of residues prior to their addition to soils. In general, changes of both the acid and alkaline phosphomonoesterase activities during incubation were closely related to those of dsDNA and ATP content (Figs. 1–3) in all soils. The fact that the dsDNA content is a sensitive measurement of microbial growth in soils after addition of organic residue (Marstorp and Witter, 1999) leads us to hypothesise that at least part of the measured enzyme activity was intra- or pericellular. In fact, extracellular enzymes stabilized by soils colloids would not be expected to respond to nutrient amendments. Our results contradicts the general assumption that short-term enzyme assays only measure the activity of the extracellular enzymes (Nannipieri et al., 2002).

The DGGE profiles showed that the three soils had different bacterial communities and the calculated Sørensen indices of similarity were 0.0, for Vallombrosa versus Romola, 0.17 for Vallombrosa versus Vicarello, and 0.17 for Romola versus Vicarello. Such differences in bacterial community composition among the soils confirm that the composition of microflora depends on the soil type and ecological conditions (Tiedje et al., 2001)

Although it is established that bacteria respond more quickly than fungi to nutrient amendments (Wagner, 1975), our monitoring of changes of the soil microflora was

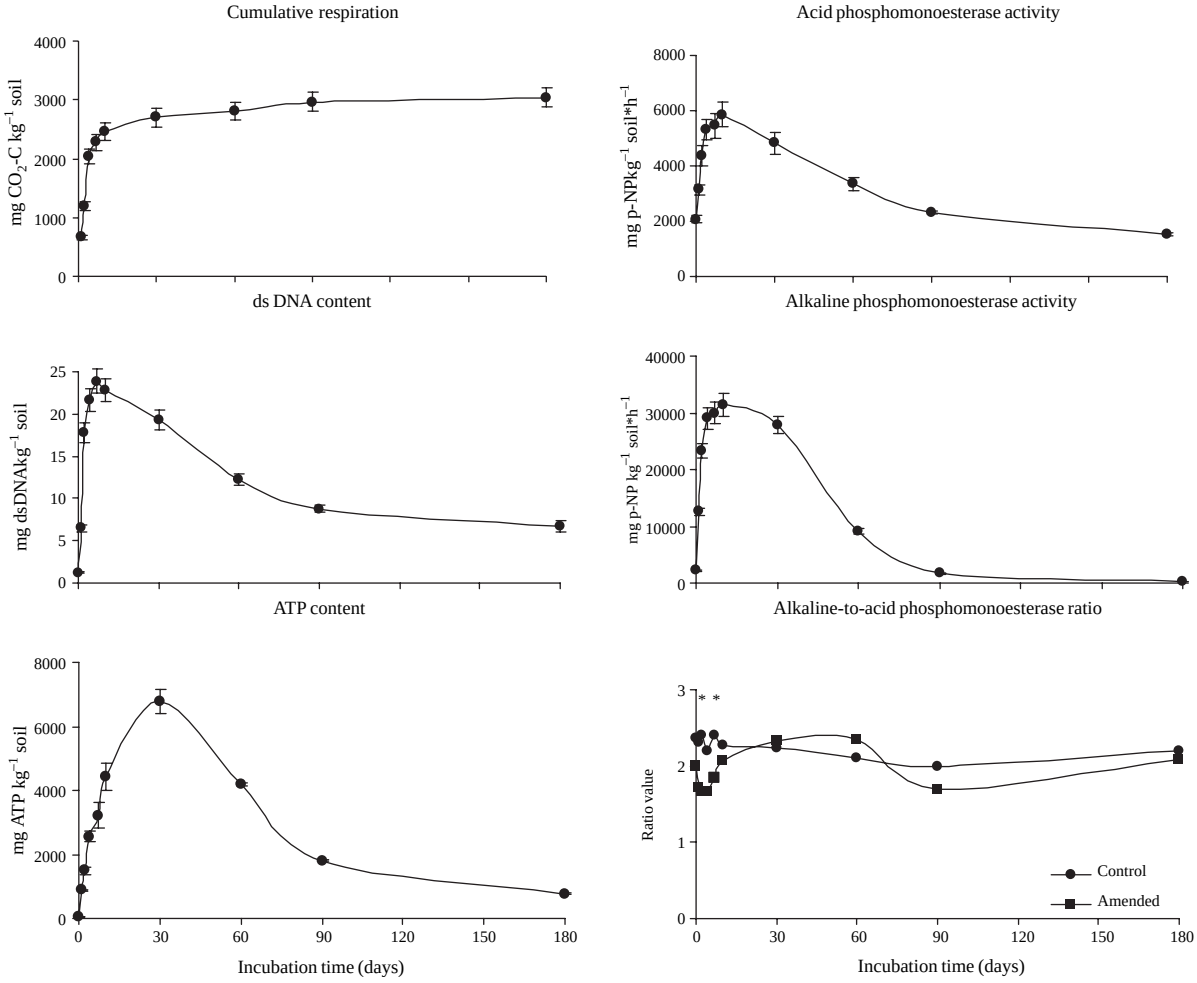


Fig. 3. Cumulative respiration, alkaline and acid phosphomonoesterase activities, alkaline-to-acid phosphomonoeseterase activity ratio, and ATP and dsDNA contents of the Vicarello alkaline soil. Values were calculated by subtracting the values of the control soils from those of the ryegrass-amended soils. Error bars represent the standard deviation of the means ($n=3$). Symbols * in the alkaline-to-acid phosphomonoeseterase activity ratio chart indicate significant differences at $P$ level of 0.05.

incomplete because the composition of the fungal community was not studied and fungal is sometimes greater than bacterial biomass (Killham, 1994). In addition, monitoring the fungal community can be important because acid phosphomonoesterases in soil are produced mainly by plant roots and fungi whereas alkaline phosphomonoesterases are produced by bacteria (Tabatabai, 1994). Protocols for monitoring the fungal communities in soil by PCR–DGGE analysis, without co-amplification of DNA from other eukaryotic organisms such as plants, algae and nematodes have been devised (van Elsas et al., 2000). Our results confirm that acidic soils are unfavorable for bacteria, because the sum of the detectable DGGE bands were 29 and 28 and 13 in the Romola neutral, Vicarello alkaline and Vallombrosa acidic soils, respectively. Of course, the validity of the approach depends on the representativeness of the detected bands.

Persistence of active phosphomonoesterases in ryegrass-amended soils might be due to their adsorption and protection against proteolysis once released as extracellular enzymes or following cell death. Active humic phosphomonoesterases complexes can be formed during humification following the degradation of organic residues (Nannipieri et al., 1996). However, acid and alkaline phosphomonoesterases may have different stabilities at different pH values. Phosphomonoesterases are homodimers containing Zn and Mg ions essential for their activity and they can be unstable at pH values markedly different from to the optimal value due to rearrangements of the enzyme subunits or loss of the metal ions (Coleman, 1992). Therefore, in acidic soils the acid phosphomonoesterase should persist longer after release than the alkaline phosphomonoesterases, whereas in the alkaline soils the opposite might occurr. The low persistence of both acid and alkaline phosphomonoesterase activities in the Romola soil, could be due to other factors such as the heavy texture and the low organic matter content of this soil (Ladd, 1978; Nannipieri et al., 2002).

There was no relationship between bacterial community composition and persistence of the phosphomonoesterase

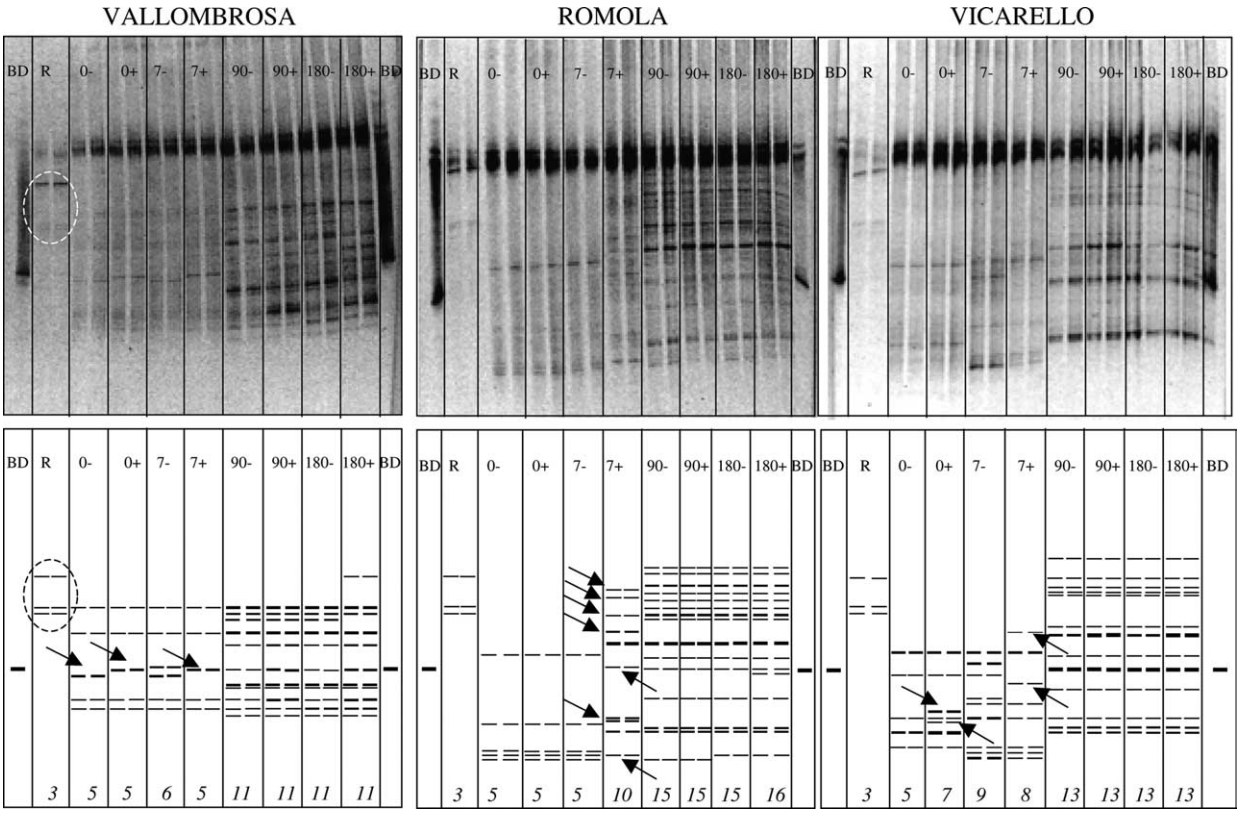


Fig. 4. Real-and schematic 16S rDNA-PCR–DGGE patterns based on total community DNA extracted from control and ryegrass-amended soils throughout the incubation period; 0, 7, 90 and 180 indicate the incubation times (days) and + and − indicate control and ryegrass-amended soils, respectively. Arrows indicate differences in the DGGE profiles. Bands in the dotted circle in the lane 'R' shows bands derived from the bacterial population associated with ryegrass. BD indicate the profile of *B. subtilis* BD 1512-marker. Each lane contains two replicates. Numbers in italics are numerically dominant bacterial populations.

activities in the different soils throughout the incubation period. Hysek and Sarapatka (1998) reported that phosphomonoesterase activity was not correlated to any specific bacterial group because these enzymes were produced by a large fraction of the soil microflora.

Our results also show that the alkaline-to-acid phosphomonoesterase activity ratio in a soil can change after organic amendments (Figs. 1–3) as previously reported (Nannipieri et al., 1979, 1983, 1996b; Frankenberger and Dick, 1983). This should be taken into account when this ratio value is used as an index of soil quality (Dick and Tabatabai, 1992; Dick et al., 2000).

In conclusion, differences in alkaline and acid phosphomonoesterase activities of soils with different pH values generated following organic amendments were related to differences in enzyme production and subsequent persistence as well as soil properties. Phosphomonoesterases with optimal pH value similar to that of soil (alkaline enzymes in alkaline soils) were produced in greater amounts and were more persistent than phosphomonoesterases with optimal pH values different from those of the soil. The comparison of DGGE profiles of the three soils showed that they were characterized by different bacterial communities; they also showed that differences between bacterial communities of control and ryegrass-amended soils only differed in the early stages of plant material decomposition. However, no definite links could be assessed between phosphomonoesterase production and composition of the bacterial community in soil. Further studies based on DGGE of bacterial subgroups and fungal populations are required to better understand changes in soil microflora occurring upon incorporation of plant residues in soils and how these changes are related to phosphomonoesterase synthesis and persistence in soils.

## Acknowledgements

The authors wish to thank anonymous referees for their valuable comments on the manuscript.

## References

Blackmer, A.M., Bremner, J.M., 1977. Gas chromatographic analysis of soil atmosphere. Soil Science Society of America Journal 41, 908–912.

Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology & Biochemistry 14, 423–427.

Ciardi, C., Nannipieri, P., 1990. A comparison of methods for measuring ATP in soil. Soil Biology & Biochemistry 22, 725–727.

Coleman, J.E. 1992 Structure and mechanism of alkaline phosphatase. Annual Review of Biophysiology and Biomolecular Structure 21, 441- 83.

Dick, R.P., Rasmussen, P.E., Kerle, E.A., 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biology and Fertility of Soils 6, 159–164.

Dick, W.A., Tabatabai, M.A., 1992. Significance and potential uses of soil enzymes. In: Metting Jr., F.B., (Ed.), Soil Microbial Ecology. Marcel Dekker, New York, NY, USA, pp. 95-127.

Dick, W.A., Cheng, L., Wang, P., 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biology & Biochemistry 32, 1915–1919.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biology & Biochemistry 9, 167–172.

Felske, A., Rheims, H., Wolterink, A., Stackebrandt, E., Akkermans, A.D.L., 1997. Ribosome analysis reveals prominent activity of an uncultured member of the class Actinobacteria in grassland soils. Microbiology 143, 2983–2989.

Frankenberger Jr., W.T., Dick, W.A., 1983. Relationship between Enzyme activities and microbial growth and activity indices in soil. Soil Science Society of America Journal 47, 945–951.

Garland, J.L., Mills, A.L., 1991. Classification and characterisation of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon source utilisation. Applied and Environmental Microbiology 61, 1458–1468.

George, T.S., Gregory, P.J., Wood, M., Read, D., Buresh, R.J., 2002. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. Soil Biology & Biochemistry 34, 1487–1494.

Griffiths, R.I., Whitely, A.S., O'Donnell, A.G., Bailey, M.J., 2002. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. FEMS Microbiology Ecology 145, 1–9.

Hysek, J., Sarapatka, B., 1998. Relationship between phosphatase active bacteria and phosphatase activities in forest soils. Biology and Fertility of Soils 26, 112–115.

Insam, H., 1997. A new set of substrates proposed for community characterization in environmental samples. In: Insam, H., Rangger, A. (Eds.), Microbial Communities: Functional Versus Structural Approaches. Springer, Berlin, pp. 259-260.

Killham, K., 1994. Soil Ecology. Cambridge University Press, UK.

Klose, S., Tabatabai, M.A., 1999. Urease activity of microbial biomass in soils. Soil Biology & Biochemistry 31, 205–211.

Ladd, J.N., 1978. Origin and range of enzymes in soil. In: Burns, R.G. (Ed.), Soil Enzymes. Academic Press, London, pp. 51-96.

Marstorp, H., Witter, E., 1999. Extractable dsDNA and product formation as measures of microbial growth in soil upon substrate addition. Soil Biology & Biochemistry 31, 1443–1453.

Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) inmicrobial ecology. Antonie van Leeuwenhoek 73, 127–141.

Nakatsu, C.H., Torsvik, V., Øvreas, L., 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil Science Society of America Journal 64, 1382–1388.

Nannipieri, P., Pedrazzini, F., Arcara, P.G., Piovanelli, C., 1979. Changes in amino acids, enzyme activities and biomasses during soil microbial growth. Soil Science 127, 26–34.

Nannipieri, P., Muccini, L., Ciardi, C., 1983. Microbial biomass and enzyme activities: production and persistence. Soil Biology & Biochemistry 15, 679–685.

Nannipieri, P., Sastre, I., Landi, L., Lobo, M.C., Pietramellara, G., 1996. Determination of extracellular neutral phosphomonoesterase activity in soil. Soil Biology & Biochemistry 28, 107–112.

Nannipieri, P., Kandeler, E., Ruggiero, P. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R. (Eds), Enzymes in the Environment. Marcel Dekker, New York, pp. 1-33.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Speir, T.W., Ross, D.J., 1978. Soil phosphatase and sulphatase. In: Burns, R.G., (Ed.), Soil Enzymes. Academic Press, London, pp. 198-250.

Sørensen, T., 1948. A method of establishing groups of equal amplitude in a plant based on similarity of species content and its applications to analysis of vegetation on Danish commons. Biologiske Skrifter 5, 1–34.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology & Biochemistry 1, 301–307.

Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomley, P.S., (Eds.), Methods of Soil Analyses, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, USA, pp. 775-833.

Tiedje, J.M., Cho, J.C., Murray, A., Treves, D., Xia, B., Zhou, J. 2001. Soil teeming with life: new frontiers in soil science. In: Rees, R.M., Ball, B.C., Campbell, C.D., Watson, C.A. (Eds.), Sustainable Management of Soil Organic Matter. CAB International, Wallingford, UK, pp. 393-412.

Torsvik, V., Goksoyr, J., Daae, F.L., Sorheim, R., Michalsen, J., Salte, K. 1994. Use of DNA analysis to determine the diveristy of microbial communities. In: Ritz, J., Giller, K.E. (Eds.), Beyond the Biomass. Wiley, Chichester, pp. 39-48.

Wagner, G.H. 1975. Microbial growth and carbon turnover. In: Paul, E.A., McLaren, A.D. (Eds), Soil Biochemistry vol. 3, Marcel Dekker, New York, pp. 269-305.

Walkley, A., Black, I.A. 1938. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37, 29-37.

Wu, J., Brookes, P.C., Jenkinson, D.S. 1993. formation and destruction of micorbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology & Biochemistry 25, 1435-1441.